MALE PHENOTYPES AND MATING EFFICIENCY IN *CAENORHABDITIS ELEGANS*

JONATHAN HODGKIN

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH England

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ABSTRACT

Mating behavior in adult male nematodes can be assayed by mating efficiency, *i.e.*, the number of cross progeny sired by males under standard conditions. Mutant males from 220 strains, representing most of the known complementation groups of *C. elegans*, have been examined for mating efficiency and for anatomical abnormalities of the specialized male copulatory organs. These data extend the phenotypic description of these mutants and indicate what anatomical and behavioral components are necessary for the ability to mate successfully. Also, mutants with specific defects in the male were sought by establishing superficially wild-type hermaphrodite stocks after mutagenesis and testing the males segregated by these stocks for mating efficiency. Forty-nine of 1119 stocks yielded abnormal males. Seventeen were characterized in detail and found to be abnormal in sensory behavior (carrying mutations in the genes *che-2* or *che-3*) or male genital anatomy (carrying mutations in one of the genes *mab-1* to *mab-10*). Four of the *mab* (male abnormal) genes affect specific postembryonic cell lineages.

THE nematode *Caenorhabditis elegans* has been the subject of several investigations in behavioral genetics (for review see WARD 1977; DUSENBERY 1980). The small size of this organism and its essentially invariant neuroanatomy have allowed reconstruction of the entire wild-type hermaphrodite nervous system from serial electron micrographs (WHITE *et al.* 1976 and personal communication). As a consequence of the short life cycle and the self-fertilizing habit of the hermaphrodite, many behavioral mutants have been isolated and characterized genetically. This paper reports some data on mating behavior in *C. elegans* and its mutants and the results of a search for mutants with specific defects in mating behavior.

C. elegans has two sexes, self-fertilizing hermaphrodite (karyotype 5AA,XX) and male (karyotype 5AA,XO). Mating behavior is observed only in the adult male, which has a more complex nervous system than the adult hermaphrodite or larvae of either sex. The complete neuroanatomy of the adult male has not been established, but all that is known is consistent with the assumption that the extra neuronal circuitry in the male controls mating behavior. This extra circuitry develops late in larval life (HODGKIN 1974; SULSTON and HORVITZ 1977;

SULSTON, ALBERTSON and THOMSON 1980) so it may be more amenable to developmental analysis than the rest of the nervous system.

Mating behavior is not required for the maintenance of a mutant stock (because hermaphrodites can self-fertilize), so in principle many mutants affecting mating can be isolated and studied. Two approaches have been taken in looking for mutants with defective mating behavior. First, mutants in most of the known complementation groups of *C. elegans* have been tested for male mating efficiency. Some mutants with extreme defects, such as paralysis or gross deformity, are unable to mate successfully, but many males with marked behavioral or anatomical abnormalities can still mate, albeit inefficiently. These tests provide extra information on the phenotype of each mutant. Furthermore, the information on mating efficiency is technically useful from a genetic point of view, because genetic experiments are sometimes facilitated by the use of homozygous males rather than the conventional use of heterozygous males (BRENNER 1974).

Second, a large number of mutagenized hermaphrodite stocks were established, and the males segregated by these stocks were tested for mating efficiency. The single hermaphrodites used to found each stock were picked as superficially wild type in phenotype, in the hope of finding mutants with defects primarily or exclusively in the male. Mutants with specific defects in mating behavior should be detected by this search. A number of male defective strains were obtained and characterized.

These results illustrate the kinds of mutant lesion that can interfere with a complex piece of behavior such as mating. Also, the results are relevant to the question of how many genes in this organism are sex limited in their expression.

MATERIALS AND METHODS

Most methods of genetic analysis were as described previously (BRENNER 1974; HODGKIN, HORVITZ and BRENNER 1979).

Mating tests: Two tests of mating efficiency were employed. One was quantitative: Six males and six *dpy-11(e224)* hermaphrodites at late L4 stage were placed together on a crossing plate (*i.e.*, a 5-cm NGM Petri dish spread with a central 1-cm spot of bacteria) and males removed after 24 hr. Total cross progeny (non-Dpy F_1) were counted. For wild-type males, the number of cross progeny is approximately proportional to the number of parental males, up to six (Figure 1). Varying the size of the spot of bacteria had little effect on mating efficiency, except that very small spots reduced the efficiency.

The other test was qualitative: Seven to ten L4 or young adult males were crossed with ten young adult *unc-17(e245)* hermaphrodites for 4 days, and total cross progeny were estimated by eye. Uncoordinated hermaphrodites were used in this test because old or mutant males are often incapable of fertilizing hermaphrodites that can move well, as shown for males of *unc-3(e151)* in RESULTS.

Origin of mutants: Most stocks were obtained from the collection established by BRENNER (1974). I am grateful to many other workers for supplying mutants, especially M. CHALFIE, G. N. COX, J. G. CULOTTI, H. F. EPSTEIN, D. HIRSH, H. R. HORVITZ, M. KUSCH, J. A. LEWIS, D. L. RIDDLE, J. E. SULSTON and R. H. WATERSTON.

Temperature-sensitive mutants were grown and tested at the restrictive temperature (25°). Experiments with all other strains were carried out at 20–22°.

Origin of males: Most *C. elegans* stocks are maintained as hermaphrodites, and males appear in these stocks only at low frequency (0.2%). To obtain enough males to examine and test, several different strategies were employed. In some cases, the mating efficiency of mutant males was high

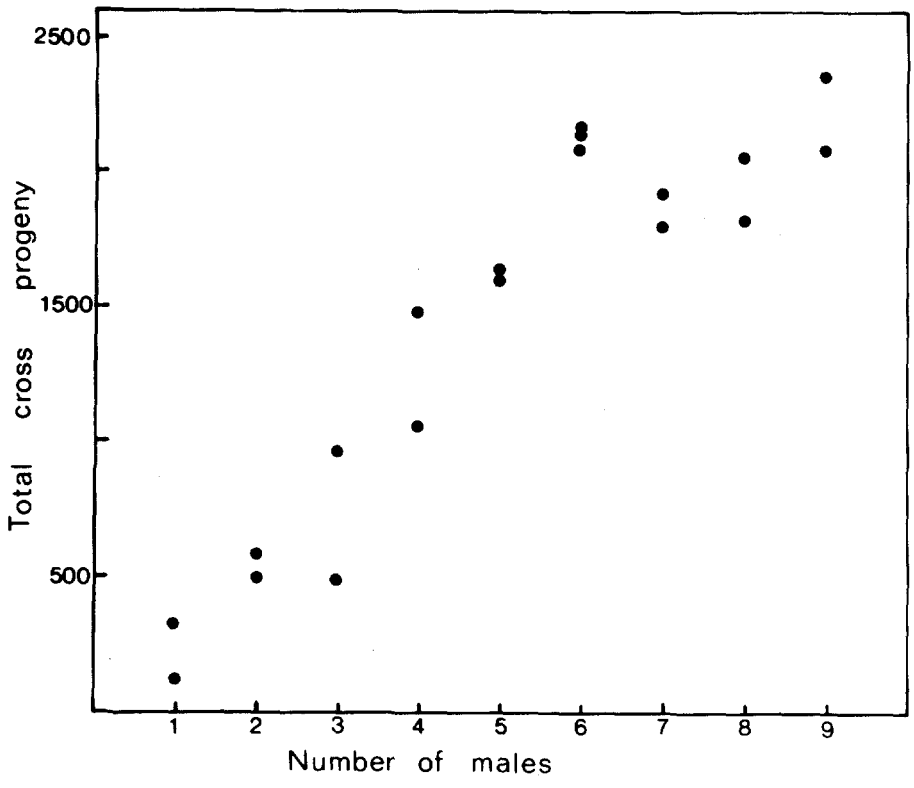


FIGURE 1.—Quantitation of mating efficiency. Six late L4 *dpy-11* hermaphrodites were crossed with one to nine late L4 wild-type males for 24 hr, and total non-Dpy progeny were counted.

enough to allow the maintenance of a homozygous male stock, starting with the rare spontaneous males. In the case of easily scored phenotypes, males were generated by backcrosses (e.g., *unc/unc* hermaphrodites × +/+ males → *unc*/+ males; *unc/unc* hermaphrodites × *unc*/+ males → *unc/unc* males). Sex-linked mutants required only a single cross (e.g., *unc* X/*unc* X hermaphrodites × +/O males → *unc* X/O males). In the case of mutants with less easily scored phenotypes, homozygous hermaphrodite stocks were subjected to heat treatment (27° for 36 hr or 30° for 6 hr). This increases the spontaneous male frequency to about 2% (P. MENEELY, personal communication). Males generated in this way from the wild-type stock N2 mate almost as efficiently as those from an N2 male stock (94% in one quantitative test). However, some mutants respond poorly to heat treatment. Another way of increasing male frequencies is by the use of *him* mutations, which increase meiotic nondisjunction of X chromosomes (HODGKIN, HORVITZ and BRENNER 1979). In many cases, mutants were tested by constructing a double mutant stock with *him-5(e1490)*, since males from this strain mate efficiently.

It should be noted that in some cases a mutant has exhibited a more complex phenotype in the male than in the hermaphrodite. This could be the result of pleiotropy, but it could also be due to the presence of linked mutations, and this possibility cannot easily be excluded in the case of genes with only one mutant allele, such as *unc-73*. In other cases, such as that of *vab-3*, the fact that several independently isolated alleles show the same complex phenotype indicates that the pleiotropy is intrinsic to the gene in question.

Isolation of male abnormal mutants: The male producing strain CB1065 *(him-2(e1065))* was mutagenized with 0.05 M ethyl methane sulfonate for 4 hr (BRENNER 1974) and allowed to produce F_1 and F_2 progeny by selfing. A total of 1621 F_2 hermaphrodites were picked, derived from 229 separate P_0 worms. Mutants isolated from the progeny of different P_0 worms are assumed to be

independent isolates. Of the 1621 hermaphrodites 502 were sterile or almost sterile, but the remaining 1119 were fertile, and their male progeny were examined for mating efficiency and male phenotype. Forty-seven stocks segregated males that sired few or no progeny.

Genetic analysis of mab *mutants:* Male abnormal *(mab)* mutants cannot be analyzed by the conventional procedures of *C. elegans* genetics, which rely on recognition of phenotypes in the hermaphrodite. However, most *mab* mutants have anatomical abnormalities in the adult male that can easily be scored at a magnification of ×100 or higher, and these mutants have been assigned to linkage groups using the anatomical phenotype. One mutant, *mab-10(e1248),* has an anatomical phenotype that is often indistinguishable from wild type. However, *mab-10* males are invariably incapable of mating, and the mutation was assigned to a linkage group on the basis of this defect. A strain, *mab-10 vab-9 II; him-5 V,* was constructed and crossed with *him-5* males to yield *mab-10 vab-9/ + +; him-5* hermaphrodites. Twenty-one Vab hermaphrodite progeny were picked and their male progeny tested for mating ability. Vab-9 males are usually capable of mating, but in this case 14 of 21 were infertile, *i.e.,* homozygous for *mab-10.* Therefore, the genes *mab-10* and *vab-9* must be loosely linked. Tests for linkage with markers on other chromosomes gave negative results.

Complementation tests were carried out between *mab* mutants with similar map locations. In some cases crosses could be carried out using homozygous *mab* male parents, because some of the mutant males are capable of mating, although at extremely low efficiency. The hermaphrodite parent carried an additional autosomal marker, to distinguish cross progeny from self-progeny. In a few tests the male parent was heterozygous (*mab/+*), and in these cases at least ten cross-progeny males were examined for abnormality.

Microscopy: Live adult worms were examined and photographed using Nomarski differential interference contrast optics, according to the method of SULSTON (1976).

RESULTS

Mating efficiency in wild-type males

Males in the last larval stage (L4) show no mating behavior, but this appears almost immediately after the larval cuticle is shed and males mate successfully (sire progeny) within 5 hr of the last larval moult at 20°. Males will mate many times over a period of as many as 6 days.

Wild-type males, although genotypically identical, exhibit individual variations in mating efficiency: Six single wild-type males crossed exhaustively with *unc-17* hermaphrodites sired, respectively, 213, 1033, 1497, 2505, 2632 and 2871 progeny; six single males crossed with *dpy-11* hermaphrodites sired 0, 244, 276, 1196, 1234 and 1824 progeny. Maximum numbers of progeny were usually sired on the second day of mating. Mating efficiency (number of progeny sired) is often much higher when a male mates with an uncoordinated hermaphrodite than when he mates with one that can move well. This is because the hermaphrodite makes no response to the male, and an active hermaphrodite can escape the attentions of a male for much of the time. The effect is pronounced if the male himself cannot move well. For example, in crosses carried out under identical conditions (ten males with ten hermaphrodites, 1-cm spot of bacteria), *unc-3(e151)* males were able to sire progeny from some mutant hermaphrodites (*unc-4, unc-13, unc-15, unc-17, unc-22, unc-51* and *unc-54*) but not from more active mutants (*dpy-5, dpy-11, sma-1, mec-7* and *unc-77*) or from wild-type hermaphrodites.

No attempt has been made in this work to provide a detailed ethological description of mating behavior or to make ethological comparisons between wild-type and mutant males. Mating has been assayed only by progeny production, because this can be measured or estimated simply and objectively.

Mating in C. elegans *mutants*

Mutants representing most of the presently known complementation groups of *C. elegans* (HERMAN and HORVITZ 1980) were examined for male phenotype and mating efficiency (Table 1). Males were examined at ×400 magnification for conspicuous anatomical abnormalities, particularly in the specialized copulatory organs of the male tail (the wild-type anatomy is shown in Figure 2), and for the presence of sperm. All contained apparently normal sperm, as expected because all of the hermaphrodite parent stocks must be able to produce functional sperm. Therefore, none of the mating defects observed are likely to be due to failures in spermatogenesis. Mating efficiency was estimated by the qualitative test in most cases. For some mutants a more accurate measure of mating efficiency was made by means of the quantitative test, and the efficiency relative to wild type is given as a percentage. In some cases more than one allele was tested.

Various mutant types (e.g., *let* and *emb* mutants, some *lin* mutants, and constitutive *daf* mutants) have not been tested because they fail to reach maturity in either sex.

Mutants are discussed by category.

Mutants with altered body shape (dpy, sma, lon, rol, sqt, bli *and* vab): Most of the dumpy (*dpy*) mutants can mate, although efficiency is usually low. These mutants are much shorter than wild type and, therefore, cannot flex their bodies as much as wild-type males. The copulatory apparatus is usually shorter than in the wild type. These differences account for the inefficient mating of dumpy males. Some of the more extreme dumpy mutants have never been observed to sire progeny.

Small (*sma*) mutants are less deformed than dumpies, but mutants in any of the four *sma* genes are unable to mate, possibly because in all four cases the copulatory spicules of the male are short and distorted.

The two long (*lon*) mutants are both about 50% longer than wild type; *lon-1* males are incapable of successful mating, whereas *lon-2* males mate well. This is correlated with bursal anatomy: *lon-1* males have very elongated abnormal copulatory bursae (Figure 3b), whereas *lon-2* bursae are only slightly elongated.

Roller (*rol*) and heterozygous squat (*sqt*/+) mutants (COX *et al.* 1980) have helical twists in their bodies such that adults tend to move along a helical axis rather than a sinusoid. This movement interferes with copulation, but, nevertheless, some of the roller mutants can mate. Homozygous squat mutants are not twisted, although they have cuticular abnormalities, and for two of the three *sqt* genes the mutant males can mate.

Blister (*bli*) mutants have cuticle abnormalities in the adult, resulting in the formation of large fluid-filled blisters at variable positions on the body. The copulatory organs of the male, in particular the cuticular fan, are preferentially affected by these mutations, and in some of the mutants (*bli-3* and *bli-5*) the whole tail region is deformed, although the tail of the hermaphrodite is relatively normal. These severely deformed blister mutants do not mate successfully.

The mutants denoted *vab* (variable abnormal) include several different kinds of mutant with gross anatomical abnormality, often with low penetrance and

TABLE 1

Mating efficiency of mutant males[a]

Gene	Linkage group	Allele tested	Mating efficiency	Source of males	Comment
ace-1	X	*p1000*	3	x	
ace-2	*I*	*g72*	4	ms	
bli-1	*II*	*e769*	0	bc	Irregular fan
bli-2	*II*	*e768*	2	bc	
bli-3	*I*	*e767*	0	bc	Variably deformed bursa
bli-4	*I*	*e937*	1	bc	
bli-5	*III*	*e518*	0	bc	Deformed bursa
cat-1	X	*e1111*	1 (1%)	x	
cat-2	*II*	*e1112*	3 (25%)	hs	
cat-4	*V*	*e1141*	2 (4%)	ms	
cha-1	*IV*	*b401*	2	ms	
che-1	*I*	*e1034*	3	ms	
che-2	X	*e1033*	0	x	Suicidal males
che-3	*I*	*e1124*	0	hm	Suicidal males
che-3	*I*	*e1253*	0	hm	Suicidal males
che-5	*IV*	*e1073*	2	hs	
che-6	*IV*	*e1126*	3	ms	
che-7	V	e1128	3	hs	
daf-3	X	*e1376*	4	x	
daf-5	*II*	*e1386*	3	ms	
daf-6	X	*e1377*	4	x, hs	
daf-10	*IV*	*e1387*	0	hs	
daf-12	X	*m20*	3	hs	
daf-13	X	*m66*	3	ms	
daf-16	*I*	*m26*	4	ms	
daf-17	*I*	*m27*	3	ms	
daf-18	*IV*	*e1375*	3	ms	
daf-20	X	*m25*	4	hs	
dpy-1	*III*	*e1*	1	bc	
dpy-2	*II*	*e8*	1	bc	
dpy-3	X	*e27*	1	x	
dpy-4	*IV*	*e1166*	3	hm	
dpy-5	*I*	*e61*	1	bc	
dpy-6	X	*e14*	0	x	
dpy-7	X	*e88*	1	x	
dpy-8	X	*e130*	1	x	
dpy-9	*IV*	*e12*	2	bc	
dpy-10	*II*	*e128*	1	hm	
dpy-11	*V*	*e224*	2	hm	
dpy-12	X	*e182*	1	x	
dpy-13	*IV*	*e184*	2	bc	
dpy-14	*I*	*e188*	0	bc	
dpy-15	*V*	*e24*	2	bc	
dpy-16	*IV*	*e225*	3	bc	
dpy-17	*III*	*e164*	2	bc	
dpy-18	*III*	*e364*	2	hm	
dpy-19	*III*	*e1259*	0	bc	t.s.
dpy-20	*IV*	*e1282*	2	bc	t.s.
dpy-21	*V*	*e428*	4 (88%)	ms	Males nondumpy

TABLE 1—Continued

Gene	Linkage group	Allele tested	Mating efficiency	Source of males	Comment
dpy-22	X	e652	0	x	Males very abnormal
dpy-23	X	e840	0	x	Males very abnormal
dpy-25	II	e817	1	hm	
dpy-26	IV	n199	4 (54%)	hm	Males nondumpy
flu-1	V	e1002	2	hs	
flu-2	X	e1003	3	x	
flu-3	II	e1001	2	ms	
flu-4	X	e1004	4	x	
him-1	I	e879	2 (1%)	hm	
him-2	I	e1065	3 (23%)	hm	
him-3	IV	e1256	2 (5%)	hm	
him-4	X	e1267	0	hm	Abnormal testis
him-5	V	e1490	4 (55%)	hm	
him-6	IV	e1423	3 (30%)	hm	
him-7	V	e1480	3 (32%)	hm	
him-8	IV	e1489	2 (9%)	hm	
him-9	II	e1487	4 (88%)	hm	
him-10	III	e1511	4 (78%)	hm	
lev-1	IV	x21	2	ms	
lev-7	I	x13	3	ms	
lev-11	I	x12	2	hm	
lin-1	IV	e1026	0	hm	(Multivulva)
lin-1	IV	e1275	1	hm	(Multivulva)
lin-2	X	e1309	2	x	(Vulvaless)
lin-3	IV	e1417	4	ms	(Vulvaless)
lin-4	II	e912	0	hm	(Vulvaless)
lin-7	II	e1413	3	hm	(Vulvaless)
lin-10	I	e1439	3	hm	(Vulvaless)
lin-15	X	e1763	0	x	(Multivulva)
lin-18	X	e620	4	ms	(Bivulva)
lon-1	III	e185	0	hm	Elongated bursa
lon-2	X	e678	4 (53%)	x	
mec-1	V	e1066	2	hs	
mec-2	X	e75	3	x	
mec-3	IV	e1338	2	ms	
mec-4	X	e1339	2	x	
mec-4	X	e1611	3	x	
mec-5	X	e1340	3	x	
mec-6	I	e1342	3	ms	
mec-7	X	e1343	4	x	
mec-8	I	e398	2	ms	
mec-9	V	e1494	2	ms	
mec-10	X	e1515	4	x	
mec-12	III	e1605	3	ms	
mor-1	III	e1071	3	hm	
mor-2	IV	e1125	3	hs	
nuc-1	X	e1392	4	x	
osm-1	X	p808	2	x	
osm-2	I	p801	2	ms	
osm-3	IV	p802	3	ms	
osm-4	IV	p821	1	hs	

TABLE 1—Continued

Gene	Linkage group	Allele tested	Mating efficiency	Source of males	Comment
osm-5	X	*p813*	1	x	Suicidal males
osm-6	V	*p811*	1	hs	
rol-1	II	*e91*	1	bc	
rol-3	V	*e754*	1	bc	
rol-4	V	*sc8*	2	bc	
rol-6	II	*e187*	0	bc	
sma-1	V	*e30*	0	hm	Variably deformed bursa
sma-2	III	*e502*	0	bc	Deformed spicules
sma-3	III	*e491*	0	bc	Deformed spicules
sma-4	III	*e729*	0	bc	Deformed spicules
sqt-1	II	*sc1*	1	hm	
sqt-2	II	*sc3*	1	hm	
sqt-3	V	*sc63*	0	hm	t.s.
unc-1	X	*e94*	2	x	
unc-2	X	*e55*	2	x	
unc-3	X	*e151*	1	x	
unc-4	II	*e120*	0	bc	
unc-5	IV	*e53*	0	bc	
unc-5	IV	*e152*	2	bc	
unc-6	X	*e78*	1	x	
unc-7	X	*e5*	0	x	
unc-8	IV	*e49*	1	hm	
unc-9	X	*e101*	0	x	
unc-10	X	*e102*	2	x	
unc-11	I	*e47*	2	bc	
unc-13	I	*e51*	0	bc	
unc-14	I	*e57*	0	bc	
unc-15	I	*e73*	0	bc	
unc-16	III	*e109*	2	bc	
unc-17	IV	*e245*	0	bc	
unc-18	X	*e81*	0	x	
unc-20	X	*e112*	0	x	t.s.
unc-22	IV	*e66*	0	bc	
unc-23	V	*e324*	1	bc	
unc-24	IV	*e138*	1	bc	
unc-25	III	*e156*	2	bc	
unc-26	IV	*e205*	0	hm	
unc-27	X	*e155*	2	x	
unc-28	IV	*e15*	0	hm	
unc-29	I	*e193*	2	bc	
unc-30	IV	*e191*	2	bc	
unc-31	IV	*e169*	1	bc	
unc-32	III	*e189*	0	bc	
unc-33	IV	*e204*	0	bc	
unc-34	V	*e315*	0	bc	Deformed spicules
unc-35	I	*e259*	2	bc	
unc-36	III	*e251*	0	bc	
unc-37	I	*e262*	1	bc	
unc-38	I	*e264*	2	bc	
unc-39	V	*e267*	1	bc	
unc-40	I	*e271*	0	bc	

TABLE 1—Continued

Gene	Linkage group	Allele tested	Mating efficiency	Source of males	Comment
unc-41	*V*	*e268*	0	bc	
unc-42	*V*	*e270*	1	bc	
unc-43	*IV*	*e408*	0	bc	
unc-44	*IV*	*e362*	0	bc	
unc-45	*III*	*e286*	0	bc	t.s.
unc-46	*V*	*e177*	2	bc	
unc-47	*III*	*e307*	2	bc	
unc-49	*III*	*e382*	2	bc	
unc-50	*III*	*e306*	1	bc	
unc-51	*V*	*e369*	0	bc	
unc-52	*II*	*e444*	0	hm	
unc-53	*II*	*e404*	0	bc	Deformed bursa
unc-54	*I*	*e190*	0	bc	
unc-55	*I*	*e402*	0	hm	
unc-56	*I*	*e403*	2	bc	
unc-57	*I*	*e406*	2	bc	
unc-58	X	*e665*	0	x	
unc-59	*I*	*e261*	0	bc	Variably deformed bursa
unc-60	*V*	*e677*	0	bc	
unc-61	*V*	*e228*	0	bc	Abnormal bursa and rays
unc-62	*V*	*e644*	0	bc	Deformed bursa
unc-63	*I*	*e384*	2	bc	
unc-64	*III*	*e246*	0	bc	
unc-65	*V*	*e351*	2	ms	
unc-67	*I*	*e713*	2	bc	
unc-68	*V*	*e540*	2	hm	
unc-69	*III*	*e587*	0	bc	
unc-70	*V*	*e524*	3	bc	
unc-71	*III*	*e541*	3	bc	
unc-73	*I*	*e936*	0	bc	Deformed spicules
unc-74	*I*	*e883*	2	bc	
unc-75	*I*	*e950*	2	bc	
unc-76	*V*	*e911*	0	bc	
unc-77	*IV*	*e625*	2	ms	
unc-78	X	*e1217*	0	x	
unc-79	*III*	*e1068*	3	ms	
unc-80	*V*	*e1272*	3	ms	
unc-81	*III*	*e1122*	3	ms	
unc-82	*IV*	*e1220*	2	ms	
unc-83	*V*	*e1408*	1	ms	t.s.
unc-84	X	*e1410*	1	x	t.s.
unc-85	*II*	*e1414*	0	hm	Variably deformed bursa
unc-86	*III*	*e1416*	2 (7%)	hm	
unc-86	*III*	*e1507*	3 (30%)	hm	
unc-87	*I*	*e1216*	0	bc	
unc-89	*I*	*e1460*	3	ms	
unc-92	*V*	*st15*	0	hm	
unc-93	*III*	*e1500*	0	hm	Abnormal cloaca
unc-94	*I*	*su177*	2	hm	
unc-95	*I*	*su33*	0	hm	
unc-96	X	*su151*	3	x	

TABLE 1—Continued

Gene	Linkage group	Allele tested	Mating efficiency	Source of males	Comment
unc-97	X	*su110*	0	x	
unc-98	X	*su130*	1	x	
unc-99	X	*su195*	1	x	
unc-100		*su149*	0	hm	
unc-102	X	*e1598*	0	x	
unc-103	*III*	*e1597*	0	hm	
unc-104	*II*	*e1265*	0	bc	
vab-1	*II*	*e2*	4	ms	
vab-2	*IV*	*e96*	4	ms	
vab-3	X	*e648*	0	x	Deformed spicules
vab-6	*III*	*e697*	2	ms	
vab-7	*III*	*e1562*	0	hm	Deformed bursa
vab-8	*V*	*e1017*	0	hm	Degenerate tail
vab-9	*II*	*e1744*	2	hm	
vab-10	*I*	*e698*	0	hm	Degenerate tail

[a] Mutant stocks are listed by gene. In most cases only the standard reference allele for a given gene was tested. Mating efficiency was estimated by the qualitative test [4 = very efficient mating (30–100% of wild-type male mating efficiency); 3 = efficient mating (10–30% of wild type); 2 = poor mating (1–10% of wild type); 1 = very poor mating (less than 1% of wild type); 0 = no detectable mating] and sometimes by the quantitative test as well (expressed as a percentage in parentheses). Males were obtained in five ways: from a male stock (ms); by a cross with wild-type males for sex-linked mutants (x); by a backcross (bc); by heat-shocking hermaphrodites (hs); or by using a *him* mutation (hm). Comments in parentheses refer to the hermaphrodite phenotype. Temperature-sensitive mutants are designated t.s.; these exhibit a mutant phenotype only at restrictive temperature (25°), and males of these mutants were grown and tested at 25°. The mutant *unc-17(e245)* was tested using *unc-15(e73)* as the hermaphrodite parent.

expressivity. Three mutants (*vab-1, vab-2* and *vab-3*) have regions of the head misshapen or dystrophic, so that they appear "notched." Two of them (*vab-1* and *vab-2*, both low penetrance) never affect male tail development, and males mate as well as wild type. The third mutant, *vab-3*, is completely penetrant, and males of this type never mate successfully. Three different *vab-3* alleles exhibit the same phenotype: the copulatory spicules are always short and deformed, and the bursal fan and rays are often abnormal. Another mutant, *vab-10*, has dystrophic regions in the neck, so that mutant worms have heads bent permanently dorsally or ventrally. In males (but not hermaphrodites), the tail region is also dystrophic, so that the copulatory apparatus is deformed. A general dystrophy of the posterior of the animal in both sexes is observed in the *vab-7* mutant. Neither *vab-7* nor *vab-10* males can mate successfully. Abnormalities of the tail cuticle of both sexes are observed in the two mutants *vab-8* and *vab-9*; *vab-9* males have almost normal tails and can frequently mate successfully, but *vab-8* males are more severely deformed and never mate successfully.

Mutants with abnormal sensory behavior (che, osm, mec, ttx *and* daf): Chemosensory (*che*) mutants fail to exhibit normal chemotaxis towards Na^+ and/or Cl^- (LEWIS and HODGKIN 1977). Two of these mutants (*che-2* and *che-3*) have marked ultrastructural changes in the endings of the sensory neurons of the head, and males of these mutant types fail to mate successfully. These

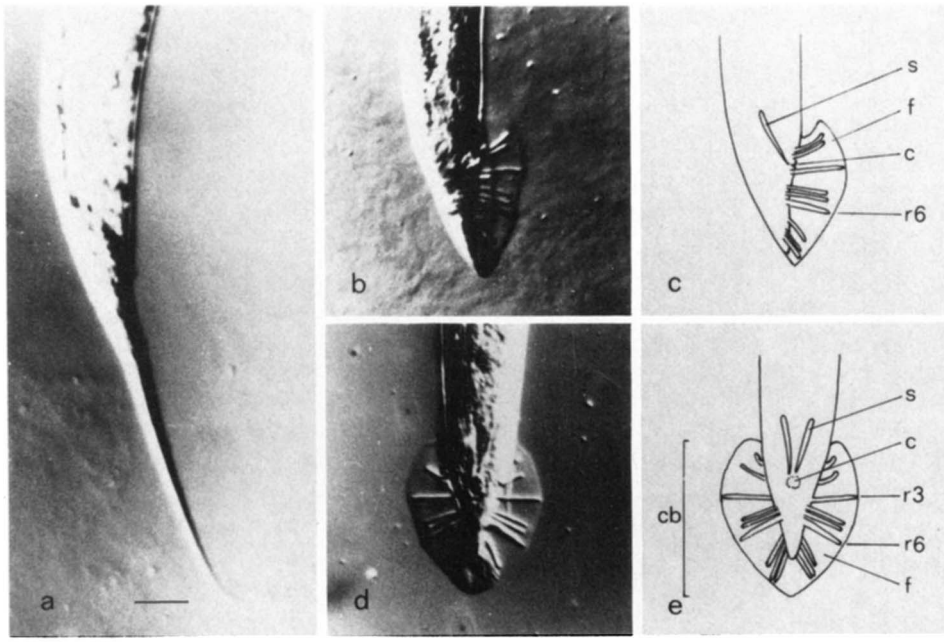


FIGURE 2.—Wild-type anatomy: (a) hermaphrodite, lateral view, (b and c) male, lateral view, (d and e) male, dorsal view. Abbreviations: c = cloaca; cb = copulatory bursa; f = cuticular fan; r3 = sensory ray 3; r6 = sensory ray 6; s = copulatory spicules. Scale bar = approximately 25 μm.

strains were originally isolated on the basis of the mating defect. It is possible that the failure to mate successfully is a result of abnormalities in the sense organs of the male tail. Other *che* mutants mate efficiently, so normal chemotaxis is not a prerequisite for mating. The *tax* chemosensory mutants isolated by DUSENBERY, SHERIDAN and RUSSELL (1975) have not been tested in this survey; some of them probably correspond to the *che* mutants.

Thermotaxis mutants (*ttx*: HEDGECOCK and RUSSELL 1975) exhibit abnormal isothermal tracking. They have not been tested in this survey, but all are capable of successful mating (E. HEDGECOCK, personal communication).

Osmotaxis mutants (*osm*: CULOTTI and RUSSELL 1978) fail to avoid high osmotic strength. Some of them mate efficiently, others very inefficiently. Mutants in the latter class (*osm-4* and *osm-5*) tend to escape from mating plates, as do the impotent *che* mutants. CULOTTI and RUSSELL (1978) have shown that all *osm* mutants are defective in chemotaxis to NaCl, so these mutants, like *che-2*, may have a general sensory dysfunction.

Mechanosensory mutants (*mec*: CHALFIE and SULSTON 1981) are defective in the response to light touch, which is mediated by six neurons containing conspicuous microtubules. All *mec* mutants mate well, and, as noted by these authors, the *mec* males do not exhibit the lethargy characteristic of hermaphrodite *mec* worms.

Some of the *daf* mutants have defective sensory behavior and mate inefficiently or not at all. These mutants are unable to form dauer larvae (RIDDLE,

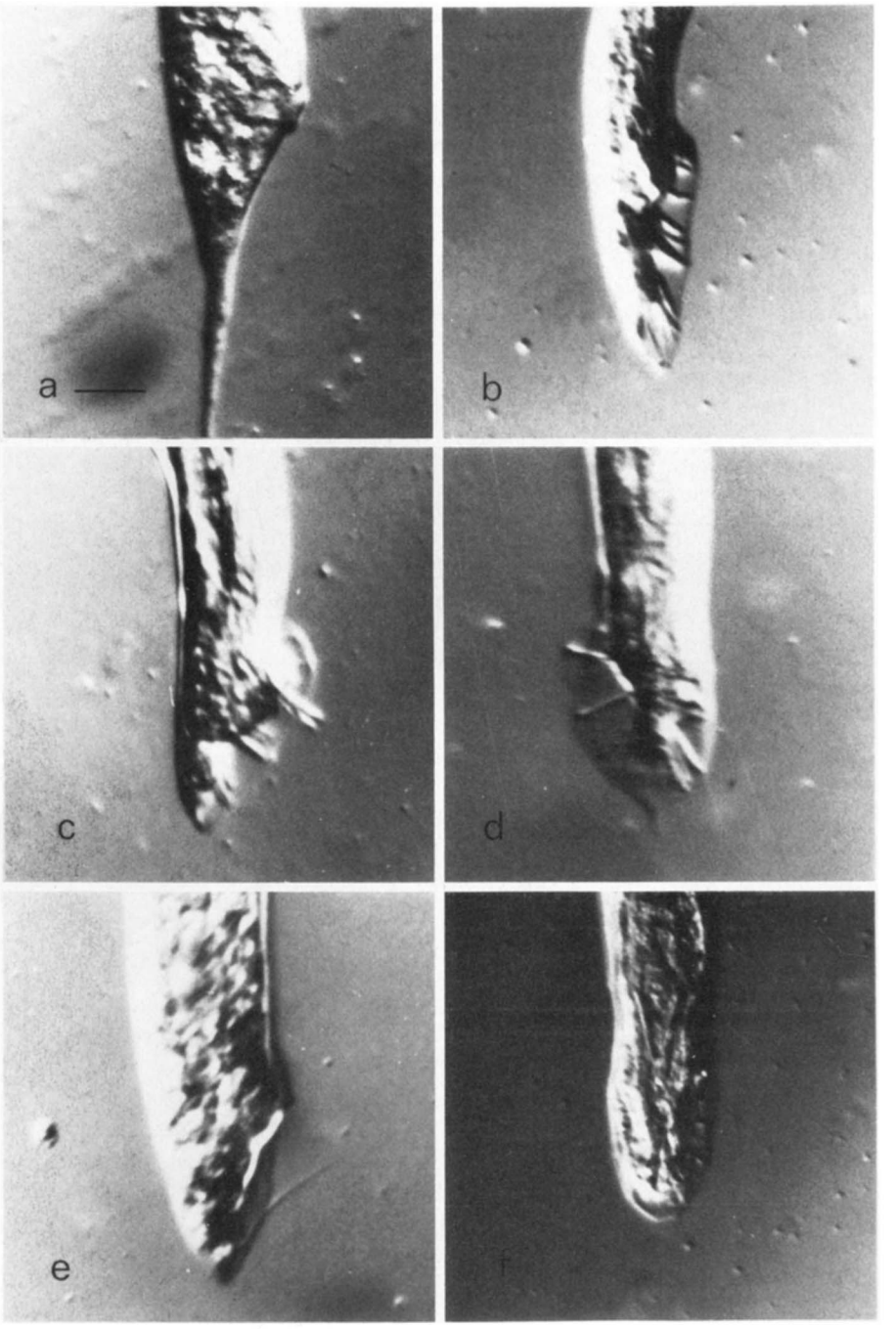
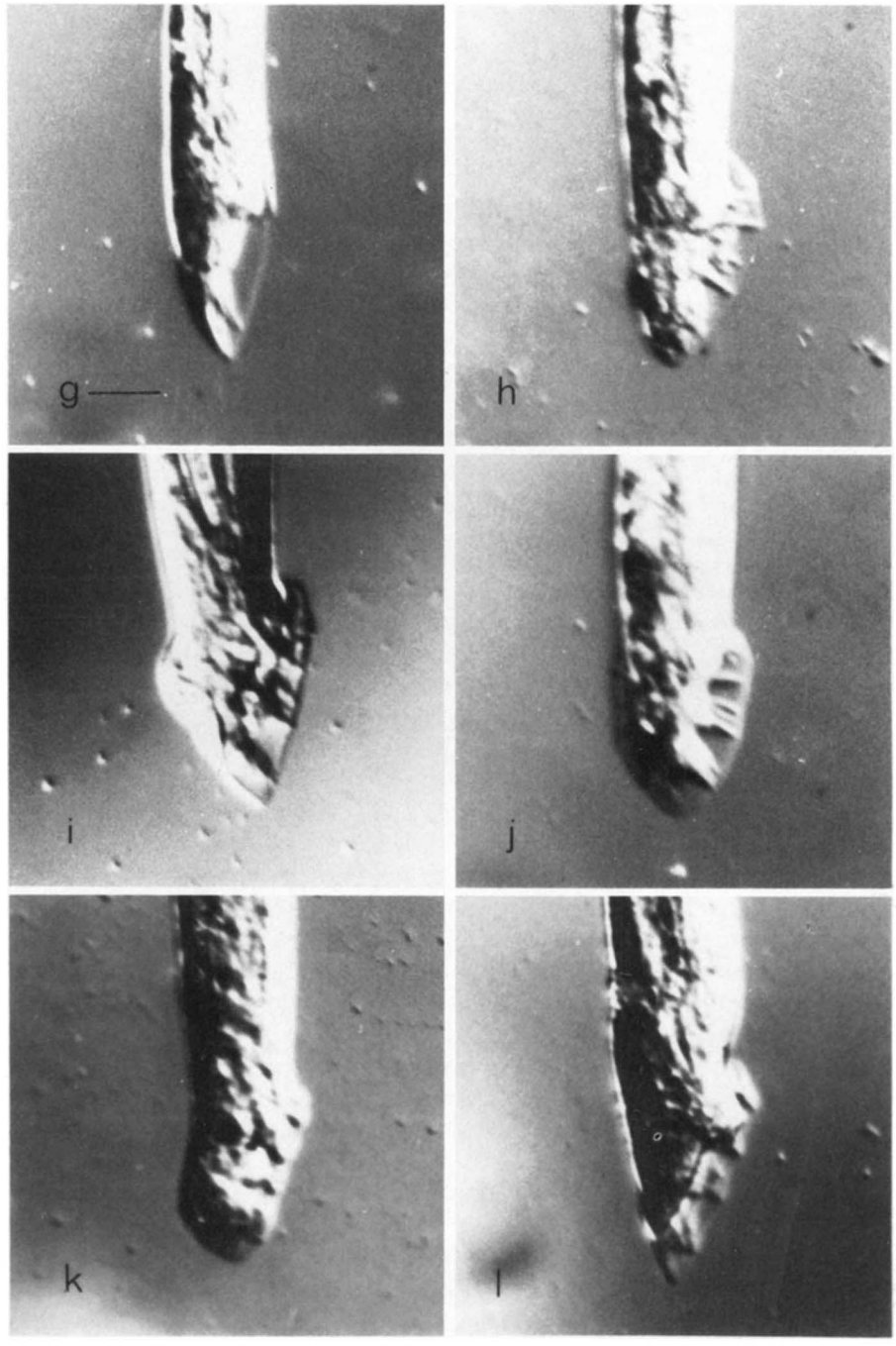


FIGURE 3.—Abnormal male genitalia (compare with Figure 2): (a) *lin-4*, lateral view (note absence of adult male characters); (b) *lon-1*, lateral view (note elongation); (c) *mab-1*, lateral view (note abnormal bursa, extended spicules); (d) *mab-2*, dorsal view (note absence of rays 4, 5, 6, 7, 8 and 9 on left side; presence of rays 7, 8 and 9 on right side); (e) *mab-3*, lateral view (note absence of rays); (f) *mab-4*, dorsal view (note swollen bursa, reduced fan and rays); (g) *mab-5*, lateral view (note absence of rays 1, 2, 3, 4, 5 and 6); (h) *mab-6*, lateral view (note swollen bursa); (i) *mab-7*, lateral view (note swollen rays and distorted bursa); (j) *mab-8*, dorsal view (note swollen bursa); (k) *mab-*



9, lateral view (note grossly deformed tail region); (l) *mab-10*, lateral view (note reduced fan). These mutants carried *him-1(e879)*, *him-2(e1065)* or *him-5(e1490)*, and in some cases *dpy-21(e428)* as well; none of these markers affects male tail anatomy. Alleles illustrated are: (a) *e912*, (b) *e185*, (c) *e1228*, (d) *e1241*, (e) *e1240*, (f) *e1252*, (g) *e1239*, (h) *e1249*, (i) *e1599*, (j) *e1250*, (k) *e1245* and (l) *e1248*. Scale bar = approximately 25 μm.

SWANSON and ALBERT 1981), the resistant stage 3 larvae that develop under starvation conditions. The failure is probably due to abnormal sensory input because it has been noted that some chemotactic mutants (*che-2* and *che-3*) also fail to form dauers, and the inability to mate may well have the same cause.

The pleiotropic mutant *unc-86* (CHALFIE, HORVITZ and SULSTON 1981) is relevant to a discussion of sensory mutants: two independent isolates are nonchemotactic (M. CHALFIE, personal communication) and also lack the mechanosensory microtubule cells. Moreover, they also lack the four cephalic companion cells that are found only in the male. H. R. HORVITZ and J. E. SULSTON (personal communication) have demonstrated a weak taxis of adult males toward hermaphrodites that may be mediated by these cells, and J. E. SULSTON (personal communication) has found that *unc-86* mutants fail to exhibit this taxis. Despite these defects, *unc-86* males mate reasonably well, so it seems that the taxis and the cephalic companions are not necessary for successful mating, although they may increase its efficiency. In soil, the natural environment of *C. elegans*, the taxis of males toward hermaphrodites may well be more important.

Neurotransmitter mutants, neuroactive drug-resistant mutants (cat, ace, lan, lev, *and* cha): SULSTON, DEW and BRENNER (1975) have used the formaldehyde-induced fluorescence of dopamine to screen for mutants with altered catecholamine distribution (*cat* mutants). Three of the nine pairs of sensory rays in the male tail have dopaminergic innervation, and it might be expected that defects in these would interfere with mating. All *cat* mutants have altered (*cat-1* and *cat-4*) or absent (*cat-2*) fluorescence in these male-specific cells and mate with reduced efficiency.

The two *ace* mutants (CULOTTI *et al.* 1981) exhibited reduced acetylcholinesterase levels, but neither has a recognizable behavioral phenotype, and both mate efficiently. The uncoordinated double mutant has not been tested. Most mutants resistant to the acetylcholinesterase inhibitor lannate are severely uncoordinated (BRENNER 1974: *unc-17* mutants) and cannot mate, but one unmapped resistant mutant, *lan-5(e239)*, is less uncoordinated and mates well. Similarly, most mutants resistant to levamisole, an acetylcholine agonist, are uncoordinated (LEWIS *et al.* 1980: *unc-29, -38, -63, -68, -74*) but these are able to mate successfully, as are other levamisole-resistant mutants (*lev-1, lev-6* and *lev-11*). Finally, a mutant altered in choline acetyl transferase activity has been isolated (*cha-1*: D. HIRSH and J. RAND, personal communication), and this also mates with reasonable efficiency, although it is somewhat uncoordinated.

Uncoordinated mutants: The uncoordinated (*unc*) mutants exhibit a great variety of behavioral defects, ranging from subtle alterations in movement to complete paralysis. Almost none of them mate efficiently, but it is of interest to determine which mutants are completely incapable of successful mating. For the purposes of this survey, the 94 *unc* genes have been classified into 16 broad phenotypic categories (Table 2). These categories are subjective, so a different observer would probably make a somewhat different classification. Mutants in the first four categories (limp paralytic, kinky paralytic, short paralytic, shaker) move so poorly that successful mating would not be expected and is not

TABLE 2

A classification of uncoordinated mutants[a]

	unc gene numbers	
	Inefficient mating	No mating
Limp paralyzed		15, 36, 45, 52, 54, 60, 97, (93)
Kinky paralyzed		13, 18
Short paralyzed		14, 33, 44, 51, 76, (73, 92)
Twitcher, shaker		22, 58
Strong coiler		5, 17, 28, 32, 69, 102, 104, (34)
Weak coiler	3, 10, 37, 56, 75, 77	
Strong kinker	11, 39, 57, 71	20, 26, 103, (85)
Weak kinker	2, 42, 83, 84	40, 41
Lev kinker	29, 38, 50, 63, 68, 74	
Backward Unc	6, 35	4, 55, (53, 59)
Forward Unc	1, 23, 24	7, 9
Shrinker	25, 30, 46, 47, 49	43
Slow	65, 82, 94, 96, 98, 99	78, 95, 100
Sluggish	16, 27, 31, 67, 80, 86	64, 87
Irregular movement	8, 70, 79, 81, 89	(61, 62)

[a] Uncoordinated mutants are listed by gene number. The gene numbers in parentheses represent mutants that exhibited abnormalities in male genital anatomy, in addition to the behavioral defect.

observed. Twitcher mutants are unable to sustain muscle contractions, so they twitch continually; this defect extends to the male-specific muscles and may, therefore, explain the inability to mate. In the remaining categories, most mutants can mate to some extent. The large categories of kinker and coiler are partly overlapping: mutants of both types are unable to propagate a smooth sinusoidal wave down the body, and coilers tend to coil up on themselves. Nevertheless, most are able to mate, as are the uncoordinated levamisole-resistant mutants which all have a similar weak kinker phenotype. Shrinkers tend to contract on both sides of the body at once when tapped on the head, but otherwise they mostly move and mate moderately well. The slow and sluggish mutants, and those with irregular movement, are the mutants with the mildest behavioral defects, and most of these are capable of successful mating.

In summary, of 94 *unc* mutants, 47 are capable of successful mating; 47 are not. Most of the latter have severe movement problems or exhibit abnormal genital development in addition to the behavioral defect (these abnormalities are noted in Tables 1 and 2). Only in a few cases (e.g., *unc-7*) is there no obvious correlation between the defective movement and the inability to mate.

Other mutant categories (daf, fer flu, him *and* lin): Dauer-defective mutants (*daf*: RIDDLE, SWANSON and ALBERT 1981) are for the most part behaviorally wild type and mate well. A few are unable to mate, perhaps as a result of abnormal sensory anatomy, as noted before.

All of the *fer* mutants so far isolated (WARD and MIWA 1978; S. WARD, personal communication) are defective in sperm production in both sexes, but some at least are able to transfer defective sperm to hermaphrodites (WARD and CARREL 1979), indicating that the defect does not affect mating behavior.

Mutants with abnormal gut fluorescence (*flu*: BABU 1974) are all capable of successful mating, but two are markedly inefficient. The mutant *nuc-1* (SULSTON 1976) lacks the major endonuclease; it has no behavioral phenotype and mates efficiently.

The *him* mutants (HODGKIN, HORVITZ and BRENNER 1979) exhibit increased rates of X chromosome nondisjunction and, hence, have high self-progeny male frequencies. Some (probably all) are meiotic mutants. They have been carefully tested for mating efficiency. Some are markedly reduced, but this is probably partly or wholly the result of sperm aneuploidy. One mutant, *him-4*, is pleiotropic, exhibiting abnormal gonad development so that the vas deferens never connects with the proctodeum, and, consequently, males are completely infertile, although sperm are produced.

The *lin* mutants exhibit a variety of alterations in the postembryonic cell lineages. Of those that reach fertile hermaphrodite adulthood, most so far isolated have abnormal vulval development (HORVITZ and SULSTON 1980), either "vulvaless" (*lin-2, -3, -4, -7, -10*) or "multivulva" (*lin-1, lin-15* and *lin-18*). Some of these mutations also affect male development (*e.g., lin-1, lin-4* and *lin-15*) but others do not (*e.g., lin-2, lin-3* and *lin-18*). Mutant *lin-4* males have a particularly striking phenotype in the adult: virtually none of the male-specific tail anatomy develops, although a mature adult male gonad is formed (Figure 3a). The phenotype has been examined in detail by CHALFIE, HORVITZ and SULSTON (1981).

A search for mutants with defective mating: A large number of stocks were established by picking apparently wild type L4 hermaphrodites from the F_2 progeny of mutagenized *him-2* hermaphrodites. The males segregated by these stocks were tested for mating ability: 47 of 1119 stocks segregated males that consistently failed to sire progeny. Fourteen of these have been characterized in detail. Of the remainder, some were lost before they could be analyzed, and most of the rest were difficult to work with because of poor growth or variability in phenotype. The 14 mutant stocks defined nine genes (two *che* and seven *mab*). The 1072 male-fertile stocks were not examined in detail for abnormalities in male anatomy, but two of them were observed to segregate a fraction of nonmating males with conspicuous abnormalities. These two stocks were heterozygous for *mab-2* and *mab-3*, respectively, and homozygous *mab* mutant stocks were obtained from them. Another *mab* mutant, *mab-7*, was obtained from a stock in the Cambridge collection, CB1453, which proved to be a double mutant, *mab-7(e1599)lin-2(e1453)* X.

The properties of the 17 mutant stocks thus obtained are summarized in Tables 1, 3 and 4. Three are defective in chemotaxis: males of these strains exhibit a conspicuous behavioral difference from wild-type males. When placed on crossing plates (5-cm NGM plates with a central 1-cm spot of bacteria in which about ten Unc hermaphrodites are feeding) mutant males spend little time in the spot of bacteria and hermaphrodites and usually attempt to crawl up the plastic sides of the dish, eventually dying of desiccation in the attempt. This suicidal behavior suggested a sensory defect, and hermaphrodites of all three strains were found to be defective in chemotaxis to NaCl. Also, hermaphrodites of the three strains had consistent abnormalities in the ultrastruc-

TABLE 3

Male-abnormal mutants[a]

Gene	Linkage group	Alleles	Hermaphrodite phenotype	Male phenotype	Mating efficiency
mab-1	*I*	*e1228*	Swollen vulva	Swollen bursa	0
		e1229	Swollen vulva	Swollen bursa	1
		e1233	Swollen vulva	Swollen bursa	1
mab-2	*I*	*e1241*	Late hypodermal divisions defective	V and T lineages defective: rays variably absent	1
mab-3	*II*	*e1240*	Apparently wild type	V, T, M, B, F lineages and gonad variably abnormal	0
mab-4	*III*	*e1252*	Swollen vulva	Swollen bursa	1
		e1247	Swollen vulva	Swollen bursa	1
mab-5	*III*	*e1239*	V divisions, Q migrations, coelomocytes abnormal	V and M lineages and gonad abnormal	0
mab-6	*II*	*e1249*	Apparently wild type	Swollen bursa	1
mab-7	X	*e1599*	Slightly dumpy	Swollen rays	1
mab-8	*II*	*e1250*	Swollen vulva	Swollen bursa	1
mab-9	*II*	*e1245*	Apparently wild type	B lineage defective; U and F lineages variably abnormal	0
mab-10	*II*	*e1248*	Slightly swollen vulva	Slightly swollen bursa	0
		e1235	Slightly swollen vulva	Slightly swollen bursa	0

[a] These descriptions are based in part on observations by J. E. SULSTON. See Table 1 for scoring of mating efficiency.

TABLE 4

Two-factor crosses for mab *mutants*

Cross (hermaphrodite × male)	No. of male progeny	
	Nonrecombinant	Recombinant
mab-1 unc-13 I × *mab-1 unc-13/++*	WT (94) MabUnc (85)	Mab (1) Unc (0)
mab-2 unc-13 I × *mab-2 unc-13/++*	WT (74) MabUnc (71)	Mab (1) Unc (1)
dpy-10 mab-3 II × *dpy-10 mab-3/++*	WT (57) MabDpy (45)	Mab (2) Dpy (0)
unc-32 mab-4 III × *unc-32 mab-4/++*	WT (224) MabUnc (ND)	Mab (22) Unc (ND)
mab-5 dpy-18 III × *mab-5 dpy-18/++*	WT (266) MabDpy (270)	Mab (17) Dpy (13)
mab-6 unc-4 II × *mab-6 unc-4/++*	WT (108) MabUnc (109)	Mab (3) Unc (2)
mab-7 +/+ dpy-3 X × *++/++*	Mab (202) Dpy (193)	MabDpy (6) WT (8)
mab-8 unc-4 II × *mab-8 unc-4/++*	WT (118) MabUnc (121)	Mab (5) Unc (2)
mab-9 dpy-10 II × *mab-9 dpy-10/++*	WT (157) MabDpy (165)	Mab (23) Dpy (28)

[a] All nine crosses demonstrate linkage to a standard marker (*dpy* or *unc*). Linkage distances are not presented because these data apply to recombination in males, not hermaphrodites. WT = wild type; ND = not determined.

ture of ciliated sensory neurons in the head (LEWIS and HODGKIN 1977; J. HODGKIN, unpublished observations). Defective chemotaxis alone cannot explain the impotence of the males, because other nonchemotactic mutants are capable of mating. Therefore, it is possible that these three mutants have

additional defects in the sensory neurons of the male tail, or elsewhere, but convincing evidence of such defects has not been obtained. These three mutants define two genes, *che-2(e1033)* and *che-3(e1124,1253)* (E. M. HEDGECOCK, personal communication).

The other mutants all exhibited abnormal genital anatomy in the adult male. Mapping and complementation tests led to the assignment of 14 mutants to ten *mab* (male abnormal) genes (Table 3). Two-factor crosses demonstrating linkage to standard markers for nine of the ten genes are summarized in Table 4. Mapping of the tenth gene (*mab-10 II*) is described in MATERIALS AND METHODS. These data show that all of these mutants behave as simple Mendelian recessives. Three-factor crosses have also been carried out with some of the mutants, allowing them to be placed more precisely on the current genetic map (data not shown).

The commonest phenotype is observed in the mutants *mab-1, -4, -6, -8* and *-10*: in all of these mutants the bursal region is swollen, and the fan and rays are concomitantly reduced. Despite this distortion, most of these mutants are capable of occasional successful mating. The degree of anatomical abnormality is not obviously correlated with mating efficiency: *mab-4* males are very abnormal but mate relatively well, whereas *mab-10* males have almost wild-type anatomy yet never mate successfully. Many of these mutants also have an abnormal vulval phenotype in the hermaphrodite (Table 3 and Figure 4). All of these mutants appear to have normal cell numbers in both sexes.

The mutant *mab-7* also has normal cell numbers but has a different phenotype: all the copulatory rays are swollen and distorted. Mutant males are capable of occasional successful mating.

The other four mutant types (*mab-2, -3, -5* and *-9*) have abnormal cell numbers and are lineage mutants. A different set of lineages is affected in each case. However, all four mutants are variable in phenotype (like other lineage mutants), and exhaustive characterization has not been attempted. Defects in the *mab-2* mutant are confined to the late hypodermal divisions of the V and T cells (SULSTON and HORVITZ 1977). In the male, these cells give rise to the nine pairs of sensory rays, and these rays are found to be variably absent in *mab-2* males. Any number between six and 18 rays are missing in mutant males, and the two sides of the animal do not show obvious correlations in the defects. Some of the mutant males are capable of mating, and these successful males always have at least five rays on at least one side of the bursa. It is difficult to identify the rays in these mutants unambiguously, but there was no obvious correlation between the presence of a particular ray and successful mating.

The ray lineages are also affected in the mutants *mab-3* and *mab-5*, but these are both pleiotropic mutants in which most of the genital structures are very abnormal or absent. The mutant *mab-5* is interesting because the T lineages are usually unaffected, so that the most posterior rays (no. 7, 8 and 9) which arise from the T lineage are present, but all of the other rays are absent. A variety of other lineages is also affected. In addition, abnormal migration of the Q cell daughters occurs consistently in both sexes (M. L. CHALFIE and J. E. SULSTON, unpublished results).

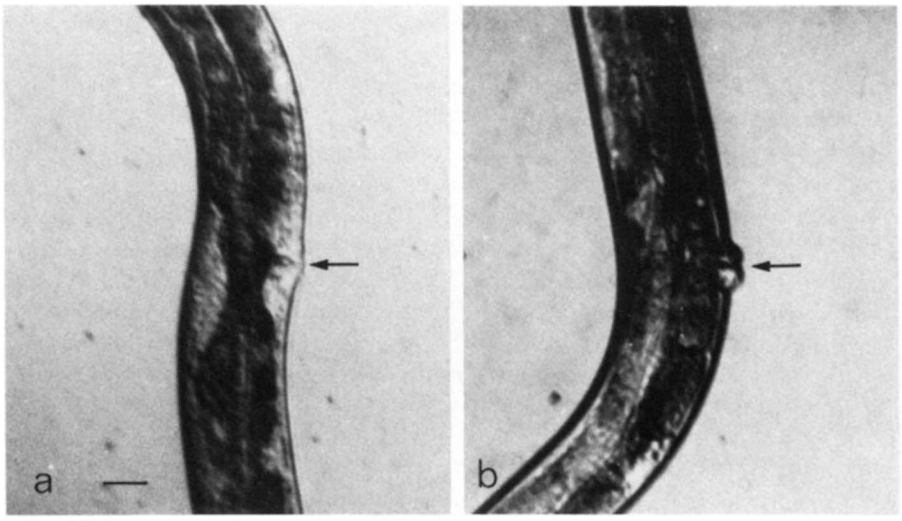


FIGURE 4.—Effect of *mab-1* on vulval anatomy: adult hermaphrodites of (a) wild type, (b) *mab-1(e1228) him-2(e1065)*. Arrows indicate vulvae. Scale bar = approximately 25 μm.

Many lineage defects are also observed in *mab-3* animals, and in this mutant both V and T cell lineages are abnormal.

The mutant *mab-9* shows a different set of lineage alterations, most conspicuous and consistent in the division of the precursor cell B and also sometimes affecting the U and F lineages. Probably, as a consequence of these division failures (which can result in blockage of the rectum), the *mab-9(e1245)* mutation is frequently lethal to males in young adulthood, and of those that survive, most are small and stunted, with very abnormal copulatory structures. The phenotype of this mutant is particularly interesting because the blast cells B, U and F (formerly B, E and F: SULSTON and HORVITZ 1977) divide only during male development and are not required for hermaphrodite development. This, therefore, appears to be a male-specific lineage mutant.

DISCUSSION

Mating behavior seems to be the most complex piece of behavior exhibited by *C. elegans*, so it is of interest to know what kinds of mutational lesion affect this behavior. The main conclusion from the survey of mating competence in *C. elegans* mutants is that successful mating is still observed in the majority of mutant types, and that only severe behavioral or anatomical lesions completely eliminate it. Paralyzed or severely uncoordinated mutants cannot mate, but most uncoordinated mutants with lesser defects in movement can mate, and so can most morphological mutants such as dumpies and longs. Moreover, the impotence of many of the remaining mutant types probably results not from the most obvious mutant defect but from abnormalities in the male genital anatomy. A frequent alteration is deformity of the copulatory spicules, observed in males of *sma-1*, *sma-2*, *sma-3*, *sma-4*, *vab-3*, *unc-34* and *unc-73*. Correct morphogenesis of the spicules depends on normal development both in their

constituent cells and in the associated muscle and body wall structures (SULSTON, ALBERTSON and THOMSON 1980), so a variety of primary lesions may result in malformation of the spicules. Males with abnormal spicules never mate successfully. However, several other alterations in the genital anatomy have less drastic effects on mating efficiency: severe deformity of the copulatory bursa is observed in the mutants *mab-1*, *mab-4* and *mab-6*, yet successful mating can still take place. Deformity (*mab-7*) or partial loss (*mab-2*) of the sensory rays also fails to prevent successful mating. Similar conclusions on the importance of different parts of the copulatory apparatus have been reached by means of laser ablation experiments. For example, ablation of the cell P10p leads to a specific loss of the hook sensillum, a sense organ located just anterior to the cloaca; males lacking the sensillum have difficulty in locating the vulva during mating but nevertheless are eventually able to mate successfully (J. E. SULSTON, unpublished observations).

Attempts to isolate mutants with specific alterations in mating behavior were largely unsuccessful: most of the impotent mutants isolated in a preliminary search were either grossly abnormal in genital anatomy or had general sensory defects. Two factors may have contributed to this failure. First, the neuronal circuitry controlling mating behavior arises from postembryonic cell lineages that also contribute to non-neuronal tissues (SULSTON and HORVITZ 1977; SULSTON, ALBERTSON and THOMSON 1980), so it might be expected that most mutations affecting these lineages would have pleiotropic effects. Also, it is likely that the neuronal circuitry is at least partly redundant, because several mutants (and some laser-operated animals) with defects in the male-specific sense organs are still capable of successful mating. The successful mating exhibited by catecholamine (*cat*) mutants demonstrates that the catecholamine cells of the male-specific nervous system are not indispensable components of the circuitry, although the relative inefficiency of *cat* males suggests that catecholamines are involved in mating behavior. If the mating circuitry were extensively redundant, then one might expect that relatively few mutations would completely prevent its functioning. Many mutations, however, might introduce subtle alterations into mating behavior. If the male neuroanatomy were known in detail, it might be possible to predict what kinds of subtle alteration to look for in mutants. Unfortunately, this neuroanatomy is more complex than the rest of the *C. elegans* nervous system.

An alternative approach to the genetic dissection of mating behavior could be provided by sex-determination mutants. For example, mutations in the gene *tra-2* cause XX animals to develop into "pseudomale" adults with most of the male-specific nervous system (e.g., cephalic companion sensilla), but these males show no interest in hermaphrodites and never exhibit mating behavior (HODGKIN and BRENNER 1977).

The search for mating-defective mutants did generate a number of novel mutants, and only some of those isolated have been adequately characterized. Most of the *mab* genes are identified by only one mutant isolate, so it is clear that this class of mutant is far from saturation. A more extensive search would probably lead to the identification of many more *mab* genes.

A significant feature of this work is that with few exceptions, mutations are expressed in both sexes of *C. elegans*. This is perhaps surprising in view of the extensive sexual dimorphism of this organism. Many of the mutants isolated on the basis of sex-specific characters, such as the multivulva mutants (*lin-1* and *lin-15*) or some of the *mab* mutants, were found to have phenotypes in both sexes, although the phenotype is usually much more conspicuous in one sex than the other. At least ten more *lin* genes affecting vulva development have been identified (C. FERGUSON and H. R. HORVITZ, personal communication), and it will be of great interest to examine the expression of these genes in the male.

Mutations in two dumpy genes (*dpy-21* and *dpy-26*) are not expressed in the male, but this is known to be a consequence of the different X chromosome dosage in males and hermaphrodites, rather than of sexual differentiation *per se* (HODGKIN 1980; J. HODGKIN, unpublished observations). Some mutations (*lin-2* and *lin-3*, *mab-6* and *mab-9*) appear to be genuinely limited in expression to one sex or the other. Further searches may reveal more mutants of this type: for example, it is likely that more *mab* genes exist. Conversely, a large number of mutations that affect egg laying have been obtained (C. TRENT and H. R. HORVITZ, personal communication), and at least some of these appear to be without effect on males. Also, there must be many genes involved in oogenesis whose expression is confined to the hermaphrodite, although their gene products are required for viability in both sexes. Genes with sex-limited expression are of interest because they could be the targets acted on by major sex-determining genes such as *tra-1* (HODGKIN 1980) in order to realize one or the other pattern of sexual differentiation (i.e., "realizator" genes in the sense of GARCIA-BELLIDO 1975). From this work it would appear that most genes are required in both sexes of the animal, even though they may have different roles to play in these two developmental paths.

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