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Genetics

Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*

(behavior/mutants/temperature/chemotaxis)

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ABSTRACT **When grown at a temperature from 16° to 25° and placed on a thermal gradient, the nematode *Caenorhabditis elegans* migrates to its growth temperature and then moves isothermally. Behavioral adaptation to a new temperature takes several hours. Starved animals, in contrast, disperse from the growth temperature. Several mutants selected for chemotaxis defects have thermotaxis defects as well; these behaviors depend on some common gene products. New mutants selected directly for thermotaxis defects have unusual phenotypes which suggest mechanisms for thermotaxis.**

Mutants with specific behavioral defects are useful in understanding the mechanisms by which genes direct the formation of a nervous system (1–4). Such mutants have been isolated in the small soil nematode *Caenorhabditis elegans* and, because the nervous system is simple (<300 cells), the mutant defects may be identified by serial section electron microscopy (1, 5–8). Mutants can also be analyzed by formal genetic techniques, such as dominance testing, complementation, and epistatic ordering, to gain insight into the structure of behavioral pathways and their development.

Mutants of *C. elegans* with general behavioral defects (1, 9) and specific chemotaxis defects (5) have been described. This paper describes some mutants with specific defects in thermotaxis. These mutants are particularly interesting because thermotaxis can be modified by experience.

MATERIALS AND METHODS

Nematodes. *Caenorhabditis elegans* [var. Bristol (strain N2)] was used for behavioral studies and mutant selection. Worms were grown monoxenically in petri plates containing nematode growth minimal medium (NGMM) agar (5) preseeded with *Escherichia coli* strain OP50 (1).

Temperature Gradients. A stable and reproducible linear temperature gradient was established by connecting two thermostatically regulated water baths, 5° and 35°, by a 61 × 10 × 1.3 cm aluminum slab tightly bolted at each end to a 10-cm aluminum cube immersed in a bath. The temperatures of the baths were stable to 0.1°. The room temperature varied from 19.5° to 20.5°. Plastic petri plates (9-cm) containing 35 ml of agar culture medium (NGMM) were placed on the aluminum gradient slab at regular intervals and the agar temperature was monitored with a glass probe thermistor. The agar surface established a uniform gradient of 0.5°/cm with an equilibration half-time of 5 min. The gradient was undistorted by edge-effects to within 1 cm of the petri plate wall.

Thermotaxis Assays. Two assays were devised: one based on the accumulation pattern of populations on a linear thermal gradient and one based on the tracks made by individual animals moving in a radial gradient.

For the accumulation assay, petri plates were placed at four positions on the aluminum gradient slab (centered at 12.9°, 17.6°, 22.8°, and 27.1°), and equilibrated for 1 hr before introducing the nematodes. The worms were washed from their growth plates with 5 ml nematode growth (NG) buffer [same inorganic ion content as NGMM plates (5)] and allowed to settle in a conical tube. The wash buffer was withdrawn and the worms were resuspended in 5 ml of buffer and settled again. These washes were performed at 20°. After removal of the second wash, the worms were resuspended in 100 μl of Sephadex G-200 Superfine gel beads swollen in buffer and then applied to the centers of the agar plates in 20-μl portions of 50 to 200 animals each. After 1 hr the nematodes were killed by inverting the plates over chloroform and their distribution was recorded.

For graphical analysis, each plate was scored for three classes of nematodes: those migrating to the warmer half of the plate (H), those migrating to the colder half (C), and those never fully leaving the point of application (usually less than 10%). The results can be expressed by the percentage going to the warmer half $100 \times H/(H + C)$ or by a "thermal preference" scale defined as $100 \times (H - C)/(H + C)$ (Fig. 3). The second scale has the advantage that 0 indicates no preference, while positive and negative values indicate preferences for higher and lower temperatures, respectively. The standard error of the mean averaged 10 scale units in repeated experiments. The sampling error, approximately $[(H \times C)/(H + C)^3]^{1/2} \times 200$ on the thermal preference scale, averages 10 scale units in the data that follow and is never more than 20 units.

For the track assay, individual worms grown at 20° were placed on 9-cm plastic petri plates containing 10 ml NGMM agar. The plates were then moved to room temperature (24°–26°) and inverted. A 20-ml glass vial filled with frozen glacial acetic acid (melting point 16.6°) was placed at the center of each plate on the plastic bottom just above the agar. As the acetic acid melted over the next 2–3 hr, the vials maintained a constant temperature of nearly 16.6° at the plate centers, creating a stable radial temperature gradient in which the worms could move. The tracks were then photographed (5, 9).

Mutant Selection. Young nematodes were mutagenized with ethylmethanesulfonate (1, 5) and allowed to self-fertilize for two generations at 16°, 20°, or 25°. A plastic tray, divided lengthwise into six channels, was filled with a slurry of Sephadex beads swollen in NG buffer and placed on the aluminum gradient slab to equilibrate. The progeny from the mutagenesis were then eluted from their growth plates,

Abbreviations: NGMM, nematode growth minimal medium; NG, nematode growth medium.

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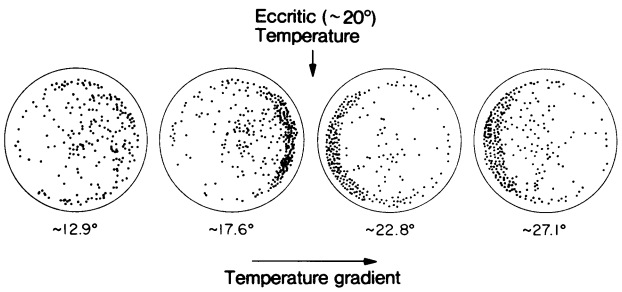

FIG. 1. The distribution of 20°-raised asynchronous adults in the accumulation assay. Animals were applied to the plate centers, run for 1 hr, chloroformed, and their final positions were recorded by dots. Summed results of five separate experiments.

washed, and suspended in a Sephadex slurry, just as for the accumulation assay, but, instead, the entire suspension from a plate (about 1000 adult worms in 150 μl) was applied to the end of the channel on the gradient. The application temperature was the same as the growth temperature. After 1 hr the channels were examined for worms migrating 10 cm from the point of application. These animals were placed on individual growth plates and raised at the same temperature as before. Successful clones were examined for behavioral defects by the usual assays. A total of 50 plates were screened, from which 64 clones were examined, yielding six independently isolated mutants.

RESULTS

Thermotaxis of normal *Caenorhabditis elegans*

Adult hermaphrodites grown at 20° migrate toward 20°. Their distribution on a linear temperature gradient is shown in Fig. 1. These same data, and similar data for 20°-grown juvenile animals, are presented graphically in Fig. 2. When grown at 16° or 25°, the nematodes distribute differently as shown in Fig. 3. 16°-raised animals migrate toward 16° from nearby temperatures while 25°-raised animals move toward 25°.

Nematodes raised at 20° orient perpendicular to the gradient when placed from 18° to 22° and move along isothermal lines. This can be seen both on the linear gradient and on radial gradients (Fig. 8A). Individuals may travel several centimeters (1–2 min) while diverging less than 1 mm (0.05°) from a linear isotherm. A worm may make several isothermal tracks in an hour which vary within a 3° range

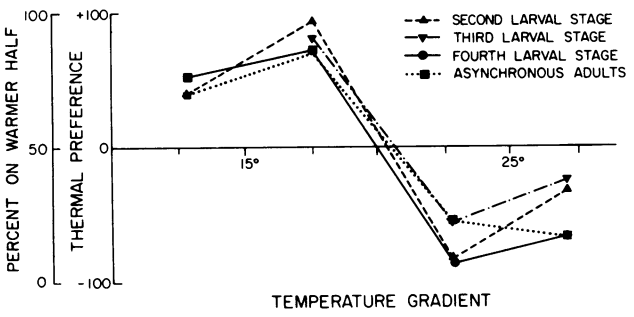

FIG. 2. The accumulation profiles of 20°-raised synchronous juveniles of three different ages and of 20°-raised asynchronous adults. The second, third, and fourth stage juveniles were from synchronous populations measured 27, 36, and 45 hr after egg-laying, respectively. The two vertical scales show the correspondence between two possible methods for expressing the results, of which the second, thermal preference, is used below. Compare to Fig. 1, which contains comparable raw data.

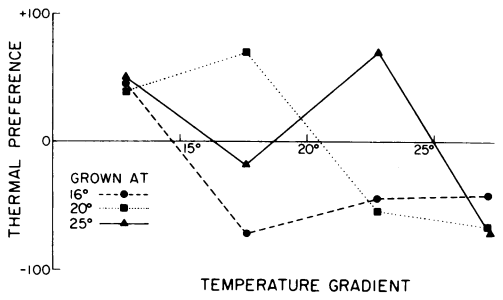

FIG. 3. The accumulation profiles of asynchronous adults raised at 16° (- - ● - -), 20° (. . . . . ■ . . . .), and 25° (—▲ —).

centered about 20°. Worms grown at 16° and, to a lesser extent, at 25° track isothermally near 16° and 25°, respectively. Worms migrating toward their eccritic (preferred) temperature along the gradient orient far less accurately than worms at the eccritic temperature moving isothermally.

Temperature-shift experiments indicate that the eccritic temperature can be reset in a period of hours. Nematodes shifted from 16° to 25° as newly laid eggs behave, when tested as adults, like normal 25°-grown animals. Adults from down-shifted eggs behave like cold-raised worms. When synchronous populations of nematodes are temperature-shifted as young adults, the thermal preferences change rapidly for 2–4 hr and more slowly thereafter (Fig. 4). The time courses are similar for up-shifts and down-shifts although the accumulation patterns during the transitions are not equivalent. The paradoxical cold-preference of 25°-raised worms is slow to appear in up-shifted worms, requiring over 8 hr.

Temperature reacclimation may require protein or lipid synthesis. Thermotaxis changes under a range of conditions involving starvation or overcrowding. Old growth plates undergo a characteristic sequence of events. As the bacterial lawn nears exhaustion, the worms form large moving aggregates or "swarms" which quickly deplete the bacteria and then disperse. In the days following swarming, many of the new larvae that hatch enter an arrested developmental stage modified for survival. These "dauerlarvae" can be recognized by their thin shape and their resistance to harsh reagents, such as 1% sodium dodecyl sulfate (R. Cassada and

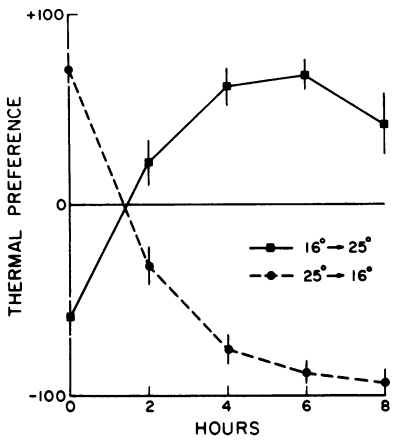

FIG. 4. Thermal preference of nematodes raised at 16° and 25° and then shifted as adults to 25° and 16°, respectively. Thermal preference was monitored at the indicated times after the temperature shift on a plate centered at 22.8° on the gradient. At this temperature 16°-raised animals show a strong negative preference and 25°-raised animals a strong positive preference, as seen here and in Fig. 3.

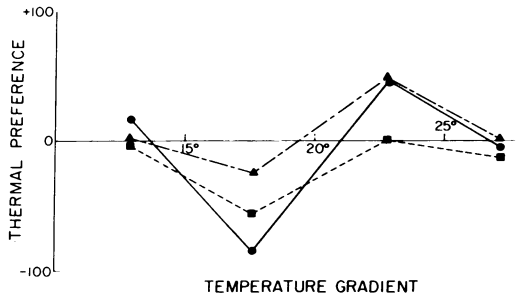

FIG. 5. The accumulation profiles of 20°-raised nutritionally deprived nematodes: from a plate 2–3 hr after the swarming stage (- - - ■ - - -), from an exhausted plate with more than 30% dauerlarvae (—● —), and from NG liquid culture (— ▲ —).

R. L. Russell, unpublished results). The accumulation profile of 20°-raised adults assayed 2–3 hr after swarming is shown in Fig. 5. Also shown are worms from very old plates (>30% dauerlarvae) and worms grown to high density in liquid culture. These nutritionally deprived worms actually disperse from 20° rather than accumulate. 20°-raised adults starved for various times prior to assay by elution from their growth plates and suspension in NG buffer show that the transition from accumulation to dispersion behavior occurs by 4–6 hr after the onset of starvation (data not shown).

Thermal responses of chemotaxis-defective mutants

Sixteen independent chemotaxis-defective mutants previously isolated in this laboratory by the technique of countercurrent separation (5, 12, 13) were examined for possible thermotaxis defects by both the accumulation and the track assays. The selection, characterization, and genetics of these mutants, each of which is defective in responding to normally attractive NaCl gradients, are described elsewhere (5).

About half the chemotaxis mutants display some defect on the thermotaxis accumulation assay when raised at 20°, and mutants assigned to the same complementation group by chemotaxis assay show similar thermotaxis phenotypes (Fig. 6). In particular, all members of the group DD74–DD79–DD80–RS6 are essentially normal in accumulation profile, whereas mutants of the group DD71–RS1–RS4 are all athermotactic. Of the two alleles DD73 and DD77, only DD73 has a detectable thermotaxis defect. This is consistent with countercurrent measurements (5) indicating that DD77 retains most of the wild-type response to NaCl whereas DD73 has none. The mutant DD75, which is actually repelled by NaCl, displays a remarkable thermophilic mistaxis when grown at 16° or 20° (Fig. 7).

Table 1. Isothermal tracking of chemotaxis-defective mutants

Classified mutants		Unclassified mutants	
Mutant	Tracking	Mutant	Tracking
DD74	+	DD72	+
DD79	+	DD75	+/−
DD80	+	DD76	+
RS6	+/−	RS3	+
		RS5	+
DD71	−	RS7	+
RS1	−		
RS4	−		
DD73	−		
DD77	+		
DD78	−		

Five or more 20°-grown individuals of each strain were tested on radial gradient plates for the presence of recognizable isothermal tracking (judged visually; see Fig. 8 for examples). Where some individuals were scored (+) and others (−) the notation (+/−) appears. Mutants are classified according to the complementation results of Dusenbery *et al.* (5).

Individual tracking assays gave results similar to the accumulation assays (Table 1). The distinction between relatively normal mutants (DD79, Fig. 8C) and completely atactic mutants (DD71, Fig. 8D) is striking.

Thermotaxis-defective mutants

Six independent thermotaxis-defective mutants were isolated and partially characterized. Each has good reproduction and locomotion, normal gross morphology, and high expression in adult hermaphrodites. All six are recessive mutations; four are autosomal and two are X-linked (EH65 and EH71).

Accumulation profiles of the mutants are shown in Figs. 6

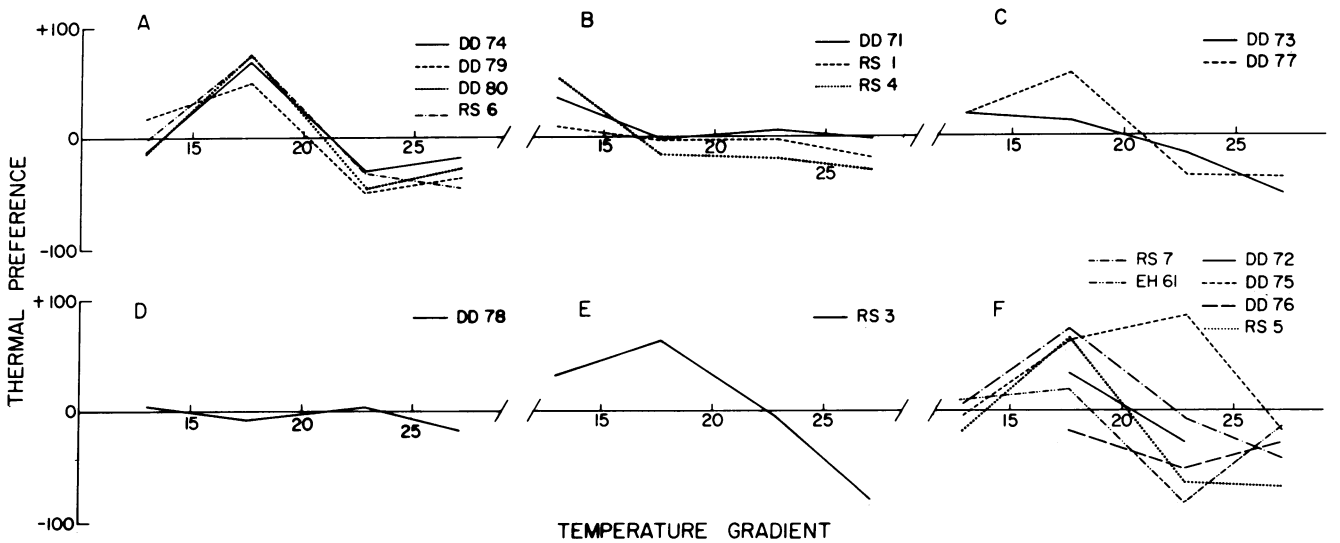

FIG. 6. The accumulation profiles of 20°-raised adults of mutants selected for defective chemotaxis to NaCl. The mutants are listed by genetic complementation groups (5) except for E and F, both of which consist of as yet unassigned mutants.

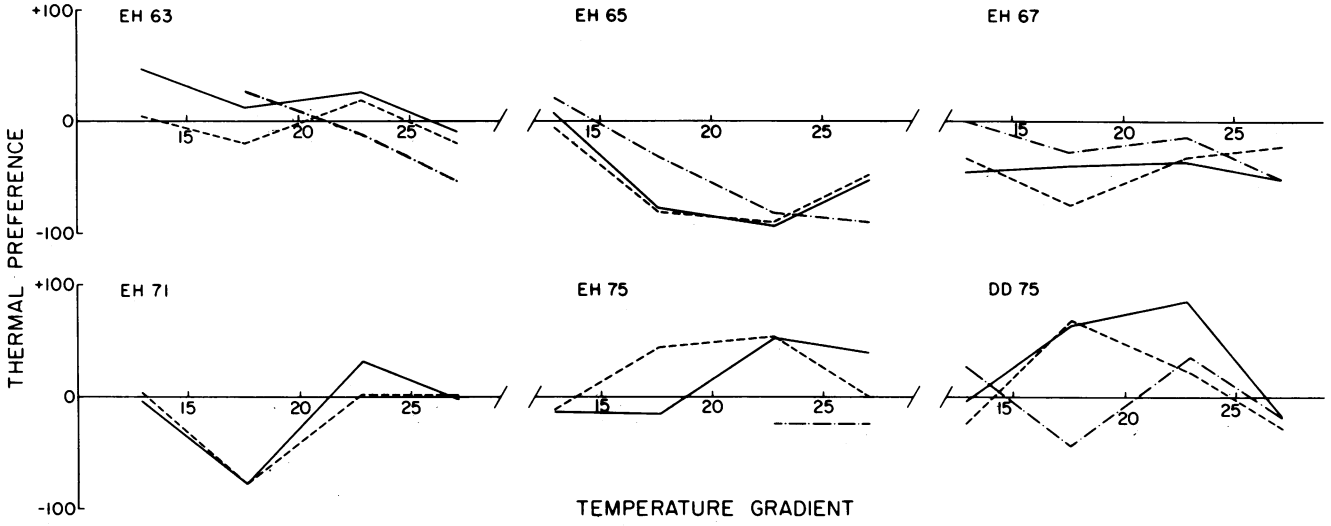

FIG. 7. The accumulation profiles of mutants selected for defective thermotaxis and of DD75: - - - - - -, raised at 16°; ——, raised at 20°; —·—·, raised at 25°.

and 7. Three of the mutants (EH61, EH65, and EH67) failed to track isothermally when raised at 20° whereas EH63, EH71, and EH75 produce recognizable isothermal tracks near 20° on the gradient. The tracking of 20°-raised EH67 is shown in Fig. 8B.

When tested for chemotactic orientation on NaCl gradients (5, 9), all but EH61 were qualitatively normal. EH61 can be reliably scored as a chemotaxis-defective mutant.

DISCUSSION

The thermal behavior of *C. elegans*, including (1) migration toward the growth temperature from nearby temperatures and (2) isothermal tracking at the growth temperature, is modified by experience. First, the eccritic temperature follows the growth temperature; the animals require several hours to adapt when the growth temperature is shifted. Second, when starved, *C. elegans* disperses from the growth temperature rather than accumulates. In a previous study, *C. elegans* and several other nematodes were reported to migrate to lethal high temperatures in a liquid suspension thermotaxis assay (11, 17). We have not seen this behavior in *C. elegans*, nor in four other soil species we examined. Our observations agree, however, with studies on *Ditylenchus dipsaci* (14–16), which accumulates at a temperature correlating to the temperature of prior storage when placed on a thermal gradient.

In a moist, organic soil subject to diurnal surface temperature fluctuations, the soil temperature varies as a propagating sine wave (about 2 cm/hr) whose amplitude decays exponentially with depth (18). A nematode in the upper few centimeters of soil may experience vertical gradients as large as 0.5°/cm and temporal gradients of 0.5°/hr. In this situation, a nematode with a fixed eccentric temperature might be driven deep into the soil within several hours, whereas *C. elegans* may regulate its vertical position by slowly changing its thermal preference. Dispersion from the growth temperature, which accompanies starvation, may allow *C. elegans* to escape from an unfavorable local environment to which it might be confined by the usual eccritic behavior. Similarly, the paradoxical diminution or reversal of thermal preference in worms raised at extreme temperatures (16° or 25°) and then displaced many degrees from the growth temperature may delimit the working range of the eccritic response. (*C. elegans* reproduces poorly above 25° and below about 13°.)

The two thermotaxis assays, population accumulation on a linear gradient and individual tracking on a radial gradient, are best suited to study migration toward an eccritic temperature and "isothermal tracking," respectively. Since several mutants (EH63, EH71, and EH75) which migrate incorrectly still make isothermal-like tracks near their growth temperature, the conceptual distinction between the two behaviors may reflect an actual separation in mechanism. Quite formally, migration to a preferred temperature may involve two opposing "drives," one for upward and one for downward thermal migration, which just balance at the eccritic temperature. If either "drive" could be separately eliminated by mutation, the resulting animal should be cryophilic or thermophilic, respectively. Several mutants have phenotypes

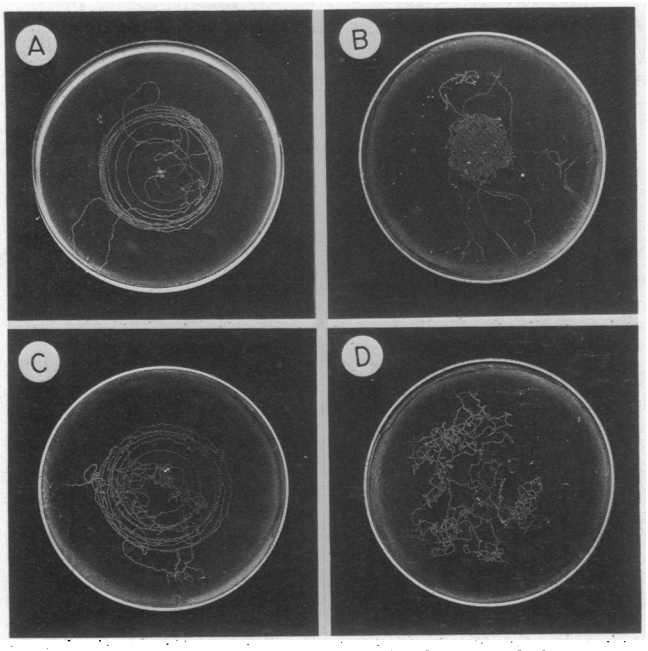

FIG. 8. Tracking of 20°-raised nematodes on radial temperature gradients: A, wild type; B, EH67 (cryophilic); C, DD79; D, DD71 (atactic).

consistent with this idea (EH65, EH67, cryophilic; DD75, EH75, thermophilic). Interestingly, the thermal response of the thermophilic mutants is still strongly modulated by the growth temperature, whereas the cryophilic mutants are insensitive to the growth temperature. This suggests that only the upward "drive" is plastic and is reset in temperature acclimation. Also, only the cryophilic mutants (EH61, EH65, and EH67) are devoid of isothermal tracking.

Since several mutants (e.g., DD71, DD73, DD78, and EH61) are defective in both chemotaxis and thermotaxis, the two responses must depend on gene products in common. Because these mutants have normal motility and normal responses to touch and to chemical repellents, it is unlikely that the gene products are involved in motor output, *per se*. The gene products may be invoked twice in separate structures involved in chemotaxis and thermotaxis, respectively, or may affect a structure involved in sensory processing after chemo- and thermoreceptor signals have converged. In the latter case, the gene products must act at a point after the hypothetical cryophilic and thermophilic drives are compared, since the jointly defective mutants (except DD75) are all athermotactic rather than mistactic. By similar reasoning, mutants with defects confined to either chemotaxis or thermotaxis (e.g., DD74, DD79, DD80, RS6, EH63, EH65, EH67, and EH75) may identify genes whose products are required even earlier in the sensory pathways, before chemo- and thermoreceptor signals have converged.

The construction and analysis of appropriate double mutants may test our predictions concerning where the gene products affect the normal stimulus–response sequence. Even more promising, the techniques of electron microscope serial-sectioning and reconstruction which have been used to analyze the sensory anatomy of *C. elegans* (7, 8) may also identify specific mutant anatomical defects. Finally, further studies on the behavioral plasticity of normal animals and of mutants may identify the site and process in cellular terms. In summary, *C. elegans* is favorable for studying the mechanisms by which genes determine behavior in a simple organism, and conversely, mutants of *C. elegans* are useful in studying the organization and function of a simple nervous system.

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