Genetic Analysis of Defecation in *Caenorhabditis elegans*

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ABSTRACT

Defecation in the nematode *Caenorhabditis elegans* is achieved by a cyclical stereotyped motor program. The first step in each cycle is contraction of a set of posterior body muscles (pBoc), followed by contraction of a set of anterior body muscles (aBoc), and finally contraction of specialized anal muscles that open the anus and expel intestinal contents (Exp). By testing existing behavioral mutants and screening for new mutants that become constipated due to defects in defecation, I have identified 18 genes that are involved in defecation. Mutations in 16 of these genes affect specific parts of the motor program: mutations in two genes specifically affect the pBoc step; mutations in four genes affect the aBoc step; mutations in four genes affect the Exp step; and mutations in six genes affect both aBoc and Exp. Mutations in two other genes affect the defecation cycle period but have a normal motor program. Sensory inputs that regulate the cycle timing in the wild type are also described. On the basis of the phenotypes of the defecation mutants and of double mutants, I suggest a formal genetic pathway for the control of the defecation motor program.

CAENORHABDITIS elegans provides several advantages for the genetic analysis of behavior. First, the complete structure of the nervous system has been determined by electron microscopy of serial sections of whole animals (WHITE *et al.* 1986). This includes probable assignments of every chemical and electrical synapse, and appears to be largely the same among individuals. Second, the nematode nervous system is simple: it has only 302 neurons that fall into 118 structural classes, and their process morphology and synaptic connections are relatively simple. Third, the nervous system is, to an astonishing degree, dispensable for life under pampered laboratory conditions (*e.g.*, Avery and Horvitz 1989), suggesting that mutants for most genes that control nervous system function and development should be viable. Fourth, behavioral mutants are easy to identify and analyze genetically, because of the many simple and stereotyped behaviors of *C. elegans.*

The nervous system of *C. elegans* acts to coordinate and regulate a wide range of behaviors including pharyngeal pumping (eating), defecation, egg laying, locomotion, foraging movements of the head, avoidance of touch, avoidance of or taxis toward a wide range of noxious or likeable compounds, a complex male mating behavior, avoidance of high temperature and thermotaxis, and a wide range of behavioral responses to the presence or absence of food. Several of these behaviors are under intensive genetic study, and more than 200 genes have been defined by mutations that appear to cause specific nervous system defects. Some genes are required for specification of certain cell fates, others are required for nerve cell differentiation, and others for various other aspects of neuron function.

The simplicity of the *C. elegans* nervous system and its relative dispensability under lab conditions, lead me to believe that most genes affecting the nervous system might be identified in classical genetic screens for behavioral mutants. Extensive searches for mutants defective in egg laying, locomotion, and touch sensitivity have been successfully undertaken (TRENT, TSUNG and HORVITZ 1983; BRENNER 1974; CHALFIE and SULSTON 1981). In addition, many mutations affecting several other behaviors such as pharyngeal pumping (L. AVERY, personal communication), dauer formation (RIDDLE, SWANSON and ALBERT 1981), and osmotic avoidance (CULOTTI and RUSSELL 1978; and my unpublished results), have been isolated. In order to identify all the genes that make a nervous system work, thorough searches must be conducted for as many behaviors as possible. Here, I describe a search for mutants defective in defecation.

MATERIALS AND METHODS

General genetic methods: The standard wild-type strain N2 and most other strains used in this work were obtained from BRENNER (1974) or the *Caenorhabditis* Genetics Center, University of Missouri. Strains carrying *unc-101(sy108)* and *unc-101(rh6)* were obtained from GREGG JONGEWARD. The alleles used are generally the reference alleles (BRENNER 1974; HODGKIN 1988), or sometimes alleles that give a similar or stronger phenotype. The parent of all the strains in this work is the Bristol *C. elegans* strain N2. Methods for culturing, handling, and genetic manipulation of *C. elegans* were as described by BRENNER (1974). Unless stated, genetic

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TABLE 1

Two- and three-factor map data

Gene	Relevant parental genotype	Phenotype picked	Recombinant genotype	Number
aex-1	*aex-1/dpy-5*	Aex	*aex-1/aex-1*	65
			aex-/aex-1 dpy-5	2
	aex-1/dpy-5 unc-13	Dpy non Unc	*dpy-5 aex-1/dpy-5 unc-13*	13
	aex-1/dpy-14 unc-29	Dpy non Unc	*dpy-14/dpy-14 unc-29*	13
			dpy-14 aex-1/dpy-14 unc-29	5
	aex-1/unc-13 lin-10	Unc non Lin	*unc-13 aex-1/unc-13 lin-10*	1
aex-2	*aex-2/lon-2*	Aex	*aex-2/aex-2*	35
			aex-2/aex-2 lon-2	10
	aex-2/lon-2 lin-2	Aex	*aex-2/aex-2*	33
			aex-2/lon-2 aex-2	11
			aex-2/aex-2 lin-2	2
	aex-2/egl-15 sma-5	Egl non Sma	*egl-15/egl-15 sma-5*	12
	aex-2/egl-15 sdc-2	Egl non Sdc	*egl-15/egl-15 sdc-2*	2
	aex-2/unc-115 egl-15	Egl non Unc	*aex-2 egl-15/egl-15 unc-115*	26
		Unc non Egl	*unc-115/unc-115 egl-15*	12
aex-3	*aex-3/lon-2*	Aex	*aex-3/aex-3*	59
			aex-3/aex-3 lon-2	22
	aex-3/dpy-3 unc-2	Aex	*aex-3/aex-3*	53
			aex-3/aex-3 unc-2	1
			aex-3/aex-3 dpy-3 unc-2	1
	aex-3/unc-1 dpy-3	Aex	*aex-3/aex-3*	53
			aex-3/aex-3 dpy-3	1
			aex-3/aex-3 unc-1 dpy-3	1
	aex-3/egl-17 unc-1	Unc non Egl	*unc-1/unc-1 egl-17*	2
aex-4	*aex-4/lon-2*	Aex	*aex-4/aex-4*	14
	aex-4/lon-2 unc-6	Aex	*aex-4/aex-4*	32
			aex-4/aex-4 unc-6	2
			aex-4/aex-4 lon-2 unc-6	2
	aex-4/unc-78 lin-18 lon-2	Aex	*aex-4/aex-4*	28
			aex-4/aex-4 lon-2	2
	aex-4/dpy-23 lon-2	Lon non Dpy	*lon-2 aex-4/lon-2 dpy-23*	6
	aex-4/unc-78 lin-18 lon-2	Lon non Unc	*aex-4 lon-2/unc-78 lin-18 lon-2*	8
			aex-4 lin-18 lon-2/unc-78 lin-18 lon-2	2
			lin-18 lon-2/unc-78 lin-18 lon-2	7
aex-5	*aex-5/dpy-5 unc-75*	Aex	*aex-5/aex-5*	25
			aex-5/dpy-5 aex-5	4
			aex-5/dpy-5 unc-75 aex-5	10
	aex-5/unc-59 unc-54	Aex	*aex-5/aex-5*	42
			aex-5/unc-59 aex-5	3
aex-6	*aex-6/dpy-5 unc-75*	Aex	*aex-6/aex-6*	24
			aex-6/dpy-5 aex-6	4
			aex-6/dpy-5 unc-75 aex-6	7
	aex-6/unc-59 unc-54	Aex	*aex-6/aex-6*	37
			aex-6/aex-6 unc-54	9
dec-1	*dec-1/lon-2*	Dec	*dec-1/dec-1*	12
			dec-1/lon-2 dec-1	11
	dec-1/unc-3 lin-15	Dec	*dec-1/dec-1*	39
			dec-1/dec-1 lin-15	1
exp-1	*exp-1/rol-6*	Exp	*exp-1/exp-1*	19
			exp-1/exp-1 rol-6	2
	exp-1/dpy-10 unc-4	Exp	*exp-1/exp-1*	14
			exp-1/exp-1 unc-4	2
			exp-1/exp-1 dpy-10 unc-4	1
	exp-1/unc-85 dpy-10	Exp	*exp-1/exp-1*	53
			exp-1/unc-85 exp-1	2
			exp-1/exp-1 dpy-10	1

manipulations and experiments were performed at 20°. This paper follows the standard *C. elegans* nomenclature (HORVITZ *et al.* 1979).

The following marker mutations were used.

LGI: *dpy-5(e61)*; *unc-13(e1091)* or *unc-13(e51)*; *lin-10(e1439)*; *unc-29(e1072)*; *unc-75(e950)*; *dpy-14(e188)*; *unc-59*(e261); *unc-54(e190)*; *sDf5*; *sDf6*; *nDf23*; *nDf24*; *eDf3*; *eDf6*; *eDf7*; *eDf9*; *eDf10*; *eDf11*; *eDf13*; *eDf16*.

LGII: *dpy-10(e128)*; *rol-6(e187)*; *unc-4(e120)*;*unc-85(1414)*; *mnDf39*; *mnDf96*.

Gene	Relevant parental genotype	Phenotype picked	Recombinant genotype	Number
exp-2	*exp-2/dpy-11*	Dpy	*dpy-11/dpy-11*	222
			dpy-11/exp-2 dpy-11	1
	exp-2/unc-46 dpy-11	Dpy non Unc	*dpy-11/unc-46 dpy-11*	8
		Dpy Exp(dom)	*dpy-11 exp-2/dpy-11 unc-46*	1
pbo-1	*pbo-1/unc-32 dpy-18*	Dpy non Unc	*pbo-1 dpy-18/unc-32 dpy-18*	8
		Unc non Dpy	*unc-32/unc-32 dpy-18*	6
			unc-32 pbo-1/unc-32 dpy-18	1[a]
	pbo-1/dpy-17 unc-32	Dpy non Unc	*dpy-17/dpy-17 unc-32*	4
		Unc non Dpy	*pbo-1 unc-32/dpy-17 unc-32*	2
	pbo-1/unc-93 dpy-17	Dpy non Unc	*pbo-1 dpy-17/unc-93 dpy-17*	10
			dpy-17/unc-93 dpy-17	4
egl-8	*egl-8/dpy-11*	Pbo	*egl-8/egl-8*	48
			egl-8/egl-8 dpy-11	12
	egl-8/dpy-11 unc-76	Pbo	*egl-8/egl-8*	27
			egl-8/egl-8 dpy-11 unc-76	16
			egl-8/egl-8 unc-76	6
			egl-8/egl-8 dpy-11	1[a]
unc-16	*unc-16/dpy-19 unc-69*	Dpy non Unc	*dpy-19/dpy-19 unc-69*	3
			dpy-19 unc-16/dpy-19 unc-69	2
	unc-16/unc-32 lin-12	Unc-32 non Lin	*unc-32 unc-16/unc-32 lin-12*	2
	dpy-19 unc-16/glp-1	Dpy non Unc	*dpy-19 glp-1/dpy-19 unc-16*	1
		Unc non Dpy	*glp-1 unc-16/dpy-19 unc-16*	4
unc-25	*unc-25/dpy-18 unc-71*	Dpy non Unc-71	*dpy-18/dpy-18 unc-71*	14
			dpy-18 unc-25/dpy-18 unc-71	8
unc-47	*unc-47/dpy-19 unc-69*	Dpy non Unc-69	*dyp-19 unc-47/dpy-19 unc-69*	8
	unc-47/sma-2	Sma	*sma-2/sma-2*	39
			sma-2/sma-2 unc-47	1
	sma-2 unc-47/unc-16	Sma non Unc	*sma-2/sma-2 unc-47*	8
			sma-2 unc-16/sma-2 unc-47	2

[a] These classes are presumed to be due to a double recombination.

LGIII: *dpy17(e164)*; *lon-1(e185)*; *unc-36(e251)*; *dpy-19(e1259)*; *sma-2(e502)*; *unc-32(e189)*; *dpy-18(e364)*; *unc-16(e109)*; *unc-47(e307)*; *unc-69(e587)*; *lin-12(n137 n720)*; *glp-1(e2141)*; *unc-71(e541)*; *unc-47(e542)*; *eT1(III;V)* (ROSENBLUTH and BAILLIE 1981).

LGIV: *unc-5(e53)*; *nT1(IV;V)* (FERGUSON and HORVITZ 1985).

LGV: *unc-34(e566)*; *unc-46(e177)*; *unc-76(e911)*; *dpy-11(e224)*; *unc-42(e270)*; *him-5(e1467)* or *him-5(e1490)*; *sDf26*; *sDf27*; *sDf28*; *sDf30*; *sDf31*; *sDf32*; *sDf33*; *sDf34*.

LGX: *lon-2(e678)*; *dpy-3(e27)*; *unc-2(e55)*; *unc-1(e1598 n1201)* or *unc-1(e580)*; *egl-17(e1313)*; *lin-2(e1453)*; *unc-3(e151)*; *lin-15(n765)*; *unc-78(e1217)*; *lin-18(e620)*; *unc-6(n102)*; *mnDf1*; *mnDf2*; *mnDf5*; *mnDf9*; *mnDf10*; *mnDf11*; *mnDf41*.

Behavioral observations were made using the following strains: CB109 *unc-16(e109)III*, CB156 *unc-25(e156)III*, CB204 *unc-33(e204)III*, CB307 *unc-47(e307)III*, CB362 *unc-44(e362)IV*, CB1197 *unc-44(e1197)IV*, *CB1260 unc-44(e1260)IV*, CB4388 *unc-89(e2338)I*, DA510 *aex-3(ad418)X*, DR1 *unc-101(m1)I*, JT3 *aex-2(sa3)X*, JT6 *exp-1(sa6)II*, JT7 *pbo-1(sa7)III*, JT9 *aex-1(sa9)I*, JT23 *aex-5(sa23)I*, JT24 *aex-6(sa24)I*, JT28 *egl-8(sa28)V*, JT32 *egl-8(sa32)*, JT48 *dec-1(sa48)X*, JT5132 + / *eT1(III)*; *exp-2(sa26)/eT1(V) let(n886)*, JT5244 *aex-4(sa22)X*, PR802 *osm-3(p802)IV*, and PR813 *osm-5(p813)X*.

Mutant isolation: Following EMS treatment (BRENNER 1974), L4 to young adult animals were picked singly to 10 cm plates seeded with bacteria. After about 40 eggs had been laid the parents were removed and the progeny F_1 grown at 25° for 3 days. The F_1 parents and their larval progeny were washed off by flooding the plate surface with M9 solution and gently rocking briefly, then pouring the liquid off. This left on the plate only the eggs from the F_1, which remained stuck to the bacterial lawn. By counting the number of eggs left on the plate after this procedure, I estimate that each F_1 parent contributed 30–40 eggs, so that a heterozygous mutation carried by the F_1 should be homozygous in eight to ten F_2 progeny. The plates were incubated at 25° for about three more days and screened visually for constipated worms. Constipated (Con) hermaphrodites were picked to fresh 5 cm plates at 25°. A candidate was kept for further analysis if some sizable fraction of its self progeny were Con. All candidates were subsequently tested for the mutant phenotype at 20° and analyzed at that temperature if possible.

Mutant mapping: Each mutation was mapped to a chromosome by mating N2 males to mutant hermaphrodites. Cross-progeny males, heterozygous or hemizygous for the defecation mutation, were crossed to two mapping strains, each carrying markers for three of the six *C. elegans* chromosomes (MT3752 *dpy-5(e61)I*; *rol-6(e187)II*; *unc-32(e189) III* and MT465 *unc-5(e53)IV*; *dpy-11(e224)V*; *lon-2(e678)X*). None of the marker mutations affect defecation. Progeny of this cross were picked and allowed to self-fertilize. From those that had inherited the defecation defect (as evidenced by segregating Con progeny), 15–30 Con progeny were picked. Self-progeny of these broods were scored for the segregation of the chromosome marker mutations. Once a mutation was mapped to a chromosome by this method, two- and three-factor crosses (Table 1) of several types (TRENT, TSUNG and HORVITZ 1983) and deficiency map-

ping (Table 2) were used to locate the mutation more accurately. A summary of these results is found on the genetic map in Figure 1.

Complementation tests: For recessive mutations that mapped to the same region, complementation tests were performed to determine allelism. N2 males were mated to hermaphrodites homozygous for mutation one, and the heterozygous (or hemizygous) male progeny were mated to hermaphrodites homozygous for mutation two and marked with a recessive *dpy* or *unc* marker. Cross progeny (nonmarker) were assessed for constipation, and in cases of apparent noncomplementation, at least one hermaphrodite heterozygous for the two mutations was picked and allowed to self-fertilize to be sure all of the progeny were mutant.

Behavioral tests: All behavioral tests were performed with mutations that had been backcrossed to the wild type at least twice. The strains used for the tests are those listed above in the strain list. When there was more than one allele of a gene, the phenotype of a selected reference allele was assessed in most detail, and the other allele(s) were observed to confirm that they shared the same mutant phenotypes. At least ten minutes prior to observation, one or a few animals were picked to a fresh seeded NG plate and allowed to calm down. Care was taken to prevent plate vibrations or air currents from affecting the animal's behavior. Observations were made at 22–24° using a Wild M5 stereo dissecting microscope at 50× magnification. Under these conditions all the major motor steps of the defecation cycle are visible. The pBoc and Exp steps are very clear and can be scored with confidence in every cycle. The aBoc step is somewhat less obvious, and is more easily confused with locomotory movements of the head region. Thus, in occasional cycles it was difficult to be certain whether or not a normal aBoc occurred, and the data for aBoc are to that extent less reliable. With practice, both the anal depressor and the intestinal muscle contractions could be observed. In some cases animals were mounted for Nomarski observation at high magnification (as previously described by SULSTON and HORVITZ 1977) to confirm details of the expulsion muscle contractions. The cycle period of the wild type was found to differ by less than 10% over the temperature range from 20° to 25° (data not shown).

Food regulation tests: Since animals are extremely reluctant to leave a bacterial lawn spontaneously, most of my observations were made with animals picked from food onto agar plates without food. This method has the difficulty that the picking itself might temporarily disrupt defecation. Therefore control experiments were performed with each strain, in which animals were picked to a plate with food and observed over time. For each strain this test showed that there is indeed a delay of one to two minutes before the first defecation, but that subsequently a normal cycle period is observed. In addition, during observations of both N2 and *osm-5* mutant strains, animals were observed to spontaneously leave the bacterial lawn long enough to confirm a dramatic lengthening of the cycle period (data not shown).

Mutants that were normal in defecation: In the process of screening for defecation defects in other behavioral mutants, a number of mutants were observed at the dissecting microscope level to have normal defecation cycles for all three motor steps, and an approximately normal cycle period. In each case a plateful of animals were observed for constipated animals (none were seen), and one or two animals were observed in detail for five to ten cycles. These mutants are: *che-3(e1124)*, *che-14(e1960)*, *daf-10(e1387)*, *egl-38(n578)*, *egl-43(n997)*, *lev-11(x12)*, *mec-3(e1498)* and *mec-3(e1612)*, *nT1*, *osm-3(p802)*, *osm-5(p813)*, *unc-1(n494 n782)*, *unc-2(e55)*, *unc-3(e151)*, *unc-4(e120)*, *unc-5(e53)*, *unc-6(e78)*, *unc-7(e5)* and *unc-7(e139)*, *unc-8(e49)*, *unc-9(e101)*, *unc-10(e102)*, *unc-11(e47)*, *unc-17(e113)*, *unc-18(e81)*, *unc-20(e112)*, *unc-24(e138)*, *unc-26(e205)*, *unc-29(e1072)*, *unc-30(e191)*, *unc-32(e189)*, *unc-35(e259)*, *unc-37(e262)*, *unc-38(e264)*, *unc-39(e257)*, *unc-40(e271)*, *unc-41(e268)*, *unc-42(e270)*, *unc-43(e408)*, *unc-46(e300)* and *unc-46(e642)*, *unc-49(e382)*, *unc-50(e306)*, *unc-53(e404)*, *unc-55(e402)*, *unc-57(e406)*, *unc-58(e665)*, *unc-59(e261)*, *unc-61(e228)*, *unc-62(e644)*, *unc-63(e384)*, *unc-69(e587)*, *unc-70(e524)*, *unc-71(e541)*, *unc-73(e936)*, *unc-74(e883)*, *unc-76(e911)*, *unc-77(e625)*, *unc-79(e1068)*, *unc-80(e1272)*, *unc-81(e1122)*, *unc-83(e1408)*, *unc-84(e1410)*, *unc-86(e1416)*, *unc-103(e1597)*, the double mutant *enu-1(ev419)*; *unc-107(ev411)*, and *vab-7(e1562)*.

Some other mutants may display subtle or inconsistent defects in defecation. It should be emphasized that many of the descriptions that follow are based on limited observations, usually six to eight cycles on two animals. Animals carrying *egl-5(n486)*, *lin-17(n671)*, *lin-17(e1456)*, or *unc-36(e251)* missed a significant fraction of Exp steps. Animals carrying *unc-1(n1167)* or *unc-68(e540)* appeared to have a weaker aBoc than normal. Animals carrying *unc-13(e1091)*, *unc-34(e566)*, *unc-67(e713)*, *unc-104(e1265)*, or *unc-108 (n501)* often had a delayed, weak or absent aBoc. Animals carrying *unc-14(e57)* or *unc-51(e369)* sometimes had delayed or absent aBoc or Exp steps. Animals carrying *unc-31(e169)* or *unc-64(e246)* had a consistently slightly sluggish aBoc contraction. Animals carrying *unc-65(e351)* or *unc-100(su115)* may have a slightly weaker than normal pBoc contraction. Animals carrying *daf-19(m86)* often had a second pBoc contraction present about 10 sec after the first. The second pBoc is weaker than the normal pBoc, and is not followed by the other motor steps (this behavior was observed in many *daf-19* animals). The defects caused by these mutations were deemed too weak or inconsistent to warrant further analysis at this time.

In addition, all body-muscle defective mutants observed have defective defecation. These include mutants for *unc-15*, *unc-54*, *unc-78* (WATERSTON, THOMSON and BRENNER 1980), and *unc-93* (GREENWALD and HORVITZ 1980). The most obvious defect in these mutants is that the defecation body-muscle contractions are weakened, but the Exp-muscle contractions also appear weak, suggesting that these genes function in the anal muscles.

Construction of double mutants: Males heterozygous (or hemizygous) for one mutation were mated to hermaphrodites homozygous for the second. Doubly heterozygous hermaphrodite progeny were allowed to self-fertilize, and progeny homozygous for one mutation were selected on the basis of its phenotype. From their progeny, homozygotes for the second phenotype were selected. In all cases the phenotype caused by one mutation could be scored independently from the other, and that mutation was homozygosed second. In all cases in which the phenotypes of either mutation could not be independently assessed in the double mutant, the presence of that mutation was confirmed by complementation testing: wild-type males were mated to double mutant hermaphrodites, and the resulting male progeny were mated to hermaphrodites homozygous for the relevant mutation and a marker mutation to distinguish cross progeny. Noncomplementation was indicated by the presence of approximately 50% mutant cross progeny.

RESULTS

Description of the defecation behavior: Defecation in *C. elegans* is achieved by periodically activating a

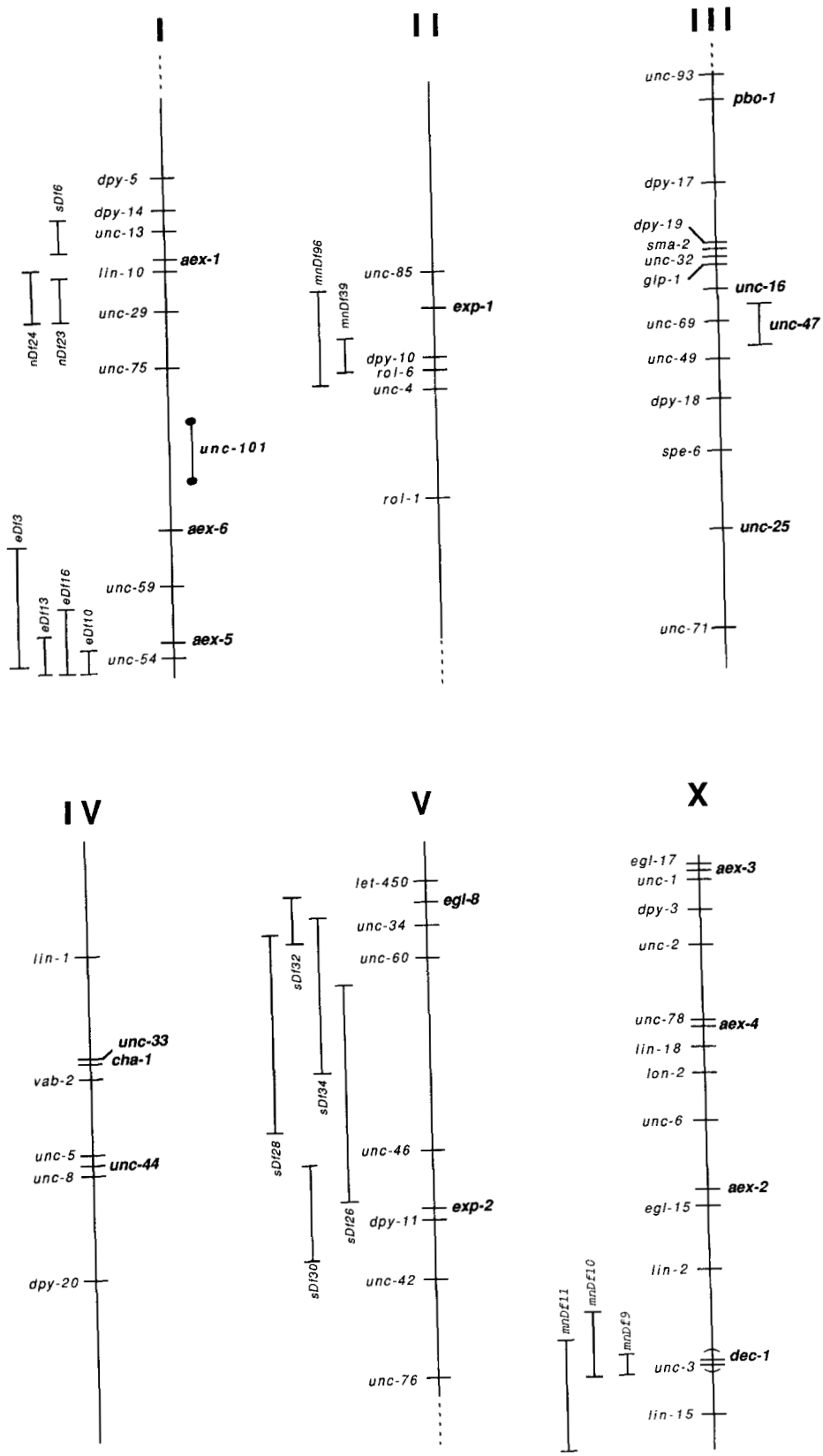

FIGURE 1.—Genetic map. Partial genetic map of *C. elegans*. Shown is the map position for each defecation defective gene discussed in the text (in bold letters to the right of the line) and marker genes and certain deficiencies (to the left of the line). Portions of linkage groups I, II, III, and V are not shown, as indicated by dashed lines. The map data are mostly from Tables 1 and 2, but some data for *unc-16*, *unc-25* and *unc-47*, and all of the data for *unc-101*, *unc-33*, *unc-44* and *cha-1* were obtained from the *Caenorhabditis* Genetics Center, Columbia, Missouri (data not shown).

TABLE 2

Map results with deficiencies

Gene	Deficiency	Complementation
aex-1	*sDf5*	+
	sDf6	+
	nDf23	+
	nDf24	+
aex-5	*eDf3*	−
	eDf6	−
	eDf9	−
	eDf10	+
	eDf11	−
	eDf13	−
	eDf16	−
aex-6	*eDf3*	+
	eDf6	+
	eDf7	+
	eDf9	+
dec-1	*mnDf1*	−
	mnDf2	−
	mnDf5	−
	mnDf9	−
	mnDf10	−
	mnDf11	−
	mnDf41	+
exp-1	*mnDf39*	+
	mnDf96	−
exp-2[a]	*sDf26*	+
	sDf27	+
	sDf30	−
egl-8	*sDf26*	+
	sDf28	+
	sDf31	−
	sDf32	−
	sDf33	−
	sDf34	+

[a] Although *exp-2(sa26)* is dominant for its defecation defect, it also appears to confer recessive lethality (see text). These complementation tests use the recessive lethal phenotype: *sa26/sDf30* is barely viable and is more severely behaviorally defective than *sa26/+*, while *sa26/sDf26* and *sa26/sDf27* animals are indistinguishable from *sa26/+*. Interpreted this way, these data agree closely with the two- and three-factor map data in Table 1.

stereotyped sequence of muscle contractions (first described by CROLL 1975). At 20–25° in the presence of plentiful food the defecation cycle period is 40 to 45 sec, with very little variation (see Tables 4 and 5 and Figure 5). Each cycle begins with the contraction of the posterior body muscles in all four muscle quadrants (Figures 2 and 3), causing the contents of the intestinal lumen to be squeezed anteriorly. About one second later these muscles relax, causing the intestinal contents to accumulate in the preanal region. Less than one second after this relaxation is complete, the body muscles near the head contract in all four muscle quadrants, driving the pharynx back into the anterior intestine (Figures 2 and 3), thereby pressurizing the intestinal contents. Just as the anterior body contraction reaches its maximum, two types of muscle near the anus are contracted simultaneously (Figure 4): the two intestinal muscles (which wrap around the outside of the posterior section of the intestine) contract to further pressurize the intestinal contents, and the single anal depressor muscle contracts to open the anus to permit expulsion. A sphincter muscle, which encircles the join between the intestine and the anus, may also be activated at this time, but is difficult to see clearly (see Figure 4 legend).

Each defecation cycle can be thought of as having three distinct steps: the posterior body muscle contraction (pBoc), the anterior body muscle contraction (aBoc), and the expulsion (Exp), which consists of the intestinal muscle and anal depressor contractions. Each of these three steps appears to be controlled by a separate set of motor neurons (S. MCINTIRE, personal communication; my unpublished observations), and all three steps are coordinately and cyclically activated from some unidentified source.

The defecation cycle is quite regular in a single animal over time, and when comparing cycles in different individuals. In both cases there is a standard deviation of only a few seconds (Tables 4 and 5). The small variation for a single animal is nearly as high as that between animals (*e.g.*, compare Figure 6 and Table 4). In the presence of bacteria, nearly every cycle is the complete sequence pBoc, aBoc, Exp, but rarely the aBoc and Exp are absent. The cycle is regulated by the presence of bacteria and the touch mechanosensory cells (see below).

Constipated mutants: Prior to this work, mutations in two genes, *unc-25* and *unc-47*, were known to specifically affect the Exp step of defecation (S. MCINTIRE, personal communication) and to cause constipation (bloating of the intestinal lumen due to poor defecation). Most of the observations on *unc-25* and *unc-47* mutants reported here were observed first by S. MCINTIRE, and generously communicated to me. The data presented here are my observations, but agree closely with those observed by McIntire. The *unc-25* and *unc-47* mutants indicated that the constipated phenotype is readily scored using a dissecting microscope: the intestinal lumen is enlarged and is clearly visible as a pale stripe running the length of the intestine. While one might guess that mutants that can't defecate should die, this appears not to be true. Even severely defecation-defective mutants are viable and fertile (see below), although somewhat slow growing. These severe mutants seem to survive by rare massive release of the intestinal contents, apparently by sheer internal pressure forcing the anus open. The fact that constipated mutants are readily scored using the dissecting microscope, and that even severely defecation-defective animals are viable, make this behavior well suited to genetic analysis.

The second-generation self progeny of mutagenized hermaphrodites were screened for visibly constipated animals. Such animals were picked, allowed to self-fertilize, and their progeny were examined for

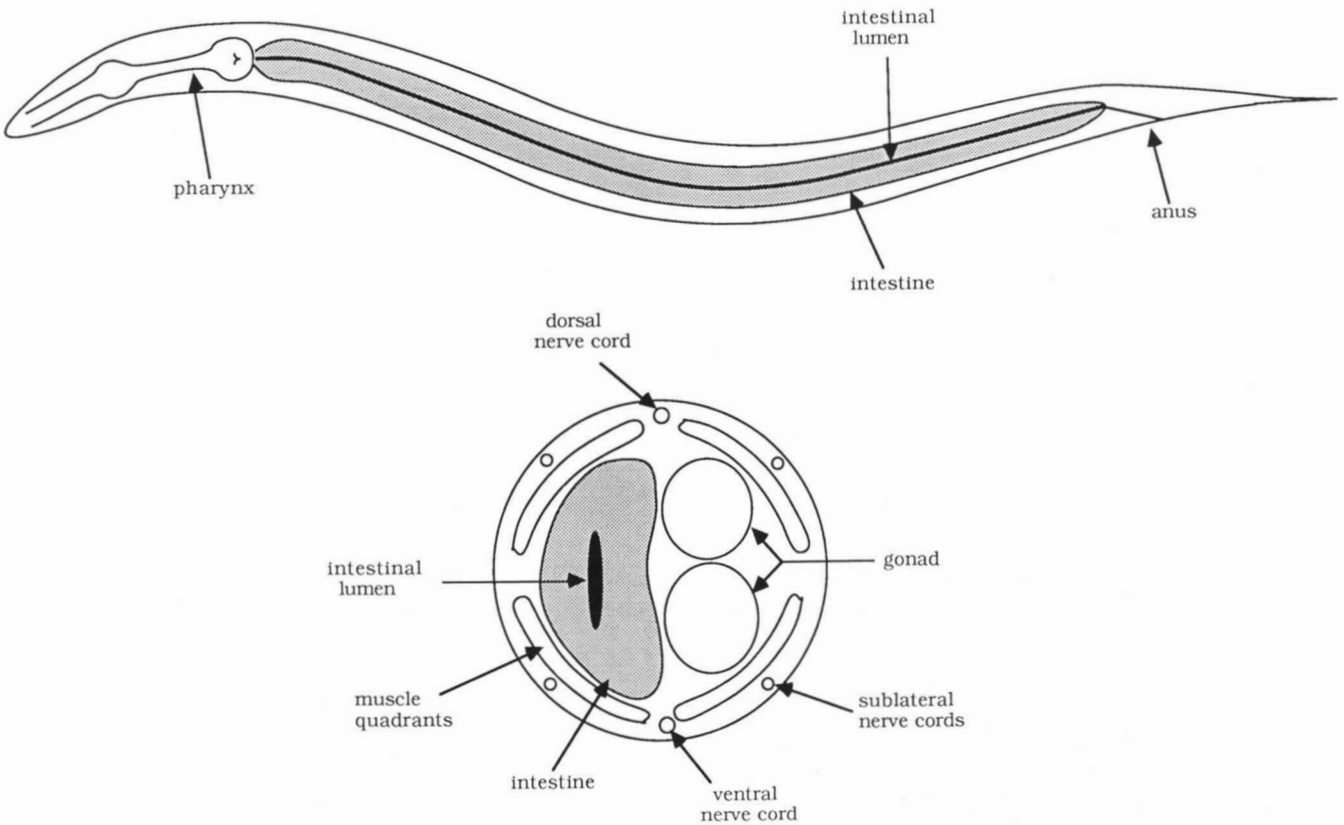

FIGURE 2.—Schematic diagram of the *C. elegans* intestinal tract. The top drawing is a lateral view, emphasizing the digestive tract. Food is pumped by the pharynx from the environment into the lumen of the intestine at its anterior end. The digested food is expelled through the anus at the posterior end. The lower drawing shows a cross section of the body, at about the middle of the anterior half of the body. The four muscle quadrants contain longitudinal fibers that are used for locomotion and for the aBoc step in defecation. A similar arrangement of muscles is found in the posterior part of the body that is used for the pBoc contraction, but the sublateral nerve cords are absent. Only selected parts of the anatomy are shown. Complete descriptions are found in WHITE *et al.* (1986).

constipation. Candidates in which a substantial fraction of the progeny were constipated were further analyzed. Independence of the mutations was ensured by the isolation of no more than one mutant from each mutagenized parent. In one case, two mutations that caused different phenotypes were isolated from one parent, and were subsequently shown to lie in different genes. About 4000 mutagenized genomes have been screened thus far, and 23 constipated mutants isolated. In addition, one EMS induced mutation, *aex-3(ad418)*, was sent to me by L. AVERY.

The 24 mutations were mapped initially to chromosomes as described in MATERIALS AND METHODS. Three-factor crosses and deficiency mapping (Tables 1 and 2) were used to determine a more-or-less precise map position for each gene. In addition, three-factor crosses were used to refine the map location of *unc-25* and *unc-47*. A partial genetic map showing the location of these genes is shown in Figure 1. All recessive mutations that mapped to the same region were tested for complementation with a representative recessive mutation in each other gene. In many cases the mutation causing the defecation defect was mapped with respect to the closest mapped genes. In these cases I am fairly confident that the mutation defines a new gene. In other cases the map position is not yet as refined, but the entire region to which the mutation mapped was examined for previously identified mutations that might define the same gene. In fact, two new alleles of *unc-25* and four new alleles of *egl-8* were identified in this screen. All of the other defecation mutations tentatively define new genes. The screens for constipated mutants are clearly not saturated, since I have isolated more than one mutation in only six of the 13 genes that are known to mutate to a constipated phenotype (Table 3).

Other defecation mutants: I have also looked through existing behavioral mutants (uncoordinated, pumping defective, egg-laying defective, etc.) for more subtle defects in defecation, which might not cause constipation. Although not previously noticed, several of these mutants have interesting defects. (For a list of mutants with normal or nearly normal defecation, see MATERIALS AND METHODS.) Mutants that were identified in this way include those carrying mutations in *unc-16, unc-33, unc-44* and *unc-101*,

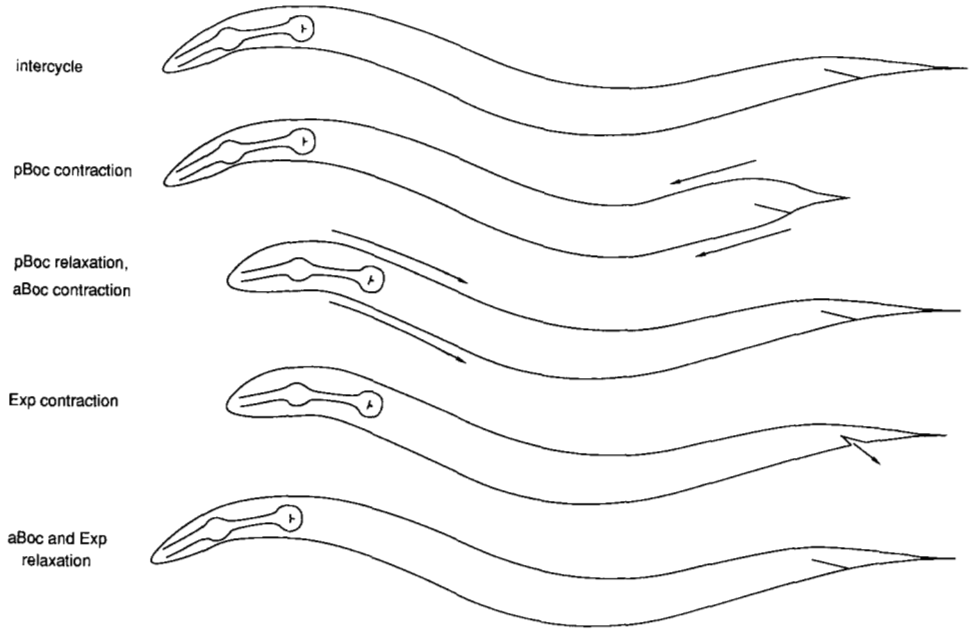

FIGURE 3.—Schematic diagram of motor steps involved in defecation. The top drawing shows a left lateral view of an animal in its typical intercycle configuration. The next three drawings depict the three steps in defecation that occur each cycle, as described in the text. Note that, unlike for locomotion, in which the dorsal and ventral muscle quadrants are alternately contracted to produce dorsal/ventral bends, the pBoc and aBoc contractions occur in all four muscle quadrants, resulting in longitudinal compression of the body in the region.

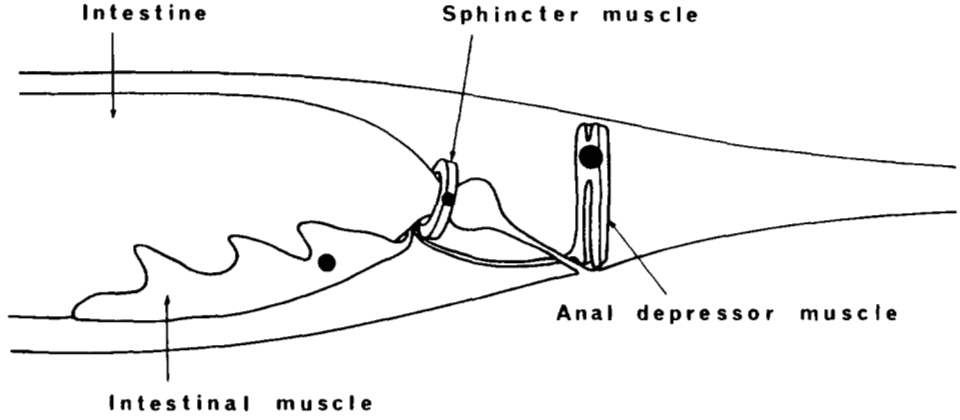

FIGURE 4.—Diagram of the anal muscles in the hermaphrodite. The three classes of muscles that control the Exp step of defecation are shown. The anal depressor muscle is H-shaped and has muscle fibers that are oriented dorso-ventrally. It is attached at its dorsal end to the body wall and at its ventral end to the dorsal-posterior wall of the anus. It contracts to lift the dorsal surface of the anus. The intestinal muscles are sheet-like and extend anteriorly along the intestine and wrap part of the way around the intestine dorsally. They contain longitudinal muscle fibers and attach to the intestine, and along their ventral edge to the body wall. The sphincter muscle encircles the join between the intestine and the anus. It contains circumferential muscle fibers. The sphincter muscle is difficult to see using a compound microscope and its movement is very fast. It is therefore uncertain what its action is, but freeze-frame video observations suggest that it stretches open (perhaps passively) during the aBoc and contracts very fast just as expulsion occurs, simultaneously with the other Exp muscle contractions (my unpublished observations). The intestinal and anal depressor muscles send arms to the preanal region where they receive synaptic input along with the sphincter muscle. All three classes of muscles are coupled together by gap junctions on these arms. The description of the morphology of these muscles is based on WHITE *et al.* (1986). Reprinted with permission from WHITE *et al.* (1986).

which are specifically defective in aBoc, and mutants for *cha-1* (choline acetyltransferase deficient), which have a longer defecation cycle period. *unc-16* was poorly mapped at the time of this study, so I used three-factor crosses to map this gene more accurately (see Table 1 and Figure 1). The number of mutations in each gene and a summary of their phenotypes are found in Table 3.

Summary of the mutants

Expulsion specific mutants: Mutations in four genes, *unc-25, unc-47, exp-1* and *exp-2*, cause a defect

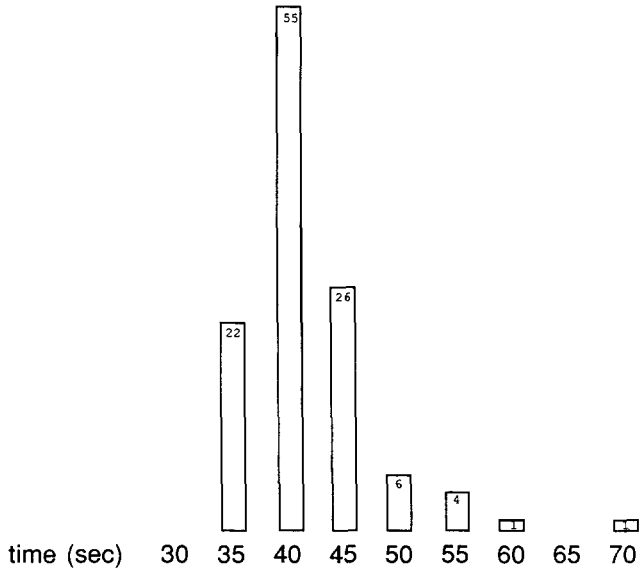

FIGURE 5.—Bar graph of the wild-type cycle period. The number of cycles of each length for thirteen wild-type animals are shown on a bar graph. Records were made at five second intervals. The mean and standard deviations for these animals are found in Table 4.

specific to the Exp step of the defecation motor program. In each of these mutants the remaining aspects of the defecation cycle are approximately normal, including the cycle period (Table 4). The typical cycle in these mutants is composed of a normal pBoc and aBoc, but the anal depressor and intestinal muscle contractions that cause expulsion are usually absent (Figure 6).

aBoc specific mutants: Mutations in four genes, *unc-16*, unc-33, *unc-44* and *unc-101*, specifically affect the aBoc step of the motor program, leaving pBoc, Exp, and cycle timing unaffected (Table 4). Mutations in these genes cause only marginal and variable constipation. Therefore, aBoc defects would not be found in the constipation mutant screen. Of these four genes, only mutants for *unc-16* are fully defective in aBoc. Mutants for *unc-33*, *unc-44* and *unc-101* lack aBoc in about half of their cycles; in the remaining half of the cycles the aBoc is either normal or somewhat weak (the amplitude of the pharyngeal motion is reduced). The fact that *unc-16* mutants, which lack any visible aBoc contraction, are at most slightly constipated indicates that aBoc is not crucial for defecation, but does seem to be required for maximal expulsion efficiency.

pBoc specific mutants: Mutations in two genes, *pbo-1* and *egl-8*, cause a fairly specific defect in the pBoc step of the motor program. In mutants for each gene, the pBoc step is usually absent or is present but weak, in that the extent of posterior body muscle contraction is greatly reduced. Mutants for each gene are moderately to severely constipated, implicating pBoc as a crucial step in the cycle. Inclusion of *egl-8* as a pBoc specific gene is somewhat uncertain, since some *egl-8* animals are also significantly Exp defective (see below). Mutants for both genes have a normal cycle period.

Aex mutants: Mutations in six genes, *aex-1* through *aex-6*, cause a defect in both the aBoc and Exp steps of the motor program, without affecting pBoc. The details of the phenotypes differ somewhat from gene to gene, and are described below for each separately. Mutants for all of the genes have an approximately normal cycle period (Table 4), and are constipated to varing degrees. Animals carrying some Aex mutations [*e.g.*, *aex-1(sa9)*] have never been observed to produce an active defecation (see below), suggesting that the defecation motor program is not essential for viability.

Cycle period mutants: Mutations in two genes, *cha-1* and *dec-1*, specifically affect the cycle period. The motor program, when it is activated, is normal in appearance, but the cycle period is much longer than normal and is more variable in duration (Table 5). *cha-1* mutants have this phenotype throughout larval growth. *dec-1(sa48)* animals have a normal cycle period as larvae and very young adults, but sometime shortly after adulthood consistently acquire a long irregular cycle.

Catalog of the genes

Exp genes:

unc-25(e156, sa4, sa25, 3 other alleles) and unc-47(e307, e542, e707): I consider these genes together because they constitute a distinct phenotypic class. Mutations in both genes cause a similar defecation defect and cause a distinctive Unc phenotype in which animals briefly hypercontract ("shrink") in response to touch. Mutations in both genes also affect levels of the neurotransmitter γ-aminobutyric acid (GABA) as detected by anti-GABA staining, implicating GABA in the control of the Exp motor step (S. MCINTIRE, personal communication). I have identified two new mutations in *unc-25*, *sa4* and *sa25*, by their constipated phenotype, while the four already existing alleles of *unc-25* were identified by their Unc phenotype. All six alleles appear very similar and result in both the Unc and Exp phenotypes (S. MCINTIRE, personal communication; and my observations), so it is clear that the two defects are caused by mutations in a single gene. Animals carrying any of the *unc-25* mutations have a very similar defecation defect, but *unc-25(e156)* animals have been most closely analyzed. *unc-25(e156)* animals lack an Exp step in about seven of eight cycles (117 of 133 cycles examined), but are quite normal in all other aspects of defecation. When an Exp is present it appears normal, and strong contractions of the intestinal muscles and anal depressor muscle are clearly visible. This similar residue of occasional normal Exp steps is seen in all *unc-25* alleles observed, and is similar to the phenotype of *unc-47* and *exp-1* mutants. It is interesting to note that the rare Exps in

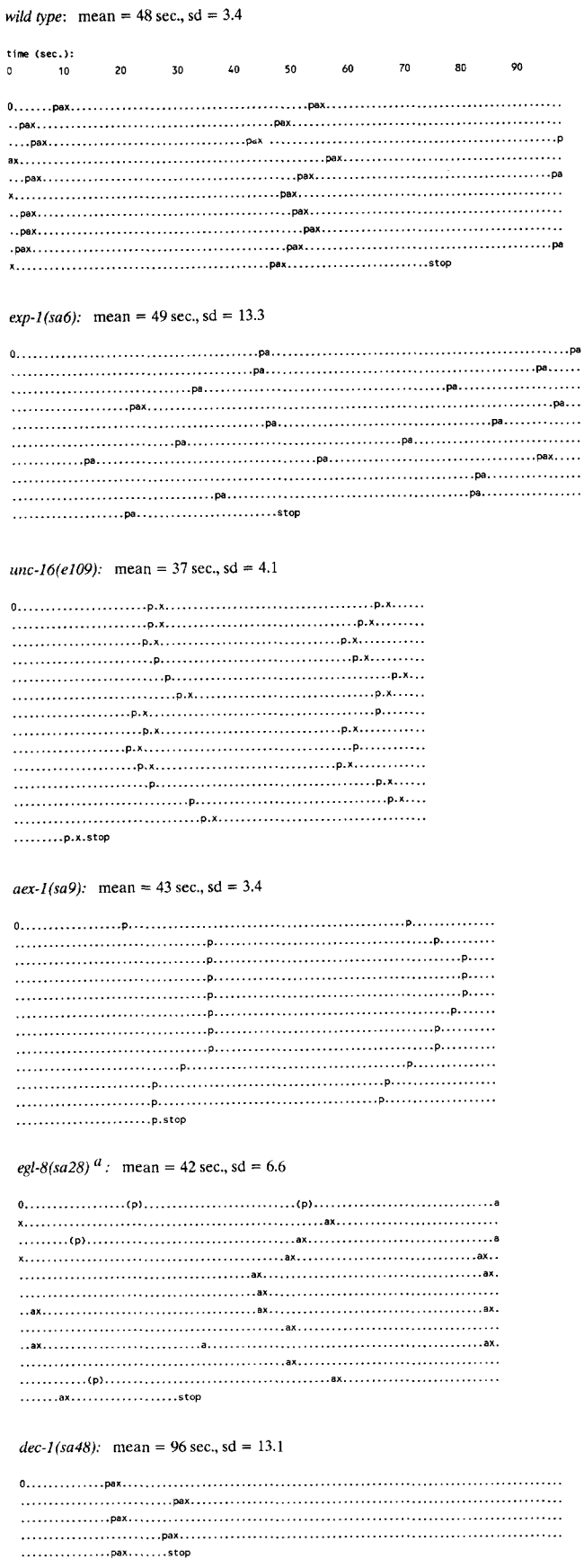

FIGURE 6.—Defecation cycles for the wild type and selected mutants. Observations on actual single animals are shown for the wild type and each class of mutant. The length of the dotted time line on each record has been selected to match twice the cycle period for the particular animal, in order to graphically emphasize the periodicity of the cycle (for *dec-1* the line is one cycle long due to the longer cycle period). The periodicity of the *exp-1* cycle is less obvious, because every time an Exp does occur (about one in six cycles), it is followed by an unusually long intercyucle (this pattern was observed in all *exp-1* animals). The mean intercycle time and standard deviation listed is, in each case, for the individual animal. These individual numbers can be compared with the average for the mutant from several animals in Table 4. For the *egl-8* record the (p) refers to the weak pBoc step characteristic of the mutant. The weak pBoc is present in each cycle but is shown only for cycles in which the time of the defecation was not marked by the aBoc and Exp steps. p = pBoc, a = aBoc, and x = Exp.

[a] This record is for the genotype *egl-8(sa28)/sDf31*. This genotype has a cycling pattern similar to *egl-8(sa28)* homozygotes (see text).

unc-25 mutants are not randomly spaced but show a clear supercycle (mean inter-Exp period = 8.6 cycles, standard deviation = 2.9).

No new mutations in *unc-47* were identified in this study. All three existing *unc-47* mutations cause phenotypes very similar to *unc-25* mutations: shrinker-Unc, and Exp usually absent (S. MCINTIRE, personal communication; and my observations). *unc-47(e307)* animals missed 105 of 127 Exp observed, a frequency that is not significantly different from that of *unc-25*.

exp-1(sa6): exp-1(sa6) causes an Exp phenotype that is identical to that caused by the *unc-25* and *unc-47* GABA-defective mutants (Table 4 and Figure 6). In 128 cycles observed in *exp-1(sa6)* animals, there were 17 normal expulsions. However, *exp-1(sa6)* animals are not shrinker-Unc, and GABA staining is at least grossly normal (S. MCINTIRE, personal communication). The similarity of the Exp phenotype with that of *unc-25* and *unc-47* mutants suggests that these genes affect a common process (also, see below). The phenotype of *exp-1(sa6)/mnDf96* (a deficiency that deletes *exp-1*) animals is similar to *exp-1(sa6)* homozygotes.

exp-2(sa26sd): sa26/+ animals display an intriguing range of phenotypes: they are egg-laying defective, pump abnormally, move abnormally, and are severely Exp defective. In 110 cycles observed *sa26/+* animals never had a normal expulsion. Occasionally they release some intestinal contents through the anus, but this release does not appear to be associated with any visible anal muscle contractions. The passive release seems to be due to sheer internal pressure in the intestine, and is seen in mutants for *aex-1* and *aex-2* as well. In mutants for all three genes, passive release is sometimes not associated with the defecation cycle, instead occurring in the intercycle period. The dominance of *sa26* is due to a gain of function, since deficiencies of the locus show none of these defects. When heterozygous to a deficiency of the locus (Table 2), *sa26* animals are barely viable and are more severely Unc. *sa26/sa26* animals are phenotypically similar to the heterozygotes but arrest growth as larvae

TABLE 3

Summary of defecation mutants

Gene	Phenotypes	Number of alleles
aex-1 I	Very rare or never aBoc or Exp, severely Con	4
aex-2 X	Very rare or never Exp, aBoc absent or mistimed, severely Con	2
aex-3 X	aBoc and Exp present about every fifth cycle, slightly Con	2
aex-4 X	aBoc and Exp rarely present, severely Con	1
aex-5 I	aBoc and Exp rarely present, severely Con	4
aex-6 I	aBoc and Exp rarely normal, possible weak aBoc more often, severely Con, slightly egg-laying defective, lethargic	1
cha-1 IV	Very long and irregular cycle period, not Con (but pumping severely deficient), severe Unc	Many
dec-1 X	Longer cycle period as adult, defecation motor steps unaffected, severely Con	1
egl-8 V	pBoc weak but normally timed, severely to moderately Con, lethargic, egg-laying defective	5[a]
exp-1 II	Exp present about every sixth cycle, severely to moderately Con, slightly egg-laying defective	1
exp-2 V	Dominant mutation, Exp rarely or never present, jerky Unc, Egl, pumping abnormal, severely Con	1
pbo-1 III	pBoc very weak to absent, normally timed when present, severely Con, very slow growing	1
unc-16 III	aBoc absent, very sluggish Unc, not Con	1
unc-25 III	Exp present about every eighth cycle, severely to moderately Con, GABA abnormal, shrinker Unc	6[b]
unc-33 IV	aBoc often absent or weak, severe Unc, process guidance defects, subteral cords absent, not Con	5
unc-44 IV	aBoc often absent or weak, severe Unc, process guidance defects, not Con	11
unc-47 III	Exp present about every eighth cycle, severely to moderately Con, GABA abnormal, shrinker Unc	3
unc-101 I	aBoc often absent, when present slightly delayed, not Con, very sluggish Unc	3

All of the mutations in *unc-16, unc-47, unc-33, unc-44, unc-101* and *cha-1* were previously identified by others. The description of *cha-1* is partly based on data communicated by J. RAND.

[a] Four alleles of *egl-8* were isolated in this study, one was previously isolated.

[b] Two alleles of *unc-25* were isolated in this study, four were previously isolated.

for unknown reasons (it is not certain that this recessive-lethal phenotype is caused by *sa26*, but the two have not been separated in mapping crosses). The pharynx in *sa26*/+ animals pumps very fast, but the muscle contractions appear incomplete. *sa26*/+ animals' locomotion is also abnormal. They often jerk rapidly forward or backward and then suddenly halt and lie motionless for many seconds. They respond to touch with an eyelash with abnormally vigorous movement interspersed with brief sudden pauses. The coordination of their body muscles during locomotion appears otherwise normal. *sa26*/+ animals are also egg-laying defective, becoming bloated with late-stage eggs but not forming bags of worms (TRENT, TSUNG and HORVITZ 1983). These behavioral phenotypes all map very close to each other, and the fact that the phenotypes are all conferred dominantly strongly suggests that a single mutation causes them. It is unlikely that the Exp defect of *sa26* results from defective muscle structure, for several reasons. First, the anal depressor muscle appears normal using polarized light microscopy, indicating a well-ordered muscle structure. Second, the broad behavioral pleiotropies caused by *sa26* are most simply interpreted as affects on neurons controlling diverse behaviors. Finally, the body muscles of *sa26* animals function normally, at least in generating coordinated contractions.

aBoc genes:

unc-16(e109): *unc-16(e109)* animals invariably lack a detectable aBoc contraction, while other aspects of the cycle, including the cycle period, are normal (Table 4 and Figure 6). The amount of material expelled by *unc-16* animals must be close to normal, since they are at most slightly constipated. *unc-16* mutants are also very sluggish and slightly dumpy. Their locomotory movements are well coordinated but are slow. Little is known about the Unc defect of *unc-16*. The fact that only one allele of *unc-16* was isolated in extensive screens for Unc mutants, despite its obvious Unc phenotype, suggests that *e109* may not reflect the null phenotype for this gene.

unc-33(e204, four other alleles) and unc-44(e1197, ten other alleles): I discuss these genes together because they form a phenotypic class for a wide range of mutant defects. The phenotype of mutants for either gene is: nearly paralyzed curly posture, slightly dumpy, PDE axon outgrowth abnormal, weak FITC uptake into the amphid neurons, phasmid process outgrowth abnormal (HEDGECOCK *et al.* 1985), slow pumping, and poor dauer formation (my observations). Mutants for either gene have very similar defects in the aBoc step: about half the time the aBoc contraction is absent, but the remainder of cyles have a normal to weak aBoc contraction that is often slightly delayed. Mutants for both genes also miss some Exp steps. The high degree of similarity between the detailed phenotypes of these two genes indicates that they function together in the same process, probably mediating neuronal process outgrowth (HEDGECOCK *et al.* 1985). *unc-33* mutants also lack the four

TABLE 4

Cycle periodicity in defecation mutants

Genotype	Mean period[a]	Standard deviation[a]	Number of cycles	Number of animals	Standard deviation of the means[b]
Wild type	42	5.7	115	13	4.7
aex-1(sa9)	37	7.1	109	10	5.8
aex-2(sa3)	48	14.8	104	10	9.5
aex-3(ad418)	44	10.7	105	10	9.2
aex-4(sa22)	31	4.0	142	10	1.2
aex-5(sa23)	42	10.9	101	10	8.6
aex-6(sa24)	37	6.9	110	10	4.2
egl-8(sa28)	46	17.9	116	10	5.3
exp-1(sa6)	45	8.5	117	10	4.5
exp-2(sa26)/+	48	14.5	109	10	9.7
unc-25(e156)	40	6.7	113	10	3.2
unc-47(e307)	47	9.2	105	10	8.0
pbo-1(sa7)	45	11.3	134	10	7.7
unc-16(e109)	36	5.8	112	10	3.9
unc-33(e204)	46	7.6	99	10	5.5
unc-44[c]	44	11.3	90	10	9.8
unc-101(m1)	39	5.8	105	10	8.0

The data include observations of at least three animals for 16 min each, and at least 8 cycle periods for the remaining animals. The ages of the animals tested were not exactly known, but in all cases they were young adults, well within the age period over which the wild type shows little variation in cycle length (see Table 5). The wild-type data are for animals approximately 70 hr old. Analysis of variance indicates that there is significant variation among individuals of most genotypes, including the wild type, but these differences are slight. Analysis of variance also shows that many of the mutants are significantly different from the wild type, but again these differences are small (as can be seen from the means in the table).

[a] Mean and standard deviation for all the recorded cycle periods.

[b] Unweighted standard deviation among the means of individual animals.

[c] Data accumulated for three alleles of *unc-44*: *e362*, *e1197*, *e1260* (alleles were not significantly different from each other).

homologous sublateral nerve cords posterior to the nerve ring (J. SULSTON, personal communication; *unc-44* mutants have not been tested for this defect). Each of the four sublateral nerve cords is composed of a process from each of five cells, and the cords run adjacent to the four body-muscle quadrants posterior to the nerve ring (Figure 2). Since this is the region in which the body muscles contract to generate the aBoc, the sublateral cords may be implicated in controlling aBoc.

unc-101(m1,sy108,rh6): Mutants for *unc-101* move extremely sluggishly and tend to coil. Each mutation causes a similar defect in aBoc, but *m1* has been analyzed in most detail. *unc-101(m1)* does not affect the cycle period or the pBoc and Exp steps. In 54 cycles observed, aBoc was absent in 25 cycles, and was present in 29 cycles. When present the aBoc was nearly always slightly delayed with respect to the Exp step. These defects are very similar to those caused by *unc-33* and *unc-44* mutations.

pBoc genes:

pbo-1(sa7): Mutants for *pbo-1* have either a very weak or undetectable pBoc, while aBoc, Exp, and cycle period are grossly normal. Animals homozygous for *sa7* are very slow growing, taking about five days to reach adulthood from conception, rather than the normal three. Their slow growth is probably not due directly to the defecation defect, since the more severe constipation caused by other mutations (for example, *aex-1(sa9)*) has only a slight effect on growth rate.

egl-8(sa28, sa32ts, sa46, sa47, n488): This gene was previously identified by the single mutation *n488* (TRENT, TSUNG and HORVITZ 1983), and I found four new alleles in this study. In *egl-8(sa28)* animals the pBoc contraction is invariably weak to very weak, but can almost always be detected. Some *egl-8* animals had normal aBoc and Exp steps, but other animals lacked an Exp in most cycles. In an animal of genotype of *egl-8(sa28)/sDf31* (deletes *egl-8*) the Exp step was present in 19 of 23 cycles, suggesting that this may be the null phenotype of mutations in *egl-8*. *sa28*, *sa47* and *n488* are strong alleles of *egl-8*, *sa46* is similar but somewhat weaker in phenotype, and *sa32* is temperature sensitive. At 15° *sa32* animals are nearly wild type, while at 25° they appear similar to animals carrying a strong allele. All five mutations also cause animals to be lethargic in movement and moderately egg-laying defective.

I have performed temperature-shift experiments with *egl-8(sa32)* animals. Adult *sa32* animals were allowed to lay eggs for 12 hours at 15° on a set of plates. Each 24 hours thereafter, for four days, one plate was shifted to 25°. After the animals reached adulthood, they were scored for constipation, the strength of their pBoc, and their egg-laying defective and sluggish phenotypes. Regardless of the animal's developmental stage at the time of temperature shift, the animals were as severely defective for all of the behaviors as the parent grown at 25°. By the time of the last shift the animals had just reached young adulthood at 15°. Seven hours after the temperature shift these animals had weak pBocs and 24 hr after the shift they were indistinguishable from *sa32* animals grown at 25°. Conversely, when young adult animals raised continuously at 25° were shifted to 15°, within 24 hours they were similar to the wild type in all behaviors. These results indicate that the *egl-8* gene is required continuously for these behaviors, and is not required specifically during development.

Aex genes:

aex-1(sa9, sa10, sa27, sa49): Each mutation in *aex-1* causes similar phenotypes, but the alleles differ somewhat in expressivity. *sa9*, the most severe mutation, causes the absence of an active Exp in every cycle (none observed in 109 cycles), but a weak aBoc may be present in occasional cycles. *aex-1(sa9)* animals are severely constipated. As with *exp-2* mutants, *aex-1(sa9)* animals passively release intestinal contents occasionally, in a manner not associated with the defecation

TABLE 5

Cycle period mutants and effects of age

Genotype	Age (hr)	Mean period	Standard deviation	Number of cycles	Number of animals	Standard deviation of the means
Wild type	50	42	4.6	68	8	3.2
	72	40	3.4	64	7	1.6
	94	46	3.5	60	7	1.6
	119	66	15.2	54	7	12.6
	142	59	14.6	43	7	13.3
dec-1(sa48)[a]	72	48	8.3	90	10	7.9
	94	99[b]	60.6	45	8	57.4
	110	112[b]	39.4	32	7	31.4
cha-1(p1152)	80–100[c]	200[b]	184	13	5	166
cha-1(cn101)	80–100	157[b]	265	18	5	456
cha-1(p1186)	80–100	>600[d]		0	3	

Means and standard deviations are as in Table 4. Analysis of variance indicates that all the *cha-1* mutants and the older *dec-1* mutants are significantly different from the wild type of the same age ($P << 0.01$).

[a] The *dec-1(sa48)* data include seven animals that were observed at each time point.

[b] These means are systematically underestimated because of occasional long cycles compared to the length of each assay (from 10 to 16 min each).

[c] The exact age of the *cha-1* animals was not determined, however they were in the range of 80 to 100 hr old, as estimated by morphology. It is not clear that age is directly meaningful for *cha-1* animals since they mature much more slowly than the wild type.

[d] One defecation was observed in a total of 30 min of observation.

cycle (see above). *sa10*, a weak *aex-1* mutation, causes aBoc and Exp to be absent in about two out of three cycles. When aBoc or Exp is absent, the other is generally also missing. Presumably because of its less severe Aex defect, *sa10* animals are only moderately to slightly constipated. The mutations *sa27* and *sa49* appear to be intermediate in severity between *sa9* and *sa10*. It is unlikely that *aex-1* mutations directly affect muscle structure: the anal depressor muscle appears normal using polarized light microscopy, the anterior body muscles are normal in structure and in their locomotory function, and the weaker alleles of *aex-1* have normal aBoc and Exp steps interspersed among fully Aex-defective cycles.

aex-2(sa3, sa21): An active Exp is always absent (in 104 cycles observed), but occasionally a passive release occurs in a manner very similar to *aex-1* and *exp-2* mutants. aBoc is absent about half the time, and when present it is often aberrantly timed. When present, the aBoc is always found within a few seconds of the expected time relative to pBoc, but can occur any time from about 3 sec *before* pBoc to a few seconds later than normal. This variability is seen for both mutations. It is simpler to suppose that it is the aBoc timing that is variable (rather than the pBoc), since the aBoc is often absent, while the pBoc shows no sign of abnormality. Both alleles result in severe constipation. *aex-2* mutations are unlikely to affect muscle structure for the same reasons given for *aex-1*, except that there are no weaker alleles of *aex-2*.

aex-3(ad418, sa5): In *aex-3(ad418)* mutants aBoc and Exp are both missing in about five out of six cycles (106 of 128 cycles observed). *aex-3(sa5)* missed aBoc and Exp in 23 of 30 cycles observed, a frequency that is not significantly different from that of *ad418*. aBoc and Exp, when present, were invariably present together (in the 128 cycles observed, clear aBoc and Exp contractions were observed 22 times, each time together). The likelihood of these data assuming uncorrelated aBoc and Exp steps is about 10^{-17}. I have no evidence as to whether the two *aex-3* mutations leave some residual gene function, or the absence of *aex-3* function does not fully abolish aBoc and Exp. Both alleles of *aex-3* display synthetic phenotypes in combination with mutations in *unc-31*: the double mutants are dauer constitutive and strongly pumping defective, phenotypes not caused by either mutation alone (L. AVERY, personal communication; my data, not shown). Double mutants between an allele of each of the other five *aex* genes and *unc-31(e169)* have been constructed, and none of these double mutants displayed this synthetic phenotype. Both mutations in *aex-3* cause mild constipation.

aex-4(sa22): In *aex-4(sa22)* animals there is an aBoc and Exp together once every six to eight cycles. In most other cycles both are absent, but occasionally a possible weak aBoc is observed without an associated Exp. *aex-4(sa22)* causes only moderate constipation, consistent with its incomplete Aex defect.

aex-5(sa23, sa41, sa42, sa43): In *aex-5(sa23)* animals there is about one active Exp in every ten cycles (eight out of 101 cycles observed). aBoc is usually absent or weak, but is sometimes present and close to normal, even in cycles without an Exp. The other *aex-5* mutations cause similar defects. All mutations cause moderately severe constipation. *aex-5(sa23)* appears similar in phenotype when heterozygous to deficiencies of the locus.

aex-6(sa24): *aex-6(sa24)* animals have a normal aBoc and Exp together about once every ten cycles (11 out

of 110 cycles observed). In about one third of the other cycles there is a weak aBoc contraction without an Exp. *sa24* animals are moderately severely constipated and are also lethargic and slightly egg-laying defective. All of these mutant phenotypes map very close to each other, but it is not proven that they are due to a single mutation.

Cycle period genes:

cha-1 (many alleles): This gene encodes choline acetyltransferase, the enzyme that synthesizes the neurotransmitter acetylcholine (RAND 1989). Strong reduced-function mutations in *cha-1* dramatically reduce synthesis of acetylcholine (RAND and RUSSELL 1984; RAND 1989). The defecation-cycle defects of *cha-1* mutants were first observed by J. RAND (personal communication). *cha-1* mutations cause variable and often very long intercycle periods (Table 5). For *cha-1(p1186)* only a single defecation was observed during 30 minutes of observation. *cha-1(p1152)* and *cha-1(cn101)* animals were less severely defective, but had mean cycle periods at least four to five times longer than normal, and were highly irregular (Table 5). It is likely that *cha-1* mutations affect the cycle period rather than the motor steps of defecation, because when a defecation was observed it appeared normal.

dec-1(sa48): Animals carrying *dec-1(sa48)* (*dec*ecation *c*ycle defective) have a normal cycle period as larvae and very young adults, but become defective as adults (Table 5). At the time they first express their cycle period defect, the *dec-1* animals are still mid-adults and appear otherwise healthy, fertile, and normal. The cycle period increases slowly with increasing age in the wild type (Table 5), initially suggesting that aging might be involved in the altered cycle period observed in *dec-1* animals. However premature aging is unlikely to explain the *dec-1* phenotype, since the change in cycle period is abrupt and results in a cycle period longer than any found in the wild type (at least up to one day post-fertility age). *dec-1(sa48)* animals are moderately to severely constipated.

Double mutants: I have constructed a variety of double mutants among pairs of defecation mutations. These doubles were mostly between mutations causing different defects. The double mutants (*aex-1;unc-16*, *exp-1;egl-8*, *exp-1;unc-16*, *unc-16unc-25*, *unc-25; aex-3*, *unc-25;egl-8*, *unc-25;unc-33*, and *unc-25;unc-44*) were always found to have the phenotype expected from the sum of the two single mutant defects. For example, the double mutants between the aBoc defective mutation *unc-16(e109)* and expulsion defective mutations in *unc-25* or *exp-1* were aBoc and Exp defective. Indeed the defecation phenotype of these doubles is very similar to that of an Aex single mutant. One of these double mutants, *unc-25(e156); aex-3(ad418)*, gave an interesting result that is more informative. *unc-25* mutants miss about seven of eight Exp steps, while *aex-3* mutants miss both aBoc and Exp together in about five of six cycles. The *unc-25;aex-3* double mutant had ten Exps in 94 cycles observed (each of these Exps was associated with a normal aBoc, as in *aex-3* alone, see above). Thus, for the Exp defect, these mutants are not synergistic, since the Exp frequency is not significantly different from that of *unc-25* alone. Other *unc-25* double mutants with *aex* genes were constructed, but the other Aex mutants were too severe to effectively assess the residual Exp frequency.

I constructed one double mutant between two members of a single phenotypic class, *exp-1* and *unc-25*. The purpose of this double mutant was to test whether the residual frequency of Exp in each single mutant (about one Exp per eight cycles) would be affected. The double mutant showed a frequency of Exp indistinguishable from each single mutant alone: *exp-1(sa6)* had 17 Exp/128 cycles; *unc-25(e156)* had 16/133; and the double mutant *exp-1(sa6); unc-25(e156)* had 7/66. This result suggests that both *exp-1* and *unc-25* affect the same underlying process, and that blocking this process causes animals to miss Exp in only seven of eight cycles.

Sensory regulation of the defecation cycle:

Food: When a well-fed worm is first removed from bacteria it immediately stops the normal defecation cycle and adopts a long and highly irregular defecation cycle (Table 6). This food regulation is presumably aimed at maximizing nutritional uptake while minimizing energy expenditure. I attempted to test what sensory inputs are important for the food regulation of defecation. To test whether chemosensation is important, I followed defecation cycles in *osm-3* and *osm-5* mutants. *osm-3* and *osm-5* mutations disrupt nearly all known chemosensory responses with high penetrance, by affecting the structure of sensory cilia (PERKINS *et al.* 1986). The defecation motor steps and the cycle period in both mutants were normal in the presence of food, and both responded appropriately to the absence of food (Table 6). From more limited observations, *che-3*, *daf-10* and *daf-19* mutants (mutants for these genes also have defective chemosensation) also appear to be approximately normal in cycle period in the presence of food (data not shown). These results suggest that chemosensory input is dispensable for food regulation of the defecation cycle.

Pumping: It seemed logical that the actual intake of food might regulate the defecation cycle. In order to test this possibility, I observed defecation in two mutants with defective pharyngeal pumping, but with normal sensory behaviors. Mutants carrying *eat-2(ad465)* pump very slowly and irregularly and therefore ingest food poorly (L. AVERY, personal communication). Mutants carrying *unc-89(e2338)* also ingest food poorly due to a defect in pharyngeal muscles (WATERSTON, THOMSON and BRENNER 1980; L. AVERY, personal communication). Both mutants have a

TABLE 6

Effects of food, chemosensory defects, and pumping defects on cycle time

Genotype	Food	Defecations per 10 min[a]	Minutes observed	Number of animals
Wild type	+	14.3	81	13
Wild type	−	0.5	56	5
osm-3(p802)	+	12.7	85	10
osm-3(p802)	−	1.4	49	5
osm-5(p813)	+	14.3	69	10
osm-5(p813)	−	1.3	48	5
eat-2(ad465)	+	10.7	71	10
unc-89(e2338)	+	10.1	94	10

All of the mutants in the presence of food have overall standard deviations, and standard deviations of the means of different animals, that are no more than 8, except *unc-89*, which had an overall standard deviation of 13.0 and a standard deviation of the means of 10.3 (standard deviations calculated on the basis of cycle period in seconds, as in Tables 4 and 5). Analysis of variance shows that *eat-2* and *unc-89* animals have cycle periods significantly longer than the wild type (for *eat-2*, F = 200, degrees of freedom 1 and 189; for *unc-89*, F = 174, degrees of freedom 1 and 174). The Mann-Whitney U test gives similarly highly significant differences.

[a] The data are presented in this form (rather than by cycle period as in the preceding tables), because defecations in the absence of food are so rare as to make a meaningful assessment of the cycle period very difficult.

cycle period that is significantly, but only slightly, longer than the wild type (Table 6). Thus, severely impaired food intake causes a significant lengthening of the defecation cycle period, but the effect is so slight as to be difficult to interpret.

Touch: One well characterized sensory stimulus in *C. elegans* is touch with an eyelash hair (CHALFIE and SULSTON 1981; CHALFIE *et al.* 1984). Touch in the head region causes a worm to move backward, touch in the tail makes a worm move forward. In order to test whether touch affects defecation, I followed the defecation cycle in an individual wild-type animal for several cycles, until a clear cycling pattern was established (Figure 7). I then touched the worm near the head at some time during the subsequent cycle and observed when the next defecation occurred. After allowing at least one further cycle to elapse to be sure of the new cycle phase, I repeated the touch procedure. The results of three rounds of such touches are shown in Figure 7. In this experiment, the touch was delivered approximately 20 sec after the preceding defecation. I found that the subsequent defecation was delayed in each case for approximately 20 sec.

A similar experiment was conducted on different wild-type animals, and with the same or different times for delivery of the touch. Table 7 shows the results of these experiments. The defecation following the touch was delayed for approximately the length of a single normal cycle, regardless of when the touch was delivered. Touch to the head appears to reset the defecation cycle clock to approximately zero or "start" (that time in the cycle just following a defecation). Touch to the tail may also reset the clock in a similar way, but less reliably (data not shown). In a control experiment, I conducted eight trials similar to those described above, with each of three *mec* mutants (*mec-4*, *mec-5* and *mec-9*). Defecation timing was unaffected by touch in each *mec* mutant, suggesting that the touch-reset phenomenon is mediated by the normal touch-cell sensory pathway (CHALFIE and AU 1989).

DISCUSSION

The defecation defects are probably due to defects in muscle activation: The normal functioning of muscles depends on two major classes of genes: those that are directly required to make the contractile apparatus of the muscle, and those that are required for regulation of contraction of the muscle by the nervous system. All of the genes discussed here are likely to affect the regulation of muscle contraction. For most of the mutants, I know that the relevant muscles *can* contract normally, either because they function intermittently in defecation, or because they are used normally in their alternate role in locomotion. Since the muscles *can* contract normally, their mutant defect must concern whether the muscles *will* contract. For mutants in three genes, *exp-2*, *aex-1* and *aex-2*, the anal depressor and intestinal muscles have never been observed to contract. Nevertheless, I think that even in these cases the genes are likely to function in regulating contraction, for reasons detailed for each gene in RESULTS.

Each motor step is activated independently from the other steps: Mutations in four genes eliminate or partially eliminate aBoc, but do not affect the timing or activity of pBoc or Exp. Mutations in four other genes eliminate or partially eliminate Exp, but have no effect on the timing or activity of aBoc or pBoc. Mutations in *pbo-1*, and much of the time *egl-8*, have a severely weakened or absent pBoc step but do not affect aBoc or Exp. These findings suggest that each of the three motor steps in defecation is independently activated, and that eliminating one step does not necessarily affect the others. The case is weakest for pBoc, since no mutant fully abolishes a visible pBoc step.

In contrast, the two muscle types that are observed to contract during the Exp step, the anal depressor and intestinal muscles, are almost always coordinately activated. All mutations that abolish one contraction also abolish the other, and for mutants in which one contraction is sometimes present, it is strongly correlated with contraction of the other. This finding is not surprising, since the anal depressor and intestinal muscles are joined by gap junctions (WHITE *et al.* 1986). It is therefore reasonable to consider these muscles as a single motor step (Exp). The gap junctions between the muscles seem not, however, to be required for their proper activation, since killing

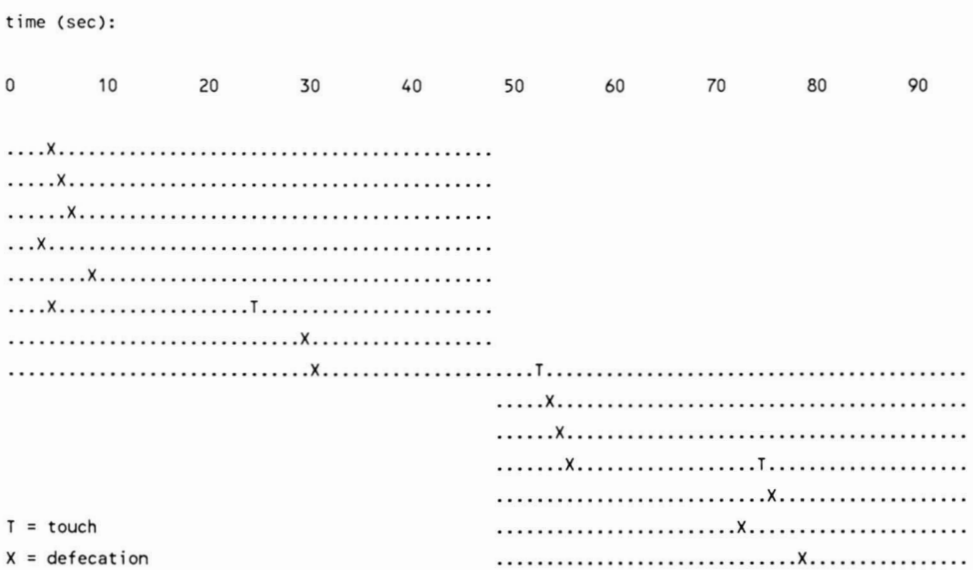

FIGURE 7.—Touch resets the cycle clock. Shown is the actual record for one wild-type animal. The dotted time line has been drawn to best display the effect of touch on the cycle: it matches the animal's cycle period, and is offset in one spot to best show the repeated reset. Individual pBoc, aBoc, and Exp steps are not shown.

TABLE 7

Touch resets the cycle clock

Time of touch from last cycle	Time to next cycle	Standard deviation	Time of apparent delay	Number of cases
20	51	7.9	23	8
25	54	6.7	31	7
30	52	9.2	34	12
35	53	9.1	40	5
Totals for all times:	53	8.2		32

All times are in seconds. Touch was usually two, or sometimes one or three, touches with an eyelash to the region just posterior to the pharynx, or in one case a vigorous tap of the plate on the table top. Records were kept for each type of stimulation and there was no significant variation among them, so the data are presented together. In two cases out of 34 total tested there appeared to be no delay of the cycle due to the touch; in both cases a relatively weak movement in response to the touch was noted. Since these two cases were clearly different (both defecated exactly at the expected time for no touch effect) they were not included in the table.

either the intestinal muscles or the anal depressor muscle with a laser microbeam leaves the other muscle class functional and correctly timed (my data, not shown).

aBoc and Exp share a functional step separate from pBoc: Despite clear evidence that aBoc and Exp are independent motor steps, mutations in six genes affect both steps. This fact suggests a model in which there is some process or neuron that coregulates these two motor steps, and that the six *aex* genes affect that process. In this hypothesis, mutations that separately abolish either Exp or aBoc, affect steps downstream from the *aex* genes (Figure 8). Another observation strongly supports this model: the occasional aBoc and Exp steps seen in *aex-3* mutants are always found together in the same cycle. The simplest explanation

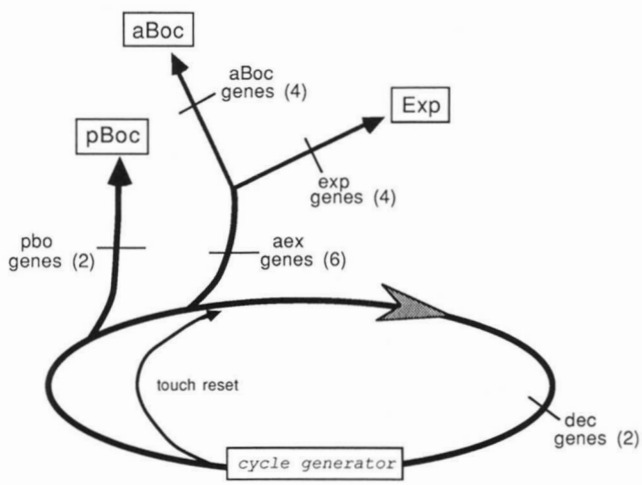

FIGURE 8.—Model of the genetic pathway for neuronal control of defecation. This arrangement of steps in defecation is the most parsimonious explanation of the mutant classes. The point in the pathway affected by each class of genes is indicated by the cross bars. The touch reset short-circuits the pathway and returns the cycle generator to "zero," or the point just after defecation. The relative positions of the *dec* genes and the touch-reset bypass are drawn arbitrarily, no tests have been performed to distinguish their order.

for this result is that *aex-3* mutations block a single step in the motor activation process, and that occasionally activation passes this block, resulting in both aBoc and Exp together.

Timing of the motor steps: The simple observation that the defecation motor steps are activated periodically and in a stereotyped timed order suggests that there is a neuronal timer that controls defecation. Various other observations support this model, most notably the reset effect of eyelash touch on the cycle

timing. In addition, mutations in two genes, *cha-1* and *dec-1*, affect the cycle period without noticeably affecting the motor actions themselves. The possibility that cyclical changes in intestinal lumen pressure account for the cycle period timing is unlikely, since mutations in many genes cause severe constipation but have a normal cycle period. If this observation were restricted to mutants for a single gene or class of genes, it might be argued that the mutant animals were defective in assessing lumen pressure, however mutations in many genes from three phenotypic classes give the same result. Nor is increased lumen pressure generated by the preceding motor step likely to explain the activation of any of the individual motor steps: *pbo*-1 mutants, which often lack any detectable pBoc contraction, activate aBoc at the correct time, and *unc-16* mutants, which lack an aBoc contraction, activate Exp at the normal time. The relative timing of the various motor steps is also unlikely to be fully explained by a long range humoral effect, since the aBoc and Exp muscles are separated by approximately 1 mm in the adult animal, and yet the time of their relative contractions is controlled with a precision on the order of 1/10 of a second. A humoral mechanism could, however, explain the control of cycle period.

Where does the cycle period timer reside? It probably does not reside in any of the component motor steps, because none of the mutants that affect the specific motor steps have a large effect on cycle period, nor do the cycle period mutants affect the motor steps. I propose that there is a separate neuron (or neurons) that acts as a timer to activate the motor neurons for the various defecation steps. Firm evidence for such a timer must await identification of both the motor neurons for the individual motor steps and neurons that affect timing but not the motor steps. Such identification can be achieved in *C. elegans* using a laser microbeam to kill identified neurons (CHALFIE *et al.* 1984; AVERY and HORVITZ 1989).

Locomotion and defecation use the same body muscles differently: One striking observation is that very few of the *unc* genes that are required for coordinated locomotion in *C. elegans* are required for pBoc and aBoc, even though both use the same body muscles. The *unc* mutants that have a normal aBoc and pBoc include several mutants, such as *unc-5* and *unc-6*, that cause severe and pleiotropic locomotory motorneuron abnormalities (E. HEDGECOCK, J. CULOTTI, and S. MCINTIRE, personal communications). This fact suggests that pBoc and aBoc activate the same set of muscles by a separate neuronal pathway from that used for coordinated locomotion.

Formal pathway for control of defecation: The finding that a large number of defecation genes fall into five simple phenotypic classes, permits the construction of a formal genetic pathway for control of defecation, found in Figure 8. The three classes of genes that are specifically involved in aBoc, Exp, or pBoc are readily interpreted as affecting independent motor processes. The class of six genes that affect both aBoc and Exp is interpreted as affecting a single process that is required for both motor steps. Finally, the class of two genes that alter the cycle period is interpreted as affecting a functionally distinct cycle period timer. The placement of these genes in the pathway is provisional, since the null phenotype is not clearly established for any of the genes.

From this genetic model, the important question arises: what do these formal steps correspond to in the real nervous system? I hypothesize that each class of mutants affects a distinct class (or classes) of neurons, and that the normal function of each of these classes is reflected in the mutant phenotypes. In particular, each of the pBoc, aBoc, and Exp classes results from defects in the motor neuron (or neurons) for their respective muscle contractions. The Aex class corresponds to an interneuron(s) that regulates both the aBoc and Exp motor neurons, and the cycle period class corresponds to an interneuron(s) that keeps time and cyclically activates first the pBoc motor neuron, and shortely thereafter the Aex interneuron. This model can be tested by searching for the hypothesized neurons, using a laser microbeam to kill individual identified neurons and observing the effects on defecation.

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