Absence of geotaxis in soil-dwelling nematodes

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Nematodes are found in almost all habitats and exhibit behaviours adapted to diverse environments and food sources. Various studies have examined vertical movement of plant- and animal-parasitic nematodes above ground in relation to survival and host-finding. The parasitic nematode, *Skrjabinoclava*, manipulates the behaviour of its intermediate host, *Corophium volutator*, to contact a potential definitive host by increasing its surface activity during the day (McCurdy et al., 1999). Several gastrointestinal nematode species position themselves to be eaten by prospective vertebrate herbivore hosts by crawling up foliage (Niezen et al., 1998). The plant-parasitic nematodes *Aphelenchoides besseyi* and *Nothanguina phyllobia* crawl to the tip of a plant, where they directly enter the plant organs (Robinson et al., 1979; Togashi & Hoshino, 2003). Geotaxis has been suggested to underlie some of these vertical movements, but many questions remain regarding such automatic responses to gravity across variable experimental conditions (Sciacca et al., 2002).

Little is known about the geotaxis of nematodes underground, despite their importance in soil ecosystems. Soil has a complicated structure and is composed of particles of various sizes and shapes, so the movement of soil-dwelling nematodes is always constrained in their natural habitats. To understand geotaxis in soil-dwelling nematodes, it is useful to investigate movements under conditions that mimic natural habitats. In addition, technical difficulties are encountered when devising equipment to investigate nematode behaviour because most nematodes are extremely small, and most nematode habitats are opaque. A recent innovation is the use of two-dimensional micro-moulded substrates, which allow for the direct observation of nematode behaviour in a soil-like structure (Otobe et al., 2004). The purpose of this study was to utilise a micro-moulded substrate to test seven nematode species for responses to gravity.

*Aphelenchoides fragariae* collected from lily (*Lilium Oriental Hybrids*) were propagated with *Botrytis cinerea* as a food source on 2% potato dextrose agar medium at 25°C. *Aphelenchus avenae* collected from glasshouse soil around tomato plants (*Lycopersicon esculentum* L.) were grown under the same conditions used to culture *A. fragariae*. *Caenorhabditis elegans* N2 (juveniles, 500-700 µm) and *Oscheius tipulae* (obtained from the *Caenorhabditis* Genetics Center, University of Minnesota, St. Paul, MN, USA) were grown on nematode growth medium agar on a diet of *Escherichia coli* OP50 at 20°C. *Meloidogyne incognita* Omei strain was reared on tomato plants, and second-stage juveniles were collected from egg masses using the Baermann funnel method. *Pratylenchus penetrans*, collected from soil around the roots of cabbage (*Brassica oleracea* L. var. capitata), was maintained at 25°C on alfalfa (*Medicago sativa* L.) callus induced by the medium of Schenk and Hildebrandt (1972). *Steinernema carpocapsae* was kindly provided by SDS Biotech K.K. (Tsukuba, Japan), and third-stage infective juveniles were used.

The micro-moulded substrate (approximately 5 × 5 mm) was made of polydimethylsiloxane as described by Otobe et al. (2004), and the structure is depicted in Figure 1. The substrate was saturated with distilled water and covered with a glass plate. Ten to 20 nematodes in 3 µl of distilled water were introduced into the substrate via a hole in the center of the glass plate, and the substrate was placed vertically in a transparent water bath at 25°C. Nematodes migrated smoothly through channels in the vertical as well as the horizontal direction and were able freely to change migration direction. Movements were recorded from the side by a CCD camera (VH-
A side view of the micro-moulded substrate showing the scale (µm) of the channels (grey) through which nematodes can move.

6200, Keyence, Japan) connected to a microscope with dim light. The movements of 50 individuals of each species were analysed using image-analysis software (Video Capture 6.5, Ulead Systems, Setagaya-ku, Japan).

The vertical distance moved by each nematode was measured between the starting point (at 0 s) and the last point (at 30-150 s after being introduced). There were three possible vertical movements: upward movement with a positive value, no vertical movement with a zero value, and downward movement with a negative value. The geotaxis index was defined as the vertical distance moved divided by the observed time (µm s⁻¹). The null hypothesis ($H_0$) was that the geotaxis index was equal to zero, and the alternative hypothesis ($H_1$) was that geotaxis index was greater or smaller than zero. To test the null hypothesis under the assumption of independence, a two-sided z-test was performed at the 1% significance level.

The two-sided z-tests indicated acceptance of the null hypothesis for all tested nematodes (Table 1), meaning that none of the seven nematodes species was geotactic, it is possible that these nematodes use other stimuli to maintain themselves within an optimal range of depths for locating food or mates. Unmodulated geotaxis requires energy and could place nematodes in a soil layer with harsh conditions.

Our findings may also be relevant to the extraction efficiency of the Baermann funnel technique. McSorley and Frederick (2004) reported that the percentage of nematodes extracted using this method varied among trophic groups, and the percentage was low for herbivores. The nematodes tested in our study represent various trophic groups but, because our tests did not reveal geotactic behaviour in these nematodes, it is likely that geotaxis is a poor explanation for the different extraction efficiencies among these groups.

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**References**

