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Behavior of Tethered *Meloidogyne incognita*

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Abstract: The tethered-nematode technique was adapted for use with second-stage juveniles of *Meloidogyne incognita.* The data demonstrate that *M. incognita* exhibits the same patterns of behavior as adults of the free-living nematode, *Caenorhabditis elegans.* The principal differences are that *M. incognita* is slower and less regular in its behavior than *C. elegans.* The frequency of normal waves is about 0.2 Hz; that of reversal waves is about 0.06 Hz. Reversal bouts last about 1 minute. In response to a change in NaCl concentration, *M. incognita* modulates the probability of initiating a reversal bout in the same manner as *C. elegans* except that it responds more slowly and is repelled instead of attracted.

Key words: chemotaxis, NaCl, root-knot nematode, nematode movement, nematode response.

To understand nematode–plant relationships, it is necessary to identify the mechanisms by which nematodes locate specific sites on a host for feeding or invasion. Chemical cues have long been considered important in attracting nematodes to hosts (13). It has been demonstrated that certain nematodes respond to specific chemicals (2,6,8,12). The mechanisms employed to migrate along a chemical gradient have been studied in a few species, but the conclusions remain controversial and the relevance to other species is not clear (6,9,12,15). A technique has been developed that has proved to be particularly informative in revealing the kinetics of the response of the free-living nematode, *Caenorhabditis elegans* (5). A nematode is tethered in a flowing liquid carrying a stimulus, and its body movements are recorded on a polygraph. This technique permits precise control of the timing of stimulation and produces a detailed record of a nematode's response. The objectives of this study were to adapt the tether technique to study second-stage juveniles of *Meloidogyne incognita* and compare their behavior to adults of *C. elegans.*

MATERIALS AND METHODS

Meloidogyne incognita was maintained on tomato (*Lycopersicon esculentum* Mill 'Rutgers'). Egg masses were removed from galled roots approximately 60 days after inoculation and placed in distilled water in a petri dish at 23 C. Egg masses were transferred daily to fresh water until new egg masses were obtained from another plant the next week. All juveniles (J2) used in these experiments were from egg masses that had been removed from the plants fewer than 10 days and had hatched within 30 hours of the test period.

The technique used for adult *C. elegans* (5) had to be modified to adapt it to the smaller, slow moving J2 of *M. incognita.* A suction pipet sufficiently small to successfully tether the small J2 was made from a 5-μl glass pipet (Yankee Disposable Micropet, Clay Adams). A vertical pipet puller (David Kopf Instruments, Model 700C) was used to draw out the tip. Heater and solenoid controls were both set at mid-scale. The closed end of the pipet was broken off as cleanly as possible so that the internal diameter at the tip was small enough to hold the J2 by the tail but not draw its body inside.

Additional modifications of the technique included increasing the magnification of the compound microscope by switching from an 8× to a 14× eyepiece and increasing the distance to the detector array. Four pairs of detectors were used in place of the two originally used. This had the advantage that placement of the nematode was less critical, and a more detailed record of the behavior was obtained. The speed of the chart recorder was reduced from 100 mm/minute to 25 mm/minute.

After being caught in the tether, the J2 was maneuvered into the chamber through which test solutions flowed. Two pumps alternated in pumping solutions through this chamber. Each experiment began with distilled water fed to both pumps. During the test period, one pump pumped deion-

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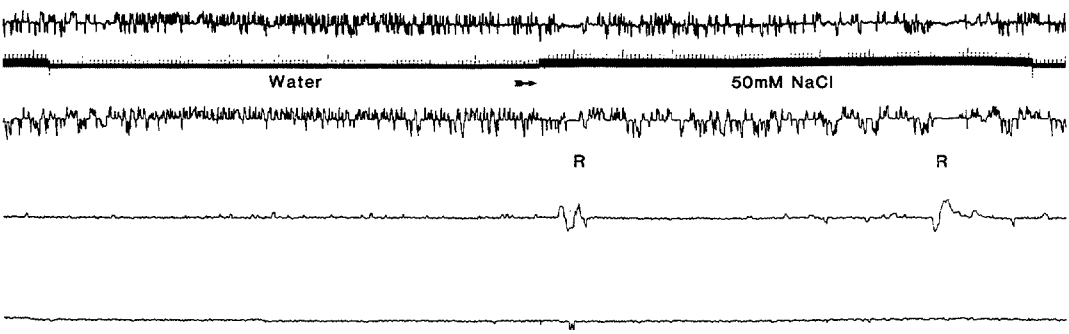

FIG. 1. Sample record of *Meloidogyne incognita* second-stage juvenile movements. The second from the top tracing is an event marker. Tics at 5-second and 1-minute intervals are clearly visible. The arrow shows direction of time. The other four tracings are records from pairs of detectors straddling the nematode at various positions along its length. They are in order with the top tracing recording from the most anterior detectors and the bottom tracing the most posterior. "R" indicates a reversal bout.

ized water past the nematode and the other pumped a solution of 50 mM NaCl. In earlier experiments, each pump was on for 10 minutes at a time, thus a complete stimulus cycle lasted 20 minutes. In order to run more stimulus cycles, later experiments employed a stimulus cycle half as long. The potassium phosphate buffer that was used in previous experiments with *C. elegans* was eliminated as it seemed to be toxic to *M. incognita*.

Recording continued until the nematode's movements deteriorated or until it escaped from the tether. Data were taken only from nematodes that appeared to be moving normally and remained on the tether for at least 10 complete cycles of stimulation. Experiments were performed at 20 C.

RESULTS

The behavior of *M. incognita* was similar to that of *C. elegans* in many respects (Fig. 1). During most of the time, fairly regular activity occurred in the two pairs of detectors near the anterior half of the nematode and there was no activity in the detectors near the posterior half. This pattern of activity is indicative of the low amplitude waves that propel untethered nematodes forward and is in contrast with periods when there is more activity in the posterior detectors than in the anterior. Examples in Figure 1 are labeled "R." Observation of nematodes exhibiting more posterior than anterior activity indicates that it is caused by waves that start at the tail and propagate forward, in contrast to the more frequent behavior. These forward propagating waves cause an untethered nematode to move backwards. The chart recording for reversal waves differs from that for normal behavior because the reversal waves are of much larger amplitude and the worm is bent sufficiently that it does not extend far enough to intercept the an-

TABLE 1. Comparison of kinetics of second-stage juveniles of *Meloidogyne incognita* and adults of *C. elegans*.

Parameter	Value		Ratio
	M. incognita	*C. elegans*	
Normal wave frequency (Hz)	0.21 ± 0.04	1.60 ± 0.10	0.13
Duration of reversal bouts (sec)	66 ± 49	13 ± 8	0.20
Number of waves/reversal bout	3.9 ± 2.1	9.3 ± 4.2	0.42
Frequency of reversal waves (Hz)	0.058 ± 0.023	0.70 ± 0.15	0.08
Ratio of frequencies (reversal/normal)	0.27	0.44	****

Mean ± SD.

Normal waves were measured from 1-minute samples selected to avoid pauses or other irregular behavior. Sixty-one such samples from 11 juveniles were used for *M. incognita* and 27 samples from 9 adults for *C. elegans*. Seventy-nine reversal bouts from 9 juveniles were used for *M. incognita* and 120 bouts from 8 adults for *C. elegans*.

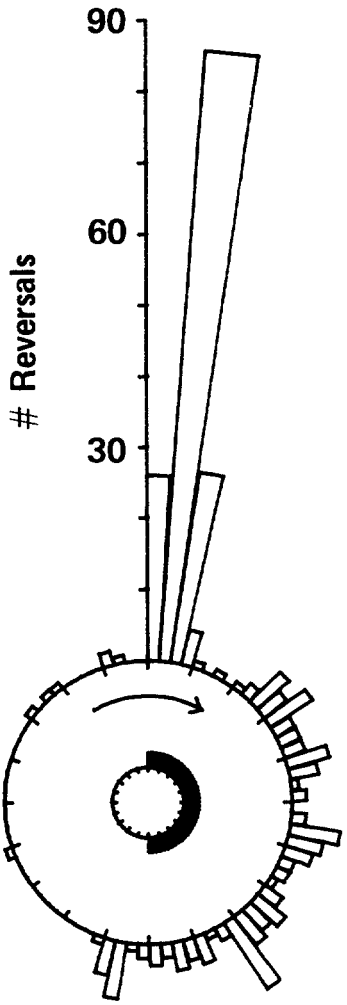

FIG. 2. Distribution of reversal bouts in second-stage juveniles of *Meloidogyne incognita* in 20-minute stimulus cycles. The tics inside the circles indicate 1-minute intervals. The inner histogram shows the presence or absence of 50 mM NaCl. The outer histogram indicates the number of reversal bouts that started in the interval in question. Altogether 282 reversal bouts from 163 stimulus cycles from three individual juveniles are included. Three-quarters of the data is from one juvenile that stayed active on the tether for 40 hours. All three nematodes had similar distributions.

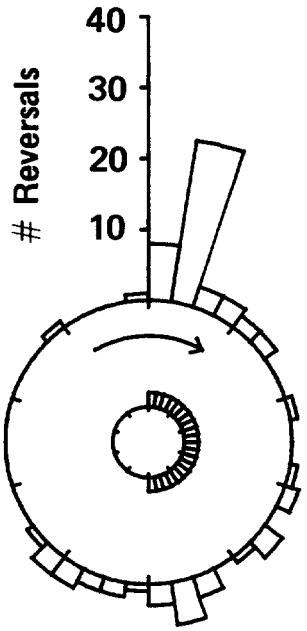

FIG. 3. Distribution of reversal bouts in 10-minute stimulus cycles. Same presentation as Figure 2. Altogether 75 reversal bouts from 50 stimulus cycles distributed evenly over three individuals is presented.

terior detectors. The reversal waves tend to occur in continuous sequences called reversal bouts. The principal differences between the observed behavior and that of *C. elegans* are that movements of *M. incognita* are much slower and less regular.

In order to quantitatively compare the two nematodes, data on several different parameters were extracted from these records on *M. incognita* and from the records used previously for *C. elegans* (5). The comparison is presented in Table 1. The overall pattern that emerges is that *M. incognita* is about 10 times slower than *C. elegans* in both normal and reversal waves. For both nematodes the frequency of reversal waves is about one-third that of normal waves. The duration of *M. incognita* reversal bouts is about five times longer than those of *C. elegans*. *M. incognita* has fewer waves in the typical reversal bout than *C. elegans*, but the difference is only by a factor of about two.

In order to study the response to chemical stimulation, the nematodes were alternately exposed to 10 minutes of 50 mM NaCl and 10 minutes of water. The recordings were then examined to determine when during a stimulus cycle reversal bouts started (Fig. 2). Immediately after the application of NaCl there was a large increase in reversal bouts. The bouts most often began between 15 and 30 seconds after application of salt. The rapid increase in frequency of reversal bouts was followed in 1 or 2 minutes by a decay to a low steady-state level which was maintained until the

salt was eliminated. About a minute after the return to water, reversal bouts were inhibited until salt was reapplied. Inhibition of reversal bouts lasted at least 9 minutes.

When the experiment was repeated with a 10-minute stimulus cycle, rather than 20-minute, the same behavior pattern was observed (Fig. 3).

Discussion

The small *M. incognita* J2 were tethered relatively easily. However, collection of data was hampered by the nematodes curling around and pushing against the pipet enabling them to extract their tails from the pipet and escape. *C. elegans* adults can also escape, but it was more of a problem with *M. incognita* J2.

Comparison of the behavior of the plant-parasitic and free-living nematodes by this technique shows remarkable similarities. For the parameters measured, the behavior of *M. incognita* could be described as similar to that of *C. elegans,* except that its movements are about 10% as rapid, which may reflect a high degree of similarity among nervous systems of nematodes. Detailed studies of the nervous systems of *C. elegans* and *Ascaris* indicate that there is practically a neuron-for-neuron correspondence (14).

Aside from the difference in speed, the movements of *M. incognita* are much less regular than those of *C. elegans,* which could be a consequence of the slow speed. The simple nervous system of nematodes may not be capable of precise coordination of slow movements, for which viscosity would have less effect in smoothing out the motion.

The mechanism by which nematodes migrate along chemical gradients is not fully understood. A variety of strategies is used by various motile organisms (1,3). The nematode most commonly studied has been adult *C. elegans,* which seems able to orient directly to a gradient by sampling on either side as its head swings back and forth (15). Such behavior has been called klinotaxis (3,7). However, in the case of *C. elegans* the orientation is not very accurate. The reversal bout (4) causes a freely moving nematode to change its direction of locomotion. Chemical stimulation of *C. elegans* adults modulates the frequency of reversal bouts in precisely the way that is optimum for causing migration along a gradient (5). Such behavior would be classified as klinokinesis (3,7) and would be a means of indirect orientation (1) assuming the turn direction and magnitude are not related to the chemical gradient. Recent reviews of the mechanisms of migration (1,3) stress that combinations of klinotaxis and klinokinesis are likely to occur. Studies of tracks of male *Heterodera* responding to sex attractants also appear to indicate both mechanisms at work (3,9).

Previous observations of relatively straight tracks of *M. javanica* J2 moving in salt gradients (10) suggest a direct mechanism such as klinotaxis. The observations reported here indicate that *M. incognita* J2 exhibit reversal bouts and demonstrate that upon stimulation the frequency of bouts is modulated in a pattern similar to that of *C. elegans.* Thus, root-knot nematodes probably are capable of klinokinesis as well as direct orientation. The significant difference between free-living and plant-parasitic nematodes is that the direction of the response is reversed and slower. *C. elegans* is attracted (15) while the root-knot nematodes are repelled by NaCl (11), and *M. incognita* responds more slowly than the free-living nematode. This suggests that the same mechanisms may be employed by a wide variety of nematodes for migrating both up and down chemical gradients.

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