

Chemotactic Responses of Male *Caenorhabditis elegans*

DAVID B. DUSENBERY¹

Abstract: Cultures of *C. elegans* containing a high proportion of males were subjected to chemotactic tests by using the method of countercurrent separation. The responses of males and hermaphrodites were determined. Both types of worms preferred Na⁺ over 1/2 Ca⁺², Cl⁻ over NO₃⁻; they were attracted to NaCl, OH⁻, cyclic AMP, pyridine, CO₂ in borate buffer (pH 8.8); and avoided CO₂ in phosphate buffer (pH 6.0), D-tryptophan, and acid. It was thus concluded that male *C. elegans* have the same chemotactic responses that hermaphrodites of this species are known to have. *Key Words:* attraction, behavior, nematode, repulsion.

It has long been supposed that chemical stimuli play an important role in the lives of nematodes, especially with regard to host and mate finding (1, 7). In spite of this

supposition, relatively little research has been reported on the behavioral responses of nematodes to identified chemicals.

Recently, a more systematic analysis of the effectiveness of various chemicals in attracting or repelling the free-living soil nematode *Caenorhabditis elegans* has been performed. Ward (8) found that this species was attracted to Na⁺, Cl⁻, OH⁻, and

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¹Schools of Biology and Physics, Georgia Institute of Technology, Atlanta, Georgia 30332. The support of the National Science Foundation (Grant GB 43561) and the assistance of Ms. Georgann Hardin are gratefully acknowledged.

adenosine 3':5'-cyclic monophosphate but not to the many other chemicals tested. In addition, he suggested that all four of the chemicals are detected by distinct receptors. Dusenbery (4) found an attraction to CO₂ (or at least one of the forms of CO₂ in aqueous solution) in the presence of pH 8.8 borate buffer, an avoidance of CO₂ in pH 6.0 phosphate buffer, and an avoidance of acid. More recently, it has been found that *C. elegans* avoids D-tryptophan and is attracted to pyridine (5, 6).

Previous studies on *C. elegans* were all performed on hermaphrodites. Therefore, a question arises as to whether males of this species have similar chemotactic behavior. Detailed anatomical investigations of the sensory neuroanatomy of the head of *C. elegans* indicate that males are nearly identical to hermaphrodites except for one additional type of sensory ending (8).

The studies reported here demonstrate that male *C. elegans* have all the chemotactic responses to defined chemicals that have been reported in hermaphrodites.

METHODS AND MATERIALS

The strain of *C. elegans* used (N2) and its methods of culture are those of Brenner (2). Male-containing cultures were generally started by introducing approximately 5 adult hermaphrodites and 10 males to a petri dish containing bacteria in a spot (approximately 1-cm diam) at the center. All the nematodes were generally found in the spot within 1 h. Their relatively high concentration in the small spot was expected to increase the probability of mating. After about 4 days at 20 C, each dish generally contained hundreds of adult worms of which roughly 20% were males.

The chemotaxis experiments were based on the method of countercurrent separation (3). In this method, a dense solution flows downward along the bottom side of an inclined tube, while a light solution, floating on the dense solution, flows upward along the top side. Several hundred nematodes are injected into the center of the tube. A response to an attractant or repellent carried in one of the solutions is determined by observing the proportion of animals which emerge from the tube with that solution.

In all experiments reported here, a pair of countercurrent tubes ("a" and "b") was run in parallel with nematodes from the same population in both tubes, but with the attractant distribution of the solutions reversed in the two tubes. An overall measure (R) of the response to the attractant was then defined as $100 (R_a' + R_b' - 1)$, where R_a' is the fraction of worms from tube "a" that were found in the solution with the higher concentration of attractant and similarly for R_b' . This measure defines a scale on which +100 corresponds to complete attraction, 0 corresponds to no response, and -100 corresponds to complete avoidance. This scale is the same measure as previously used (4). In the present series of experiments, hermaphrodite and male nematodes were counted separately, and separate responses were calculated for hermaphrodites and males from the same population.

In all chemotaxis experiments, 0.5% methylcellulose (Fisher 1500 centipoise) was present in both light and dense solutions to increase the nematodes' swimming ability. The light solutions contained 10 µg/ml of the dye Light Green SF Yellowish to allow visualization of the flow patterns, and the dense solutions contained 2% sucrose to produce the density difference. In addition to the previously mentioned materials, one of the following sets of stimulus chemicals and buffers was added, according to the particular experiment being performed. These were: (i) NaCl [10mM NaCl in one solution; (ii) Na⁺ vs. 1/2Ca²⁺ [10mM NaNO₃ in one solution and 5mM Ca(NO₃)₂ in the other. The NaNO₃ was treated as the stimulus in determining the sign of the response]; (iii) Cl⁻ vs. NO₃⁻ [10mM KCl in one solution and 10mM KNO₃ in the other. The KCl was treated as the stimulus in determining the sign of the response]; (iv) OH⁻ [1mM KOH in one solution and 50mM NaCl in both. Under these conditions there is no response to K⁺ (4)]; (v) cAMP [1mM adenosine 3':5'-cyclic monophosphate in one solution and 5mM K₂HPO₄ + 5mM KH₂PO₄ in both solutions]; (vii) Pyridine [1mM pyridine in one solution and 5mM K₂HPO₄ + 5mM KH₂PO₄ in both solutions]; (viii) CO₂

(borate) [1mM NaHCO₃ in one solution and 10mM sodium borate buffer of pH 8.8 in both solutions]; (viii) CO₂ (phosphate) [1mM NaHCO₃ in one solution and 25mM potassium phosphate buffer of pH 6.0 in both solutions]; (ix) D-tryptophan [5mM D-tryptophan in one solution and 5mM K₂HPO₄ + 5mM KH₂PO₄ in both solutions]; (x) H⁺ (unbuffered) [1mM HNO₃ in one solution and 50mM NaCl in both. Under these conditions there is no response to NO₃⁻ (4)]; (xi) H⁺ (phosphate) [6mM HNO₃ in one solution and 5mM K₂HPO₄ + 5mM KH₂PO₄ + 50mM NaCl in both solutions]; (xii) H⁺ (citrate) [15mM HNO₃ in one solution and 10mM potassium citrate buffer of pH 6.0 + 50mM NaCl in both solutions].

RESULTS AND DISCUSSION

The chemotactic responses of males and hermaphrodites from the same cultures to 12 different tests are presented in Table 1. In each test, the response of the males was nearly as strong or stronger than that of the hermaphrodites. On the average, the males responded somewhat more strongly than the hermaphrodites. This difference may result from a greater swimming ability of the males (which are longer for their thickness than the hermaphrodites). This hypothesis has been tested by using strain E678, a mutant isolated by Brenner in which hermaphrodites also have a greater length for a given thickness (2). Hermaphrodites of this strain responded more strongly than N2 hermaphrodites in these same tests. Thus, there is no reason to conclude from these experiments that males are any different in their sensory abilities than hermaphrodites. This finding is in accordance with the observation that the sensory anatomy of both forms is similar (9).

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TABLE 1. Chemotactic Responses of *Caenorhabditis elegans*.

Experiment	Response*	
	Males	Hermaphrodites
NaCl	+100	+100
"	+100	+90
Na ⁺ vs. 1/2Ca ⁺²	+100	+98
"	+100	+92
Cl ⁻ vs. NO ₃ ⁻	+95	+95
"	+96	+88
OH ⁻	+75	+79
"	+44	+52
cAMP	+75	+96
"	+82	+46
Pyridine	+66	+81
"	+73	+60
CO ₂ (borate)	+72	+9
"	+100	+78
"	+98	+63
CO ₂ (phosphate)	-100	-95
"	-92	-57
"	-100	-100
D-tryptophan	-77	-87
"	-71	-87
H ⁺ (unbuffered)	-94	-92
"	-93	-66
H ⁺ (phosphate)	-100	-71
"	-100	-86
H ⁺ (citrate)	-100	-100
"	-100	-96

*Each line represents the results of testing one culture, containing both males and hermaphrodites, in one experimental situation by using a pair of tubes as described in *Materials and Methods*. Each experiment was performed two or three times with different cultures being used each time. The results for each culture are presented to provide an indication of the variations found in these experiments.

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