Behavioral Responses of *Meloidogyne incognita* to Small Temperature Changes

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Abstract: Small, rapid temperature changes were generated by incandescent radiation, and behavioral responses of *Meloidogyne incognita* juveniles were recorded with high time resolution by computer tracking. Temperature changes away from the preferred temperature resulted in decreases in the rate of movement and increases in the rate of change of direction, whether the changes were toward warmer or cooler temperatures. These behavioral changes lasted about 30 seconds. Temperature changes toward the preferred temperature caused the response rates to change in the opposite directions, and the behavioral changes persisted for several minutes. These results demonstrate that nematodes can respond to a purely temporal thermal stimulus in a manner consistent with efficient indirect orientation or klinokinesis. The rate of temperature change was estimated to be of the order of 10^{-4} C/second, suggesting that the nematodes detected a change of about 0.001 C.

Key words: behavior, computer tracking, klinokinesis, *Meloidogyne incognita*, root-knot nematode, temperature, thermotaxis.

There is much interest in unraveling the mechanisms used by various organisms to migrate effectively in a stimulus field. Basic questions are how the field is sensed and how the organisms move in the preferred direction. Two simple alternatives for sensing the field are simultaneous measurement by receptors separated in space and measurement of temporal changes as the animal moves through the field. The basic alternatives for responding are that the animal orients directly by making turns that are biased with respect to the direction of the stimulus field (klinotaxis) and that migration occurs indirectly by modulating the frequency of turns that are not oriented to the field (klinokinesis) (1,4,14).

Nematodes are capable of direct orientation to chemical gradients (15,20,21). Other experiments demonstrate that nematodes modify the frequency of turning behavior in response to temporal changes in concentration in such a way that indirect orientation would be efficient (7,12,17).

In response to thermal gradients, nematodes are among the most sensitive organisms (11,18). Infective juveniles of *Meloi-* *dogyne incognita* (Kofoid & White) Chitwood can migrate in thermal gradients of less than 0.001 C/cm (J. A. Diez, M. J. Pline, and D. B. Dusenbery, unpubl.). Previous experiments demonstrating isothermal tracking (13) suggest that nematodes are capable of direct orientation to thermal gradients, at least when the gradients are steep. The hypothesis that nematodes may also respond by indirect mechanisms using temporal changes was tested in experiments reported here and found to be supported.

MATERIALS AND METHODS

Meloidogyne incognita was propagated on greenhouse-grown tomato plants as previously described (12). Egg masses were removed from the roots and maintained at 23 C. Second-stage juveniles used in these experiments were collected within a day of hatching.

Rapid responses to temperature shifts were followed using a computer tracking system similar to that previously described (8,9) except that an IBM XT computer with a PCVision camera interface (Imaging Technology, Woburn, MA) was used. The position (trailing edge) of each nematode was determined every 3 seconds. Locomotion was defined as the sum of the distance moved in the X and Y directions. The discrepancy from the actual average

distance moved for 200 nematodes moving in random directions was a relatively constant overestimate of 27%. Since in this study it was the changes in the rate of locomotion that were of interest, this discrepancy is not significant. The number of changes in direction of locomotion (reversals) were recorded as a change in direction of locomotion of more than 90 degrees (9). This measurement does not provide an accurate measure of the frequency of reversal bouts or other turning behavior (6,7), but changes in this parameter were thought to provide an indication of changes in the frequency of these behaviors.

Rapid temperature changes were generated using a 100-W spot lamp operated at low power by a household dimmer switch so that the lamp glowed dimly. The lamp, positioned about 30 cm from the nematodes and directed at them, was turned on and off to generate the temperature changes. With the dimmer switch set at full power, the initial rate of temperature change was measured as 3.6×10^{-3} C/second with a miniature thermistor (YSI 520 probe); it fell to half the initial rate in about 3 minutes. Heating and cooling rates were nearly identical. A thermopile was used to determine that the radiant energy at low power was 0.038 of radiant energy at full power. These measurements indicated that the initial rate of temperature change to which the nematodes were subjected was about 1×10^4 C/second. Since there were many uncertainties in this estimate, it was probably reliable only to a factor of three or so.

RESULTS AND DISCUSSION

Second-stage juveniles of *M. incognita* changed their movements within a few seconds of a small temporal change in temperature (Fig. 1). In a spatial temperature gradient, juveniles from eggs kept at 23 C moved toward a preferred temperature of about 26 C (J. A. Diez et al., unpubl.). If the temperature was above the preferred temperature during temporal stimulation, locomotion decreased when the temperature was raised (Fig. 1A) and increased when it fell (Fig. 1B). The opposite responses were observed when the temperature was below the preferred temperature (Fig. 1C, D). Although the signal from measuring reversals was noisier, it decreased (Fig. 1E) when locomotion increased and increased (Fig. 1F) when locomotion decreased. Opposite changes in locomotion and reversal frequency were also found in similar experiments using carbon dioxide as stimulus (17). These changes were all in the direction expected if the response was one that led nematodes to efficient migration in spatial gradients using undirected responses (klinokinesis).

Since it has been demonstrated that a nematode without ocelli can respond to visible light (5), it is important to consider whether light rather than heat could be the stimulus. The observation that the sign of the response was reversed when the ambient temperature was below the preferred temperature as compared to above it indicates that heat was the stimulus.

The nematodes adapted to these stimuli within a few minutes. Adaptation to an aversive change causing a decrease in locomotion and an increase in reversals was faster than the opposite response to a favorable change. This demonstrates that the nematodes responded to the temporal aspects of the stimulus. Whereas the lamp may have generated spatial thermal gradients, if the nematodes were responding to temperature differences between two body parts, say head and tail, for every individual with a warmer head there would be another with a warmer tail and the average response for the randomly distributed group would be the same for lamp on as lamp off.

Although the temperature changes were very slow (about 1×10^{-4} C/second), responses were evident within 10 seconds, indicating a total temperature change of less than about 0.001 C was detected by the juveniles. In similar experiments with other organisms, the lowest reported rate of temperature change is 0.015 C/second, for paramecium (16). Experiments on the
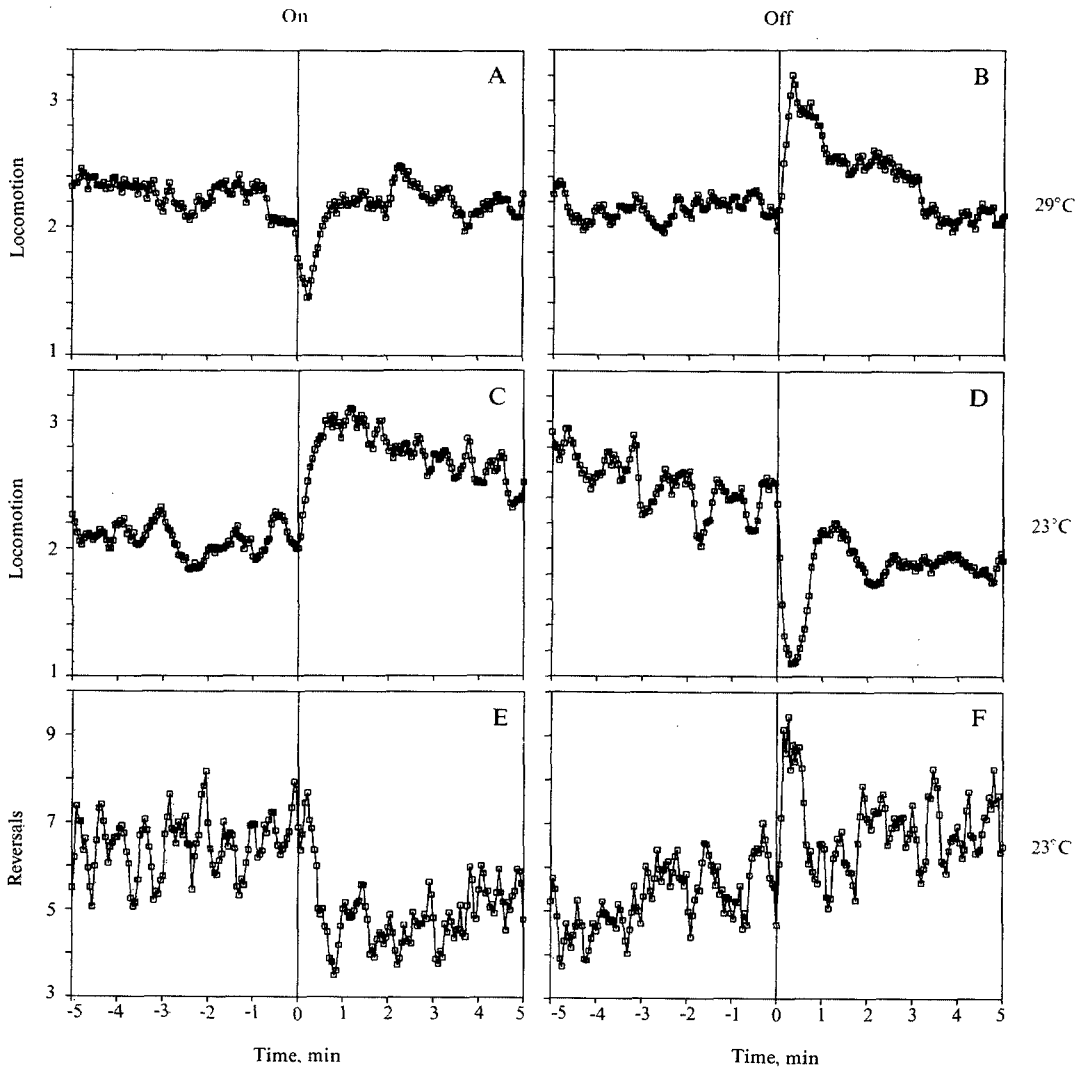
FIG. 1. Changes in behavior of second-stage juveniles of *Meloidogyne incognita* caused by changes in temperature. Temperature changes were generated by turning a red glowing incandescent lamp on (left side plots) or off (right side plots) at zero time. Experiments A and B were conducted at an ambient temperature of 29 C; the others were conducted at 23 C. Generally about 200 nematodes were tracked simultaneously with the position of each established every 3 seconds. Each plot is the average of three experiments smoothed with a running average of three. Locomotion (A–D) is the average per nematode of the sum of the number of pixels moved in the X and Y directions during the 3-second interval. At the magnifications used pixels are separated by about 33 μm. Number of reversals, changes of direction of more than 90 degrees, recorded in each interval per 100 nematodes is plotted (E, F).

infra-red detectors of rattlesnakes (3) indicate that a temperature change of 0.001 C is on the threshold of detection. In the studies with snakes, the rate of change is apparently 100-fold larger with a correspondingly shorter time period than observed here. Since the threshold for the nematode response was not determined, it is likely that it is even more sensitive than the snake.

These results can be related to movement in a thermal gradient as follows. The rate of movement of *M. incognita* is roughly 10 μm/second whether one considers the rate of migration of a group, the average movement of an individual, or the back

and forth movements of the head during crawling. In a spatial gradient of 0.001 C/cm (near threshold) (J. A. Diez et al., unpubl.), this rate of movement would produce a rate of temperature change on the order of 1×10^{-6} C/second, substantially lower than the rate used in the present study. A similar sensitivity to the rate of change can be calculated for the slower moving amoeboid slime mold (2):

$$0.04 \text{ C/cm} \times 2 \text{ mm/hr} = 2 \times 10^{-6} \text{ C/sec.}$$

This organism, however, is thought to respond via spatial comparisons (tropotaxis) rather than temporal comparisons (19).

With regard to the mechanism of migration, the fundamental distinction is whether the organism orients to the gradient using directed turns or simply employs a baised random walk. The free-living nematode *Caenorhabditis elegans* can respond directly to a thermal gradient, since it can follow isotherms without significant deviation (13). It has been demonstrated here that in *M. incognita* the frequency of reversals is modulated in just the way necessary to make undirected responses efficient. Thus, both mechanisms are probably used.

It appears that nematodes use much the same mechanisms for responding to chemical and thermal gradients. Both *C. elegans* adults (7,9) and *M. incognita* juveniles (12,17) respond to changes in NaCl or CO_2 concentration by modulating the frequency of reversals in a manner similar to that demonstrated in Figure 1E, F, although the free-living nematode moves about 10 times faster than the plant parasite. The similarity extends to the observation that increases in reversal frequency adapt faster than decreases. There is evidence that *C. elegans* can make both directed (21) and undirected (7) responses to chemical gradients. In addition, the thermal response of *C. elegans* shares specific gene requirements with responses to chemical stimuli (10,13). Thus, it is suggested that the mechanisms nematodes employ for response to thermal stimulation are very similar to those employed for response to chemical stimulation.

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