Differing preferences of Antarctic soil nematodes for microbial prey

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Abstract

We tested the preferences of three nematode taxa, *Geomonhystera villosa, Plectus* spp. and *Teratocephalus* spp., extracted from moss at Signy Island in the Maritime Antarctic, for two microalgae, three microfungi and six heterotrophic bacteria, each also from soils at Signy Island. Choice test experiments on water agar medium, in which nematodes were enumerated in wells containing microbes at 24 and 48 h, indicated that there were differing preferences between nematodes for distinct prey. *G. villosa* was significantly attracted to the alga *Chlorella* cf. *minutissima* and the fungus *Mortierella hyalina*, and was more attracted to all algae and fungi than either of the other two nematodes. Both *G. villosa* and *Teratocephalus* spp. were attracted to an actinobacterium. *Plectus* spp. were significantly attracted to the alga *Stichococcus bacillaris* and bacteria with close taxonomic affinities to *Arthrobacter*, *Pseudomonas* and *Polaromonas*. Experiments using 0.5 µm diameter fluorescent beads indicated significantly increased ingestion by nematodes in the presence of each of these microbes compared with controls, except by *Plectus* spp. in the presence of *S. bacillaris*. We conclude that complex trophic interactions may occur in apparently simple Antarctic soil food webs.

Key words: Algae / bacteria / food webs / fungi / trophic interactions

1. Introduction

Nematodes are dominant components of Antarctic soil food webs. Not only are they abundant, with up to 10^6 individuals present per m^2 of soils in the Maritime Antarctic [11], but they also play important ecological roles in these soils by acting as consumers, typically of bacterial prey, and also as predators of other nematodes, rotifers and protozoa [21]. Furthermore, nematodes are the top predators in what are thought to be the simplest soil food webs encountered on Earth, those occurring in the McMurdo Dry Valleys of Continental Antarctica [7]. Previous studies have concentrated on Antarctic nematode taxonomy, ecophysiology and biogeography [2,7,10,11,15,26]. However, despite their potentially large ecological influence, little is known of the feeding preferences of Antarctic soil nematodes [11]. Direct observations suggest that a species of *Mesodorylaimus* at Signy Island in the Maritime Antarctic may feed upon coccoid algae and dead collembola [21]. *Coomansus gerlachei* has also been shown to feed upon algae, fungal hyphae and spores, arthropods, rotifers and tardigrades in Signy Island soil [21]. Furthermore, laboratory studies have shown that a yeast and bacteria isolated from the McMurdo Dry Valleys support the growth of *Scottnema lindsayae* Timm. extracted from the same habitat [14]. However this is the only information available on the specific feeding preferences of Antarctic soil nematodes.

Here we report a study which aimed to determine the preferences of the three most abundant soil nematode taxa at Signy Island, *viz. Geomonhystera villosa* (Bütschli) Andrássy, *Plectus* spp. and *Teratocephalus* spp., for 11 microbes, each isolated from Signy Island soil. These nematodes are currently classified as microbivores and are assumed to feed predominantly on bacteria, although their diet might potentially include algae and filamentous fungi [21]. We also tested for significant differences between nematodes' preferences for microbes to assess whether apparently simple Antarctic soil food webs consist of complex patterns of trophic interactions.

2. Methods

2.1. Extraction of nematodes

Nematodes were extracted from turves of the moss *Sanionia uncinata* (Hedw.) Loeske *ex* Nitardy collected from 100 m to the east of the British Antarctic Survey's research station on Signy Island (60º 42' 34" S, 45º 35' 36" W) in austral summer 2001. The moss was frozen at -20 ºC shortly after collection and was transported to the UK. Small (*c.* 5 g fresh weight) pieces of frozen moss were placed

onto muslin resting on a 2 mm rigid mesh in the mouth of a plastic funnel held by a retort stand. A plastic tube attached to the funnel's spout was blocked with a clip and spring water was added to the funnel until a thin layer covered the moss. Nematodes that passed through the muslin were collected after 24 h by releasing the clip. They were sorted under a dissecting microscope (6-50 x magnification) into *G. villosa*, *Plectus* spp. (typically *P. antarcticus* de Man) and *Teratocephalus* spp. (typically *T. tilbrooki* Maslen) and were stored in spring water at 4 ºC for a maximum of 3 d until required for experiments.

2.2. Isolation of microbes from soil

Microbes were isolated from soil under *S. uncinata* taken from the same location at which moss was collected for nematode extraction. Microalgae were isolated on Bold's Basal (BB) medium (Sigma-Aldrich, St Louis, MO, U.S.A.). Dilution series of 1 g (fresh weight) of soil were prepared in sterile 10 mM phosphate buffer, 100 μl aliquots of the 10^{-4} dilution were spread onto BB medium in 90 mm single vented Petri dishes and the dishes were incubated in the light at *c*. 4 ºC. A single colony of the alga *Stichococcus bacillaris* Nag.*,* and two separate colonies of the alga *Chlorella* cf. *minutissima*, were isolated onto BB medium after 28 d and were kept in the light at 4 ºC until required for experiments. Algal isolates were free of bacteria. The shapes and dimensions of each alga are shown in Table 1.

Microfungi were isolated by a modification of the Warcup soil plate method [24]. Briefly, small volumes of soil (*c*. 5 mg fresh weight) were placed into non-vented 90 mm Petri dishes, cooled broad-spectrum Czapek-Dox agar medium was placed onto the soil and the dishes were incubated at 4 ºC for 21 d. Single colonies of the three commonest fungi, *Mortierella hyalina* (Harz) W. Gams, *Penicillium aurantiogriseum* Dierckx. and *Trichoderma viride* Pers.: Fr., were isolated onto potato dextrose agar medium and kept at 4 ºC until required for experiments. The shapes and dimensions of the conidia of each fungus are shown in Table 1.

Bacteria were isolated on R2A (Oxoid Ltd., Basingstoke, U.K.), a broad spectrum bacteriological agar medium. Dilution series of soil were prepared as for algae, and 100 μl aliquots of the 10^{-4} dilution were spread onto the medium in 90 mm single vented Petri dishes. The dishes were incubated in the dark at 4 ºC and individual colonies of the commonest six morphotypes of bacteria were isolated onto R2A medium after 28 d.

2.3. Taxonomic placement of bacteria

Bacteria were taxonomically placed by comparing 16S rRNA sequences with those in a public access database. A single colony of each isolate was placed in 50 μl of sterile water. Cells were then vortexed and subjected to five freeze-thaw cycles (-80 ºC and 80 ºC, each for 2 min). The resulting supernatant was cleaned for polymerase chain reaction (PCR) amplification with GFX DNA purification columns (Amersham Biosciences U.K. Ltd., Chalfont St Giles, U.K.).

PCR amplification was performed using 43 μl of ABgene 1.1x ReddyMix PCR Master Mix (ABgene, Epsom, U.K.) with 20 pmole of each primer and *c.* 10 ng of template DNA in a final volume of 50 μl. The amplification program consisted of one cycle of 95 ºC for 5 min, 30 cycles of 94 ºC for 1 min, 56 ºC for 1 min, 72 ºC for 70 sec and a final extension step of 72 ºC for 10 min. Bacterial rDNA was targeted with the primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') [6] and 1500R (5'-AGAAAGGAGGTGATCCAGCC-3') [4]. PCR products were purified using GFX DNA purification columns and sequenced. Sequence reactions were run on a Megabace 500 sequencer (Amersham Biosciences U.K. Ltd.) using the 8F primer. Nucleotide sequences obtained from the bacteria have been deposited in the EMBL Nucleotide Sequence database (http://www.ebi.ac.uk/embl). The accession numbers of the deposited sequences are AJ557831*,* AJ557832, AJ557833, AJ557834, AJ557835 and AJ557836. The bacteria from which these sequences were obtained are hereafter referred to as B1, B3, B6, B7, B9 and B16, respectively. The closest phylogenetic affiliations of the bacteria to named species or genera in the EMBL database are shown in Table 1, along with cell shapes and dimensions.

2.4. Preference experiment

Nematodes were offered a choice of microbes in a 'cafeteria' design [23] on lake water agar (LWA) medium. The medium consisted of 0.2 μm-filtered water from an oligotrophic lake at Signy Island, 0.5% technical agar (agar no.3, Oxoid Ltd., Basingstoke, U.K.) and 50 μg ml^{-1} of the bacteriostatin ampicillin (Sigma-Aldrich, Gillingham, U.K.). Preliminary experiments indicated that ampicillin added to the LWA medium reduced bacterial growth on its surface and improved attraction of nematodes to microbial prey offered within the cafeteria design, relative to LWA medium without ampicillin. These experiments also indicated that the inclusion of ampicillin in the medium was more effective at reducing bacterial growth than rinsing nematodes in bacteriostatin solution prior to preference experiments [12]. With the regular addition of bacteria to cultures, nematodes could be kept for at least

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four weeks on LWA medium to which ampicillin had been added without showing altered behaviour, reproduction or mortality. The LWA medium was poured to a depth of 1.5 mm into 48 single-vented 90 mm Petri dishes. After the layer of LWA medium had solidified, six sterile plastic cylinders of 12 mm diameter and 15 mm length were placed vertically and equidistantly onto the medium under a sterile hood. The centre of each disk was 30 mm from the centre of the Petri dish. A second layer of LWA medium was then gently poured to a depth of 5 mm. After this layer of medium had solidified the plastic cylinders were removed, leaving six wells of 12 mm diameter and 5 mm depth in the LWA medium (Fig. 1 a).

Three alternate wells in each Petri dish were plugged with 12 mm diameter and *c.* 4.5 mm thick discs of R2A medium. These wells are hereafter referred to as control wells. The remaining three wells were plugged with discs of R2A medium (Fig. 1 b), of the same dimensions as those in control wells, on which microbes had been grown for 7 d in the light at 15 ºC. Twelve Petri dishes received alternate plugs inoculated with *C. minutissima* strains 1 and 2 and *S. bacillaris*, 12 dishes received alternate plugs inoculated with *M. hyalina*, *P. aurantiogriseum* and *T. viride*, 12 dishes received alternate plugs inoculated with B1, B3 and B6, and a further 12 dishes received alternate plugs inoculated with B7, B9 and B16. Hyphae and conidia were present on the plugs of medium on which fungi were grown. The total volumes of cells offered to nematodes, estimated from the product of the mean volume of each cell (calculated from standard geometric formulae, assuming right circular cylinders for rods and spheres for cocci) and the mean number of cells per unit area of medium from which plugs were cut, did not differ within each of the three groups of microbes (all $P>0.05$). There was also no difference between the total cell volumes ± s.e.m. of algae and bacteria in each well (29.5 H 10^7 ± 1.8 x 10^7 μm^3 and 21.2 x 10^7 ± 1.8 H 10^7 μm^3, respectively; $P>0.05$). However, the mean volume ± s.e.m. of fungal hyphae and conidia (*c.* 7.4 x 10^7 ± 1.2 x 10^7 μm^3) in each well was significantly lower than the volumes of algal and bacterial cells presented to nematodes ($P<0.01$).

After the plugs of medium had been added to each of the 48 dishes, the LWA medium was gently flooded with a *c.* 1 mm deep layer of 0.2 μm-filtered lake water to ensure that less motile nematodes could move across the agar medium surface. The tops of the R2A medium plugs were *c.* 0.5 mm lower than the level of LWA medium in each Petri dish, ensuring that excess microbial cells did not distribute across the surface of the medium when the layer of lake water was added. Groups of 50 adult *G. villosa*, *Plectus* spp. or *Teratocephalus* spp. of similar size, each of which had been washed in

three changes of sterile lake water, were pipetted into the water at the centre of each of four Petri dishes containing plugs of R2A agar medium inoculated with either algae, fungi or one of the two combinations of bacteria. The dishes were incubated in the dark at 15 ºC to ensure rapid movement of nematodes towards microbial prey [12]. Nematodes in each well were counted at 24 and 48 h with a stereomicroscope (6-50 x magnification). The dishes were handled carefully to avoid displacement of nematodes from the wells.

2.5. Ingestion of fluorescent beads

When the preference experiment indicated significant attraction of nematodes to microbes, experiments using fluorescent beads were performed to test for increased ingestion by nematodes in the presence of prey. Spring water (0.2 μm-filtered) was added to a depth of 1 mm to colonies of the appropriate microbes growing on R2A medium and the surfaces of the colonies were gently rubbed with a sterile glass spreader. Aliquots (0.5 ml) of the water, containing c. 2.5 x 10^6 algal and bacterial cells and c. 1.2 x 10^6 fungal conidia, were dispensed into tissue culture plate wells and 2 μl of a suspension of Tetraspeck fluorescent beads (0.5 μm diameter; Molecular Probes Inc., Eugene, OR, U.S.A.), with respective excitation and emission maxima at 505 and 515 nm, was added to each well. Controls consisted of 0.5 ml of 0.2 μm-filtered spring water, taken from the surface of uninoculated R2A medium, with 2 μl of fluorescent beads suspension. Treatment and control wells each received c. 3 x 10^6 beads. Individual nematodes were introduced into each of 10 control and treatment wells and the tissue culture plates were incubated in the dark at 15 ºC. The number of fluorescent beads ingested by each nematode was counted after 24 h using epifluorescence at 1000 x magnification.

2.6. Statistical analyses

One way ANOVA was used to compare (i) the numbers of nematodes remaining on the agar medium surface with those accumulated in control wells and wells containing microbial prey and (ii) the numbers of nematodes accumulated in control wells with those accumulated in wells containing microbial prey. Control values entered into the latter analyses were the mean numbers of nematodes in the two control wells either side of each replicate well containing the appropriate microbial prey. Repeated measures ANOVA was used to determine the main effects of time, and the main and interactive effects of prey type and nematode genus or species, on nematode accumulation in wells.

Control values were eliminated from these analyses. One way ANOVA was also used to compare the numbers of fluorescent beads accumulated in the guts of nematodes in the presence of microbial prey with the numbers accumulated in the guts of nematodes in water controls.

3. Results

3.1. Responses to time

No main effect of time was found on the numbers of nematodes accumulated in wells containing microbial prey (Table 2): numbers of nematodes in the wells remained the same at 24 and 48 h.

3.2. Responses to algae

Significantly more nematodes remained on the LWA medium surface than accumulated in control wells and wells containing algae (mean numbers per dish = 35.4 and 14.6, respectively; $P<0.001$). However, there was a strong main effect of algae on nematode accumulation in wells (Table 2): more nematodes accumulated in wells containing *C. minutissima* strain 2 than in wells containing *C. minutissima* strain 1 or *S. bacillaris* (mean numbers per well ± s.e.m. = 4.5 ± 0.7, 1.5 ± 0.2 and 2.6 ± 0.4, respectively). There was also a highly significant main effect of nematode genus on the numbers of nematodes accumulated in wells containing algae (Table 2): *G. villosa* was more frequently recorded in algal wells than *Plectus* spp. or *Teratocephalus* spp. (mean numbers per well ± s.e.m. = 4.8 ± 0.7, 2.5 ± 0.3 and 1.3 ± 0.3, respectively). Furthermore, there was a significant interactive effect of alga and nematode genus on the numbers of nematodes accumulated in wells (Table 2): there were significant differences between nematode genera in the isolates of algae that they were attracted to.

A comparison of the mean numbers of nematodes accumulated in wells containing individual algae with those in adjacent control wells indicated significant attraction to two algal isolates. *G. villosa* was significantly attracted to *C. minutissima* strain 2 and *Plectus* spp. were attracted to *S. bacillaris* (Fig. 2 a, b). However only *G. villosa* ingested a significantly greater number of fluorescent beads in the presence of algal cells compared with water controls (Fig. 2 a, *inset*): *Plectus* spp. did not ingest more beads when presented with *S. bacillaris* (Fig. 2 b, *inset*).

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3.3. Responses to fungi

Significantly more nematodes remained on the LWA medium surface than accumulated in control wells and wells containing fungi (mean numbers per dish = 38.3 and 11.7, respectively; *P*<0.001). There was also no main effect of different fungi on nematode accumulation in wells (Table 2). However there was a strong main effect of nematode genus on the numbers of nematodes accumulated in wells containing fungi (Table 2): more *G. villosa* individuals were attracted to wells containing fungi than either *Plectus* spp. or *Teratocephalus* spp. (mean numbers per well ± s.e.m. = 4.0 ± 0.6, 2.4 ± 0.3 and 1.0 ± 0.2, respectively). There was no interactive effect of fungus and nematode genus on the numbers of nematodes accumulated in wells containing fungi (Table 2). Only one significant trophic link between nematodes and fungi was recorded: *G. villosa* was significantly attracted to *M. hyalina* (Fig. 2 c) and ingested a greater number of fluorescent beads compared with controls when presented with conidia of the fungus (Fig. 2 c, *inset*).

3.4. Responses to bacteria

There was no difference between the numbers of nematodes remaining on the LWA medium surface and those accumulated in control wells and wells containing bacteria (mean numbers per dish = 28.0 and 22.0, respectively, *P*>0.05). There were no significant main effects of bacterium or nematode genus on the accumulation of nematodes in wells (Table 2). However, there was a strong interactive effect of bacteria and nematode genus on nematode accumulation in wells (Table 2): there were significant differences between nematode genera in the isolates of bacteria that they were attracted to.

A comparison of the numbers of nematodes accumulated in wells containing individual bacteria with those accumulated in adjacent control wells indicated that both *G. villosa* and *Teratocephalus* spp. were attracted to B16 (Fig. 2 d, e). Both of these nematodes ingested more fluorescent beads in the presence of B16 than in their absence (Fig. 2 d, e *insets*). Higher numbers of *Plectus* spp. accumulated in wells containing B3, B7 and B9 than accumulated in control wells (Fig. 2 f-h) and more fluorescent beads were ingested by *Plectus* spp. in the presence of these bacteria than in their absence (Fig. 2 f-h, *insets*).

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4. Discussion

Our experiments indicated significant differences between nematodes in the algal and bacterial prey that they were attracted to. The finding that Antarctic soil nematodes exhibited differing preferences for microbial prey is an important observation because it suggests that they are apparently not generalist feeders, as has been previously assumed [21]. Selective, rather than generalist, feeding by nematodes on microbial prey may have important implications for analysing the flow of energy through soil food webs, particularly if microbivorous nematodes do not specifically feed on bacteria or fungi, as has been assumed in previous work [14]. Furthermore, selective feeding by nematodes on microbial prey may alter the outcome of interspecific competition between microbes and potentially has implications for determining microbial distribution in soil. Differing preferences for microbial prey have similarly been shown in marine ecosystems, in which different nematode species have been shown to exhibit selective preferences for distinct diatoms and bacteria [12,23]. These differences in the attraction of nematodes to distinct microbial species, and, in the case of *Geomonhystera* in the current study, to specific strains of microbes, are likely to be owing to chemotaxis towards different types or concentrations of water-soluble compounds produced by microbes, such as cyclic nucleotides or ammonia [3,25].

The laboratory-based approach taken in this study was necessary to determine trophic interactions between members of a soil microbial food web. However the artificial nature of the experiments probably influenced nematode response to microbial prey. For example, the use of a two-dimensional agar medium surface on which to offer prey to nematodes may have influenced their behaviour, relative to the three dimensional matrix in which they locate prey in the natural environment [1]. The growth of prey on a relatively nutrient-rich medium is also likely to have altered their attractiveness to nematodes, relative to prey growing in nutrient-limited conditions in soil. Furthermore, a relatively high temperature (15 ºC) was used in our experiments to ensure rapid movement of nematodes towards microbial prey. This temperature is regularly experienced by organisms inhabiting the upper layers of Antarctic soils during cloudless periods in summer, but does not persist for more than a few hours each day [9]. A previous study has, however, found that temperature does not influence nematode preference for different microbial prey [23].

Another artificial aspect of our study was the inclusion of a bacteriostatin in the agar medium used in the preference experiment. It was necessary to use the bacteriostatin because bacterial contaminants present on the surfaces of nematodes, which had been extracted directly from field-

collected material, grew on the agar medium and reduced the attraction of nematodes to microbes in wells. Previous studies [12,23] have not adopted this approach, probably because nematodes were extracted from long-term gnotobiotic cultures fed on specific strains of bacteria or diatoms, reducing the numbers of contaminants present on nematodes. However, we were unable to adopt this approach in our experiments: although we have developed cultures of *Plectus* spp. from Signy Island soil, we have failed to develop either *G. villosa* or *Teratocephalus* spp. cultures, despite repeated attempts to do so.

Despite the artificial experimental conditions used in our study, common elements of the microbial food web present in Signy Island soil were used in the experiments. Previous work has found 70% of nematodes extracted from moss at the island to consist of *G. villosa* and species of *Plectus* and *Teratocephalus* [11]. Furthermore, the microbial prey presented to nematodes in our study are commonly present in the natural environment. For example, *S. bacillaris*, *Arthrobacter*, *Bacillus*, *Chlorella* and *Mortierella* spp. are all frequently isolated from Signy Island soils [5,8]. *S. bacillaris*, *Chlorella* and *Mortierella* spp. are also common representatives of eukaryotic rDNA clone libraries constructed from Signy Island soil (B. Lawley, *pers. comm.*). In addition, the numbers of microbial cells offered to nematodes in our study were similar to those encountered in the field: algal and bacterial cell counts at soil surfaces range from 1×10^3 to 8.5×10^6 cm^{-2} and 4×10^9 to 3×10^{10} cm^{-2}, respectively [5,20], which broadly correspond to the mean numbers of cells of these microbes presented to nematodes in our study (1.1×10^7 and 7.0×10^8 cm^{-2}, respectively).

Our data in part confirm current views on the feeding habits of Antarctic nematodes. *Plectus* spp. and *Teratocephalus* spp. are typically classified as bacterial feeders [27] and our study suggested that these nematodes only feed upon bacteria, and not on algae and fungi. However, *Geomonhystera* spp. are classified as bacterial feeders and substrate ingesters [27] and our data indicate that *G. villosa* should now also be classed as a unicellular eukaryote feeder in the scheme presented by Yeates et al. [27]. Given that *G. villosa* has wider mouthparts than *Plectus* spp. or *Teratocephalus* spp. (mean stoma widths of the nematodes are *c*. 7, 5 and 3 μm, respectively [2,10]), it is not surprising that it was more attracted to algae and fungi than the other two nematodes: with the exception of the bacterium B1, algal and fungal cells were larger than the bacteria offered to nematodes.

Previous studies have recorded heterogeneous spatial distributions of nematodes in Antarctic soils [11,18]. The primary factors responsible for these clumped distributions are thought to be

increased temperature and water availability, which lead to the development of favourable microhabitats for nematodes in soil [11,18]. However, discrete microhabitats in soils also favour the development of distinct microbial populations, for example by affinities for different organic substrates [21]. In agreement with previous studies [11,16,18,19], our data therefore suggest that attraction to different populations of microbial prey present in discrete microhabitats may be a potentially important factor governing the heterogeneous distribution of nematodes in Antarctic soils.

Our observation that nematodes exhibited differing preferences for microbial prey suggests that complex trophic interactions may exist in apparently simple Antarctic soil food webs. This observation has potentially important implications for predicting the responses of these food webs to imminent climate change. The simplicity of food webs in Antarctic soils, and particularly those of Continental Antarctica, is thought to increase their vulnerability to environmental change [7]. However, given the strong theoretical link between resilience to perturbation and food web complexity, and in particular connectance [17], the finding that these food webs may exhibit reduced connectance is likely to influence their response to environmental change. At present it is not possible to accurately predict whether low connectance within Antarctic soil food webs increases or decreases their resilience to perturbation. This question, and the influence of other aspects of food web architecture on the response of model Antarctic food webs to environmental change, is a focus for ongoing research in our laboratory.

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Table 1

Taxonomic placement and morphology of microbial prey

prey type	name	shape	length of rod / diameter of coccus (μm) [a]	width of rod (μm) [a]
alga	*Chlorella* cf. *minutissima* strain 1	coccus	3.0-(3.7)-8.0	
	Chlorella cf. *minutissima* strain 2	coccus	3.0-(4.0)-7.0	
	Stichococcus bacillaris	rod	3.0-(5.6)-10.0	2.0-(2.1)-3.0
fungus	*Mortierella hyalina*	coccus [b]	4.0-(4.7)-5.5	
	Penicillium aurantiogriseum	coccus [b]	2.8-(3.0)-3.5	
	Trichoderma viride	coccus [b]	3.0-(3.1)-3.5	
bacterium	B1 (*Bacillus macroides*, 95%, 180) [c]	rod	6.0-(23.3)-80.0	1.2
	B3 (*Arthrobacter*, 99%, 720) [c]	rod	0.8-(1.0)-1.2	0.5
	B6 (*Frateuria*, 95%, 754) [c]	rod	1.0-(1.2)-1.8	0.3
	B7 (*Pseudomonas*, 98%, 684) [c]	rod	1.0-(1.5)-2.0	0.4
	B9 (*Polaromonas*, 98%, 659) [c]	rod	1.0-(1.2)-1.5	0.3
	B16 (*Frigoribacter*, 90%, 670) [c]	rod	0.5-(0.6)-0.8	0.2

[a] $n = 50$, values are minimum-(mean)-maximum, except for widths of bacteria which are approximate values

[b] conidia only

[c] names, percentages and numbers in parentheses are the closest phylogenetic affiliations of bacteria to named species or genera, the similarities to these taxa and the sequence length in base pairs

Table 2

Main effects of time, and main and interactive effects of prey type and nematode genus, on the numbers of nematodes accumulated in wells containing microbial prey in preference experiment. Data were analysed by repeated measures ANOVA.

main and interactive effects	d.f.	*F*	*P*	
time	1,27	2.16	0.154	
alga	2,27	14.36	<0.001	***
nematode	2,27	19.74	<0.001	***
alga x nematode	4,27	8.91	<0.001	***
time	1,27	2.17	0.152	
fungus	2,27	0.40	0.676	
nematode	2,27	5.95	0.007	**
fungus x nematode	4,27	0.44	0.776	
time	1,60	1.51	0.233	
bacterium	5,60	1.75	0.138	
nematode	2,60	0.55	0.582	
bacterium x nematode	10,60	3.42	<0.001	***

Figure legends

Fig. 1. (a) Transverse section through Petri dish used in the preference experiment (b) Plan view of dish showing positions of control wells (◯) and wells containing microbial prey (⊛).

Fig. 2. Numbers of nematodes accumulated in control wells (☐) and wells containing microbial prey (■) in the preference experiment. Nematode / prey combinations are shown above each Figure. Values are means of four replicates ± s.e.m. Data in (b), (d) and (f) were recorded at 24 h, data in other Figures were recorded at 48 h. Note that y-axes of all Figures are identically scaled. *Insets*: numbers of fluorescent beads ingested per nematode in the absence (☐) and presence (▨) of prey. Values are means of 10 replicates ± s.e.m. Differences between control and treatment means in Figures and insets are indicated by n.s. $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

(a)

water layer   lake water agar   R2A plug

(b)

(a) *Geomonhystera /*
Chlorella strain 2

(b) *Plectus /*
Stichococcus

(c) *Geomonhystera /*
Mortierella

(d) *Geomonhystera /*
B16

(e) *Teratocephalus /*
B16

(f) *Plectus /*
B3

(g) *Plectus /*
B7

(h) *Plectus /*
B9

No. nematodes per well

No. beads ingested