Fundam. appl. Nematol., 1997, **20** (2), 157-163

Nematode movement along a chemical gradient in a structurally heterogeneous environment. 1. Experiment

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Accepted for publication 5 February 1996.

Summary – The interaction between soil structural heterogeneity and chemical gradients, and their effect on the movement of free-living nematodes was investigated. Four experimental treatments were used. These consisted of a nematode *(Caenorhabditis elegans)* on a homogeneous layer of nutrient agar in a Petri dish, with or without a localised bacterial food source *(Escherichia coli)* acting as an attractant. Structural heterogeneity was then introduced by adding a monolayer of sand grains onto both of the homogeneous treatments. All trails were recorded using time-lapse video, and subsequently digitised prior to analysis. Turning angle distributions and the fractal dimension of the trails were calculated for each treatment. There was a statistically significant effect ($P \leq 0.01$) of all treatments on the movement of the nematode. In the presence of the attractant, nematode movement was more linear and directed towards the bacterial source. Structural heterogeneity caused the nematode to have more linear movement compared to a homogeneous environment. The fractal dimension of the nematode trails was significantly higher ($P \leq 0.01$) for the treatment without structure or bacteria, than for the other treatments. The results, for the first time, quantify the degree to which nematodes carry out random foraging type behaviour in a homogeneous environment and produce more directed non-random movement in the presence of attractant. Finally, when structure is present the foraging strategy becomes more of an avoidance strategy, allowing the nematode to escape structural traps, such as " dead-end " pores, and then continue to react to attractant gradients.

Résumé – ***Déplacement des nématodes suivant un gradient chimique dans un milieu à structure hétérogène. 1. L'expérimentation*** – L'interaction entre l'hétérogénéité structurale et les gradients chimiques, ainsi que leur influence sur le déplacement des nématodes, ont été étudiées. Trois dispositifs expérimentaux ont été utilisés qui comprennent un nématode *(Caenorhabditis elegans)* placé sur une couche homogène de milieu nutritif gélosé dans une boîte de Petri avec ou sans présence d'une source bactérienne de nourriture *(Escherichia coli)* utilisée comme attractif. L'hétérogénéité structurale est réalisée en ajoutant des grains de sable en une seule épaisseur dans chacun des traitements homologues. Toutes les traces ont été relevées à l'aide d'un dispositif de vidéo à séquences temporelles et les données digitalisées avant analyse. Les répartitions des angles de changement de direction et les dimensions fractales des traces sont calculées pour chaque traitement. Il se révèle un effet statistiquement significatif ($P \leq 0.01$) de tous les traitements sur le déplacement des nématodes. En présence d'un produit attractif, le déplacement du nématode est plus linéaire et dirigé vers la source bactérienne. L'hétérogénéité structurale provoque un déplacement plus linéaire que dans le cas d'un milieu homogène. La dimension fractale des traces du nématode est significativement ($P \leq 0.01$) plus élevée pour les traitements sans sable ni bactéries que pour les autres traitements. Ces résultats permettent, pour la première fois, de quantifier le degré auquel les nématodes *i)* utilisent un comportement de recherche de nourriture au hasard dans un milieu homogène et *ii)* adoptent un déplacement mieux orienté en présence d'un produit attractif. Finalement, lorsqu'une hétérogénéité est présente, la stratégie de recherche de nourriture devient plutôt une stratégie d'évitement permettant au nématode d'échapper aux « pièges » structuraux, tels les pores en cul-de-sac, et de pouvoir ainsi continuer à réagir à l'attraction.

Key-words : chemical gradient, foraging strategy, fractal dimension, nematode movement, reversals, turning angle distribution.

One of the major factors governing the movement of nematodes in soil is the physical arrangement of mineral and organic particles (Griffiths *et al.*, 1995). The spatial and temporal arrangement of such particles acts to control the distribution of water and gases in the soil (Crawford *et al.*, 1993). However, the effect of the soil structural environment on microbial processes is not adequately understood. At present, the best knowledge we have relates to how pore (or aggregate) sizes, with associated moisture regimes, affect the movement of protozoa, and nematodes (Wallace, 1968; Croll & Sukhdeo, 1981; Young *et al.*, 1994). How the actual physical framework of the soil, and the heterogeneity of that framework, impacts on soil-microbe interactions remains largely an unknown.

The movement of nematodes is influenced significantly by chemical gradients spreading through the soil (Ward, 1978; Dusenbery, 1985*b*, 1987), from decomposing organic matter and plant roots. It is important therefore to take account of how structural heterogenei-

ty affects the movement of nematodes and the distribution of chemical gradients, and the resultant interaction. No-one has attempted to do this in any quantitative assessment, primarily because of the difficulties in observing processes in soil at the micro-organism scale, and the lack of any experimental systems which are able to simulate such interactions.

It is the intention of this paper to quantify the effect of the interaction between nematode movement along a chemical gradient and a structurally heterogeneous environment. We examine the role of heterogeneous structures in affecting nematode movement under various experimental treatments.

Materials and methods

Organisms used

The movement of *Caenorhabditis elegans* was examined in response to the presence of a population of *Escherichia coli*. *C. elegans* nematode populations were maintained on 3 % (w/v) nutrient agar (NA, Oxoid Ltd.). Stock cultures of *E. coli* were maintained on LB agar (Oxoid Ltd.).

Preliminary observations of the nematodes showed that they were at their most mobile 2 to 3 days after hatching (third stage juvenile); therefore all nematodes used in this study were of that age. Typical body dimensions were 1 mm in length and 30 μm in width.

Experimental treatments

To analyse the effects of structural heterogeneity on nematode movement, four experimental treatments were selected. The experimental unit was a single nematode placed right of centre on a homogeneous layer of nutrient agar in a 9 cm Petri dish. The different treatments consisted of experimental units with or without a bacterial food source *E. coli*, acting as the attractant (placed left of centre), and with or without the addition of structural heterogeneity in the form of sand grains.

A culture of *E. coli* was grown overnight in LB broth at 37 °C, and used to inoculate an experimental Petri dish of NA. A 1 cm-long streak of *E. coli* was approximately 4.5 cm distant from where the nematode would be placed and allowed to grow overnight at 22 °C. Structural heterogeneity was then introduced by adding a monolayer of acid washed sand grains (diam. 112-250 mm), into each of the homogeneous treatments. This was done by soaking a piece of sterile velvet with sterile distilled water, pressing it firmly onto a single layer of sand grains and then transferring it to the Petri dish in a similar manner. This partially embedded the sand grains into the agar, and produced plates with approximately 40 % pore space between the sand grains at the agar surface (see Fig. 1, for example). A single nematode was picked from a culture plate, washed in sterile 0.5 % saline solution for approximately 1 min and placed on the Petri dish on the opposite side from the bacteria. A lid, with a thin layer of agar poured on the inside, was then placed on the Petri dish. The thin layer of agar prevented droplet formation due to condensation from building up inside the covered Petri dish which would hamper clear observation during the time-lapse photography.

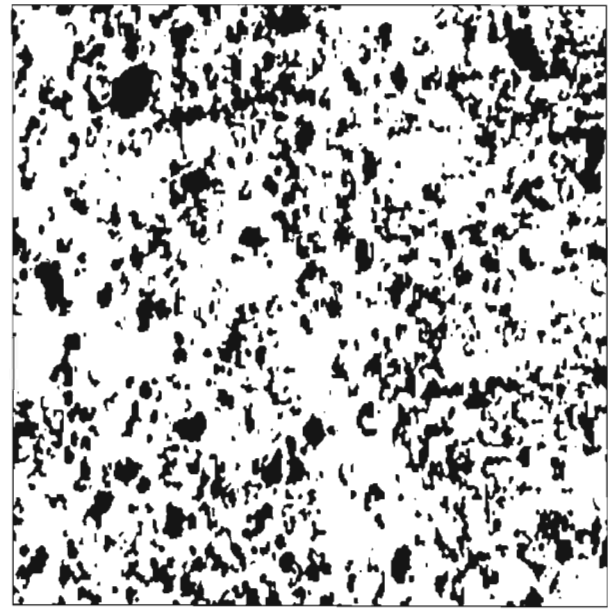

Fig. 1. *Example of a digitised structure from one of the experimental treatments. Black represents sand grains, white pore space.*

Therefore, four experimental treatments were investigated : *i)* a nematode on homogeneous agar (" homogeneous nematode "); *ii)* a nematode on homogeneous agar with bacteria (" homogeneous with bacteria "); *iii)* a nematode on agar with a layer of sand grains (" heterogeneous nematode ") and *iv)* a nematode in the presence of sand grains and bacteria (" heterogeneous with bacteria ").

Image capture

A time-lapse video system (Alphey *et al.*, 1988) was used to record the movement of a single nematode moving in the previously described conditions. Darkfield illumination (Dusenbery, 1985*a*) was used to improve contrast between the nematode and the background. Twenty replicate Petri dishes were used for each treatment. Video images were analysed, using in-house digitising software, to obtain the final digitised nematode trails. The trails were discretised in the digitising process by identifying points along the nematodes path separated by typically 1 s of real time.

Turning angle distributions

Trails were rediscretised, to remove any bias in the initial discretisation caused by uneven spacing between digitised points, by taking the original sequence of

points and generating a new sequence with a constant step length (R) between them (Fig. 2). Bias was thus removed by discretising all the trails from each treatment with the same step length R.

The choice of R depended upon the trail being rediscretised, however, in order to combine trails from the same treatment, to generate the turning angle distribution, the same R must be used for all trails within any given treatment. The criterion upon which R was chosen was that the smallest rediscretisation step will have the most information. This was taken to be equal to half of the largest distance between successive points in the original trail discretisation (Bovet & Benhamou, 1988). Therefore, the closer the points were originally digitised the smaller the choice of R in the rediscretisation.

Turning angle distributions were then generated by calculating the angle between successive line segments (length R) on the trail (Bovet & Benhamou, 1988) as shown in Fig. 2B. The angular range chosen was $-\pi$ radians to π radians, and all angles were computed with respect to the previous angle. Therefore the resulting turning angle distributions are for changes in angles.

The Kolmogorov-Smirnoff test (Hollander & Wolfe, 1973) was used to test whether any two sets of results could be described by the same distribution. This was done by calculating the cumulative turning angle distributions for all four treatments, then subtracting specific pairs of distributions for comparison. Thus, any significant differences between the turning angle distributions from all of the experimental treatments would be detected.

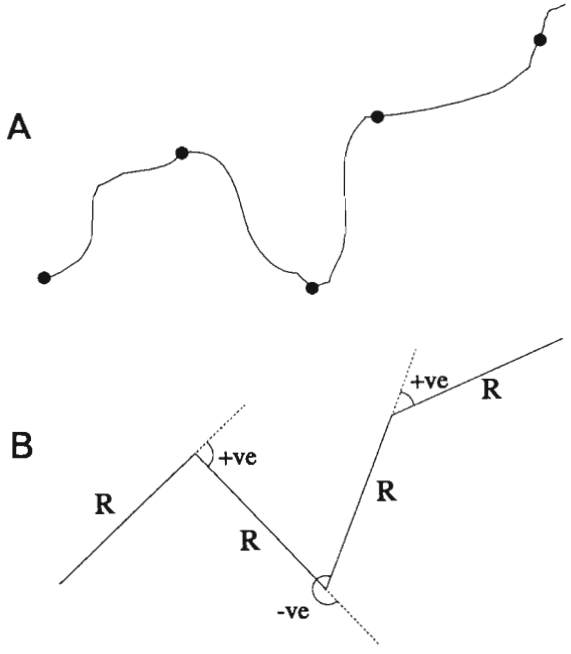

Fig. 2. *Methodology for quantifying the turning angle distribution of nematode trails. (See text for details).*

FRACTAL DIMENSIONS

To examine further the digitised nematode trails, the fractal dimension of nematode trails from each treatment was calculated. To ensure that the dimensions computed were representative, each trail had to contain at least 150 points. By this criteria five trails, from each treatment, were rejected. The method was similar to that used by Dicke and Burrough (1988), who examined the tortuosity of animal trails, commonly known as Richardson's plot (Mandelbrot, 1977).

The fractal dimension is essentially a statistical technique which measures the space-filling efficiency of objects (Young & Crawford, 1992), and the method for measuring it is described briefly below.

The number of " rulers ", *N(r)*, of size *r*, that it takes to cover each nematode trail is calculated for a range of *r*'s. If the trail is a statistically self-similar fractal, then a *log-log* plot of *r vs N(r)* will produce a gradient which is the fractal dimension *D*. The lowest dimension we would expect is 1 (the dimension of straight line) which is indicative of a relatively linear trail. Where *D* tends to 2, the trail will be more rugged, covering a large area in a short distance, although, in our case it is unlikely we would get close to 2 as this implies the nematode has visited every point on the Petri dish. Hence the fractal dimension is a quantitative measure of the roughness of the nematode trails. *D* tending to 1 would be indicative of biased, directed movement.

To compare the fractal dimensions, the Mann-Whitney test (Hollander & Wolfe, 1973) was used, at the 99 % level of confidence. This test allows the median of two sets of independent data to be compared for significant difference.

Results

GENERAL OBSERVATIONS

Typical examples of nematode trails produced in each of the four different treatments are presented in Fig. 3A-D. The nematodes were observed to be carrying out two distinct types of behaviour, *i)* looping/spiralling, as seen in Fig. 3A, and *ii)* reversals.

Typically the trails through structure take longer to reach the bacterial source, due to the restrictive pore network. How much longer depended on the density of the structure. For the structures used here it was found that the nematodes took approximately twice as long.

TURNING ANGLE DISTRIBUTIONS

Fig. 4 shows four pairs of turning angle distributions generated from the experimental treatments, all four paired distributions proved to be significantly different ($P \leq 0.01$). The tests proved that the distributions being compared were significantly different when taken as whole distributions. To obtain a clearer picture of the

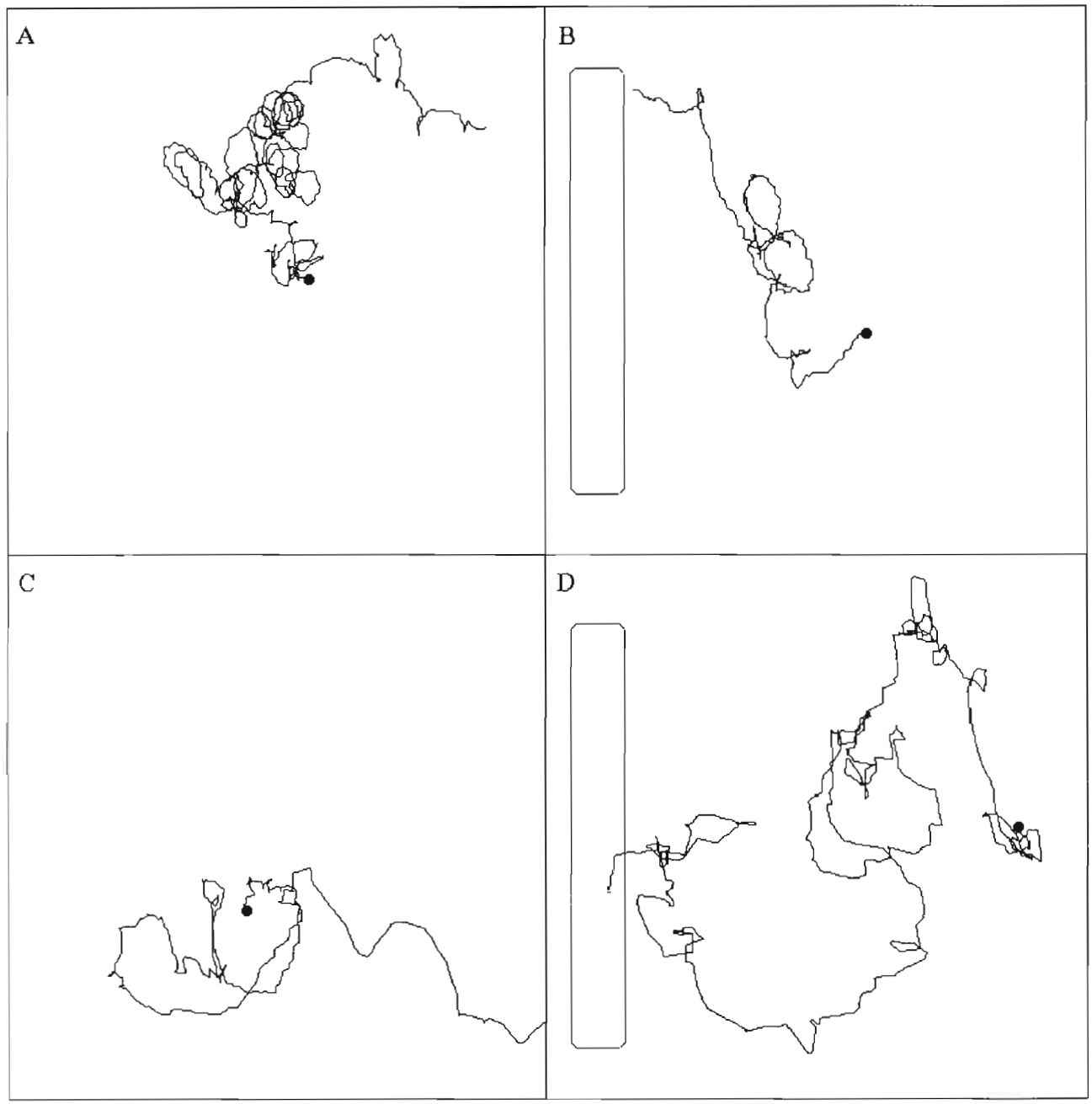

Fig. 3. *Digitised nematode trails. A : Homogeneous (nutrient agar with no bacterial source); B : Homogeneous with bacteria shown approximately on the left; C : Heterogeneous (nutrient agar with a monolayer of sand grains [not shown]); D : Heterogeneous with bacteria, shown approximately on the left, (● denotes starting position).*

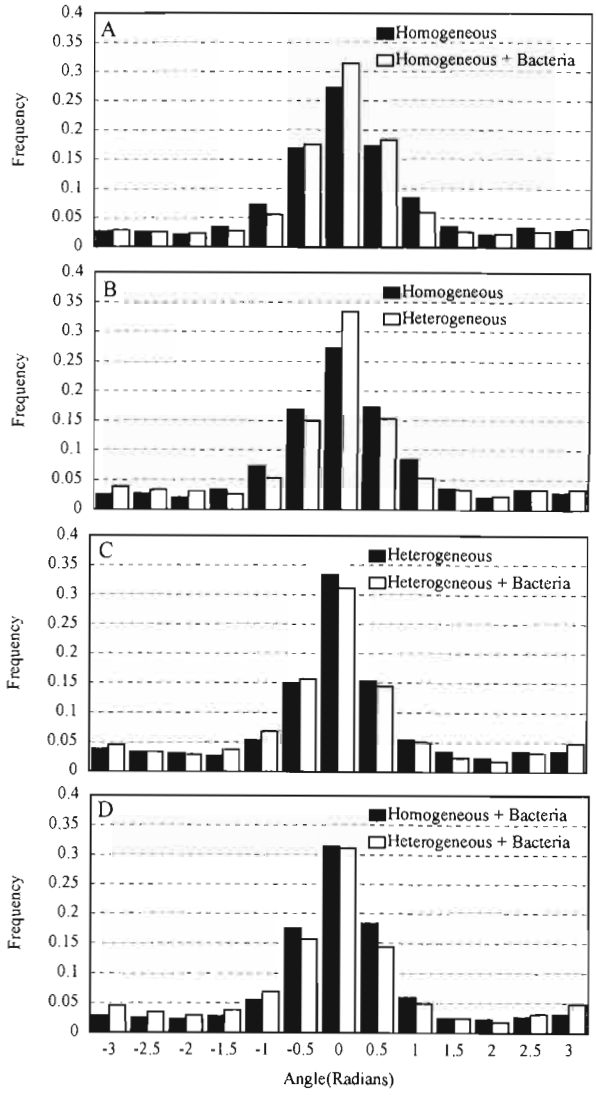

Fig. 4. *Turning angle distribution comparisons. All frequency distribution comparisons shown are statistically significant ($P \leq 0.05$) different from one another.*

meaning of the differences in the distributions we also compare individual angles belonging to each distribution.

Fig. 4A shows the comparison between the homogeneous nematode distribution with/without a bacterial source. There is more directed movement for a nematode with a bacterial source, as indicated by the sharper distribution when compared with the non-bacterial distribution, *i.e.,* the zero radians peak is 15 % larger for the bacterial distribution. The nematode with a bacterial source will move in the same direction more often because the attractant gradient emanating from the bacteria will cause a more biased and directed movement.

The impact of heterogeneity on the nematode without bacteria is seen by the comparison given in Fig. 4B between the homogeneous and heterogeneous distributions. The homogeneous case leads to more changes in direction in the smaller angles, 10 % more than in the heterogeneous case, whereas the heterogeneous case has an increased tendency for larger changes in direction (for the negative angles) and more moves without changing direction.

Fig. 4C shows the comparison between the heterogeneous nematode distributions with/without a bacterial source. The heterogeneity has masked the effect of the attractant gradient, although the turning angle distributions are statistically different ($P \leq 0.01$). Both structural heterogeneity and chemical gradients give a similar distribution favouring linear movements. Yet, the direction of the movement is always biased towards the bacterial source, presumably as a consequence of the presence of a chemical gradient, and this is not the case for structural heterogeneity on its own.

The final comparison between heterogeneous and homogeneous nematode distributions with bacterial source (Fig. 4D) shows that they both have the same frequency of moving without changing direction, although, in the homogeneous case, the nematode changes direction by a small angle 10 % more often than in the heterogeneous case. The latter distribution is skewed to the left, (*i.e.,* the frequency of most of the positive angles is consistently lower than that of its negative), implying the nematode will change direction by a negative angle more often. One point to remember is that since the trails were always recorded with the nematode to the right of the bacteria, then the nematode will move left more often than moving right, *i.e.,* towards the bacteria. As a result of this it will carry out a reversal towards the right more than the left – this will typically generate a negative angle.

Fractal dimension

The nematode trails from the homogeneous environment, without a bacterial source, have the greatest fractal dimension (Table 1), as a consequence of the forag-

Table 1. *Fractal dimension of nematode trails ± standard errors (If the errors are followed by a different letter then the fractal dimensions are significantly different [$P \leq 0.01$]).*

Trail type	Dimension ± S.E.
Homogeneous	1.219 ± 0.024 *a*
Homogeneous + Bacteria	1.079 ± 0.025 *b*
Heterogeneous	1.083 ± 0.022 *b*
Heterogeneous + Bacteria	1.079 ± 0.014 *b*

ing behaviour (looping/spiralling), which causes the trail to be more dense and localised (*i.e.,* more space filling). The bacterial attractant has a significant effect on the fractal dimension of the nematode trail as does the heterogeneous structure when taken separately. When combined, the structure masks the effect of the attractant gradient.

Discussion

It is not the intention of this work to examine the nature of the attractant gradient emanating from the *E. coli*. We do recognise that cases exist for the attractant to either be in a gaseous or water-soluble form. In our experimental system the mono-layer of sand would have interacted with either attractant : either restricting the movement of a gas over the surface of the agar; or by restricting the movement of a water-soluble compound within the agar. However, it is worth noting that the speed of the response of nematodes to attractant depends largely on the diffusivity of any attractant. A gas through air would diffuse at a rate that is at least 10^4 times faster than a water-soluble compound, or a gas through liquid (Marshall & Holmes, 1979). Whilst a dramatic increase in the transport rate of a water-soluble compound may occur with significant conductance, in our experimental set-up this would not have been the case. The uniqueness of our work lies not in empirical or semi-quantitative observations which marks other work on behavioural patterns of nematodes, but in a more rigorous quantitative analysis of nematode trails.

Croll (1970) has stated that the looping track followed by nematodes may be thought of as a type of foraging behaviour, whereas the reversals, which involve repeated forward and backward movements over the same area, are a means of changing direction. From Fig. 3B, and the comparable fractal dimensions, it is evident that there is a change from a foraging type behaviour (as in Fig. 3A) to a more directed movement, most probably due to the influence of a chemical gradient, diffusing from the bacterial source. This is in agreement with other workers (Grewal & Wright, 1985; Dusenbery, 1986*a*; Bargmann *et al.,* 1993). A semi-empirical approach of nematode movement has been adopted by Croll and Blair (1973), in an attempt to simulate the movement of nematodes. Obtaining parameters for larval stage, activation of larvae, initial direction of track, and form of track, a stochastic model was developed that was able to generate nematode trails based on a random selection of the distribution parameters. The simulated trails are reasonably similar to the observed trails, and do provide a useful observational tool. However, the semi-empirical nature of the model does not lend itself to any functional link with soil nor attractant parameters.

Where physical structure, in the form of sand grains, was present the foraging behaviour was not as apparent (Fig. 3C). However, there may be another type of behaviour which could be termed, " hugging ", where the nematode appears to loop or curl round individual sand grains many times. The hugging is difficult to see in these static representations of the nematodes movements. This behaviour may purely be an artefact of the classical sinusoidal wave movement, usually associated with nematode movement (Croll & Sukhdeo, 1981), being restricted by the contours of the structure. Alternatively, it may be due to the nematode being held by the surface tension of the fluid layer around the sand grain.

The movement of nematodes between sand grains led to more linear movements, even without the presence of an attractant. The reason for this maybe due to the restrictive pore network minimising the looping behaviour of the nematode. However, if a blocked pore is encountered then a rapid withdrawal is followed by a large change in direction. This may also be interpreted in terms of foraging behaviour. Whilst moving through structure the nematode cannot forage in its usual manner instead it moves in a more random fashion guided by the structure. The nematode in a homogeneous environment will have a small change in direction more often than in the heterogeneous environment because it is free to forage (*i.e.,* loop) which is a succession of small changes in angle.

Experimental and analytical techniques have been directed to examine the effects of structural heterogeneity on nematode movement with and without the presence of bacteria. The results have shown that nematodes in a homogeneous environment (*i.e.,* agar plate) carry out random foraging type behaviour which involves looping around many imaginary origins. This behaviour changes in the presence of an attractant gradient to a more directed linear, non-random, movement. The inclusion of structural heterogeneity, in the form of a mono-layer of sand grains, modifies significantly the foraging behaviour, and the nematode follows a trail which is dictated by the structure, thus taking longer to reach the bacterial source. However, by modifying the foraging strategy, the nematode effectively moves in a biased random manner restricted by structure, *i.e.,* moving much shorter distances than without structure, with a random element aiding the nematode in finding a path through the structure. Therefore, the foraging strategy changes to an " avoidance " strategy in a structured environment which aids the nematode in escaping structural " traps ". The persistence of a random element in the nematodes movement, in all of the experimental treatments observed, is clear. This persistence is advantageous to a nematode which is faced with structural (or indeed chemical/biological) traps, whereby the nematode is able to switch from a biased foraging strategy to a semi-biased strategy. This allows a greater degree of freedom for escape, but still permits the nematode to identify the advantageous chemical gradients towards food sources. In such complex heterogeneous structures

as soil, such a mix of strategies is vital to the nematode survival and population spread. In soils, structural heterogeneity may also affect the diffusion pathways of the attractant. This, together with relevant interactions, is dealt with in the next paper (Anderson *et al.*, 1997).

Acknowledgements

The authors would like to thank Ms Alexandra Holmes for technical assistance, and Dr Christine Hackett (Biomathematics & Statistics Scotland) and Dr Dick Brown (Dept. Mathematics & Computing) for statistical advice. We are also grateful to the referees for their constructive comments. The Scottish Crop Research Institute is funded by The Scottish Office Agriculture, Environment & Fisheries Department.

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