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Extremely Sensitive Thermotaxis of the Nematode *Meloidogyne incognita*

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Abstract: Eggs of the root-knot nematode *Meloidogyne incognita* were acclimated to 23 C. Newly hatched second-stage juveniles migrated toward higher temperatures when placed in shallow thermal gradients averaging 23 C. The threshold gradient for this response was below 0.001 C/cm, with a best estimate of 4×10^{-4} C/cm. Calculations of physical limitations on thermotaxis indicate that this sensitivity is well within the limits of what is physically possible.

Key words: behavior, *Meloidogyne incognita,* root-knot nematode, temperature, thermotaxis, threshold.

A variety of micro-organisms and small animals migrate in shallow thermal gradients (16). Extensive experiments with *Paramecium* (13,15,19), a slime mold (1,14,17), and nematodes (2,4,8,9,12,18) have previously suggested thresholds for migration in thermal gradients as low as 0.03 C/cm, but these responses were not well characterized.

Recent discoveries demonstrate that infective juveniles of the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood also respond to shallow thermal gradients. Peculiarly, they move toward a preferred temperature that is several degrees C above the temperature to which they are acclimated (J. A. Diez and D. B. Dusenbery, unpubl.). Here we report measurements of the threshold gradient for this response. This nematode is sensitive to much shallower thermal gradients than has been reported for any organism.

MATERIALS AND METHODS

Meloidogyne incognita was propagated on tomato plants (11). Egg masses were removed from roots and incubated in a shallow dish of water at 23 C for at least 6 days before use. Second-stage juveniles were collected for use within 1 day of hatching.

Thermal gradients were established by connecting an insulated aluminum channel between two thermostatically controlled water baths in a constant temperature room. The aluminum channel (107 cm long, 5 cm wide, 5 cm high, 6.4 mm thick), with welded aluminum plates submerged in the water baths at both ends, was insulated on the bottom and sides with 3 cm of urethane foam and covered with a 1.5-cm-thick removable foam strip.

The recorder outputs of two YSI telethermometers with thermistor probes (Yellow Springs Instrument Co., Yellow Springs, OH) were connected in series (head to head) to obtain the high sensitivity needed to measure very shallow thermal gradients along the channel. One probe was placed in the cooler water bath as a reference. A chart recorder measured at high sensitivity the difference in temperature between this probe and the other placed along the gradient. The accuracy of the temperature differences was estimated to be ± 0.001 C. Gradient temperatures were measured at 10-cm intervals, and intermediate temperatures were established by interpolation. By adjusting the temperature of one of the baths, thermal gradients ranging from 0.0002 to 0.06 C/cm were established.

Approximately 150–200 nematodes were placed in the centers of small plastic trays (62 mm long, 22 mm wide, 4 mm deep) containing 3 or 4 ml of 2% agar. The trays were covered and placed end to end in the aluminum channel. The channel was covered with a strip of insulation, and the nematodes were allowed to migrate on the

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agar surface for 2 hours. Their distribution was then recorded.

Nematodes were counted in each of 12 equal sections along the length of the plastic trays, using a template under a dissecting microscope. Numbers were weighted to reflect the number of sections over which the nematodes had moved from the center. The weighted numbers of nematodes moving down a gradient were subtracted from those moving up the gradient, divided by the sum of all weighted numbers, and multiplied by 100 to yield the response (R):

(see below)

where N_1 is the number of nematodes found in the section closest to the end with higher temperature and N_{12} the number found in the section closest to the end with lower temperature. This response index has maximum and minimum values of 100 and −100, indicating the migration of all nematodes to the warmest and coolest sections of agar, respectively. The response index is zero if the nematodes are distributed symmetrically around the center starting position.

Results and Discussion

Juveniles from egg masses acclimated to 23 C were placed on gradients with temperatures between 22 and 24 C. Our previous studies demonstrated that this led to high positive responses. The results from 67 individual experiments using gradients of varying steepness are presented in Figure 1. To establish a threshold for this response, a straight line relationship between response and the log of the gradient was fitted (least squares) to the data. The intersection of the fitted line with the zero response level defined the threshold. The linear fit was considered satisfactory, since the data did not appear to extend much below threshold or into a maximal response plateau.

The best estimate of the threshold defined in this way was about 4×10^{-4} C/cm. If the intersection of the zero response level with the 99% confidence contours are considered, the threshold is below 8×10^{-4} C/cm.

The most sensitive thresholds for migration in thermal gradients previously reported are about 0.04 C/cm for the slime mold *Dictyostelium discoideum* (1,17) and about 0.03 C/cm for the plant-parasitic nematodes *Ditylenchus dipsaci* and *Pratylenchus penetrans* (9). The limit we have observed for *M. incognita* of less than 0.001 C/cm is one to two orders of magnitude less than these reported values. The comparison to *D. discoideum* is reliable, since the experiments with it have defined its threshold quite well. Other organisms have not been tested as thoroughly, so it is not certain that *M. incognita* is exceptionally sensitive.

This high degree of sensitivity has been confirmed by experiments in which the rate of movement was measured by computer tracking before and during a small temperature change of about 1×10^{-4} C/sec. A clear response was obtained within 10 seconds, during which the temperature has changed only about 0.001 C (7).

Since this response is more sensitive than any previously observed, it is important to consider whether the observations are consistent with basic physical constraints. One of the limitations to consider is whether thermal changes of the relevant size and time course could penetrate to an interior receptor without damping out. If temperature varies sinusoidally with a period (p) at a surface, the amplitude of temperature changes decays exponentially with depth (3). The depth over which the decay is 1/e (37%) is given by the

$$\text{damping depth} = (p\ D_T/\pi)^{1/2}$$

where D_T is the thermal diffusivity. If the thermal diffusivity is that of water (1.4 ×

$$R = 100 \times \frac{[6(N_1 - N_{12}) + 5(N_2 - N_{11}) + 4(N_3 - N_{10}) + 3(N_4 - N_9) + 2(N_5 - N_8) + (N_6 - N_7)]}{[6(N_1 + N_{12}) + 5(N_2 + N_{11}) + 4(N_3 + N_{10}) + 3(N_4 + N_9) + 2(N_5 + N_8) + (N_6 + N_7)]}$$

10^{-3} cm^2 sec^{-1}) and the period is 1 second, the damping depth is 200 μm. Since the fastest relevant movements in these nematodes takes more than a second (11), the thermal stimulus can easily penetrate to the center of a 14-μm-d nematode.

What is the magnitude of thermal fluctuations occurring in a thermoreceptor over behavioral time periods? The information is contained in the correlation formula for temperature at different times and locations (10). Taking the square root and converting to heat capacity per unit volume, one obtains the following:

$$\langle \Delta T(r, t)\Delta T(r', t')\rangle^{1/2} = T\left(\frac{k_B}{c_v}\right)^{1/2}\left(\frac{c_v}{4\pi k|t - t'|}\right)^{3/4} \cdot \exp\left(-\frac{c_v|r - r'|^2}{8k|t - t'|}\right)$$

where ΔT (r, t) is the difference between the temperature at position r and time t, and the mean absolute equilibrium temperature, T (298 K assumed here), k_B is Boltzmann's constant (1.38×10^{-23} J K^{-1}), c_v is the heat capacity per unit volume (4.2 J cm^{-3} K^{-1} for water), and k is the thermal conductivity (6.2×10^{-3} J sec^{-1} cm^{-1} K^{-1} for water).

This formula indicates that thermal fluctuations are essentially independent of distance for

$$|r - r'|^2 \ll 8k|t - t'|/c_v$$

For water and a time interval of 1 second the temperature fluctuations fall off by only 7% at a distance of 300 μm. For a volume with maximum extension within this distance, the average over the volume will be approximately the same as for any points within. Thus, simple substitution of the appropriate values for water into the above formula indicates that for a receptor less than 300 μm d, the thermal fluctuations on a 1-second time scale will be about 10^{-8} K. This is two orders of magnitude lower than the temperature change expected in 1 second for a nematode moving along a thermal gradient of 0.001 C/cm. A more detailed argument indicates that physical constrains limit thermotaxis of these nematodes to gradients greater than 1×10^{-7} C/cm (5). In either case, it is clear that thermal fluctuations are small enough to be consistent with the observed sensitivity.

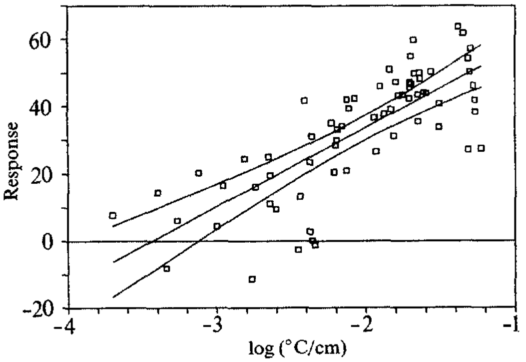

FIG. 1. Response of nematodes as a function of steepness of thermal gradient. Lines are the linear regression with 99%, 2-tailed confidence limits for mean values. Regression equation is $R = 81 + 24 \log$ (C/cm). Zero response level intercepts the regression line at −3.44 and the lower confidence limit at −3.1. These values correspond to gradients of 3.6×10^{-4} and 7.9×10^{-4} C/cm, respectively.

What is this extreme sensitivity used for? Other experiments (J. A. Diez and D. B. Dusenbery, unpubl.) demonstrate that these animals move toward a temperature that is several degrees above the temperature to which they have been acclimated and acclimation occurs in less than a day. Computer modeling of this behavior suggests that in the dynamic thermal environment of soil this response causes them to move toward a particular soil depth that may be optimal for locating the roots of host plants (6, unpubl.). Thermal gradients probably do not lead the nematodes directly to roots because the gradients produced by metabolic heat are predicted to be small compared with those produced by changes in sunlight and cooling of the earth's interior. Although the sensitivity demonstrated here is not the ultimate of what is physically possible, it is sufficient to almost always detect the thermal gradients present in soil (5).

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