Vertical migration by the infective larvae of three species of parasitic nematodes: is the behaviour really a response to gravity?

J. SCIACCA[1], A. KETSCHEK[1], W. M. FORBES[1], R. BOSTON[2], J. GUERRERO[1], F. T. ASHTON[1], H. R. GAMBLE[3] and G. A. SCHAD[1]*

[1] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA*
[2] *Clinical Studies, NBC, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348, USA*
[3] *US Department of Agriculture, Agriculture Research Service, Parasite Biology, Epidemiology and Systematics Laboratory, Building 1040, Room 103, BARC-East, Beltsville, MD 20705, USA*

SUMMARY

Vertical migration by infective larvae (L3) of 3 species of nematodes was investigated. Upright truncated agarose cones were used to test upward migration, and comparable truncated cone-shaped agarose hollows were used to test downward migration. Flat agarose plates were control surfaces. When placed at the bases of upright cones, 74% of *Ancylostoma caninum* L3 migrated up, whereas only 16·5% migrated down the indented cones; this latter value was not significantly different from larval migration on flat plates. *Strongyloides stercoralis* L3 also migrated upward in significant numbers (80%). These larvae also failed to migrate downward under normal conditions. However, when the bottoms of the indented cones were 3–5 °C warmer than the tops, 75·5% of *S. stercoralis* L3 migrated down. In contrast, *Haemonchus contortus* L3 showed no tendency to crawl up or down cones, when compared with normal crawling behaviour on flat plates. Thus, L3 of *A. caninum* and *S. stercoralis* exhibited negative geotaxis, crawling against the pull of gravity, while *H. contortus* did not. The biology of these parasites may explain these behavioural differences.

Key words: *Ancylostoma caninum*, geotaxis, *Haemonchus contortus*, *Strongyloides stercoralis*, thermotaxis.

INTRODUCTION

Vertical migration by the infective larvae of several species of parasitic helminths has been described (Augustine, 1921; Payne, 1923*a*; Peters, 1928; Buckley, 1940; Schroeder & Beavers, 1987; Duncan, Dunn & McCoy, 1996). Some of these behaviours have been interpreted to be geotactic in nature, while others were assumed to be responses to other environmental stimuli, or simply random crawling modified by other environmental conditions. Africa (1931) described the behaviour of *Nippostrongylus muris* infective larvae (L3) in which they climbed onto projections on the soil surface, but carefully avoided interpreting the behaviour as a geotactic response. Cunningham (1956) described the tropisms (taxes) of various stages of this same nematode, observing the direction of migration in inclined closed tubes. While other stages showed a tendency to migrate down, L3s of this species were found to move upward. Infective larvae of the entomopathogenic nematode *Steinernema robravis* were found to migrate to the soil surface in cultures (Duncan *et al.* 1996), a behaviour these authors describe as negative geotaxis. Downward migration by these larvae was also observed; presumably, the larvae moved in search of moisture. Likewise, L3s of 5 other species of insect-parasitizing nematodes were found to migrate upward in soil columns (Schroeder & Beavers, 1987).

Hookworm and trichostrongyle L3s are known to migrate out of faeces and up on surrounding grass and on the walls of culture dishes (Augustine, 1921; Payne, 1922, 1923*b*; Rees, 1950; Crofton, 1954). It has been questioned whether this behaviour is, in fact, a response to gravity (Croll, 1970). Payne (1923*a*), observed the upward migration of *Necator americanus* L3 in soil, but concluded that the effect of water in the soil was far stronger than that of gravity. Crofton (1954) emphasized that surface tension forces are 10^4 to 10^5 times greater than gravitational forces acting on a small nematode, thus dismissing the possibility that gravity was the stimulus for the migratory response. Buckley (1940), using microscope slides on which a thin layer of damp sand was deposited, observed

* Corresponding author: University of Pennsylvania, School of Veterinary Medicine, 3800 Spruce Street, Rosenthal Building, Room 212, Philadelphia, PA 19104, USA. Tel: +215 898 6680. Fax: +215 573 7023. E-mail: schad@vet.upenn.edu

that L3s of the small strongyles of horses, *Trichonema* spp., migrated downward, but only at 13–14 °C. Surprisingly, he found that these migrations occurred on slides tilted with a slope of less than 1 in 8. Furthermore, he observed the larvae actively turning and crawling down these slopes and interpreted this migration as being influenced by gravity, not other stimuli. Crofton (1954), unable to confirm Buckley's results, felt that vertical migrations were actually random crawling. He further suggested that when a larva crawled up on a grass blade, it would be restricted in its freedom to turn around, and thus would be more likely to continue to crawl upward. Broadbent & Kendall (1953), using Crofton's data, proposed a 'Random Walk' hypothesis, postulating that channels of varying widths would restrict a larva's ability to turn.

Consequently, the narrower the channel, the more likely the larva would continue to crawl in one direction. In experiments conducted by Rees (1950), infective larvae of *H. contortus* were observed to climb up grass blades when conditions were environmentally favourable, but to turn and migrate down when conditions began to turn harsh. Infective larvae of *Ostertagia* spp. were found to show seasonal migration patterns: out of the soil and up on grasses during the spring, and back into the soil mat in the autumn (Al Saqur *et al.* 1982), but this behaviour may not be a response to gravity.

In the present work, we investigated the vertical migratory behaviour of L3s of two skin-penetrating species, *Ancylostoma caninum* and *Strongyloides stercoralis*, and of those of the passively-ingested nematode, *Haemonchus contortus*. Vertical migration by these larvae is an important part of their host-finding behaviour; this behaviour could be influenced by gravity and might be mediated by sensory neurons, as it appears to be in miracidia of trematodes (Brooker, 1972). Although nematodes lack statoliths or other obvious gravity receptors (Croll, 1972), they might still be able to detect gravitational pull, perhaps by integrating signals from their amphids and phasmids.

MATERIALS AND METHODS

Infective larvae

Strains of *Ancylostoma caninum* and *Strongyloides stercoralis* were maintained in dogs at the University of Pennsylvania School of Veterinary Medicine. Faeces containing the eggs of *A. caninum* were collected from an infected dog and used to make charcoal cultures in 100 mm Petri dishes, which were incubated at 26 °C. Infective 3rd-stage larvae (L3) were harvested from 7-day-old cultures using the Baermann method. Likewise, the faeces from a dog infected with *S. stercoralis* were made into cultures, and incubated at 26 °C. The L3s were collected from 5-day-old cultures using the Baermann method or, alternatively, they were simply washed off the covers of the Petri dishes containing these cultures. A wild-type strain of *Haemonchus contortus* originating in Louisiana was maintained in sheep at the US Department of Agriculture, Beltsville, MD. These larvae were reared in cocultures at 26 °C and recovered by Baermannization. Infective larvae of *H. contortus* were stored at 4 °C; these were kept at room temperature for 28 h prior to use to allow them to acclimatize to this temperature (Li *et al.* 2000).

Substratum preparation

To provide test surfaces on which to observe the vertical migratory behaviour of L3 larvae, truncated conical substrata were made using agarose. An 'upright' truncated cone was used to assay for a negative geotactic response. Conversely, an 'indented' cone, a truncated-cone-shaped hollow, was used to assay for a positive geotactic response. A flat plate was used as a control surface on which to observe random migration of the larvae, and to confirm that no unintentional stimuli from the external environment were operative.

To make the substrata, a mixture of 5% agarose (w/v) in water was heated to boiling in a microwave and allowed to stand until any bubbles in the mixture rose to the surface. The mould used to make the upright and indented cones was the conical bottom portion of a 50 ml centrifuge tube (Falcon 2098, 50 ml Opticul polypropylene tube), the top 98 mm cylindrical portion having been removed. Upright cones were formed by pipetting 1·5 ml of agarose into the cone mould and allowing it to harden; the resulting cone was then removed from the mould and placed apex up in the bottom half of a 35 mm Petri dish (Corning Glass Works #25050-35, 35 mm × 10 mm). An additional 1 ml of warm agarose was used to surround the base of the agar cone (Fig. 1A). Indented cones were formed by pipetting 9 ml of warm agarose into the bottom half of a 35 mm Petri dish in which an empty cone mould, apex down, was centred, thus forming a cone-shaped hollow. After the agarose cooled, the mould was removed (Fig. 1B). The sides of both the upright and the indented cones were inclined at an angle of approximately 60° with respect to horizontal. Flat plates were prepared by pipetting 5 ml of agarose into a 35 mm Petri dish and allowing it to cool (Fig. 1C).

Wells approximately the size of the area at the bottom of the indented cone (approximately 6 mm diameter × 1 mm deep) were cut in the apex of the upright cones and in the centre of the flat plates (Fig. 1A–C). The wells in each of the 3 types of plates were filled with approximately 15 μl of distilled water. The water acted as a 'trap' for any larvae
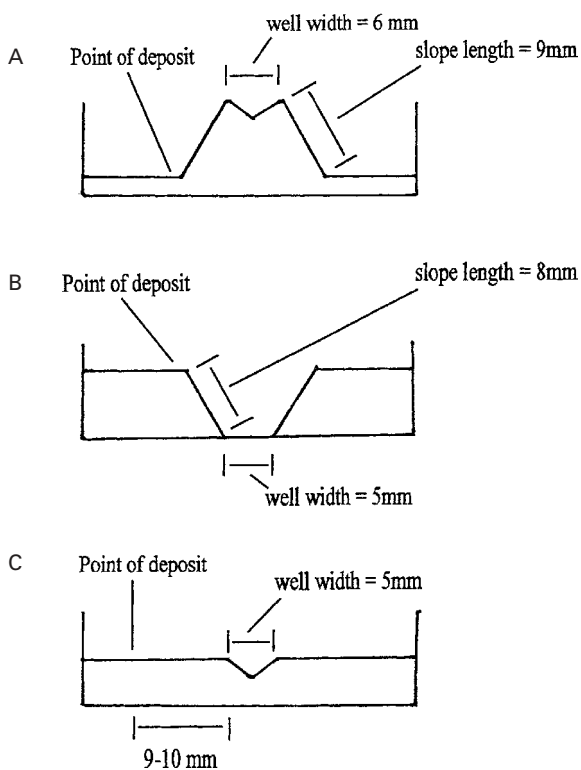
Fig. 1. Cross-sectional diagrams of the 3 agarose test substrata, made of 5% (w/v) agarose (see text), that were used to test vertical migration of the larvae. The dimensions of the 2 types of cones are given; their sides slope at 60° with respect to the horizontal. Approximately 15 μl of water was placed in the wells shown in each type of substratum. (A) Upright cone: the larvae were placed on the flat surface surrounding the cone, very close to its side. (B) Indented cone: the larvae were placed on the flat surrounding surface very close to the transition to the sloping side of the cone. (C) Flat plate: the larvae were placed 9–10 mm from the well at the centre of the plate.

that made it to any of the wells. The larvae could enter the droplet of water but could not break the surface tension of the droplet to exit. This was important because a known number of larvae could be deposited on the plate and any larvae that made it to the well would be counted only once. When the indented cones were prepared, a meniscus was formed above the level of the horizontal agarose because of the surface tension between the warm agarose and the outside of the conical mould. This meniscus was removed with a scalpel, so that there was a clean transition between the horizontal surface and the sloping wall of the indented cone.

Experimental procedure

Experiments were conducted using larvae of the 3 species tested in groups of approximately 5 (5–6) or sometimes 10 (9–11). The larvae were deposited near the base of the upright cone and any excess liquid was removed with a micropipette (Fig. 1A). Likewise, larvae were deposited near the transition between the flat agarose surface and the sloping wall of the indented cone (Fig. 1B). Larvae were deposited onto the flat plate at a distance of 9–10 mm from the outer edge of the central well (Fig. 1C). The start time for observation of migratory behaviour for all 3 types of plates was recorded as the time when the excess liquid was removed, after which the larvae were free to crawl on the agarose surface. Experiments were terminated after 20 min for *A. caninum*, and after 15 min for the other 2 species.

During experiments, the tops of the Petri dishes were removed, exposing the larvae to the environment in the laboratory; including ambient light and any heat radiating from the illuminator in the stage of a Leica MZ8 microscope. Since *S. stercoralis* L3s react to even small temperature gradients (Lopez *et al.* 2000), the temperature at the top surface and in the well at the bottom of the indented cones was monitored, when testing the migratory behaviour of this species. To control for temperature variation, the indented cones were immersed in water baths, either cooled with ice to approximately 20 °C, or warmed to approximately 37 °C. The temperature at the upper surface and in the well was measured at the beginning and end of each 15-min experiment using a YSI Model 47 Tele-Thermometer fitted with a surface probe (Yellow Spring Instrument Co.). The temperature in the water baths was kept within 1 °C of the starting temperature by adding ice or warm water, as needed.

To determine if a thermotactic response would override other migratory behaviour in *S. stercoralis* L3s, indented cones were placed on a Multi-Blok Heater (Lab-Line, Inc.) and the temperature was adjusted so that the temperature at the bottom of the cones was 3–5 °C higher than that on the surface. Migratory behaviour was observed for 15 min as described above.

Statistical analysis

Data were analysed using generalized linear modelling and applying the binomial distribution. In this approach, design variables were portrayed as indicator variables and reference states, or treatments, were assigned as appropriate for the analysis. Results were expressed in terms of odds ratios of failure (succumbing to the well) for the treatment of interest relative to the reference state or treatment. To check the robustness of all analyses, distributions less sensitive to the homogeneity assumptions of the binomial family were employed, specifically, the negative binomial and Poisson families (Collett, 1996). The statistical software STATA, Release 7, 2001 (Stata-Corp, College Station, TX, Stata Press) was used for the analysis (procedures used: blogit and glm), and a *P* value for the clarity of definition of the odds ratio of less than 0·05 was considered indicative of an actual difference between the 'interest' treatment and

Table 1. *The vertical migratory behaviour of* Ancylostoma caninum *infective larvae tested at ambient temperature by placing them on upright or indented (hollow) cones, or flat plates made of 5% (w/v) agarose (see text)*

(Experiments were terminated after 20 min. Migratory behaviour on upright cones was significantly different from that on indented and flat plates ($P < 0.001$), however behaviour on indented cones and flat plates did not differ ($P = 0.097$). The odds ratio for indented cones relative to upright cones was 0.070 ± 0.022, and for flat plates relative to upright cones was 0.035 ± 0.014 (here $\pm$ denotes standard error of odds ratio). The odds ratio of indented cones relative to flat plates was 0.505 ± 0.208.)

	No. of replicates	Total no. of worms placed on agarose	Total no. of worms found in well	Mean % $\pm$ S.D.
A Upright cones	20	127	94	74.0 ± 17.9
B Indented cones	17	121	20	16.5 ± 13.1
C Flat plates	15	110	10	9.1 ± 11.1

the reference treatment. Wald tests for the significance of terms on the respective logistic models were based on the logistic coefficients and their errors.

RESULTS

Vertical migratory behaviour of Ancylostoma caninum *infective larvae*

When placed at the base of a moist upright agarose cone (Fig. 1A), L3s of *A. caninum* migrated upwards. Most larvae reached the top of the cone, where they became trapped in the water-filled apical well. As shown in Table 1A, 94 (74%) of 127 larvae reached the top in 20 min or less. In contrast, *A. caninum* larvae placed near the lip of the indented cones (Fig. 1B), generally did not move downward (Table 1B). Those larvae that did migrate downward (16·5%) reached the bottom in 20 min or less.

To determine the number of larvae that might be expected to reach the centre of either cone simply by random migration, larvae were placed on the flat plates described above (Fig. 1C), and the number migrating into the water-filled centre trap was recorded. As shown in Table 1C, 10 (9%) of the 110 larvae placed on the flat plates reached the centre.

Vertical migratory behaviour of Strongyloides stercoralis *infective larvae*

When placed at the base of upright cones, 88 (80%) of 110 third-stage larvae of *S. stercoralis* migrated to the top of the cone and into the well in 15 min or less (Table 2A). In several experiments, all of the larvae placed at the base of the cone reached the well.

In preliminary experiments with *S. stercoralis* L3s, using the indented cones at ambient temperature, some of the larvae migrated down the sloped sides of the cone, to become trapped at the bottom. To determine if this behaviour was the result of unintentional warming of the bottom of the cone by the light source of the Leica MZ8 microscope,

the temperature was measured on the top surface of the indented cone and in the well at the bottom at the beginning and at the end of each of 8 replicates. The initial temperature in the well varied between 2 °C lower and 1·5 °C higher than that on the surface. Under these conditions, only 1 of the 42 larvae tested migrated to the bottom of the well (Table 2B). Worms were often observed starting down the sides of the hollow cone and then making a 'U-turn' and crawling back up to the flat surface. There was also a tendency for some worms to follow in the tracks of other larvae.

To control temperature more precisely, indented cones were immersed either in cooling water baths (16–23 °C) or warming baths (33–39 °C). Of 66 larvae tested on uniformly cooled cones (11 replicates), none migrated down into the wells (Table 2C), nor did any of the 38 larvae placed on uniformly warmed indented cones (6 replicates, Table 2D).

To determine the number of L3s of *S. stercoralis* that might reach the traps of the upright or indented cones as a result of random, undirected crawling, larvae were placed on the flat plates described above, and the number that migrated into the centre well and became trapped was recorded. As shown in Table 2E, 13 (11·8%) of the 110 larvae placed on the flat plates reached the centre.

To determine if *S. stercoralis* L3 migration to the bottom of the indented cones in the preliminary experiments might have been attributable to thermotaxis, several plates were placed on a Multi-Blok Heater, which was adjusted so that the temperature at the bottom of the wells was 3–5 °C higher than that on the flat upper surface of the plate. Under these conditions, 37 (75·5%) of 49 larvae migrated to the bottom of the indented cone (Table 2F).

Vertical migratory behaviour of Haemonchus contortus

When *H. contortus* L3 were placed at the bases of upright cones, 8 (9·8%) of 83 worms migrated up the

Table 2. *The vertical migratory behaviour of* Strongyloides stercoralis *infective larvae was tested by placing them on upright or indented (hollow) cones, or flat plates made of 5 % (w/v) agarose (see text)*

(Experiments were terminated after 15 min. Ambient temperature, except as noted. Migratory behaviour on upright cones was different from that on indented cones (except for F, the behaviours on indented cones were compiled into a single group because of their commonality of response) and on flat plates ($P < 0·001$ in each case), but was not different from that on indented cones F ($P = 0·524$). Behaviour on flat plates and on indented cones (except for F) was different ($P = 0·005$), and the behaviour on indented cones B, C and D was different from that on indented cones F ($P < 0·001$). The odds ratio of indented cones (except F) relative to upright cones was $0·0017 \pm 0·0018$, and flat plates relative to upright cones was $0·034 \pm 0·013$, and indented cones F relative to upright cones was $0·77 \pm 0·32$. The odds ratio of the flat plates relative to indented cones (except F) was $19·4 \pm 20·3$ and indented cones F relative to indented cones excluding F was 447 ± 472.)

	No. of replicates	Total no. of worms placed on agarose	Total no. of worms found in well	Mean % ± S.D.
A Upright cones	15	110	88	80·0 ± 27·1
B Indented cones	8	42	1	2·4 ± 5·8
C Indented cones*	11	66	0	0
D Indented cones†	6	38	0	0
E Flat plates	15	110	13	11·8 ± 18·1
F Indented cones‡	7	49	37	75·5 ± 36·0

* Indented cones were immersed in cooling water baths so that there was no temperature gradient between the top and the bottom well; temperature range of replicates: 16–23 °C.

† Indented cones were immersed in warming water baths so that there was no temperature gradient between the top and the bottom well; temperature range of replicates: 33–39 °C.

‡ Indented cones were placed on a warming plate so that the wells were 3–5 °C warmer than the tops of the cones, thus producing a thermal gradient (see text).

cone and were trapped (Table 3A). Some worms crawled part way up the cone and then crawled around the cone, without migrating either farther up or back down to the flat surface.

Likewise, when *H. contortus* L3s were placed on the indented cones, they showed very little tendency to migrate down to the well at the bottom; only 8 (17·0%) of the 47 worms tested did so (Table 3B). When placed on the flat control plates, these larvae crawled 'randomly', often reversing direction; only 3 (3·4%) of 87 tested migrated into the wells at the centres of the plates (Table 3C).

DISCUSSION

Organisms that exhibit geotactic behaviour generally have at least 1 gravity receptor. The usual sense organs, statocysts, typically have a heavy object, the statolith, enclosed in a fluid-filled chamber innervated by mechanosensory neurons (Frankel & Gunn, 1960). Nevertheless, many organisms, including paramecia, zoospores of fungi, miracidia and cercariae of trematodes, lack statocysts, but do show geotactic behaviour (Brooker, 1972; Dryl, 1974; Cameron & Carlile, 1977; Haas, 1994).

According to a number of authors, the miracidia of several trematodes show geotactic behaviour (reviewed by Brooker, 1972). In addition to simple ciliated nerve endings, which are believed to be mechanoreceptors, and ciliated pit sensilla suggesting chemoreceptors, the miracidia of most trematodes have a conspicuous pair of lateral papillae. In the lateral papillae of these larvae of *Fasciola hepatica* and *Schistosoma mansoni*, the cilia, whose structure suggests that they are sensory in nature, are in close proximity to elaborate membrane elevations. It has been postulated that mechanical stimulation of the cilia by these evaginations would provide the larva with information about its orientation with respect to gravity (Brooker, 1972). Trematode cercariae also respond to gravitational fields as part of their adaptations for transmission success. While some species may be responding to light, others do, in fact, appear to be responding to gravity, rather than other stimuli (Haas, 1994). Thus, even in the absence of obvious gravity-sensing organs, many trematode larvae still show geotactic behaviour.

When placed near the bases of upright agarose cones, a great majority of infective larvae of both *A. caninum* and *S. stercoralis* crawled up to the top of the cones. The infective larvae of both these species often show nictitating behaviour, extending themselves vertically from particulates on the substratum, presumably in attempts to contact a passing host (Augustine, 1921; Granzer & Haas, 1991; Fine *et al.* 1997). However, nictitation was not observed on the upright cones, because the larvae that reached the top became trapped in the water wells. Furthermore, the surface of the 5% agarose cones was most likely too wet to permit the larvae to break the surface film.

Table 3. *The vertical migratory behaviour of* Haemonchus contortus *infective larvae was tested by placing them on upright or indented (hollow) cones, or flat plates made of 5% (w/v) agarose, at ambient temperature (see text)*

(Experiments were terminated after 15 min. Migratory behaviour on upright cones did not differ from that on indented cones ($P=0\cdot234$) nor did behaviour on upright cones differ from that on flat plates ($P=0\cdot111$). However, behaviour on indented cones and that on flat plates did differ ($P=0\cdot013$). The odds ratio for indented cones relative to upright cones was $1\cdot90\pm1\cdot02$, and for flat plates relative to upright cones was $0\cdot33\pm0\cdot23$. For flat plates relative to indented cones, the odds ratio was $0\cdot17\pm0\cdot12$.)

	No. of replicates	Total no. of worms placed on agarose	Total no. of worms found in well	Mean% ± S.D.
A Upright cones	19	82	8	$9\cdot8\pm19\cdot2$
B Indented cones	10	47	8	$17\cdot0\pm22\cdot2$
C Flat plates	16	87	3	$3\cdot4\pm9\cdot2$

Crofton (1954) claimed that vertical migration is basically only random crawling modified by environmental conditions. Thus, a larva crawling on the soil surface might encounter a grass blade or a veined leaf, on which narrow bands of moisture would provide vertical pathways for the larva to crawl. In our experiments, larvae crawling on a flat surface, specifically the smooth surface of an agarose cone, encountered not a narrow pathway, but a broad surface, uniformly moist, on which they could crawl in any direction they chose. A significant number chose to crawl up, indicating that they must be responding to a stimulus. Temperature was essentially constant, and there was no difference in the chemical *milieu* from the bottom to the top of the cone. The water in the well at the top, besides acting as a trap for the larvae, kept the top of the cone from drying out, so that surface moisture was essentially constant, as well. The cones were evenly illuminated. Furthermore, these larvae were not found to be phototactic (W. M. Forbes, unpublished observations). Consequently, the most logical explanation is that the larvae of *A. caninum* and *S. stercoralis* were responding to gravity.

As these nematodes lack statocysts, how might they detect the pull of gravity? It is possible that one or more of the ciliated dendritic processes in the amphidial channel, or those in the phasmidial channel, might sense gravity by making contact with each other or with the wall of the channel in which they are located, in a process similar to that proposed by Brooker (1972) for the miracidia of trematodes.

When *A. caninum* L3 were placed on the indented cones, a small percentage migrated to the well in the bottom. The number of larvae which did migrate down was slightly, but not significantly, greater than the number which crawled to the well in the centre of the flat control plates. The light source in the stage of the Leica microscope may have warmed the well of the indented plates slightly, with a few worms responding thermotactically to this

temperature gradient (Granzer & Haas, 1991; Bhopale *et al*. 2001).

The behaviour of *S. stercoralis* L3 on indented cones, where they started down the sloping sides, but turned and crawled back up, suggests that they were also responding to gravity, but that the response was not immediate. The larvae took additional time to 'decide' that they were not going in the direction they wanted to go.

While the number of *A. caninum* L3 that migrated to the bottom of the indented cones was not significantly different from the number reaching the centre of the flat plates, *S. stercoralis* L3 did show some tendency to migrate to the bottoms of these cones in preliminary experiments. Presumably, the warmth of the illuminator in the stage of the Leica Wild MZ8 microscope was a factor in this observed behaviour with *S. stercoralis* L3s being more sensitive to small temperature gradients than L3s of *A. caninum* (Li *et al*. 2000; Lopez *et al*. 2000; Bhopale *et al*. 2001). When there was very little temperature difference between the top and bottom of the indented cones, very few L3s migrated down and when there was no temperature difference, no larvae migrated down. However, when the bottom of the indented cones was 3–5 °C warmer, appreciable numbers of larvae migrated to the bottom, indicating that thermotactic behaviour overrides geotaxis.

Presumably to make contact with a potential host, L3s of *A. caninum* and *S. stercoralis* position themselves on upward projections of the substratum (Granzer & Haas, 1991; Ashton, Li & Schad, 1999). By nictitating, especially when they receive signals that a host is near, they increase the likelihood of making contact with that host and prepare themselves for transfer (Granzer & Haas, 1991; Sciacca *et al*. 2002). Negative geotactic behaviour would be an important part of the host-finding behaviour of these 2 skin-penetrating species and nictitation may well be another part of this behaviour. However, other behavioural responses may, at times, override

geotaxis. When environmental conditions become harsh, L3s of at least some species retreat to sheltered areas (Ashton *et al.* 1999), while those of other species are trapped (Wakeshima, 1933).

In sharp contrast to the behaviour of L3s of *A. caninum* and *S. stercoralis*, very few L3 larvae of *H. contortus* migrated to the tops of upright cones, and few migrated down the walls of indented cones. On flat plates, the behaviour of these larvae suggested random crawling, with frequent reversals of direction; very few (3·4% of 87 worms) migrated to the centre well of these plates. If *H. contortus* L3s do not undergo geotaxis, then it is possible that they either are attracted to moisture, as are the L3s of *A. ritzemabosi* (Barraclough & French, 1965), or exhibit rheotaxis, crawling against a gentle flow of dew down grass blades, as do the L3s of the rice white-tip nematode, *Aphelenchoides besseyi* (Adamo, Madamba & Chen, 1976). Large numbers of *H. contortus* L3s migrate up on grass blades at dawn, when humidity is high and the grass is moist, then withdraw to the soil during the day when humidity is lower, thus avoiding dessication. At dusk, there is a second upward migration, but generally fewer worms crawl up at this time (Rees, 1950). These migrations appear to be mediated by light, temperature and humidity, but our observations indicate that geotaxis is not part of the behaviour of this species.

The environment within the host, when compared to the external environment, is comparatively constant. Thus, helminth parasites may recognize and respond to only a few variables in this environment (Sukhdeo & Sukhdeo, 1994). In the otherwise highly variable environment occupied by the free-living infective larva, gravity is one stimulus that is constant. As part of their host-finding behaviour, infective larvae of some species appear to respond to the influence of gravity. This response, not readily discernible in the presence of other environmental stimuli, can be observed under controlled laboratory conditions.

REFERENCES

ADAMO, J. A., MADAMBA, C. P. & CHEN, T. A. (1976). Vertical migration of the rice white-tip nematode, *Aphelenchoides besseyi*. *Journal of Nematology* **8**, 146–152.

AFRICA, C. M. (1931). Studies on the activity of the infective larvae of the rat strongylid, *Nippostrongylus muris*. *Journal of Parasitology* **17**, 196–206.

AL SAQUR, I., BAIRDEN, K., ARMOUR, J. & GETTINBY, G. (1982). Population study of bovine *Ostertagia* spp. infective larvae on herbage and in soil. *Research in Veterinary Science* **32**, 332–337.

ASHTON, F. T., LI, J. & SCHAD, G. A. (1999). Chemo- and thermosensory neurons: structure and function in animal parasitic nematodes. *Veterinary Parasitology* **84**, 297–316.

AUGUSTINE, D. L. (1921). Investigations on the control of hookworm disease. IX. On the position of the infective hookworm larvae in the soil. *American Journal of Hygiene* **2**, 172–176.

BARRACLOUGH, R. M. & FRENCH, N. (1965). Observations on the orientation of *Aphelenchoides ritzemabosi* (Schwartz). *Nematologia* **11**, 199–206.

BHOPALE, V. M., KUPPRION, E. K., ASHTON, F. T., BOSTON, R. & SCHAD, G. A. (2001). *Ancylostoma caninum*: The Finger Cell neurons (AFD) mediate thermotactic behavior by infective larvae of the dog hookworm. *Experimental Parasitology* **97**, 70–76.

BROADBENT, S. R. & KENDALL, D. G. (1953). The random walk of *Trichostrongylus retortaeformis*. *Biometrics* **9**, 460–466.

BROOKER, B. E. (1972). The sense organs of trematode miracidia. In *Behavioural Aspects of Parasite Transmission* (ed. Canning, E. U. & Wright, C. A.), pp. 171–180. Academic Press, Inc., London, UK.

BUCKLEY, J. J. C. (1940). Observations on the vertical migration of infective larvae of certain bursate nematodes. *Journal of Helminthology* **18**, 173–182.

CAMERON, J. N. & CARLILE, M. J. (1977). Negative geotaxis of zoospores of the fungus Phytophthora. *Journal of General Microbiology* **98**, 599–602.

COLLETT, D. (1996). *Modeling Binary Data*. Chapman & Hall, London, UK.

CROFTON, H. D. (1954). The vertical migration of infective larvae of strongyloid nematodes. *Journal of Helminthology* **28**, 35–52.

CROLL, N. A. (1970). *The Behaviour of Nematodes*. Edward Arnold Ltd, London, UK.

CROLL, N. A. (1972). Behaviour of larval nematodes. In *Behavioural Aspects of Parasite Transmission* (ed. Canning, E. U. & Wright, C. A.), pp. 31–52. Academic Press, Inc., London, UK.

CUNNINGHAM, F. C. (1956). A comparative study of tropisms of *Nippostrongylus muris* (Nematoda: Trichostrongylidae). *Biological Studies of the Catholic University of America* **36**, 1–24.

DRYL, S. (1974). Behavior and motor response of Paramecium. In *Paramecium: a Current Survey* (ed. Van Wagtendonk, W. J.), pp. 165–218. Elsevier Scientific Publishing Co. Amsterdam.

DUNCAN, L. W., DUNN, D. C. & McCOY, C. W. (1996). Spatial patterns of entomopathogenic nematodes in microcosms; Implications for laboratory experiments. *Journal of Nematology* **28**, 252–258.

FINE, A. E., ASHTON, F. T., BHOPALE, V. M. & SCHAD, G. A. (1997). Sensory neuroanatomy of a skin-penetrating nematode parasite *Strongyloides stercoralis*. II. Labial and cephalic neurons. *Journal of Comparative Neurology* **389**, 212–223.

FRANKEL, G. S. & GUNN, D. L. (1940, revised 1960). *The Orientation of Animals*. Dover, New York and Oxford University Press, Oxford, UK.

GRANZER, M. & HAAS, W. (1991). Host-finding and host recognition of infective *Ancylostoma caninum* larvae. *International Journal for Parasitology* **21**, 429–440.

HAAS, W. (1994). Physiological analyses of host-finding behaviour in trematode cercariae: adaptions for transmission success. *Parasitology* **109**, S15–S29.

LI, J., ZHU, X., BOSTON, R., ASHTON, F. T., GAMBLE, H. R. & SCHAD, G. A. (2000). Thermotaxis and thermosensory neurons in the infective larvae of *Haemonchus contortus*, a passively ingested nematode parasite. *Journal of Comparative Neurology* **424**, 58–73.

LOPEZ, P. M., BOSTON, R., ASHTON, F. T. & SCHAD, G. A. (2000). The neurons of class ALD mediate thermotaxis in the parasitic nematode, *Strongyloides stercoralis*. *International Journal for Parasitology* **30**, 1115–1121.

PAYNE, F. K. (1922). Investigations on the control of hookworm disease XI. Vertical migration of infective hookworm larvae in the soil. *American Journal of Hygiene* **2**, 254–263.

PAYNE, F. K. (1923 a). Investigations on the control of hookworm disease XIV. Field experiments on vertical migration of hookworm larvae. *American Journal of Hygiene* **3**, 46–58.

PAYNE, F. K. (1923 b). Investigations on the control of hookworm disease XXX. Studies on factors involved in migration of hookworm larvae in soil. *American Journal of Hygiene* **3**, 547–583.

PETERS, B. G. (1928). On the bionomics of the vinegar eelworm. *Journal of Helminthology* **6**, 1–38.

REES, G. (1950). Observation on the vertical migrations of the third stage larvae of *Haemonchus contortus* (Rud.) on experimental plots of *Lolium perenne* S24, in relation to meteorological and micrometerological factors. *Parasitology* **40**, 127–143.

SCHROEDER, W. J. & BEAVERS, J. B. (1987). Movement of the entomogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. *Journal of Nematology* **19**, 257–259.

SCIACCA, J., FORBES, W. M., ASHTON, F. T., LOMBARDINI, E., GAMBLE, H. R. & SCHAD, G. A. (2002). Response to carbon dioxide by the infective larvae of three species of parasitic nematodes. *Parasitology International* **51**, 53–62.

SUKHDEO, M. V. K. & SUKHDEO, S. C. (1994). Optimal habitat selection by helminths within the host environment. *Parasitology* **109**, S41–S55.

WAKESHIMA, T. (1933). Experimental studies on the tropisms of the mature larvae of ancylostomadae. II Report: Especially on the hydrotropism of *Ancylostoma caninum*. *Journal of the Medical Association of Formosa* **33**, 125–127.