CHEMOTAXIS-DEFECTIVE MUTANTS OF THE NEMATODE *CAENORHABDITIS ELEGANS*[1]

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ABSTRACT

The technique of countercurrent separation has been used to isolate 17 independent chemotaxis-defective mutants of the nematode *Caenorhabditis elegans*. The mutants, selected to be relatively insensitive to the normally attractive salt NaCl, show varying degrees of residual sensitivity; some are actually weakly repelled by NaCl. The mutants are due to single gene defects, are autosomal and recessive, and identify at least five complementation groups.

THAT genes play a major controlling role in the formation and functioning of nervous systems can hardly be questioned (BENTLEY 1971; BENZER 1971; BRENNER 1973, 1974; DELONG and SIDMAN 1970; KUNG and ECKERT 1972; MACAGNO, LOPRESTI and LEVINTHAL 1973), yet relatively little is known of the mechanisms by which this control is exerted. One approach toward discovering these mechanisms is through the study of a simple nervous system which happens to occur in a genetically tractable organism. In this case the simplicity of the nervous system offers the hope that it may become relatively well understood, while the genetic tractability offers the promise of genetic variants which may be used to explore the roles of genes in nervous system formation and function.

As BRENNER (1973, 1974) has pointed out, an organism which has very promising characteristics for this type of study is the soil nematode *Caenorhabditis elegans*, which has fewer than 300 neurons. It reproduces rapidly (generation time of about 3 days) and because most individuals are self-fertilizing hermaphrodites, induced recessive mutations are readily exposed. In addition, rare spontaneously-occurring males may be maintained, so that sexual crosses can be used for genetic mapping and complementation testing.

Mutations affecting the behavior of this animal seem very likely to affect its nervous system as well, and hence "behavioral mutants" are sought for the purposes outlined above. In order that such mutants be most easily analyzed, it is desirable that they should affect a relatively limited part of the animal's nervous system and behavior. Among the several behaviors which *C. elegans* demonstrates, chemotaxis is particularly attractive for genetic study because it permits a direct means of selecting and characterizing mutants with relatively specific behavioral defects.

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WARD (1973), using radial chemical gradients formed in Sephadex slurries, has reported that *C. elegans* is attracted to 3′,5′ cyclic AMP and to a number of common inorganic salts. DUSENBERY (1973, 1974), using the technique of countercurrent separation, has also shown that *C. elegans* is attracted to a number of common salts, and in addition, that *C. elegans* avoids acid and may be either attracted to or repelled by CO_2, depending upon the conditions used. This demonstrated variety of *C. elegans*' chemotactic responses affords flexibility both for isolation and for characterization of defective mutants.

We report below the isolation and partial characterization of an initial set of mutants, defective in response to NaCl, selected by countercurrent separation.

MATERIALS AND METHODS

Nematodes: ***Caenorhabditis elegans*** var. Bristol (strain N2) was from the Caltech stock collection (originally obtained from S. BRENNER); it was grown monoxenically, with *Escherichia coli* strain OP50 as food source, on NGMM-containing petri plates.

Bacteria: OP50 is a uracil-requiring derivative of *E. coli* strain BB, from the strain collection of the Medical Research Council Laboratory of Molecular Biology, Cambridge, England. It is used because on NGMM plates, which contain little uracil, it forms a lawn which is thick enough to feed *C. elegans* well, but not thick enough to interfere with observation of the nematodes. It was routinely grown in tryptone broth containing 10 g Difco Batco-Tryptone, 5 g NaCl, and 60 mg NaOH per liter.

Media: NGMM was prepared by mixing 17 g Difco Bacto-Agar, 3 g of NaCl, 2.5 g of Difco Bacto-Peptone, and 975 ml of H_2O, autoclaving, and subsequently adding 1 ml of 0.5% cholesterol in ethanol, 2 ml of 0.5M $CaCl_2$, 1 ml of 1.0M $MgSO_4$, 25 ml of 1.0M potassium phosphate buffer, pH 6.0, and 1.25 ml of Squibb Mycostatin, 50,000 units/ml (all sterile). The medium was routinely distributed to plastic petri plates, 100 × 15 mm, 40 ml per plate. After hardening, such plates were seeded with 0.1–0.2 ml of an overnight tryptone broth culture of OP50 bacteria and this was allowed to grow into a lawn before nematodes were added.

S medium was prepared by mixing 5.85 g of NaCl, 1.0 g of K_2HPO_4, 6.0 g KH_2PO_4, 1.0 ml of β-sitosterol (10 mg/ml in Tween 80), and 1 liter H_2O, autoclaving, and subsequently adding 3.0 ml of 1.0M $MgSO_4$, 3.0 ml of 1.0M $CaCl_2$, 10 ml of 1.0M potassium citrate buffer (pH 6.0), and 10 ml of a trace metals solution containing 0.69 g $FeSO_4 \cdot 7\,H_2O$, 1.9 g disodium EDTA, 0.20 g $MnCl_2 \cdot 4\,H_2O$, 0.29 g $ZnSO_4 \cdot 7\,H_2O$, and 0.025 g $CuSO_4 \cdot 5\,H_2O$ per liter.

Tracking medium contained per liter 15 g Difco Bacto-Agar, 2.5 ml Tween 20, and 100 ml of 0.1M HEPES or MOPS buffer, brought to pH 7.2 with NH_4OH. It was autoclaved, cooled until it cleared, and then rapidly distributed to plates.

Mutagenesis: The mutagenesis techniques we have used are based on those worked out in the laboratory of DR. SYDNEY BRENNER (BRENNER 1974). The objective of the method is to treat the nematodes (which are self-fertilizing hermaphrodites) at such a time during development and with such a dose of mutagen that one obtains relatively healthy adults bearing populations of mutagenized gametes, both eggs and sperm. We have found that this can be accomplished by treating animals which have grown for 28 to 36 hours at 22–23° after hatching. After being washed in S medium, about 5000 such animals were suspended in 5 ml of S medium containing 0.1% glucose and 1% ethyl methane sulfonate and incubated in a petri dish for 4 hours at 20°. After mutagenesis they were thoroughly washed with S medium and transferred to fresh NGMM plates for a short (approximately 1-hour) recovery period.

Growth and harvesting: Since the mutagenic treatment occurs well after sperm-cell and egg-cell precursors have become developmentally distinct, any one induced mutational event cannot generate both mutant eggs and mutant sperm. Consequently the first-generation progeny from mutagenized animals, even though produced by self-fertilization, are at most, heterozygous for an induced mutation (see Figure 1). However, because of the self-fertilizing nature of these ani-

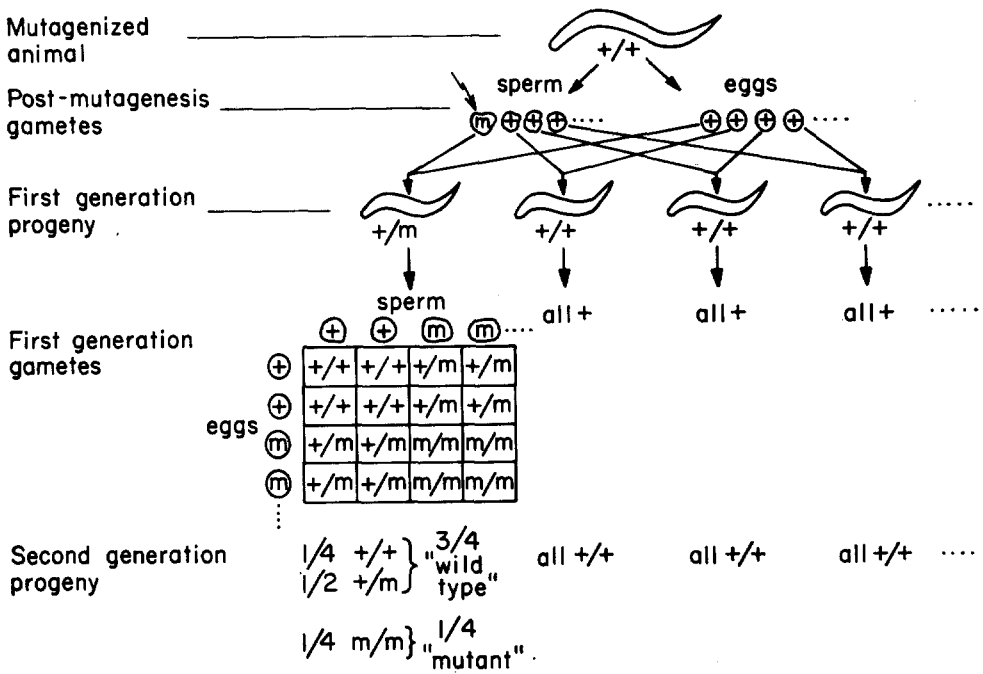

FIGURE 1.—Production of homozygous mutant progeny after mutagenesis of *C. elegans*. A mutational event giving rise to a single mutated gamete produces a single first generation progeny animal heterozygous for the mutation. Since each type of gamete produced by the animal has a probability of 0.5 of carrying the mutant allele, and *since these gametes fertilize each other without exchange to other individuals*, one-fourth of this heterozygote's progeny will be homozygous for the mutation.

mals, one-quarter of the second generation progeny derived from such a first-generation mutational heterozygote should be mutant homozygotes expressing the mutant phenotype. To allow expression of these homozygotes, a post-mutagenesis growth period of two generations was allowed. Two mutagenized, recovered individuals were used to start each growth culture, which was then incubated for about 10 days at 16° or 18° on NGMM plates pre-seeded with an OP50 bacterial lawn. After growth, the 10^4–10^5 nematodes on each plate were washed off and rinsed in sterile distilled water, using 25 mm Millipore type SC filters (nominal pore size, 8 microns). In the case of plates with unusually large numbers of animals only a fraction were taken; on the average 2×10^4 animals were taken from each plate.

Selection: Mutagenized, grown animals were selected for insensitivity to NaCl in a countercurrent separation apparatus (DUSENBERY 1973). In this apparatus two solutions of different compositions are made to flow stably past one another in opposite directions, in an inclined chamber. Animals injected at a neutral position migrate in the flow direction of the favored solution and exit from the chamber with this solution. For mutant selection, both solutions contained 0.7% methyl cellulose (Fisher 1500 centipoise) to increase the swimming efficiency of the worms; the lighter solution contained in addition 10 μg/ml of the marking dye light green SF yellowish, while the denser one contained 2.0% sucrose (to establish the required density difference) plus 10 mM NaCl as attractant. NaCl was used in the bottom (dense) layer so that mutants could be isolated from the top layer; with this arrangement, mutants with simple motor defects should sink to the bottom layer and not be isolated. The countercurrent chambers were 6 mm

I.D. cylindrical glass tubes 61 cm long, inclined at an angle of 45°, and the solutions were pumped in at approximately 0.2 ml/min as previously described (DUSENBERY 1973). The washing and countercurrent separations were performed in a room with temperature of 18°.

The 2-ml suspension of mutagenized, grown nematodes from one plate was injected into the center of one of nine countercurrent tubes and at least an hour was allowed to elapse before another suspension was injected into that tube. This provided adequate time for the bulk of the animals to emerge from the apparatus. After 3 to 6 plates of animals had been sequentially injected into each tube and allowed to emerge from the apparatus, the numbers of animals in some or all of the 18 collection vessels were determined and the animals from all 9 light solutions (potential mutants) were separately recovered. This was usually done by filtering the animals out of the methyl cellulose solution using a 48-mm SC filter in a Millipore Sterifil filter holder. The animals were then transferred from this filter in 10 ml sterile distilled water to a 25-mm SC filter, from which they were transferred to an NGMM plate with OP50 bacterial lawn by simply touching the filter to the plate, worm-side down. The resulting cultures, each representing one line, were allowed to grow up at 18° and the selection procedure was repeated separately for each line. After 3 or 4 rounds of selection each line was either cloned or discarded, depending on whether it exhibited abnormal chemotaxis.

Testing: Countercurrent tests of chemotaxis to NaCl were carried out using a pair of tubes with 10 mM NaCl in the light solution of one tube (a) and the dense solution of the other (b). The conditions were otherwise as described above for mutant selection, except that in some cases 0.5% methyl cellulose was used, with tubes inclined at 25° and a pumping rate of approximately 0.3 ml/min. (These differences had no apparent effect on the data obtained.) To correct for slight gravitational effects, a quantitative measure of the chemotaxis response, R, was defined as $100\ (R_a' + R_b' - 1)$, where R' is just the fraction of worms exciting with the NaCl-containing solution in the indicated tube. This definition creates a scale on which +100 corresponds to complete attraction, 0 corresponds to no response, and −100 corresponds to complete avoidance. Control experiments with no NaCl in either solution gave R values in the range from −10 to +10.

Tracking tests of individual responses to NaCl were carried out at 20° by a method slightly modified from that of WARD (1973). One μl of 5M NaCl was applied to the center of a 100 × 15 mm petri dish containing 4 ml of solidified tracking medium, and a period of 5–6 hours was allowed to establish a radial diffusional gradient. A single animal was then transferred from a normal culture plate to a spot about 1.5 cm from the center and allowed to run for an additional 5–6 hours. The animal's movement deformed the agar surface, leaving tracks whose refractive properties allowed them to be visualized as dark lines when the plate was placed about one inch above a uniform white surface under a nearly parallel beam of light from a photographic enlarger. Photographs were made by substituting photographic paper for the white surface.

Double blind tracking tests were performed by establishing separate tracking plates for 24 wild-type and 24 mutant individuals, marking the lids of the plates to distinguish mutant from wild type, and allowing a separate person (not the observer) to randomize the grouping of the plates and to place them in a visually isolated container. For scoring, the plates were handled individually; the lid was removed inside the container and the unmarked plate bottom was removed from the container, examined, and its tracks scored as normal or defective. The plate bottom was then returned to the container, the lid was put back on, and the plate was placed in a separate portion of the container as part of a "normal" or "defective" group. After all plates had been scored, the container was opened and the lids of each group were examined to determine the numbers of correct and incorrect assignments. Since this test is potentially subject to some bias based on the observer's knowledge that equal numbers of mutant and normal individuals comprise the sample, a second kind of test was performed on one mutant (RS1). In this case, 48 plates of each type were established, and a subset of 48 was drawn from these in a randomly determined ratio of mutant:wild type. The actual ratio was 8 mutants: 40 wild type, and all 48 plates were correctly scored, even though the observer knew nothing of the actual ratio.

Crosses: Crosses were performed by placing 3 young males and 3 young hermaphordites on a 35 × 10 mm petri dish containing 5 ml of NGMM medium and seeded with an OP50 bacterial

lawn. After 2 days at 20°, these parents were removed, and two (or sometimes three) days later, progeny were examined, tested, and used for further crosses. When hermaphrodite progeny were subsequently used to produce progeny by self-fertilization, an attempt was made to take young individuals which would not have much chance to be inseminated by their male siblings; however, since the genotype of these males was always identical with that of the desired hermaphrodites themselves, occasional cases where cross fertilization did occur were not treated differently from the others.

RESULTS

Mutant isolation: Of the many known chemical responses of *C. elegans* (WARD 1973; DUSENBERY 1974), the response to NaCl was selected for initial examination, primarily for two reasons. Firstly, the response of the wild type is strong and sensitive, so that a defective mutant response should be easily detected, and secondly, Na^+ and Cl^- ions are likely to be detected by different receptors (WARD 1973), so that mutants defective in response to both are likely to harbor interesting defects not confined to a single peripheral receptor.

It was assumed, in designing a selection procedure for mutants insensitive to NaCl, that the mutation frequency, even after chemical mutagenesis, would probably be sufficiently low to require a strong selective method. Preliminary tests of various methods showed that countercurrent separation (DUSENBERY 1973) was, for bulk populations, the method of choice, giving a predicted selection ratio of about 20:1 for mutants relative to wild type. This ratio, although high, still required the use of several successive selection cycles to enrich the proportion of mutants from an initial low level to the point where mutant cloning might be feasible. Accordingly, a procedure which accomplished these successive cycles was designed; the expected course of selection for this procedure is presented in Table 1. In this procedure, the successive selection cycles are separated by intervening growth periods, an approach which is potentially troublesome but nonetheless desirable. The potential difficulty arises because of the opportunity for accumulation of multiple mutations during the growth periods, possibly leading to a mutant phenotype which is multigenically determined. The advantage, however, is that relative to other procedures without intervening growth periods, this procedure involves at least eight times less work for a comparable yield of mutants. Accordingly, the procedure was adopted with the caveat that any mutants isolated with it would be carefully examined to determine whether they carried single or multiple mutations.

A population of near-adult *C. elegans* hermaphrodites was mutagenized with ethyl methane sulfonate, and 225 post-mutagenesis cultures were grown and harvested as described in MATERIALS AND METHODS. To ensure an independent origin for every isolated mutant, these cultures were grouped to provide 45 independent lines which were carried separately through three to four cycles of selection and growth (see MATERIALS AND METHODS). As Figure 2 shows, the behavior of these lines during the selection procedure corresponds quite well to the predictions of Table 1. In the first two selection cycles, the behavior of all lines was not very different from normal. In the third cycle, however, several lines showed clear chemotaxis defects, and these were confirmed in the fourth cycle.

TABLE 1

Expected course of the selection process

Time	Number of individuals of genotype +/+	+/m	m/m	Percent of animals recovered in the light solution
At inoculation of initial plates	10	0	0	..
After first generation of growth	1×10^3	1	0	
After second generation of growth	1×10^5	50	25	
After first selection	1×10^3	0	5	1.0
After intervening growth period	2×10^4	0	100	
After second selection	2×10^2	0	20	1.1
After intervening growth period	2×10^4	0	2×10^3	
After third selection	2×10^2	0	400	2.7
After intervening growth period	6.7×10^3	0	1.33×10^4	
After fourth selection	67	0	2.7×10^3	13.6

This table represents the expected course of selection in one line derived from 5 plates inoculated with two mutagenized worms each. It was assumed that in this line one initial gamete carrying a recessive mutation (*m*) to non-chemotaxis contributed its genome to the line, that the mutant worms grew at the same rate as the others, and that selection recovers 1% of non-mutant and 20% of mutant worms. These recovery figures are based on the observed behavior of normal *C. elegans* under selection conditions (i.e., with 10 mM NaCl in the heavy solution of the countercurrent apparatus) and on the assumption that mutant worms would distribute in the selection just as normal *C. elegans* does in the absence of an attractant in either solution.

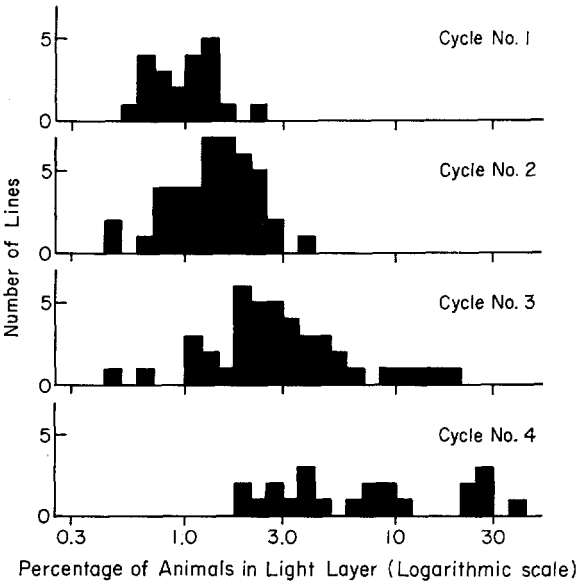

FIGURE 2.—Behavior of mutagenized lines during selection. The number of lines with a given percentage of animals in the light (NaCl-free) layer is plotted against that percentage. In the first cycle an arbitrary sample of 21 of the 45 lines was counted. In the second cycle all 43 lines were counted (two pairs had been pooled). In the third cycle all 42 lines were counted (one had been lost). In the fourth cycle 23 lines selected from those which had more than 1.5% in the light solution of the third round were counted.

Seventeen lines which showed clear defects in cycles three and four, and which grew well, were "cloned" by starting new cultures from single individuals. Since approximately five generations of self-fertilization had occurred since mutagenesis, it was assumed that these individuals were homozygous at virtually all loci (although potentially carrying more than one induced mutation). All clones were checked by countercurrent separation (using paired tubes as detailed in MATERIALS AND METHODS) to ensure that they had been initiated by mutant individuals. From four lines, single clones were started and all were mutant; for the remaining thirteen lines, two clones were started for each. In eleven cases both clones were mutant, and in two cases one was mutant and one normal. Thus, in total, 28 of the 30 initiating individuals turned out to be mutant, indicating that the selection procedure had, as predicted, been very efficient in enriching for mutants.

A rough estimate can be made for the frequency of the selected mutations by considering that 17 mutants were isolated from a total of 45 lines, in each of which approximately 1000 first generation progeny were produced from approximately 2000 gametes. By this estimate the frequency of gametes carrying a selectable mutation was about 2×10^{-4}; however, since comparable selections were not done on unmutagenized populations, it cannot be said to what extent this relatively high mutation rate is dependent upon mutagenesis.

Mutant characterization: For further work, a single clone was chosen from each of the original 17 lines and used to start a strain. The countercurrent responses of these 17 strains to NaCl are presented in Table 2A, along with concurrently determined wild-type responses; the mutant responses range in strength from approximately half that of wild type, through zero responses, to substantial responses in the opposite direction (avoidance of NaCl) confirming that the mutant strains are indeed altered. The five duplicated measurements indicate the relatively high degree of repeatability of the countercurrent method. Individuals from the mutant strains were also tested in a tracking assay (see MATERIALS AND METHODS). Wild-type *C. elegans* makes tracks which are highly concentrated in the center of the tracking plates, where the concentrations of NaCl is highest (Figure 3A-C), whereas most of the mutants make markedly different tracks with little or no preference for the center (Figure 3 D–F). To determine how reliably this assay could be used to distinguish a given mutant strain from wild type, double blind experiments were performed. In general, mutant and wild-type individuals could be quite reliably distinguished, as shown by the data of Table 2B. RS5, however, could not be distinguished at all, and the reliability of distinction was somewhat less for RS4 and DD72 than for the others.

The general reliability and relative simplicity of the tracking assay made possible a genetic characterization of most of the mutants. For each of these mutant strains, wild-type males were crossed with mutant hermaphrodites (Figure 4A), and a number of progeny males and hermaphrodites were individually tested by the tracking assay. The male progeny are especially pertinent, since all or nearly all of them result from cross-fertilization, while a variable fraction of the hermaphrodites result from self-fertilization. In these tests, virtually all of

TABLE 2

Mutant responses to NaCl

	A. Countercurrent separator Response (R) of		B. Tracking plates Fraction of individuals identified as wild type	
Mutant strain	Mutant strain	Wild type	Mutant strain	Wild type
DD74	−1, −5	+89, +93	1/25	24/24
DD79	+4	+96	0/24	24/24
DD80	−4	+93	0/24	22/24
RS6	−4	+100	0/24	23/24
DD71	−20, −39	+97, +89	0/25	23/24
RS1	+9	+99	1/50	50/50
RS4	+6	+90	4/24	23/24
DD73	0	+93	1/25	24/24
DD77	+49, +69	+96, +96	2/24	23/24
DD78	−20	+100	0/24	24/24
DD72	+23, +42	+97, +96	2/25	20/25
DD75	−46	+93	0/24	24/24
DD76	+18, +33	+96, +96	0/24	24/24
RS3	+14	+93	0/24	23/24
RS5	+29	+99	22/24	20/24
RS7	+43	+90	1/24	23/24

Note: In countercurrent separation, each mutant strain was tested in parallel with two to three other mutant strains and wild type. The score of the wild type is given beside that for each mutant strain. The method of countercurrent separation and the definition of R, the response measurement, are described in MATERIALS AND METHODS. Tracking plates were scored by the double blind method described in MATERIALS AND METHODS; note that the fraction given is the fraction of *mis*-identification for the mutant strains, but the fraction of *correct* identifications for the wild type. The fractions are presented as the actual numbers of individuals scored. Mutants are grouped according to the complementation results of Table 4.

the males and some of the hermaphrodites showed normal tracking (Table 3A), suggesting that each mutant was autosomal and recessive. (If the mutant were a sex-linked recessive, it would be expressed in the hemizygous male progeny.) When progeny males of cross A were backcrossed to mutant hermaphrodites (Figure 4B), the resulting male progeny were, as expected, about one-half normal and one-half chemotaxis-defective (Table 3B). This result shows that each tested mutant is capable of expression in the male, and thereby confirms the assignment of each mutant as autosomal and recessive. When chemotactically normal hermaphrodite progeny of cross A were allowed to self-fertilize (Figure 4C), the resulting progeny hermaphrodites were composed of normals and defectives in the ratio of approximately 3:1 (Table 3C). This result confirms that the chemotaxis defect in each tested strain is due to a single gene alteration, an important result in view of the multiple rounds of growth and selection employed in isolating the mutants.

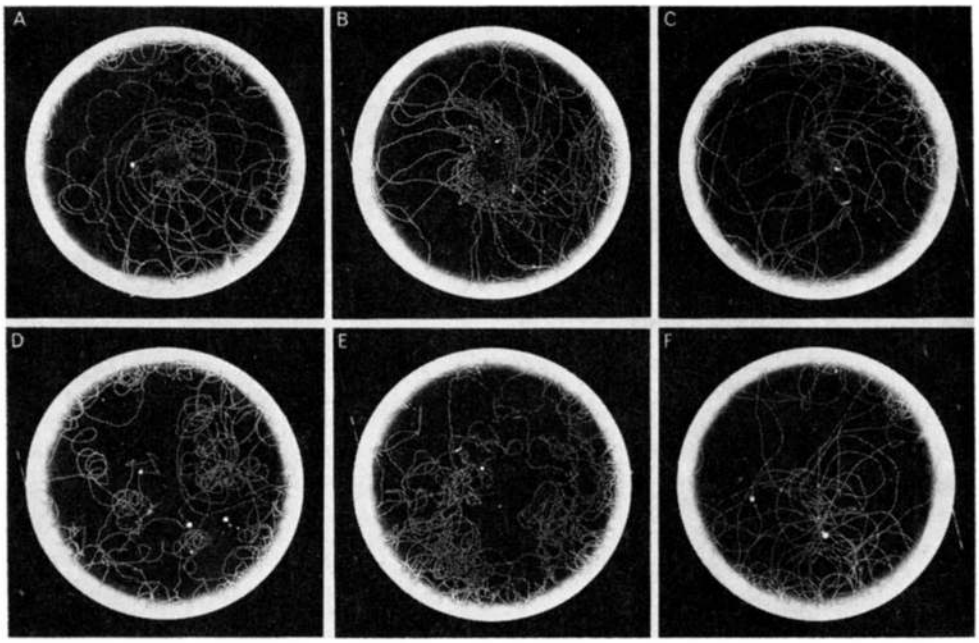

FIGURE 3.—Tracks of wild-type and selected mutants. Tracks were formed in response to a radical diffusion gradient of NaCl, as described in MATERIALS AND METHODS. A, B, C, Three different wild-type individuals; D, DD74; E, DD71; F, DD77.

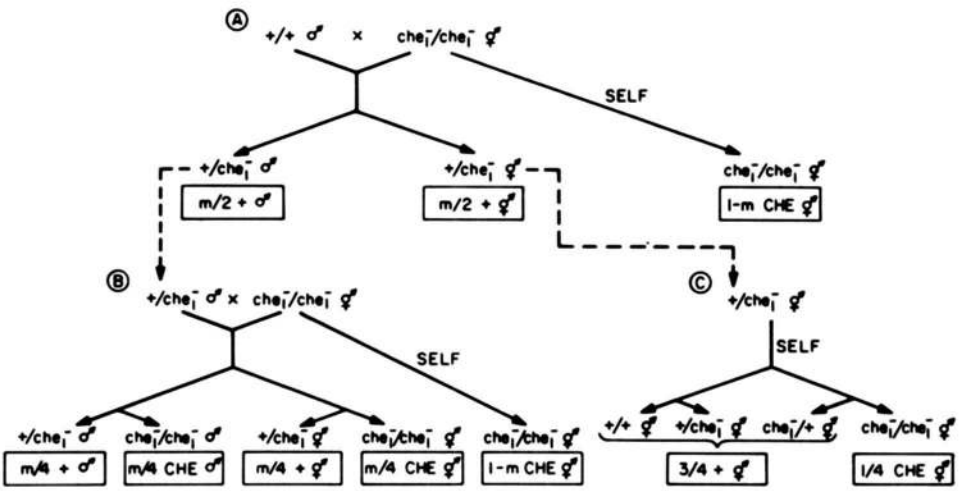

FIGURE 4.—Expected segregation behavior for an autosomal, recessive mutation. For each cross, self-fertilization progeny are indicated at right, cross-fertilization progeny at left. Phenotypes and their expected frequencies are indicated in boxes under the corresponding genotypes; + is normal taxis, CHE is defective taxis. The variable *m* is just the fraction of progeny derived by mating (cross-fertilization). A, cross of wild type ♂ with homozygous mutant ♂; B, cross of heterozygous ♂ from cross A with mutant ♂ homozygous for same mutation; C, self-fertilization of heterozygous ♂ from cross A.

TABLE 3

Segregation behavior of mutants

Mutant strain	Fraction of individuals identified as wild type in Progeny of cross A Males	Hermaphrodites	Progeny of cross B Males	Hermaphrodites	Progeny of self-fertilization C 1	2	2′
DD74	24/24	4/40	11/20	33/48		††	
DD79	25/25	3/30	8/20	34/48		††	§
DD80	25/25	10/45	9/20	33/48		††	
RS6	25/25	10/45	12/20	36/48		††	§§
DD71	24/24	7/45	11/20	64/96		††	§
RS1	3/3	6/30	2/9	39/48		††	§§
RS4	5/5	—	—	—			
DD73	24/24	20/45	13/20	73/96		††	§§
DD77	24/25	12/40	17/20	35/48		††	§
DD78	25/25	12/45	12/20	73/96		††	§§
DD72	24/27	21/60	—	—			
DD75	10/10	7/40	13/20	38/48		††	§§
DD76	22/23	8/45	—	30/48	*	††	
RS3	25/25	16/45	36/65	77/96		††	§§
RS7	29/29	5/30	—	76/95		††	§§

All identifications were performed by tracking tests, and fractions are given as in Table 2. Crosses A and B and self-fertilization C refer to Figure 4. Occasional non-wild-type males in column A may result either from mistaken scoring or, more rarely, from inadvertant mating between the cross A ♀ parent and a rare mutant ♂ present in its culture of origin. (Such males arise at a frequency of about 1 in 2000.) The significance of thee segregation data of column C is assessed in the columns to the right—in column 1, under the assumption that the mutant defect affects only a single gene (expected fraction of wild-type progeny: 3/4); in column 2, under the assumption that the mutant defect results from recessive mutations in two genes, *both* of which must be homozygous to give the defect (expected fraction of wild-type progeny: 15/16); and in column 2′, under the assumption that the mutant defect results from recessive mutations in two genes, *either* of which, if homozygous, gives the defect (expected fraction of wild-type progeny: 9/16). In all cases, no symbol indicates no significant deviation from expectation, one symbol indicates deviation significant at the 5% level, and two symbols indicate deviation significant at the 1% level.

To determine the number of genes identified by the set of mutant defects, complementation tests were performed by crossing mutant hermaphrodites of one type with homozygous mutant males of another type, obtained from cross B (Figure 5). All possible pairwise tests were attempted, and where possible each test was performed in both possible parental orientations. In general, male progeny from such tests were either all normal or all defective, permitting the two parental mutants to be unambiguously assigned as either complementing or non-complementing. The results are presented in Table 4 for ten mutant strains, in nine of which homozygous mutant males could be easily maintained and used for testing. The results are internally consistent and show that these ten mutants identify four complementation groups. Hermaphrodites from each of the remaining six mutants were tested against, and shown to complement with, mutant males from each of the nine male-fertile strains. These additional six mutants,

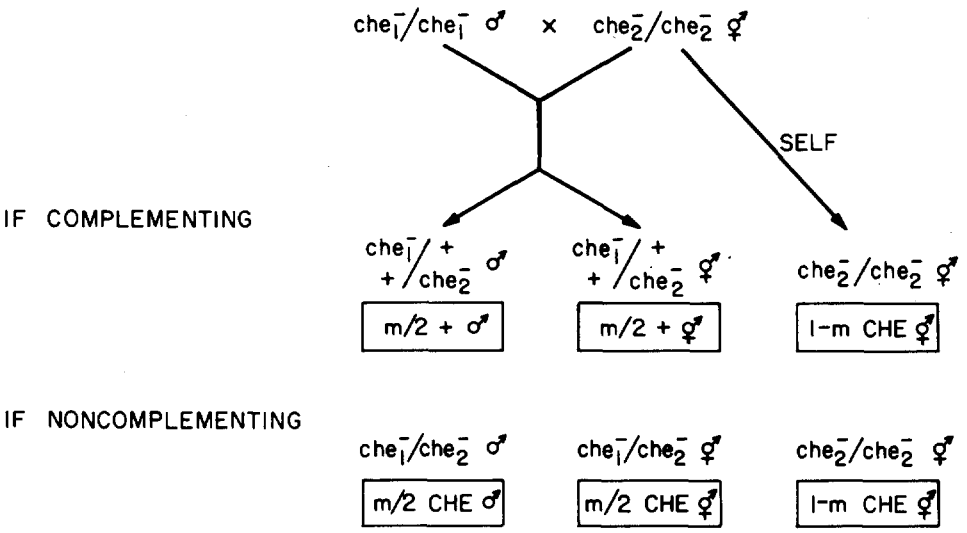

FIGURE 5.—Expected phenotypes in complementation tests. Symbols as in Figure 4. In practice, only ♂ progeny are scored, and should be either all + (if the two mutations complement) or all CHE if they do not).

therefore, show that one or more additional complementation groups have been identified, bringing the total to at least five. However, because of the difficulties of maintaining males of these mutant strains, we have so far been unable to determine just how many additional groups they identify.

TABLE 4

Complementation properties of mutants

	DD78	DD77	DD73	RS4	RS1	DD71	RS6	DD80	DD79	DD74
DD74	+[2]	+[2]	+[2]	+[2]	+	+[2]	—[2]	—[2]	—	—
DD79	+	+[2]	+[2]	+[2]	+	+[2]	—[2]	—	—	
DD80	+	+[2]	+[2]	+[2]	+	+[2]	—[2]	—		
RS6	+[2]	+[2]	+	+[2]	+	+[2]	—			
DD71	+[2]	+	+[2]	—[2]	—	—				
RS1				—						
RS4	+	+	+[2]	—						
DD73	+[2]	—[2]	—							
DD77	+[2]	—								
DD78	—									

Note: Homozygous ♂ ♂ of one genotype were mated with homozygous ♀ ♀ of the other, and 10 ♂ progeny were scored in tracking tests. If 9 or 10 were scored as wild type, complementation was judged to have occurred, and a "+" is entered in the table. If 1 or 0 were scored as wild type, non-complementation was judged to have occurred, and a "—" is entered in the table. Occasional intermediate results, probably due to rare prior mating by the ♀ parent, were discarded and the tests repeated. A superscript 2 indicates that the test was performed in both possible parental orientations, with the same result. No inconsistencies were observed.

DISCUSSION

The above results demonstrate that the technique of countercurrent separation can be used to isolate chemotaxis-defective mutants of the nematode *Caenorhabditis elegans*. They also indicate that at least the majority of the NaCl-insensitive mutant strains isolated in this fashion harbor defects which are due to single, recessive, autosomal genes.

The initial set of 17 mutants has identified at least 5 genes whose products are required for normal NaCl chemotaxis. Since independent repeat mutations in three of these genes have been identified in the initial mutant set, the total number of genes which can easily mutate to give NaCl insensitivity may be relatively small. Presumably this restriction is due in part to the choice of an attractant, NaCl, whose component Na^+ and Cl^- ions are probably detected by different receptors (WARD 1973).

The behavioral scope of the mutant defects has been examined further, using countercurrent separation, tracking assays, and some other behavioral tests currently in use in this laboratory. A full description of the results will be published elsewhere, but a few central points should be mentioned here. Firstly, for each mutant at least one, and usually two or three, different chemotaxis responses remain unaltered, showing that the mutants have not simply lost, for instance, a motor control program required for all responses. Secondly, mutants in different genes show different behavioral defects, some responses being lost by some mutants and retained by others; this suggests that the products of these genes play different roles in the stimulus-response sequence. And thirdly, several mutants, in addition to their chemotaxis defects, show defects in thermotaxis as well (HEDGECOCK and RUSSELL 1975), showing that these two different response modalities depend upon some gene products in common.

The structural basis of the mutant defects is of considerable interest, and is currently being examined by serial section electron microscopy, using techniques which have been used to establish an anterior sensory anatomy of wild-type *Caenorhabditis elegans*. (WARE *et al.* 1975; WARD, THOMSON, WHITE and BRENNER, 1975).

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