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Selective attraction of marine bacterivorous nematodes to their bacterial food

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ABSTRACT: This paper explores the role of selective attraction to food in determining the spatial (micro)distribution of closely related nematode species. The attractiveness of 3 different bacterial strains to 4 species of Monhysteridae, *Diplolaimelloides meyli*, *Diplolaimella dievengatensis*, *Monhystera* sp. and *Geomonhystera disjuncta*, was studied in a multiple choice design. In our study area, the 4 nematode species considered are associated with *Spartina anglica* detritus decay and have partially overlapping microhabitat preferences. As they all belong to the same feeding guild, they are potential competitors for food. Each of the 4 nematode species was attracted to the bacterial strain B1, but important interspecific differences were noted in the nematodes' response to live or heat-killed bacteria, to bacteria at different cell densities or of different age, and to the filtered supernatant of B1 culture. While the responses of *D. meyli* to the Gram-positive bacteria *Halobacillus trueperi* and to the Gram-negative *Escherichia coli* were similar, *D. dievengatensis* and *Monhystera* sp. were preferentially attracted to *H. trueperi* and *E. coli*, respectively. This opposite preference influenced both the numbers and their relative abundances of *D. dievengatensis* and *Monhystera* sp. inside bacterial patches in experiments with a mixed 2-species nematode inoculum. Bacterial cell density strongly influenced the nematode response, with *D. meyli* invariably preferring the highest cell densities offered, while *D. dievengatensis* and *Monhystera* sp. had a peak response at lower cell densities. Though chemotaxis is suggested as an underlying mechanism, the nature of the nematodes' response remains unproved. The present results strongly support the importance of food patchiness in determining the heterogeneous distribution of nematodes, and extend the concept in such a way as to allow for small differences in microhabitat choice between closely related species. They also support the view that nematodes are specialist feeders, though they probably select spots where suitable food is plentiful rather than individual food particles. Finally, the present study offers a baseline for an understanding and further study of patterns of succession among nematode species associated with decaying *Spartina anglica* detritus in terms of highly specific relationships with different strains, growth stages, and densities of bacteria involved in the mineralization of *Spartina anglica*-derived organic matter.

KEY WORDS: Nematodes · Bacteria · Estuarine · Recruitment · Taxis · Chemotaxis · Microdistribution · Species succession

INTRODUCTION

The meiofauna of marine and estuarine sediments is almost invariably dominated by nematodes. Densities in fine-grained intertidal and shallow subtidal sediments average 10^6 ind. m^{-2}, representing a biomass of roughly 0.2 to 2 g C m^{-2} (Heip et al. 1985). An enigmatic feature of marine nematode communities is their often high species diversity. It is not uncommon to find 50 species in a 10 cm^3 core, and, for example, some 800 species have been reported for the North Sea alone (Vincx 1989). In the deep sea, diversity may even be considerably higher (Lambshead 1993). By contrast, studies trying to streamline this high diversity into functional groups or trophic guilds have arrived at a limited number of categories (Wieser 1953, Jensen 1987, Moens & Vincx 1997). It has been inferred that nematodes are specialist feeders (Tietjen et al. 1970, Tietjen & Lee 1973, 1977), but observations on selected taxa of the different feeding guilds have indicated a mainly mechanistic food particle selection and significant opportunism (e.g. prey-switching) in the feeding behaviour of several species (Moens & Vincx 1997).

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Meiofauna in general and nematodes in particular have a strongly heterogeneous small-scale distribution; the size of their patches may be considerably smaller than the surface area covered with the traditionally deployed 10 cm^2 meiofauna cores (Findlay 1981, 1982). Lee et al. (1977) used a 'cafeteria' setup based on the multiple choice design of Gray (1966b) to demonstrate that food patches scattered around a central meiofauna inoculum attract strongly varying numbers of meiofauna and different meiofaunal taxa, depending on the type of food offered. They concluded that selective recruitment to food spots may be a major factor driving the heterogeneous field distribution of the meiofauna. In a similar approach, Trotter & Webster (1984) demonstrated that 3 dominant nematode species from kelp holdfasts were differentially attracted to several types of bacterial and microalgal food. The preferences so illustrated revealed a good agreement between the seasonal abundance pattern of each nematode species and of its preferred food. Decaying organic matter has been shown to attract some nematode species and to repell others (Buerkel 1901, Gerlach 1977, Riemann 1986, Lorenzen et al. 1987, Prein 1988, Olafsson 1992). Gravid females of *Metoncholaimus scissus* strongly recruited to mycelia of certain marine fungi (Meyers & Hopper 1966, 1967).

In a series of pioneering studies, Gray documented the role of bacteria in determining the horizontal distribution of some interstitial archiannelids, a gastrotrich, and a harpacticoid copepod, and demonstrated a highly differential attractiveness among bacteria from the meiofauna's natural habitat (Gray 1966a, b, 1967a, b, 1968, Gray & Johnson 1970). It was concluded that bacterial films on sand grains differentially attract meiofaunal organisms, and that the response of the meiofauna is mainly directed at characteristics of the bacterial cell wall, rather than to a product or products released by the bacteria into their environment (Gray & Johnson 1970). Such a response would imply a 'tactile chemical sense' of the meiofauna as defined by Crisp & Meadows (1963). Contrary to this interpretation are studies on mainly terrestrial and plant-parasitic nematodes exhibiting a mainly chemotactic response to a variety of inorganic ions, organic molecules, pheromones, bacteria, and bacteria- or degradation-associated compounds (see 'Discussion' for references).

The present study aims at elucidating the potential role of a taxis, i.e. a directed movement, towards patches of preferred food in determining the small-scale spatial heterogeneity in nematode abundance and species composition. This paper reports on the taxis of 4 monhysterid nematodes towards bacteria. *Diplolaimelloides meyli* Timm, 1966, *Diplolaimella dievengatensis* Jacobs et al. 1990, *Monhystera* species Bastian 1865, and *Geomonhystera disjuncta* (Bastian 1865) Jacobs 1987 all occur in a 1 m^2 sampling quadrant at the edge of the Paulina salt marsh, situated near the mouth of the Westerschelde Estuary, SW Netherlands, where they are mainly associated with decaying plant material. They are all considered deposit feeders (Wieser 1953, Jensen 1987, Moens & Vincx 1997), feeding predominantly on the bacterial flora associated with the plant detritus (Bouwman et al. 1984). A year-round field survey of selected microhabitats in the salt marsh (Moens unpubl.) suggests significant habitat overlap between the species. As such, they are potential competitors for food.

In this paper, we focus on differences in the response of different nematode species (1) to different strains of bacteria, (2) to bacteria sampled from differently aged cultures, (3) to different densities of bacteria, (4) to bacterial growth medium, and (5) to substances released by the bacteria. The first of these is studied in order to elucidate the potential of different species of bacteria—e.g. those associated with specific types of salt marsh detritus—to differentially attract nematodes. The second and third test the hypothesis that nematode species may preferentially respond to bacterial cues characteristic of specific stages of detritus decay. The fourth and fifth aim at a preliminary characterization of the nature of the nematode response. Furthermore, the hypothesis that any taxis will be influenced by the abiotic environment is tested using incubations under different temperature regimes.

MATERIALS AND METHODS

Cultivating the nematodes. A detailed description of the methods employed in the isolation, maintenance, and monospecific, agnotobiotic[1] cultivation of the nematodes studied is given elsewhere (Moens & Vincx 1998). Briefly, spot plates were prepared by the inoculation of small samples of plant litter (*Spartina anglica* and *Fucus vesiculosus*) and sediment from the Paulina salt marsh (Westerschelde Estuary, SW Netherlands) onto sloppy (0.75%) bacto-agar layers prepared with modified Killian nutrient medium (von Thun 1966). Monospecific, agnotobiotic cultures of each species were established by manual transfer of a few tens of specimens from the spot plates to a 1% bacto-nutrient agar (bacto and nutrient agar in a weight/weight ratio of 4/1) dissolved in artificial seawater (ASW) (Dietrich & Kalle 1957) with a salinity of 25 psu. Bacteria cotransferred from the spot plates served as food. Stocks were kept at 20°C in the dark. By the start of the presently reported experiments, *Geomonhystera disjuncta* had been in permanent culture for more than 6 mo, the other species for more than 1 yr.

[1] Containing unidentified associated (micro-)organisms

The 4 nematode species can reach densities of hundreds of individuals per ml of agar, and as a result of the intense microbial activity in the plates, the agar gradually becomes more fluid. This eventually results in (semi-)liquid cultures dominated by juveniles that do not fully mature anymore, probably as a result of crowding. When at this stage food is added as a dense suspension of *Escherichia coli*, growth briefly resumes, resulting in densely populated cultures dominated by adults and third (J3) and fourth (J4) stage juveniles. Aliquots of such cultures were used in all experiments with *Diplolaimelloides meyli*, *Diplolaimella dievengatensis* and *Monhystera* sp. *Geomonhystera disjuncta* were hand-picked or rinsed off from the surface of cultures.

Before experiments, nematode aliquots were washed with sucrose in a final concentration of 40% (w/w) to remove most adhering bacteria and culture medium (Sulston & Brenner 1974, modified according to pers. comm. of Dr J. Vanfleteren), subsequently rinsed 4 times in ASW, and finally resuspended in ASW. Streptomycin sulphate and benzylpenicillin were added in final concentrations of 5000 µg ml^{-1} and 5000 units ml^{-1}, respectively, to block growth of bacteria still present in the nematode inocula. Aliquots from this nematode suspension were then used for experiments.

Cultivating the bacteria. In each of the experiments performed, 1 of the following 4 bacterial cultures were used: (1) A batch culture isolated from stocks of the nematode *Diplolaimelloides meyli*; this batch culture contained 4 bacterial strains (as determined from observations of colony morphology), 2 of which were dominant, grown in 2.5% heart infusion broth dissolved in ASW with a salinity of 30 psu (buffered to a pH of 7.5 to 8 with 5 mM Tris-HCl). (2) Strain B1 was isolated from this batch culture using standard procedures and cultured on the same medium. Both batch cultures and B1 cultures were grown at room temperature in 250 ml Erlenmeyer flasks on a rotary shaker. For experiments, aliquots of these bacterial cultures were pipetted onto quadrant plates (see 'General experimental design and statistical data analysis'). Alternatively, bacteria were harvested from the cultures by centrifugation (15 min at 8000 rpm [5200 × *g*]) and subsequently rinsed 3 times with and resuspended in ASW. The supernatant obtained after the first centrifugation was also used for further tests.

(3) Stock cultures of the Gram-positive bacterium *Halobacillus trueperi* strain BTM1 and (4) the Gram-negative *Escherichia coli* strain LMG2092T were cultivated on 2% marine nutrient agar (Difco). For experiments, selected colonies of these bacteria were inoculated in marine broth (Difco) in 250 ml Erlenmeyer flasks on a rotary shaker and allowed to grow for 24 h at 28°C. Bacteria were then harvested by centrifugation and subsequently washed 3 times with and resuspended in physiological water (PW). The supernatant obtained after the first centrifugation was also used for experiments.

General experimental design and statistical data analysis. The experimental setup used in this study was a modified quadrant plate design (Fig. 1) coined from the quadrant plate design of Andrew & Nicholas (1976) and from the cafeteria design of Trotter & Webster (1984), which in turn are both modifications of Gray's (1966b, 1967a, b) multiple choice setup. Candidate attractants and controls were spotted crosswise around a central nematode inoculum on sloppy bacto-agar layers. There was always a total of 4 spots surrounding the inoculum, including the candidate attractants and at least 1 control spot. The spots and inocula were 100 or 200 µl aliquots of a candidate attractant, control or nematode culture, with an average distance between the centers and edges of the nematode inoculum and the test spots of 3.5 and 2.5 cm, respectively.

Sloppy agar layers were prepared by pouring 12 ml of a 0.5% bacto-agar (Difco) into 9 cm diameter petri dishes exposed on a perfectly flat surface. The low agar concentration enabled the nematodes to easily penetrate the agar, as preliminary experiments proved this was important, particularly to *Diplolaimella dievengatensis* and *Geomonhystera disjuncta*. The nematodes were inoculated about 1 h after spotting the candidate attractants. The nematode inocula were allowed to evaporate for about 15 min under a laminar flow hood, because nematodes often were unable to escape the surface tension of the inoculum drop. The petri dishes were then incubated at 20°C in the dark, except when noted otherwise, and the numbers of nematodes in each spot as well as in the sectors between the spots (intersects) counted after 24 h. Preliminary observations showed that the nematodes dispersed or moved towards an attractant after seconds or minutes, occasionally after a few hours, with a stable response almost invariably having been reached after 24 h. Once inside a preferred spot, most nematodes tended to stay inside it or make only small excursions in its immediate vicinity (see also Andrew & Nicholas 1976). A preliminary experiment was run to ascertain that the position and orientation of the petri dishes inside the incubator did not influence the dispersal of the nematodes.

All results have been expressed as relative % of nematodes recovered from spots or intersects. Only the nematode %, inside candidate attractive spots (including the control spot[s]) were retained for statistical analysis, and the % were adjusted to give composition, i.e. their cumulative abundance equals 100%. As such, the weight of all replicates in a replicated statistical test

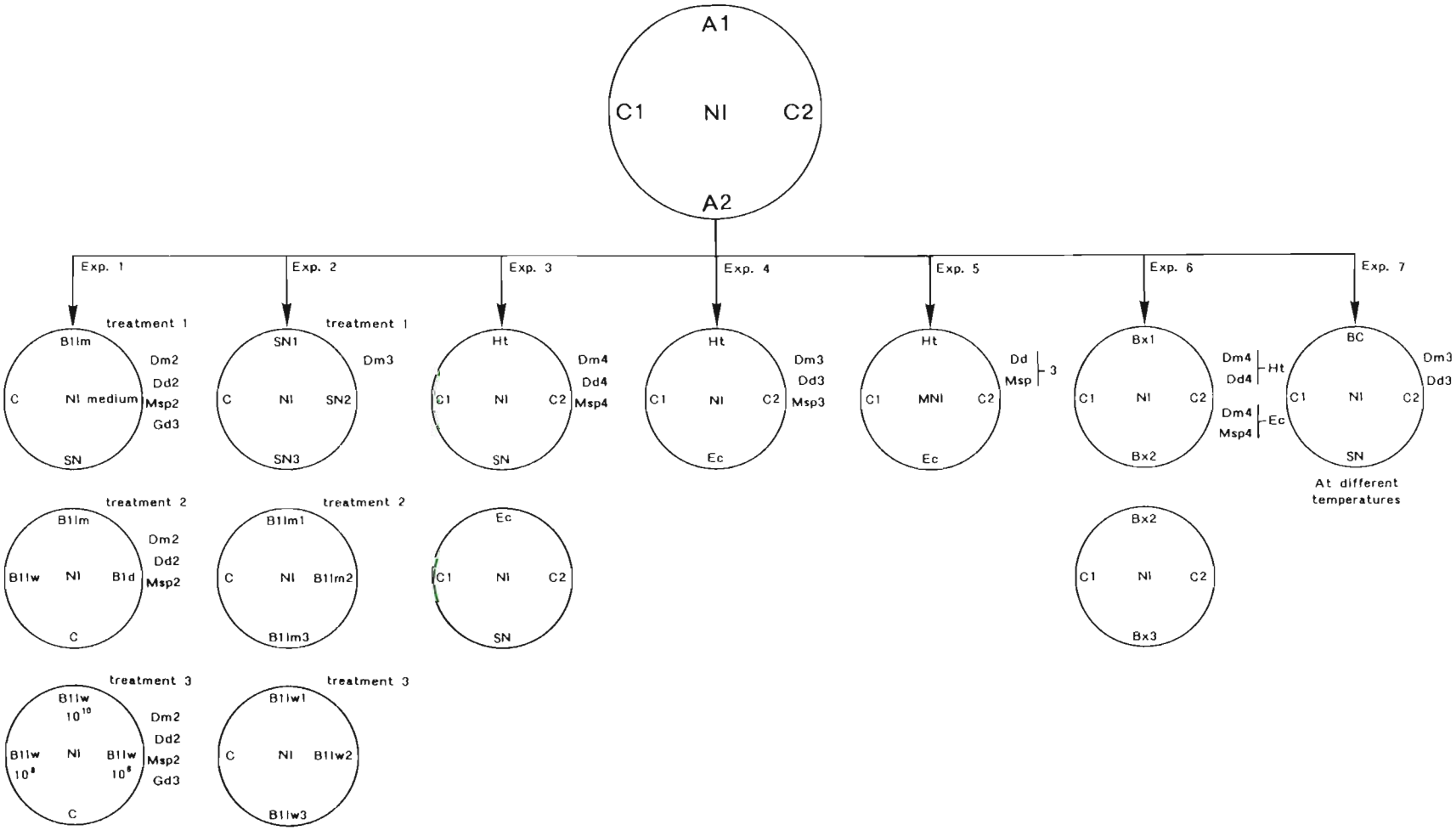

Fig. 1. General schematic representation of the modified quadrant plate design used in the present experiments and flow diagram of the setup of each individual experiment. NI = nematode inoculum, A1 and A2 = candidate attractants, C1 and C2 = control spots. The identity of the different spots in each experiment is given with the following abbreviations: MNI refers to a mixed nematode inoculum, consisting of 73% *Diplolaimella dievengatensis* and 27% *Monhystera* sp. B1, Ht, and Ec are the bacterial strains B1, *Halobacillus trueperi* BTM1, and *Escherichia coli* LMG2092T, respectively; B1lm and B1lw are live bacteria in medium and in water (washed), respectively; B1d are heat-killed bacteria; B1lm1, B1lm2, and B1lm3 are live B1 in medium from 1, 2, and 3 d old cultures, respectively; B1lw1, B1lw2, and B1lw3 similarly refer to live bacteria in water; SN refers to the filtered supernatant of the bacterial culture, and SN1, SN2, and SN3, then, are the supernatant fractions of 1, 2, and 3 d old culture, respectively. 10^x refers to bacterial densities used. Bx1, Bx2, Bx3, etc. also refer to bacteria at different cell densities, with x1 = 10^9 cells ml^{-1}, x2 = 5×10^8 cells ml^{-1}, x3 = 10^8 cells ml^{-1}, etc., in descending order, following the densities specified in the 'Materials and methods' BC are bacteria from a batch culture consisting of at least 4 different strains. Annotations to the right of the circles indicate species (Dm, *Diploma cloides meyli*; Dd, *Diplolaimella dievengatensis*; Msp, *Monhystera* sp.; Gd, *Geomonhystera disjuncta*) and number of replicates used per treatment

is equal. Replicated G-tests for goodness of fit to a chi-square distribution, i.e. with 25% of the nematodes inside each of the 4 spots, were performed in order to determine significant deviations from the expected 1/1/1/1 distribution. Heterogeneity G (G_H) was determined as an indication of whether the observed distributions differed among replicates of 1 treatment. Emphasis was, however, on the pooled G (G_P), as a measure of overall deviation from the expected distribution over all replicates of 1 treatment (Sokal & Rohlf 1995). Unplanned, pairwise comparisons were performed by computing G_P at a critical probability of $\alpha' = \alpha/k$, with k equal to the number of intended tests (Bonferroni approach, Sokal & Rohlf 1995). Since each interspot comparison was potentially meaningful, these unplanned tests were performed at an α level of 0.005 ($<0.05/6$), ensuring an experimentwise α of < 0.05.

Response of the 4 monhysterid nematode species to the bacterial strain B1. ***Expt 1:*** Each nematode species was subjected to 3 different treatments (Fig. 1). In the first treatment, the 4 spots around the nematode inoculum were 200 µl aliquots of (1) a 48 h old B1 culture (= live B1 in medium), (2) supernatant—filtered over a 0.22 µm millipore filter—of the same B1 culture, (3) sterile heart infusion broth medium, and (4) sterile ASW of 30 psu. The second treatment consisted of a B1 culture spot and an ASW control as in the first series, a heat-killed (1 h at 70°C) aliquot of the same B1 culture, and B1 washed and resuspended in ASW to remove culture medium. The third treatment had spots of B1 in ASW at densities of 10^{10}, 10^8 and 10^6 cells ml^{-1}, respectively, and an ASW control.

Expt 2: The attraction of *Diplolaimelloides meyli* to strain B1 at different growth stages was tested using 3 treatments. The 4 spots surrounding the central nematode inoculum were 100 µl aliquots (2×10^9 cells ml^{-1}) from cultures grown for 24, 48 and 72 h. In treatment 2, these aliquots consisted of live B1 in medium. In treatment 3, they were washed bacteria (= bacteria from that culture but resuspended in ASW), and in treatment 1 they consisted of culture supernatant. ASW was used as the control in all treatments (Fig 1).

Attraction to a Gram-positive and a Gram-negative bacterium. ***Expt 3:*** The attraction of *Diplolaimelloides meyli*, *Monhystera* sp. and *Diplolaimella dievengatensis* to the bacteria *Halobacillus trueperi* and *Escherichia coli* was studied. *Geomonhystera disjuncta* was omitted from this and all subsequent experiments, as well as from treatment 2 in Expt. 1, because of an infection of the stock cultures. For each nematode species, quadrant plates were spotted with *H. trueperi* in PW (= washed bacteria) and with the filtered culture supernatant at opposite sides, and with 2 spots of PW as controls, and incubated at 20°C in the dark for 24 h. A second treatment was run with *E. coli* (Fig. 1).

Expt 4: Each petri dish was inoculated with a spot of washed *Halobacillus trueperi* and one of *Escherichia coli* at opposite sides of the nematode and with 2 control spots (Fig. 1) to directly infer any nematode preference for either bacterial species over the other. Bacterial inocula contained 2×10^8 cells ml^{-1} throughout this experiment.

Expt 5: The nematode species with the strongest relative preference for *Halobacillus trueperi*, i.e. *Diplolaimella dievengatensis*, and the one with the highest relative preference for *Escherichia coli*, i.e. *Monhystera* sp., were spotted in a mixed 2-species inoculum between opposing spots of washed *H. trueperi* and *E. coli* at equal cell densities (3×10^8 cells ml^{-1}) (Fig. 1). After 24 h, the total number of each species in each bacterial spot was noted, as well as the relative proportions of both species in each spot.

Expt 6: The effect of bacterial density on the attractiveness of a spot of *Escherichia coli* to *Diplolaimelloides meyli* and *Monhystera* sp. was studied in the following way. For each nematode species a series of quadrant plates was prepared with spots of decreasing bacterial density. The first plate of such a series had a spot of 10^9 and one of 5×10^8 cells ml^{-1} at opposite sides, as well as 2 control spots; the second had opposed spots of 5×10^8 and 10^8 cells ml^{-1}; the series was continued down to 10^3 cells ml^{-1} with the following density pairs: 10^8 and 10^7, 10^7 and 10^6, 10^6 and 10^5, and 10^5 and 10^3 (Fig. 1). The effect of bacterial density on the attractiveness of *H. trueperi* to *D. meyli* and *D. dievengatensis* was tested in a parallel experiment.

Impact of temperature on the attraction of nematodes to their bacterial food. ***Expt 7:*** Aliquots of *Diplolaimelloides meyli* and of *Diplolaimella dievengatensis* were inoculated amidst 200 µl spots of live bacteria in medium of a 48 h old batch culture, supernatant of this culture, and 2 ASW controls (Fig. 1). Three replicate petri dishes for each species were incubated in the dark at each of the following temperatures: 5, 10, 15, 20, and 25°C. After 24 and 48 h, the nematode numbers inside each spot, in the intersects, and in the inoculum spot were counted in order to assess any temperature-induced differences in the taxis response and in the activity level of the nematodes.

RESULTS

Response of the 4 monhysterid nematode species to the bacterial strain B1

Expt 1

The results of the experiments on the attraction of the 4 nematode species to the bacterial strain B1 are

Table 1. Relative recruitment percentages of the 4 monhysterid nematode species to the unidentified bacterial strain B1. Averages and standard deviations of 2 or 3 replicates per treatment are given. nd = not determined. Washed bacteria are live culture aliquots washed and resuspended in ASW (see 'Materials and methods'). All bacterial spots had cell densities of 10^{10} cells ml^{-1}, except for bacteria 1/100 and bacteria 1/10000, which had densities of 10^{8} and 10^{6} cells ml^{-1}, respectively

Treatment 1	Live bacteria	Bacterial medium	Supernatant	Control	Total intersects	Central inoculum
Monhystera sp.	41.5 ± 2.12	0 ± 0	5 ± 4.24	13 ± 2.83	21.29 ± 4.39	19.42 ± 0.14
Diplolaimelloides meyli	37 ± 2.83	9.5 ± 4.95	16 ± 4.24	9.5 ± 0.71	23.77 ± 2.14	4.6 ± 0.97
Diplolaimella dievengatensis	12.5 ± 0.71	1 ± 1.41	12 ± 1.41	17 ± 4.24	17.69 ± 10.08	36.45 ± 14.33
Geomonhystera disjuncta	27.2 ± 8.3	1.5 ± 1.7	7.4 ± 3	14 ± 8.2	7.6 ± 4.7	39.3 ± 10.5
Treatment 2	Live bacteria	Heat-killed bacteria	Washed bacteria	Control	Total intersects	Central inoculum
Monhystera sp.	10 ± 4.24	3 ± 2.83	27 ± 2.83	7.5 ± 3.54	8.31 ± 0.18	43.81 ± 2.13
Diplolaimelloides meyli	23.5 ± 0.71	35.5 ± 2.12	20 ± 2.83	5 ± 0	13.06 ± 1.68	3.49 ± 1.78
Diplolaimella dievengatensis	13.5 ± 16.26	0 ± 0	7.5 ± 17.68	4.5 ± 3.54	11.45 ± 8.41	42.14 ± 14.19
Geomonhystera disjuncta	nd	nd	nd	nd	nd	nd
Treatment 3	Washed bacteria	Bacteria 1/100	Bacteria 1/10 000	Control	Total intersects	Central inoculum
Monhystera sp.	37.5 ± 12.02	13 ± 9.9	10.5 ± 2.12	4.5 ± 0.71	3.35 ± 1.28	31.59 ± 7.06
Diplolaimelloides meyli	33 ± 11.31	23 ± 4.24	14.5 ± 4.95	12 ± 5.66	10.28 ± 0.02	5.58 ± 2.51
Diplolaimella dievengatensis	14.5 ± 4.95	15.5 ± 7.78	24.5 ± 7.78	8.5 ± 7.78	7.13 ± 0.65	29.8 ± 1.74
Geomonhystera disjuncta	33.5 ± 13.03	13.7 ± 7.27	9.1 ± 1.94	7.1 ± 2.05	13.6 ± 3.91	23 ± 7.11

summarized in Table 1. In the first treatment, live bacteria in medium attracted significantly higher numbers of *Monhystera* sp., *Diplolaimelloides meyli* and *Geomonhystera disjuncta*, but not of *Diplolaimella dievengatensis*, than did control spots ($p \ll 0.001$). *D. meyli* was the single species not to be repelled by bacterial growth medium and to be attracted to supernatant of bacterial culture. The attractiveness of the supernatant was lost when diluted with an equal volume of ASW, or after heating (1 h at 60°C) or autoclaving (15 min at 1.1 atm and 120°C) (data not shown). In the second treatment, washed bacteria were more attractive to *Monhystera* sp. than unwashed culture aliquots ($p \ll 0.001$), while both attracted similar numbers of *D. meyli*. The latter species, however, significantly preferred heat-killed over live bacteria ($p \ll 0.001$). The response in *D. dievengatensis* was highly heterogeneous among replicates ($p \ll 0.001$), but this species was repelled by heat-killed bacterial cells in both replicates ($p < 0.001$). In the third treatment, the highest cell density attracted significantly more nematodes than did lower densities in all nematodes except *D. dievengatensis*. The response was density dependent over the entire range of observed densities in *D. meyli* ($p \ll 0.001$), while the response of *Monhystera* sp. and *G. disjuncta* to the lower 2 densities did not differ ($p > 0.05$). *D. dievengatensis* preferred the lowest cell density over the 2 higher ones ($p < 0.005$).

Expt 2

Live B1 in medium from 3 d old culture attracted more *Diplolaimelloides meyli* than did bacteria from 2 d old cultures ($p < 0.005$), but differences between 1 and 2 d old or between 1 and 3 d old culture aliquots were not significant (Fig. 2). No differences were

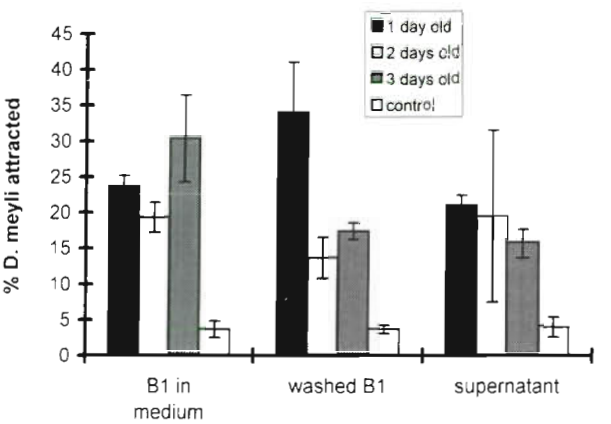

Fig. 2. *Diplolaimelloides meyli*. Effect of bacterial culture age on the attractiveness of culture aliquots, of washed bacteria, and of filtered culture supernatant to the nematode. Means and standard deviations of 3 replicates per treatment are given

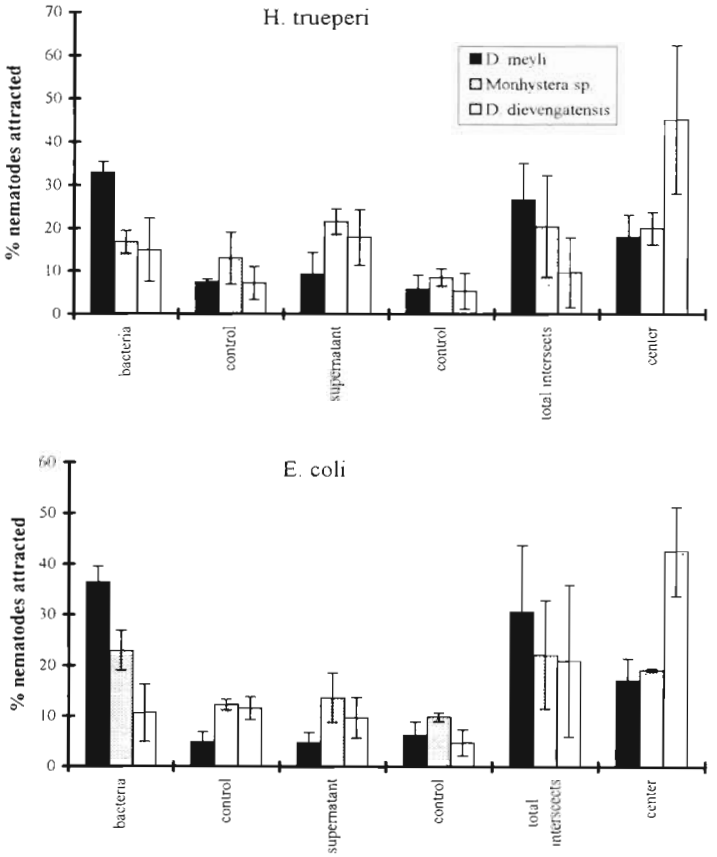

Fig. 3. *Diplolaimelloides meyli, Monhystera* sp. and *Diplolaimella dievengatensis*. Relative recruitment percentages of the 3 monhysterid nematode species to cells and filtered supernatant of cultures of the bacteria *Halobacillus trueperi* and *Escherichia coli*. Means and standard deviations of 4 replicates per treatment are given. See 'Materials and methods' for details on treatments, incubation conditions and bacterial cell densities used

found between the numbers of *D. meyli* reaching supernatant of 1, 2 or 3 d old cultures. However, washed bacteria from 1 d old culture attracted twice as many *D. meyli* than did bacteria from 2 and 3 d old cultures ($p \ll 0.001$) (Fig. 2).

Attraction towards a Gram-positive and a Gram-negative bacterium

Expt 3

Diplolaimelloides meyli moved to both washed *Halobacillus trueperi* and *Escherichia coli*, but not to their respective supernatant fractions (Fig. 3). *D. dievengatensis* showed a small but significant ($p \ll 0.001$) positive response to both cells and supernatant of *H. trueperi* but not of *E. coli*. By contrast, *Monhystera* sp. reacted only to *E. coli* cells.

Expt 4

When simultaneously presented with the 2 species of bacteria, *Diplolaimelloides meyli* was equally attracted to both; *Diplolaimella dievengatensis* significantly ($p \ll 0.001$) preferred *Halobacillus trueperi* over *Escherichia coli*, while *Monhystera* sp. exhibited the opposite preference ($p \ll 0.001$) (Fig. 4). In the former 2 species, there was significant heterogeneity among replicates ($p < 0.005$). Pooled data suggested a preference of *D. meyli* for *H. trueperi* over *E. coli*, but omission of the deviant replicate from the analysis overruled this effect. Replicate heterogeneity did not affect the observed preference of *D. dievengatensis* for *H. trueperi*.

Expt 5

In a mixed inoculum of *Diplolaimella dievengatensis* and *Monhystera* sp. simultaneously offered *Halobacillus trueperi* and *Escherichia coli*, similar numbers of *Monhystera* sp. were found in both bacterial spots. *D. dievengatensis*, on the other hand, was 3 times more abundant in spots of *H. trueperi* than in spots of *E. coli* (Fig. 5). As a consequence, the relative percentages of *D. dievengatensis* and *Monhystera* sp., which in the inoculum were 73 and 27, respectively, decreased to 62.8 for *D. dievengatensis* in *E. coli* spots and to 22.8

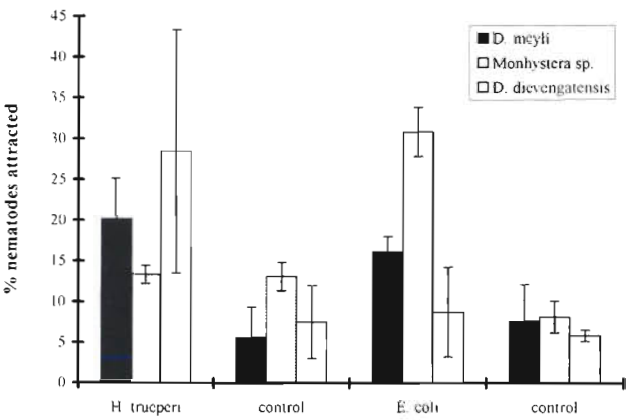

Fig. 4. *Diplolaimelloides meyli, Monhystera* sp. and *Diplolaimella dievengatensis*. Relative recruitment percentages of the 3 monhysterid nematode species to the bacteria *Halobacillus trueperi* and *Escherichia coli* offered simultaneously. Data are means and standard deviations of 4 replicates per treatment. See 'Materials and methods' for details on treatments, incubation conditions and bacterial cell densities used

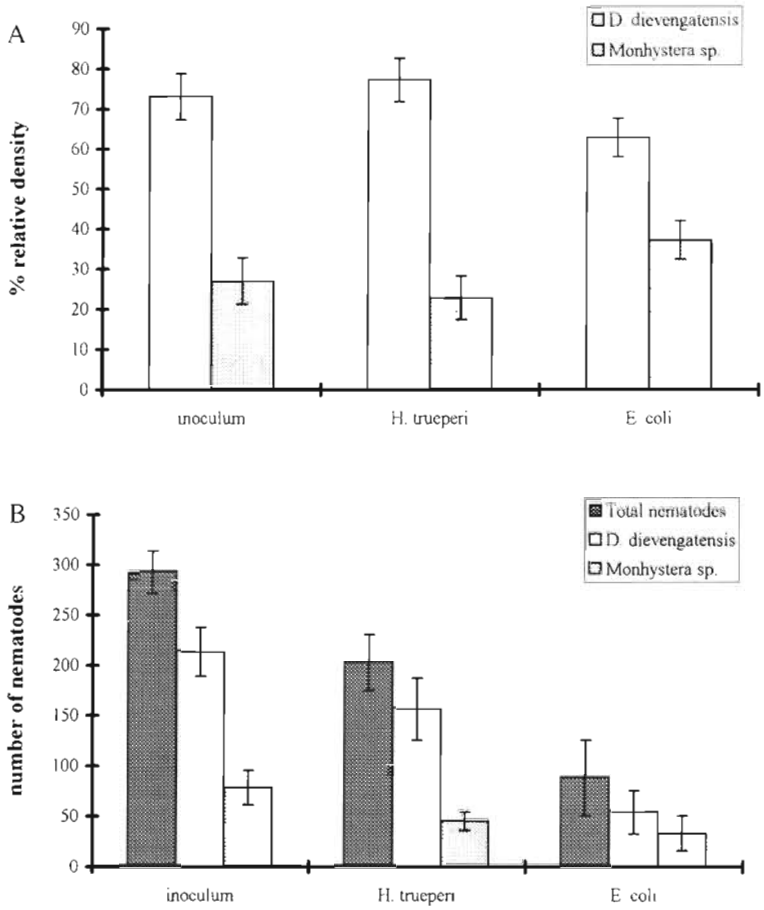

Fig. 5. *Diplolaimella dievengatensis* and *Monhystera* sp. (A) Relative densities and (B) absolute numbers of the nematodes inside spots of the bacteria *Halobacillus trueperi* and *Escherichia coli* in incubations with mixed inocula of both nematodes between 2 bacterial spots at opposite sides. Means and standard deviations of 3 replicates are shown

for *Monhystera* sp. in spots of *H. trueperi*, and increased to 77.2 for *D. dievengatensis* in spots of *H. trueperi* and to 37.2 for *Monhystera* sp. in spots of *E. coli*. The differences between relative nematode abundances in bacterial spots were significant ($p < 0.005$), as were the relative depletion of *D. dievengatensis* and the relative enrichment of *Monhystera* sp. in the *E. coli* spots compared to the inoculum ($p < 0.001$). The percentage increase and decrease of *D. dievengatensis* and *Monhystera* sp., respectively, in the *H. trueperi* spots relative to the inoculum, though occurring in all 3 replicates, was not statistically significant ($p > 0.05$).

Expt 6

In trials with different densities of bacterial cells, *Diplolaimelloides meyli* consistently preferred the highest cell density (10^9 cells ml^{-1}) over lower ones (Fig. 6). The response was, however, not entirely density dependent over the whole range of densities tested, with a fairly density-independent attraction in the intervals of 10^8 to 5×10^8 cells ml^{-1} and 10^3 to 10^7 cells ml^{-1}. A sharp decline at densities below 10^8 cells ml^{-1} was obvious. *Diplolaimella dievengatensis*, however, preferred a cell density of 5×10^8 ml^{-1} over the higher (10^9) and lower ones. The rest of its response was broadly similar to that of *D. meyli*, with a fairly density-independent response in similar intervals, and with a steep decline at the transition from 10^8 to 10^7 cells ml^{-1} (Fig. 6). By contrast, *Monhystera* sp. showed a density-dependent response over the whole interval tested, with peak numbers reaching spots of 10^7 *Escherichia coli* ml^{-1}; higher and lower bacterial densities recruited less *Monhystera* sp. (Fig. 6).

Impact of temperature on the attraction of nematodes to their bacterial food

Expt 7

After 24 h, the respective percentages of nematodes that had moved out of the inoculum spot at 5, 10, 15, 20, and 25°C were approximately 2, 20, 20, 50, and 70% for *Diplolaimelloides meyli* and only 0, 4, 12, 12, and 12% for *Diplolaimella dievengatensis* (data not shown). In the latter species, however, the inocula had not evaporated and many individuals were at the edges of the drops, unable to escape the surface tension. After 48 h, the respective percentages were approximately 5, 65, 75, 90, and more than 95% for *D. meyli*, and less than 1, 5, 10, 40, and 70% for *D. dievengatensis* (Fig. 7). A parallel response was noted for the numbers of *D. meyli* inside the bacterial spots after 24 h; after 48 h, however, half of the nematodes had reached the bacterial spots at all temperatures from 10 to 20°C. At 5 and 25°C, this was less than 5 and more than 75%, respectively. In *D. dievengatensis*, less than 10% of the nematodes had reached the bacterial spots at temperatures up to 15°C, even after 48 h, and the bacterial spots were not significantly more attractive than were the controls. At 20 and 25°C, however, approximately 35 and 60% of the nematodes were inside the bacterial spots after 48 h, while the controls at these temperatures contained equal numbers of nematodes as those at the lower temperatures.

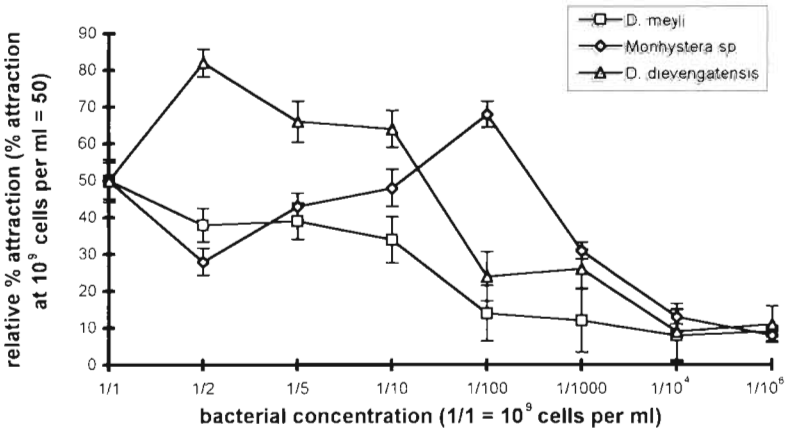

Fig. 6. *Diplolaimelloides meyli*, *Monhystera* sp. and *Diplolaimella dievengatensis*. Influence of cell density on the recruitment of the 3 nematode species to bacterial spots. Recruitment at the highest cell density was normalised to 50%. In reality, it averaged 57, 24, and 24% in *D. meyli*, *Monhystera* sp., and *D. dievengatensis*, respectively. Recruitment percentages at the lower cell densities were extrapolated by comparison of data at a particular cell density to the mean recruitment at the previous density, with densities ranked from highest to lowest. Each data point represents the mean of 3 replicates. Error bars show the variance as a percentage of the mean. Since in all but the highest and lowest cell densities, 2 separate series of 3 replicates each were counted, the highest of both variances is shown

In a first experiment, we show that the bacterial strain B1 is attractive to all 4 monhysterid nematodes tested, but that the conditions under which this bacterium attracts nematodes largely differ. This highly differential response suggests that even 1 single bacterial species might be able to influence a community of the 4 nematodes studied to form patches of at least 3 different relative species compositions, depending on the 'condition' of the bacterial food. The observation that *Diplolaimelloides meyli* responded differently to bacteria sampled from cultures of different age further supports the potential of bacteria in different phases of growth to differentially attract nematodes. Cells from cultures in exponential growth phase are preferred over cells from older cultures by *D. meyli*. Evidence for an impact of bacterial (nutritional) status on the migration of nematodes was also pre-

DISCUSSION

A considerable body of literature exists on the response of mainly plant-parasitic and terrestrial nematodes to a variety of external stimuli, including electrical, mechanical and chemical stimuli and factors such as temperature and light (reviews in Croll 1970a, Dusenbery 1980, Coomans & De Grisse 1981, Huettel 1986, Perry 1996). Analogous information on marine or brackish-water nematodes is, however, scant, and limited to observations on the attractiveness of CO_2 to *Adoncholaimus thalassophygas* (Riemann & Schrage 1988) and the recruitment of different major meiofaunal taxa or different nematode species, belonging to different feeding types, to patches of candidate food or towards sediment impregnated with different species of bacteria or unicellular algae (see 'Introduction' for references). This study demonstrates a highly species-specific marine nematode response to food and shows that one candidate food organism may be, depending on its condition, attractive, unattractive or even repulsive to the same nematode species.

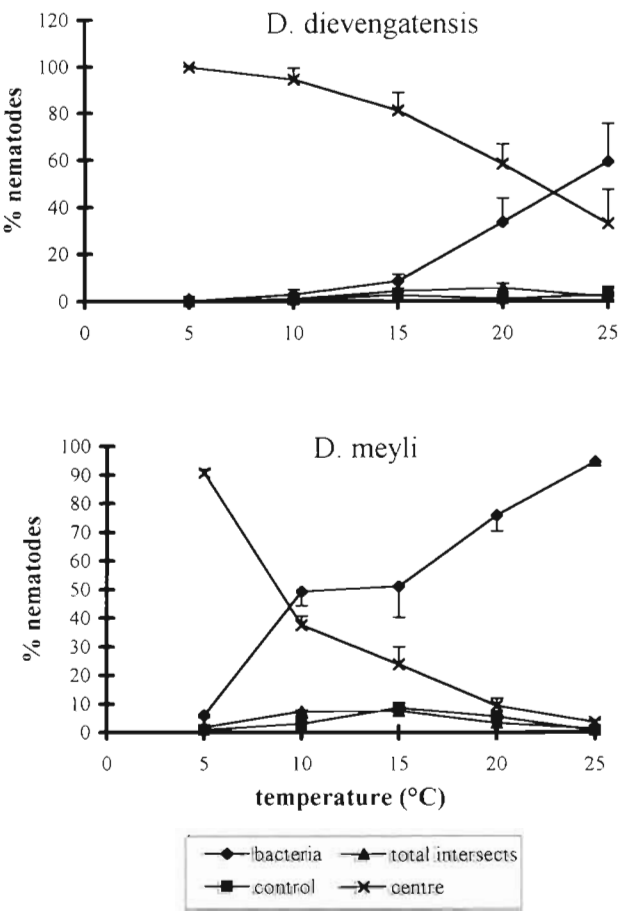

Fig. 7. *Diplolaimella dievengatensis* and *Diplolaimelloides meyli*. Recruitment response of nematodes to inocula of the bacteria *Halobacillus trueperi* at different temperatures and after a 48 h incubation. Data are averages and standard deviations of 3 replicates

sented for the terrestrial *Caenorhabditis elegans* (Grewal & Wright 1992).

In a second step, we focused on the response of nematodes to different species of bacteria. Three different types of response were noted for the 3 Monhysteridae studied: no clear preference (*Diplolaimelloides meyli*), a preference for the Gram-negative bacterial strain over the Gram-positive one (*Monhystera* sp.), and the reverse (*Diplolaimella dievengatensis*). In summary, *Halobacillus trueperi* was attractive to all 3 Monhysteridae, but was the preferred source for only 1; *Escherichia coli* elicited a positive response from only 2 nematode species, and was the preferred source for 1 of these. A differential attractiveness of different bacterial species to terrestrial nematodes (Andrew & Nicholas 1976, Jansson & Nordbring-Hertz 1983, Grewal & Wright 1992), and to free-living stages of the insect-parasitic *Neoaplectana carpocapsae* (Pye & Burman 1981) has been noted previously. *Caenorhabditis elegans* showed either a strong, an intermediate or a weak positive response to different bacteria and was repelled by others (Andrew & Nicholas 1976, Grewal & Wright 1992). However, none of the cited studies tested for the degree of response or preference in multiple choice experiments with 2 or more strains offered simultaneously, nor were bacteria standardized to cell densities. This study therefore eliminates the possibility that the observed preferences might be due to a density-dependent response. However, there still remains a chance that the choice of a non-specific growth medium for the 2 bacterial strains could have affected the (nutritional) quality of the bacterial populations in our experiments, and as such influenced their attractiveness to nematodes.

Surprisingly, in tests with a mixed nematode inoculum consisting of *Diplolaimella dievengatensis* and *Monhystera* sp., the latter species did not exhibit the same preference for *Escherichia coli* over *Halobacillus trueperi* that was observed in mono-specific nematode inocula. *D. dievengatensis*, on the other hand, did show the same pattern of response. We repeated this experiment with observations of the nematodes' migration after shorter incubations, and found that *D. dievengatensis* responded more rapidly to its preferred source (i.e. *H. trueperi*), while *Monhystera* sp. started migrating somewhat later. Initially, this migration was mainly directed versus the *E. coli* inoculum, but many individuals reversed before reaching this spot and started migrating in the opposite direction. We suggest that the tracks of *D. dievengatensis* in some way influenced *Monhystera* sp., setting out a pattern that directed a larger-than-expected fraction of this species' inoculum to the *H. trueperi* spot. Inspite of this, the hypothesis that different bacterial spots would be colonised by differently composed nematode assemblages was corroborated by our results: *D. dievengatensis* increased its dominance over *Monhystera* sp. in the *H. trueperi* spots, but decreased relative to *Monhystera* sp. in *E. coli* spots. In addition, the total numbers of nematodes reaching food spots heavily depended on the nature of the bacteria, with *H. trueperi* invariably attracting far higher nematode numbers than *E. coli*. While previous studies have already reported on the differential potency of a number of bacterial strains to attract *Caenorhabditis elegans* (see above), the present results demonstrate that 1 bacterial strain can at the same time elicit a positive response from one nematode, but not from another, closely related species. The observed preferences are unlikely to relate to preferences for either Gram-positive or Gram-negative bacteria, since, for example *D. dievengatensis*, the species with the most pronounced preference for *H. trueperi*, has successfully been cultivated on diets of Gram-negative bacteria (Vranken et al. 1984).

Gray (1968) found no significant influence of bacterial density on the attractiveness of bacteria to the harpacticoid *Leptastacus constrictus*, while Gray & Johnson (1970) did note a significant correlation between the number of attractive bacteria and the response of the gastrotrich *Turbanella hyalina*. The differential density-dependent response of the 3 nematodes used in our experiments to bacteria offers an attractive basis for explaining observed microhabitat preferences in terms of a succession of species on detritus in different stages of decay. As such, *Diplolaimelloides meyli* could somewhat presumptuously be considered as the species that may most readily respond to the early breakdown of plant litter, at a stage where concentrations of highly labile organic carbon and correspondingly high densities of bacteria are available. *Diplolaimella dievengatensis* and *Monhystera* sp. might then be envisaged as preferentially associated with later stages of leaf litter decay, i.e. with generally more refractory material and lower overall bacterial densities. Although our results provide no direct evidence for this relation, it is noteworthy that *in situ*, *D. meyli* is most abundant on decaying leaves still attached to the stems of *Spartina anglica* and other macrophytes, whereas *D. dievengatensis* and *Monhystera* sp. are more typical of the sediment around the roots of macrophytes, where they could be associated with the burial of plant litter. *Geomonhystera disjuncta* takes a position that is more similar to that of *D. meyli*, but prevails at lower temperatures (Moens unpubl.). It is also interesting to note that in monoxenic cultures of *G. disjuncta* and *D. dievengatensis* on the bacterial strain *Alteromonas haloplanktis* ISC_2, the former nematode needed high food levels, whereas the latter (erroneously referred to as *Monhystera microphthalma*

in the original article) thrived on cell densities as low as 10^6 to 10^7 bacteria ml^{-1} (Vranken et al. 1984). Furthermore, the density-dependent response also indicates that the mere presence of a bacterial cue may be insufficient to trigger a nematode response: not only may the concentration of the stimulant be too low, it may also be too high.

It is by no means surprising to find that both the overall motility and the taxis of nematodes towards bacteria are strongly dependent on temperature. In general, the activity pattern of both species studied agrees well with patterns of temperature dependence as established from life cycle studies (Vranken 1985, for *Diplolaimella dievengatensis*; Moens unpubl. for *Diplolaimelloides meyli*) and from measurements of respiration (Moens unpubl.). All these data point at temperature optima in between 20 and 30°C for both species, with *D. dievengatensis* perhaps preferring slightly more elevated temperatures than *D. meyli*. Whereas both nematodes appear to have a similar temperature optimum, their activity at lower temperatures (up to 15°C) as revealed by their migration away from the inoculum spot differs, with *D. meyli* remaining more motile than *D. dievengatensis* at 10 to 15°C. However, since similar numbers of nematodes were recovered from control spots and intersects at all temperatures after a 48 h incubation, the distinctly higher numbers and proportions of nematodes inside bacterial spots at the higher temperatures suggest that the efficacy with which both nematodes respond to the bacterial spots is also temperature dependent. This can be explained either by a better perception by the nematodes of the bacterial stimulus, or alternatively by an increased stimulus production by the bacteria in the plates.

This paper demonstrates that free-living marine nematodes migrate in a directed way towards patches of food. In the absence of an attractive source, nematodes showed a random movement on and in the agar. Deviations from this 'random walk' behaviour consisted of clustering in groups of several tens of individuals in the inoculum spot or migration of adult males to female J4 and adults. Clustering of nematodes in aqueous suspensions has been noted previously and has been ascribed mainly to mechanistic interactions among individuals in dense suspensions (Doncaster & Webster 1968, Croll 1970b); in our experiments, *Diplolaimella dievengatensis* showed the strongest tendency to aggregate, probably because the *D. dievengatensis* inocula contained on average the highest nematode densities. Consequently, the overall response in *D. dievengatensis* was less than in the other nematodes studied.

There is a general consensus, based on morphological and experimental evidence, that chemotactic factors emanating from prey or host organisms govern the primary food-finding mechanisms in nematodes (Croll & Sukhdeo 1981, Zuckerman & Jansson 1984); both 'taste' (most studies) and 'smell' (Bargmann et al. 1993) are involved in this chemotaxis. From the present observations on the nematodes' migration up bacterial cue gradients, it is likely that the recruitment in our experiments also resulted from a chemotactic response, and, since the time allowed for the establishment of gradients emanating from the attractive spots was relatively short (sometimes less than 1 h) and the nematodes' response often instantaneous, both soluble and volatile substances may have been involved. Nevertheless, the nature of the stimulus or stimuli that guide bacteriophagous nematodes to their food is hitherto unknown. The only bacteria-associated semiochemical of which the involvement in nematode chemotaxis has repeatedly been demonstrated is CO_2 (Klingler 1965, Edmunds & Mai 1967, Croll 1970a, Dusenbery 1974, Pline & Dusenbery 1987, Riemann & Schrage 1988). The attraction of free-living nematodes to a variety of inorganic ions (Ward 1973, Dusenbery 1974, 1976a), cyclic nucleotides (Ward 1973), and other organic compounds (Dusenbery 1975, 1976b) has been documented. A remarkable specificity of the response has been noted, e.g. D-tryptophan repelled *Caenorhabditis elegans* (Dusenbery 1975) and electrophysiologically stimulated the parasitic *Syngamus trachea* (Riga et al. 1995), while L-tryptophan elicited no response from *C. elegans* (Dusenbery 1975).

Although observations of the nematodes' behaviour in our experiments are highly suggestive of a true taxis response, the possibility of a random food-finding strategy where nematodes stay inside a suitable food spot after a chance encounter cannot be entirely excluded on the basis of this type of experiment alone. In a heterogeneous environment such as the benthos, a random food-finding strategy would be disadvantageous, particularly in organisms with an overall low vagility (White 1978). Even in organically enriched sediments of the Westerschelde Estuary, on average less than 3% of the sediment consists of organic matter, of which only part is a potential food source for the meiofauna. The difficulties in monoxenically rearing marine bacteriophagous or herbivorous species (see Moens & Vincx 1998 for a review) are evidence of highly specific nutritional requirements, which are met by only a few food organisms, and the efficient finding and recognition of these suitable foods can therefore be considered vital to the nematodes' reproductive success.

The assay method used in our experiments is a rapid and suitable method for primary assessment of the response of many marine and brackish-water nematodes, be they cultivated or extracted from sediment

samples, to a variety of candidate food sources. It is, however, much less applicable when aiming at the identification of particular single stimuli involved in the nematode response. For that purpose, individual nematodes need to be studied in a proper gradient of the stimulus. Candidate stimuli can preferentially be administered to the center of a petri dish containing a homogeneous agar or sephadex layer, and after appropriate time has been allowed for the establishment of a radial gradient, single nematodes can be inoculated to the edges of the dish and their response noted (Ward 1973, Riga & Webster 1992). Additional information on the nematodes' response can be obtained from detailed observations of their migration, e.g. by photographing nematode tracks (Riddle & Bird 1985, Riga & Webster 1992) or by video-monitoring of their movement (Dusenbery 1983, 1992, Pline & Dusenbery 1987, Anderson et al. 1997a). Totally different approaches towards the study of nematode responses to chemicals have been countercurrent separation (Dusenbery 1973, 1974) and electrophysiological stimulation of tethered worms (Riga et al. 1995, Perry 1996).

The present results reconcile the seemingly controversial observations of a high selectivity (Tietjen et al. 1970, Tietjen & Lee 1973, 1977) and a mainly mechanistic and rather unselective food ingestion (Moens & Vincx 1997) in the feeding of marine nematodes. We suggest that many nematode species select spots where suitable food is abundant from a distance, but may feed rather non-selectively therein. Their response to food is thus highly selective, but their ingestion may be less so.

Chemotaxis is considered an important factor underlying the patchiness of benthic harpacticoid copepods, though passive dispersal through hydrodynamic forces may be of equal importance (Fleeger et al. 1995, and references therein). The nematodes studied here are typical 'Aufwuchs' species, and probably subject to considerable hydrodynamic disturbance, as most of their habitat is inundated at high tide. In a similar habitat, the densities of *Diplolaimelloides bruciei* were significantly reduced by flooding (Alkemade et al. 1994). The capacity to efficiently find suitable feeding spots may therefore be vital to nematodes living in these dynamic environments. It is as yet unclear at what distances food spots can be recognized and how the nematodes move towards them. Jensen (1981) demonstrated that, next to movement on or through a substrate, some nematodes may show a chemotactically driven swimming behaviour.

Food is clearly not the only factor underlying the patchiness of benthic and Aufwuchs environments. Nematodes have been shown to respond with almost incredible accuracy to temperature gradients (Hedgecock & Russell 1975, Thomas 1995) and to use temperature patterns for their positioning in soils (Dusenbery 1988, 1989, 1996, Robinson 1994). Plant parasites may orientate along redox-induced gradients (Bird 1959) or electric fields (Robertson & Forrest 1989) around plant roots. They may respond to subtle changes in pH (Ward 1973), and structural heterogeneity, which is omnipresent in a benthic environment, has recently been shown to interact with chemotaxis to give complex patterns of attraction to bacterial cues (Anderson et al. 1997a, b). There can be little doubt that taxis is not restricted to nematode-bacteria interactions, but equally mediates the response of nematodes with other feeding strategies. Attraction of nematodes to exudates from yeasts and fungi (Balanovà et al. 1979) and repulsion from slime molds (Kessin et al. 1996) have been demonstrated. The principle illustrated here may therefore generally govern nematode-food interrelations in the benthic environment, and as such deserves further study.

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