Spatial structuring and frequency distribution of the nematode *Steinernema feltiae* Filipjev

D. A. BOHAN*

IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF, UK

(*Received 19 November 1999; revised 10 April 2000; accepted 13 April 2000*)

SUMMARY

The frequency distribution of first generation, *Steinernema feltiae* Filipjev parasitic stages was over-dispersed with the majority of hosts containing few or no parasitic stages, whilst a few hosts contained a great many. Because of high extraction efficiency, the frequency distributions of the parasitic stages and the infective stages in the soil were assumed to be directly related. To explain the frequency distribution of the parasites it was therefore necessary to account for the frequency distribution of the *S. feltiae* infective stages in the soil. The infective stages were spatially aggregated into 30 cm diameter patches at the site of host death. These patches were randomly distributed approximately 1 m apart. At the 1 m scale, the pooled counts of infective stages were randomly distributed. Thus, in contrast to the frequency distributions, the spatial structuring of *S. feltiae* changed with the spatial scale of the interaction. This dynamic spatial structuring means that the majority of samples taken would contain few or no infective stages, whilst a few soil samples would contain a great many. Thus, the spatial structuring of the infective stages generates the over-dispersed frequency distribution of the *S. feltiae* in the soil. Hosts, encountering infective stages from this spatial distribution will, therefore, show an over-dispersed frequency distribution of *S. feltiae* parasitic stages.

Key words: SADIE, spatial sampling, spatial distribution, frequency distribution.

INTRODUCTION

Work on the distributions of parasites has generally taken a parametric statistical approach, concentrating on the frequency distributions of parasitic stages in the host population. Typically, the distributions are over-dispersed and have been described using a variety of frequency distributions, most notably the Negative Binomial (Pennycuick, 1971; Schmid & Robinson, 1972). The over-dispersion of parasites in the host population is of profound importance, having a marked effect on parasite fitness, through parasite reproduction and the probability of parasite mortality (Keymer & Slater, 1990; Gregory, Keymer & Clarke, 1990; Smith & Guerroro, 1993), and parasite-induced morbidity and mortality of the hosts (Anderson & Gordon, 1982; Anderson, 1987; Smith & Guerroro, 1993). Importantly, mathematical approaches have shown that parasite frequency distributions may be responsible for cycles and crashes in host numbers (Anderson & May, 1978; May & Anderson, 1978; Hudson & Dobson, 1997), and that by changing the frequency distribution of parasites, host population cycles and crashes may be prevented (Anderson, 1994; Anderson & May, 1985, 1991; Hudson, Dobson & Newborn, 1998).

Over-dispersed frequency distributions are believed to be the result of a dynamic tension between demographic processes producing demographic stochasticity (Anderson *et al*. 1982). These processes include within-host parasite mortality, immunological responses and parasite density-dependent host death, all of which tend to generate under-dispersion, whilst individual differences in host behaviour and immunity and the reproduction of the parasites within the host, tend to generate over-dispersion (Anderson *et al*. 1982). The heterogeneous distribution of infective stages in the environment external to the host is also thought to play a role in generating over-dispersion, and it has been presumed that parasite over-dispersion results from a spatial aggregation of the infective stages in the environment (Anderson, 1993). However, although it is thought to be important, the explicitly spatial distribution and dynamics of infective stage parasitic helminths have largely been ignored.

Natural infestations of the insect pathogenic nematode *Steinernema feltiae* Filipjev are a good system for investigating the spatial distribution of infective stages. Test hosts, such as larvae of the wax moth *Galleria mellonella* L., can be used to bait defined samples of soil to extract the infective stages, following infection, as parasites; individuals which are defined as parasitic stages. As *G. mellonella* larvae have little or no immunity to infection by *S. feltiae* (Dunphy & Thurston, 1990; Bohan, 1995; Bohan & Hominick, 1996), host immunity to infection does not prevent the establishment of parasitic stages. Thus, in experimental systems with a soil of pure

* Tel: +44 1275 549294. Fax: +44 1275 394007. E-mail: David.Bohan@bbsrc.ac.uk

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

sand, extraction efficiencies approaching 90% are possible (Bohan, 1995), yielding parasitic stage counts in the host which directly reflect the infective stages in the soil.

It is important to state that bait extractions may not yield counts of all infective stage *S. feltiae*. Those parasitic stages present in the hosts will reflect only of those infective capable of infecting a host at the time of the experiment or sampling. Infective stages of *S. feltiae* exhibit the phenomenon of 'infectiousness', where only a proportion of individuals may infect, possibly for physiological reasons (Bohan, 1995; Bohan & Hominick, 1995, 1996, 1997*a*, *b*; Fairbairn *et al*. 2000). Over time, this phenomenon could have a marked effect on the spatial and frequency distribution of parasites, but this is a question best investigated using mathematical approaches beyond the scope of this paper. Rather, all field samples for nematodes using baiting are best viewed as a snap-shot of the infectious individuals present in the soil at the time of sampling.

Field samplings for steinernematid nematodes have been conducted, and where it is possible to estimate the sample mean and variance these show the frequency distributions of the infective stages to be over-dispersed (Koppenhöfer *et al*. 1998). Most notably, an adaptive sampling approach using transects, where locales of high abundance were returned to for re-sampling, showed the background distribution of the *S. feltiae* infective stages to be Poisson distributed with an over-laid set of peaks in abundance (Spiridonov & Voronov, 1995). This over-dispersed distribution was interpreted as being due to a Poisson background of old infective stages with new infective stages forming the peaks in abundance (Spiridonov & Voronov, 1995).

In this study I have investigated spatial dynamics and dynamics of infective stage *S. feltiae* in a sand dune system, with a soil of almost pure sand that will allow high efficiencies of extraction. An explicitly spatial sampling approach using grids of sampling points was conducted to establish the relationships between the population dynamics of the parasite–host system, the spatial structuring of the *S. feltiae* infective stages and the frequency distribution of the parasitic stages in a test host population, at the time of sampling.

MATERIALS AND METHODS

Life-cycle of Steinernema feltiae

Nematodes of the genus *Steinernema*, are obligate pathogens of a broad range of insect species (Poinar, 1990). The life-cycle of the steinernematids is similar in all hosts (Poinar, 1979, 1990). Juvenile infective stages of the nematodes reside in the soil awaiting contact with a suitable insect host. On contact, the nematodes penetrate into the haemocoel of the insect through body openings such as the mouth, anus and spiracles (Poinar, 1979). Once in the haemocoel, the nematodes regurgitate cells of a symbiotic bacterium, of the genus *Xenorrhabdus*, which are stored in a modified bursa of the nematode alimentary tract. Over the next 24 to 48 h, these bacteria multiply causing a septicaemia that is fatal to the host. It is on this soup of bacterial cells and host products that the infective stages feed and when mature reproduce sexually to form a second and subsequently a third generation of nematode juveniles (Poinar & Leutenegger, 1968). Food limitation and density cues within the host cadaver may then trigger the formation of many thousands of infective stages (Fan, 1989), from this third generation, which leave the depleted cadaver to enter the soil (Popiel, Grove & Friedman, 1989; Fodor, Vecseri & Farkas, 1990). There the infective juveniles may persist for in excess of 1 year, awaiting another host (Curran, 1990).

Field site

The field sampling was conducted in a degenerate sand dune at Dùn à Beard, Ross of Mull, UK. The site was chosen for a soil of almost pure sand, which afforded easy sampling and would be expected to give efficiencies of extraction, for the steinernematid infective stages in the soil, approaching 90% (Bohan, 1995). At sampling, in May 1996, the site was sparsely covered by mixed vegetation of unidentified grasses and sedges, and sphagnum moss. No naturally occurring, nematode-infected insects were noted at the site, but unidentified lepidopteran larvae were present on the vegetation and a coleopteran larva was found in one soil sample.

Sampling protocol

Two spatial scales of sampling were conducted at Dùn à Beard (Fig. 1). At the smallest spatial scale, a uniform grid of 11×11 sample points was employed, with a between-sample spacing, or spatial scale, of 10 cm. This gave a total 10 cm scale grid dimension of 1 m × 1 m, made up of a total of 121 sample points. The 10 cm grid was nested within a larger grid of 4×10 sampling plots of 1 m^2 area. The 39 1 m sampling plots were each sampled 6 times, with the location of each sampling point within the plots being determined by random numbers drawn from the Uniform Frequency distribution, giving 234 sampling points. Six samples from the 10 cm scale grid were chosen at random to contribute to the larger, 1 m scale grid. This gave a total number of 355 sampling points across the design.

The soil samples were taken using a 3 cm diameter soil augur. At each sampling point the augur was used to remove a soil core. Sections were taken from the cores, between 2 cm and 6 cm from the surface of

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

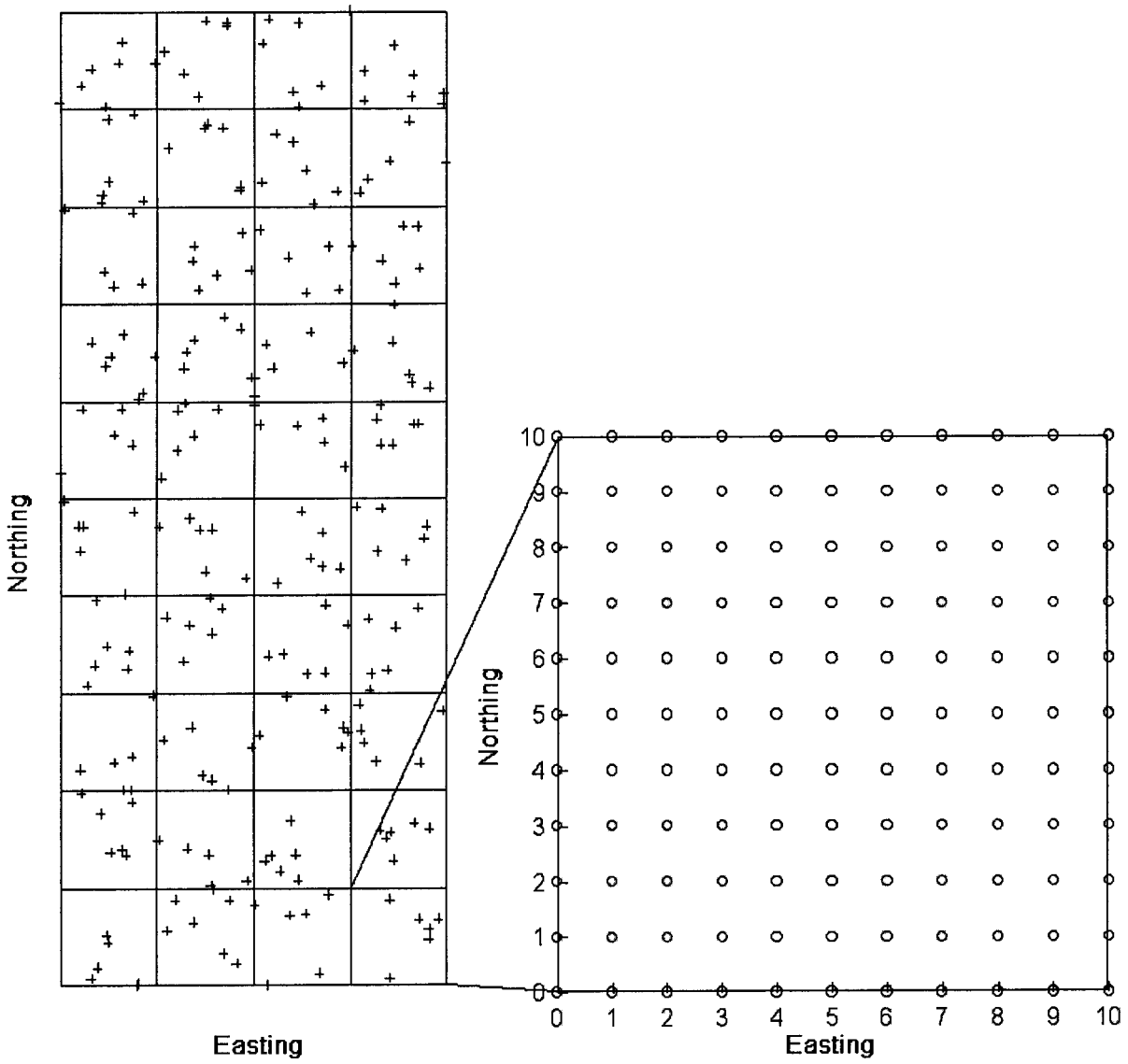

Fig. 1. Plan of the nested, spatial grids used to sample for entomopathogenic nematodes at Dùn à Beard. At the lowest spatial scale, a grid of 11×11 samples was used, with a between-samples pacing of 10 cm denoted by the open circles. This grid was nested within a larger scale grid of 4×10 sampling plots of 1 m². Each 1 m² plot was randomly sampled 6 times, using statistics from the Uniform distribution, denoted by the crosses. Six samples from the 10 cm grid were chosen at random to contribute to this larger spatial grid.

the soil, to give 30 ml soil samples. These soil samples were then placed into small plastic bags, individually marked with the sample location, and removed to the laboratory. To extract the steinernematid infective stages, 3 larvae of the Great Wax Moth, *Galleria mellonella* L., were introduced into each soil sample as test hosts. The larvae were exposed to the soil sample at 20 °C, for 72 h, in a darkened incubator. *G. mellonella* larvae have low immunity to *S. feltiae* infection (Bohan & Hominick, 1996), and the *S. feltiae* infective stages readily infect this host. The larvae were then removed from the samples and stored for a further 48 h, at 20 °C, to allow any parasitic stage nematodes within the insects to develop to adulthood. All insects were then dissected in Lumm's Ringer solution, and the number of all parasitic stages was determined for each cadaver. Summing the numbers of parasitic stages within the 3 cadavers, from each soil sample, produced a total count of the available infective stage nematodes in each soil sample. It should be noted that the use of 3 *G. mellonella* larvae, per soil sample, does not result in a significant increase in extraction efficiency over a single test host (Bohan & Hominick, 1996). Rather, 3 larvae were used to ensure that the variability of extraction was reduced to a minimum (Bohan & Hominick, 1996).

Statistical analysis

Standard parametric techniques were used to describe the count data. The fitting of the Negative Binomial frequency distribution to the count data was done in Genstat 5 for Windows, Version 4.1.

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

Table 1. Sample mean and variance for *Steinernema feltiae* parasitic stages infecting test *Galleria mellonella* across the 10 cm and 1 m scale grids and the pooled 30 cm (see Fig. 3) and 1 m scales

(Statistics for the Negative Binomial distribution fitted to the 10 cm and 1 m scale parasitic stage data.)

Scale	$\bar{x}$	s^2	k (S.E.)	$\chi^2_{\text{D.F.}}$	P
10 cm	11·57	388·20	0·401 (0·054)	$\chi^2_6 = 6{\cdot}50$	$P > 0{\cdot}10$
1 m	3·01	60·44	0·214 (0·027)	$\chi^2_3 = 3{\cdot}89$	$P > 0{\cdot}10$
30 cm a	118·56	15021·28	(See Fig. 3)		
30 cm b	135·78	6223·44	(See Fig. 3)		
30 cm c	138·22	6538·94	(See Fig. 3)		
30 cm d	128·11	7710·36	(See Fig. 3)		
1 m pooled	18·05	664·31			

Table 2. Spherical, omni-directional kriging model statistics for the spatial distributions of *Steinernema feltiae* infective stages across the 10 cm sampling grid

Range	31·22 cm
Sill Variance	2·01
Nugget Variance	0·55
F	$F_{2,9} = 53{\cdot}87$
P	$P < 0{\cdot}001$
Variance accounted for	90·6 %

To determine the spatial scales upon which the infective stage nematodes reside, the Geostatistical technique of kriging was used in Genstat (Pannatier, 1996). Kriging uses a conceptually similar approach to auto-correlation, calculating the variance between sample counts against the distance between samples. Changes in variance with distance may then be modelled and used to indicate the spatial scales upon which the abundance of individual infective stages was similar. Prior to all kriging analysis, the count data were normalized using the $\ln(x+1)$ transformation.

The analysis of infective stage spatial structuring and distribution, at the scales determined by the kriging analyses, was conducted using the recently developed suite of Spatial Analysis by Distribution IndicEs (SADIE) algorithms (Perry, 1995*a*, *b*). Using a transportation algorithm developed from the linear programming literature (Kennington & Helgason, 1980), these find the shortest total distance to regularity for the observed sample by moving the sampled 'individuals' between the sample points until the same number is achieved for each (Perry, 1995*b*). A specified number of simulations is then conducted, where the observed counts are randomly assigned to new sample points, and the distance to regularity calculated for the randomization, to achieve a distribution of permuted distances to regularity. In this way a sample may be assigned an index of aggregation (I_a) and a probability of aggregation (P_a) based upon comparison of the observed distance to regularity with the distribution of permuted distances to regularity. Values of I_a in excess of unity denote spatial aggregation, those approximating unity indicate randomness and those less than unity indicate regularity (Perry, 1995*a*). It should be noted that SADIE tests, of spatial distribution and association, are two-tailed, and P_a should be interpreted accordingly. For all SADIE analyses, 1000 randomization simulations were conducted with a standard random seed.

RESULTS

Of the 355 sample points, 198 were found to be positive for nematode infective stages. Of these infective stage individuals from 5 sampling points were sent to Dr A. Reid (CABI Bioscience, UK Centre, Egham) for identification by Restriction Fragment Length Polymorphism (RFLP) analysis of the Internal Transcribed Spaced (ITS) region of the small ribosomal subunit gene (Reid, Hominick & Briscoe, 1997). Using this species-specific method, nematodes were identified as *S. feltiae*.

The parametric distribution of the counts of *S. feltiae* were found to be highly over-dispersed, both at the 10 cm and 1 m spatial scales (Table 1). These count data conformed to the Negative Binomial distribution (Table 1). The clumping parameter of the Negative Binomial, k, was small at both scales indicating that most sample points had few or no infective stages, whilst at a few sample points high numbers of *S. feltiae* were present.

A SADIE analysis of the *S. feltiae* count data, at the 10 cm scale, showed that the spatial distribution of these counts was highly aggregated ($I_a = 1{\cdot}33$, $P_a < 0{\cdot}001$), suggesting that the infective-stage nematodes were spatially aggregated into structures, or groupings, at this scale. Using the 10 cm count data, a spherical, omni-directional kriging model indicated that these structures were circular and approximately 30 cm in diameter (Table 2). The presence of

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

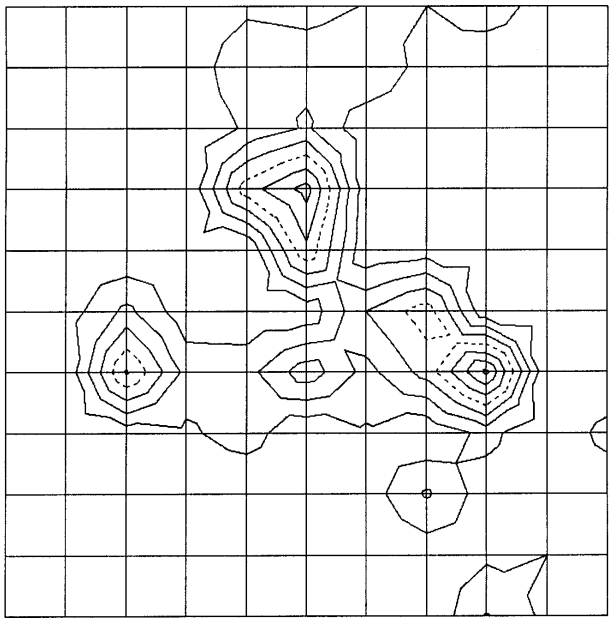

Fig. 2. A contour plot of the abundance of *Steinernema feltiae* infective-stage nematodes across the 10 cm grid presented in Fig. 1. The intersections of the grid indicate the sampling points of the 10 cm grid. The contours are spaced at 15 individuals, with a dotted contour at 60 individuals, and indicate nematode abundance from a minimum of 0 to a maximum of 109 infective stages.

Table 3. SADIE statistics for the spatial distribution of *Steinernema feltiae* in the pooled sampling, across the 30 cm scale, from Fig. 3

Pool	I_a	P_a
a	1·02	0·42
b	1·24	0·07
c	1·00	0·44
d	0·87	0·84

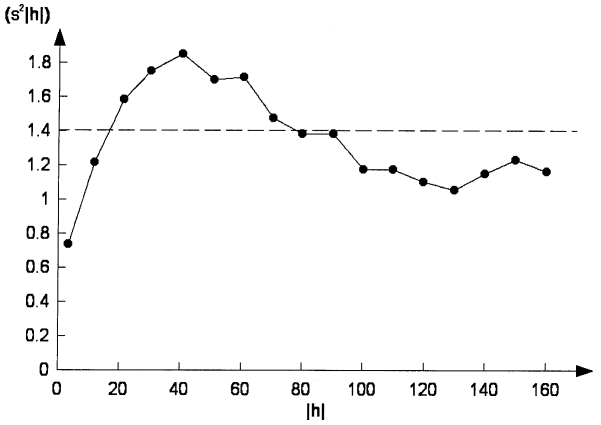

Fig. 4. Omni-directional variogram for the change in sample variance ($s^2|h|$) with between-sample distance ($|h|$ in centimetres). The plot is of infective stage variance with between-sample distance across the 10 cm grid and the random samples of the 1 m² grids. At distance in excess of 1 m, sample variance decreases indicating that samples spaced at this distance are correlated, thus suggesting the inter-group scale.

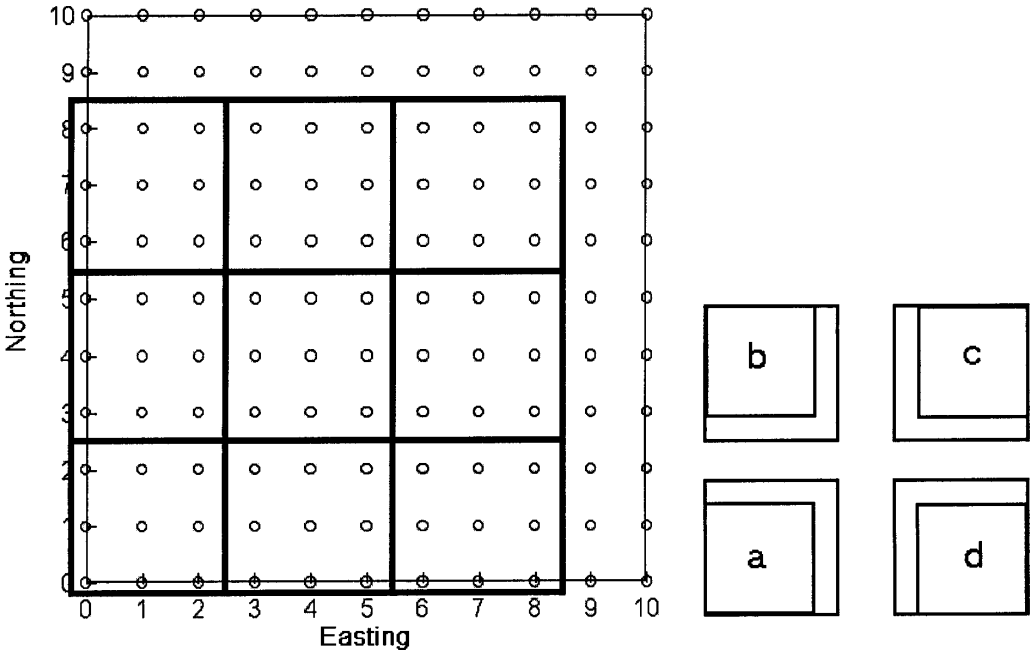

Fig. 3. Schematic for the process by which the samples from the 10 cm grid, in Fig. 2, were pooled to form a 30 cm spatial scale. A 3 × 3 grid was overlaid onto the 10 cm scale and the samples in each cell of the grid were pooled. The 3 × 3 grid was them moved from position **a** to position **b**, the data pooled, and so on to **c** and finally **d**. This allowed 4 separate SADIE analyses to be conducted, that included all 10 cm sampling data, to analyse the spatial distribution of *S. feltiae* infective-stage abundance at the patch scale.

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

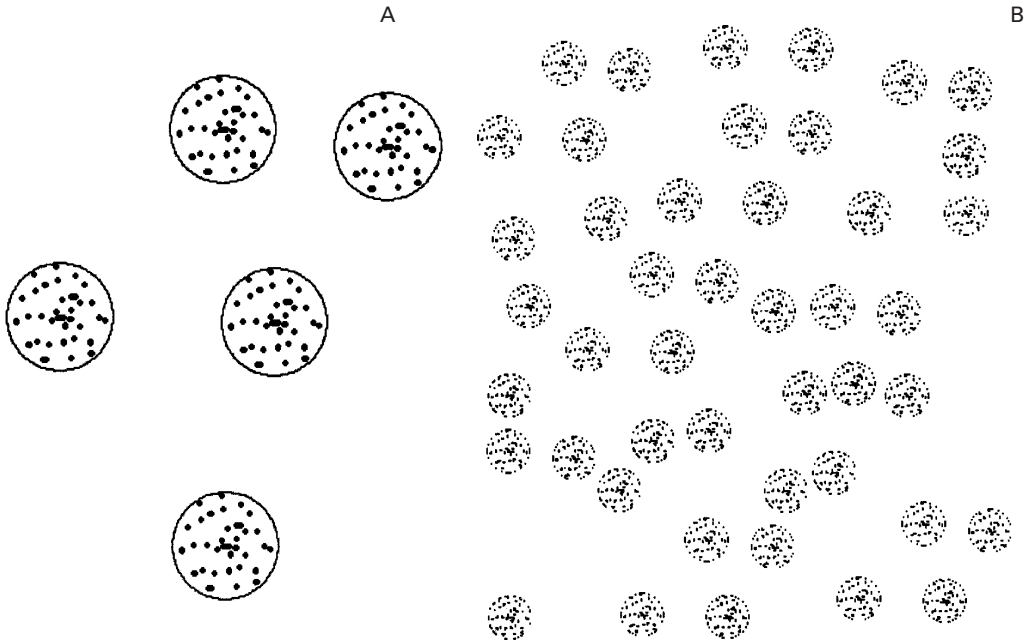

Fig. 5. Schematic diagram of the spatial structuring of the entomopathogenic nematode *S. feltiae* in a sand dune system at Dùn à Beard. (A) Individual infective-stage nematodes were spatially aggregated into patches of approximately 30 cm in diameter. These patches were positioned at the point of host death and were separated from other patches by an inter-patch distance, of approximately 1 m, that is some function of the distance a host moves from the point of infection to the point of death. The patches were distributed in a spatially random manner, suggesting that the infected host moves at least 1 m in a random direction from the point of infection to the point of death. (B) At the higher spatial scale of 1 m, groups of patches were found to show a strong tendency to spatial randomness. This randomness was due to the pooled effects of infected hosts moving from their points of infection to death and so indicated something of the movement of the whole host population. The mechanism for this movement was unknown but may have included host food resources such as plants.

groupings of individual infective stages was supported by a contour plot of the 10 cm sampling grid, showing strong 'island-like' groupings of infective stage *S. feltiae* individuals (Fig. 2).

To investigate the spatial distribution of the infective stage island-groups, the counts at the 10 cm scale were pooled to produce a *post hoc* 30 cm scale of *S. feltiae* counts. This was done by overlaying a 3×3 grid of plots onto the 10 cm scale (Fig. 3). The infective stage counts from the 9 sample points within each plot were then pooled, by summation, to achieve a 3×3 grid of counts for the 30 cm scale. This was done 4 times, shifting the position of the 3×3 grid to include all sample points (Fig. 3). The pooled counts of infective stages were found to be highly over-dispersed on each occasion (Table 1). However, for all 4 attempts the SADIE analyses showed that the spatial distribution of the pooled counts was random (Table 3). Thus, at the 30 cm scale, the observed groupings of *S. feltiae* infective stages were randomly distributed in space.

Extending the evaluation of variance to higher spatial scales, by including the infective stage count data from both the 10 cm and 1 m scales, suggested further spatial structuring (Fig. 4). Beyond a between-sample point distance of approximately 1 m, a distinct fall in variance was noted. This reduction in sample variance, of 37%, would indicate that samples spaced at this distance were correlated, and could indicate the between-group scale for the *S. feltiae* infective stages.

At the 1 m spatial scale, the 6 random sample points in each 1 m^2 plot were pooled. These counts were again found to be highly over-dispersed (Table 1). A SADIE analysis of the distribution of the counts showed, however, that the infective stages were randomly distributed at this scale ($I_a = 1{\cdot}189$, $P_a = 0{\cdot}179$). Thus, despite the parametric over-dispersion of the infective stage count data at all spatial scales, the spatial distribution of infective stages resolved from aggregation on the 10 cm scale, through randomness across the 30 cm scale to a spatially random pattern across the highest spatial scale of 1 m (Fig. 5).

DISCUSSION

This analysis shows that the frequency distribution of *S. feltiae* parasitic stages was over-dispersed at all spatial scales and conformed to the negative binomial model. I have argued that as *G. mellonella* have low immunity to *S. feltiae* infection and the extraction efficiency of infective stages from sandy soil approaches 90% (Bohan, 1995), the distribution of parasitic stages can be assumed to be directly related to the distribution of the infective stages in the soil

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

samples. Thus, that the majority of *G. mellonella* were found to contain few or no parasites, whilst a few hosts had a great many, would mean that the majority of soil samples had few or no infective stage *S. feltiae*, whilst a few soil samples had a great many. To explain the over-dispersed distribution of parasitic stages, it becomes an imperative to account for the frequency distribution of infective stages in the soil. Why did the majority of soil samples contain few or no infective stage *S. feltiae*, whilst a few soil samples had a great many?

Following reproduction within the host, *S. feltiae* infective stages emerge and enter the soil (Poinar, 1979). At the 10 cm scale the spatial distribution of these infective stages was found to be highly aggregated into approximately circular, island-like groups, with a diameter of about 30 cm, structures that presumably represented the sites of host death and emergence of the infective stages. If this were the case, then the 30 cm infective stage structures would suggest both a spatial scale for the infective stage population, of 30 cm, and a field scale for infective stage dispersal, of 15 cm. Spridonov & Voronov (1995) similarly estimated the dimension of a population-patch to be between 15 cm and 20 cm in sandy soil.

Further spatial analyses indicated that these *S. feltiae* patches were randomly distributed, in the soil at Dùn à Beard, at an average distance of 1 m apart. One credible mechanism for this spatial distribution of population-patches was infected host movement. Infected hosts could have carried the *S. feltiae* from the patch at which the host was infected to the site of host mortality, and the formation of a new patch. To produce the observed random spatial distribution and spacing of the patches, the infected hosts would have had to disperse in a random manner for a net distance of the order of 1 m. Thus, although this undoubtedly underestimates the actual distances that infected hosts may migrate, the 1 m host dispersal dimension would suggest a practical minimum distance of host dispersal at Dùn à Beard.

At the highest spatial scale examined, of 1 m, the distribution of the *S. feltiae* infective stages was found to be random. The samples from within each 1 m^2 cell were pooled, for analysis, including *S. feltiae* individuals from different nematode patches, each formed by the dispersal of a distinct host. The spatial analysis of infective stage distribution, at this scale, therefore reflected the cumulative effects of infected host movements. Thus, it would seem that large-scale dispersal in the host population has a randomizing effect upon the spatial distribution of the infective stages. What could cause this random spatial pattern is unknown, but it may have included the spatial distribution of host food resources.

In the description proposed here, the observed spatially structured and dynamic mosaic of *S. feltiae* infective-stage patches was maintained by a balance of nematode, infected host and infected host population movement, coupled through nematode reproduction. Such a dynamic spatial structure has important properties, most notable being the 'space' it affords. At the spatial scale of the nematode patch, in particular, the relationship between the dimensions of the patch and infected host migration means that although there may be many thousands of individual *S. feltiae* infective stages within a locale much of the soil environment may be devoid of infective stages. The majority of samples taken from this spatial structure would yield few or no infective stages whilst a few samples would have high numbers of *S. feltiae*; an over-dispersed frequency distribution of infective stages.

Under more natural situations, hosts do not become parasitized through the spatially structured sampling protocol adopted here. Rather parasitism would naturally result from host and infective stage movement, contact, and infection. Hence, because the scales of host dispersal were found to be greater than the scales of infective stage movement, it may be argued that hosts sample for the infective stages, at the spatial scales of host movement. Put simply, the hosts were doing most of the work. In such a case, then a pattern of host movement that approximated this sampling protocol would produce the observed, over-dispersed sample of *S. feltiae* infective stages. Indeed, another property of this open structuring is the expectation that for most conceivable patterns of host dispersal and sampling the resulting frequency distribution of infective and parasitic stages would be over-dispersed.

That the spatial structuring of infective stages and most conceivable patterns of host movement lead directly to over-dispersed samples of infective and parasitic stages might suggest that these are quite general findings for parasites. However, differences in behaviour and transmission between systems may be qualitatively important, potentially limiting these findings to *S. feltiae* and similar systems.

S. feltiae infective stages have been described as adopting 'ambushing' strategies to infect potential hosts (Grewal *et al.* 1993*a*, *b*; Lewis, Gaugler & Harrison, 1992, 1993). In essence, ambushers sit-and-wait for host items to come into contact before infecting, and the relatively small infective juvenile to host scales of movement found for *S. feltiae* may reflect this approach. By contrast, 'cruisers' actively search through the soil for hosts, and for some species the scale of infective juvenile movement may exceed the scale of host movement. Local population patches of infective stages would then merge into one another tending to produce a homogeneous distribution. Where this occurs, then the explicit spatial distribution of the infective stages would fail to explain over-dispersed distributions of parasitic stages.

In many zoo-parasitic systems, in contrast to *S.*

https://doi.org/10.1017/S0031182099006551 Published online by Cambridge University Press

feltiae, host death is not obligatory. This may allow repeated exposures of hosts to infective stages, and parasite burdens that consist of parasites from many different infection bouts. Should high rates of mixing and transmission also occur, such as for some hookworms for example, effectively Poisson distributions of parasitic stages are predicted (Grenfell, Dietz & Roberts, 1995). The repeated bouts of infection tend to average-out the spatial heterogeneity of contact between the infective stages and host. Thus, explanations for over-dispersed distributions of hookworm parasites rely upon between-host differences in susceptibility or immunity to infection (Grenfell *et al*. 1995).

As noted in the Introduction section, demographic stochasticity may also have an effect on the over-dispersion of parasitic stages. For infective stages in the soil environment, possibly the most important source of demographic stochasticity would be the reproductive output of the host, effected through differences in host size (Poinar, 1979, 1990; Fan, 1989) or immune reactions (Dunphy & Thurston, 1990). Importantly, though, stochasticity in reproductive output generally has a quantitative effect, leading to an increase in the over-dispersion of the parasitic stages. Qualitatively, however, the changes in the frequency distribution of the infective stages that result from reproductive stochasticity would be unimportant to the distribution of parasitic stages in the host population. If all patches contained similar numbers of infective stages, but were spatially distributed in the manner described here, then the frequency distribution of parasitic stages sampled by the host would be over-dispersed, as many samples would contain few or no infective stages and a few samples would contain high numbers.

These findings indicate that the spatial structuring of the infective stages was the most important determinant of the frequency distribution of *S. feltiae* parasitic stages, explaining why 'the majority of soil samples contain few or no infective stage *S. feltiae*, whilst a few soil samples had a great many'. Whilst in other, more typical, zoo-parasitic systems transmission and behaviour could act to qualitatively change the importance of infective-stage spatial structuring to explain over-dispersed frequency distributions of parasites, in many systems the explicitly spatial structuring and dynamics of infective stages will be an underlying generator of over-dispersion, being relatively insensitive to reproductive stochasticities and patterns of host movement. Interestingly, these findings also show that over-dispersed frequency distributions of parasitic stages do not result from aggregated spatial distributions of infective stages in the soil. Rather, the over-dispersed frequency distributions obscure much of the infective-stage spatial structuring and dynamics in the environment. Here it would be impossible either to describe the dynamic spatial structuring of *S. feltiae* or how this changes with scale, or predict the properties of the structuring from the over-dispersed frequency distribution alone.

This research could not have been completed without the enormous help of Dr Stuart Barlow and Dr Aldo Poiani, who thought they were going on holiday to Mull but ended up sampling. Thanks lads. I would like to thank Professor Peter Hudson, Dr Matthew Evans and Dr Alex Reid for their support during this work, two anonymous referees for their comments and Dr Rachel Norman, Professor David Glen and Dr Mikhail Semenov for their discussions.

REFERENCES

ANDERSON, R. M. (1987). The role of mathematical-models in helminth population biology. *International Journal for Parasitology* **17**, 519–529.

ANDERSON, R. M. (1993). Epidemiology. In *Modern Parasitology* (ed. Cox, F. E. G.), pp. 75–116. Blackwell Scientific Publications, Oxford.

ANDERSON, R. M. (1994). Mathematical studies of parasitic infection and immunity. *Science* **264**, 1884–1886.

ANDERSON, R. M. & GORDON, D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

ANDERSON, R. M., GORDON, D. M., CRAWLEY, M. J. & HASSELL, M. P. (1982). Variability in the abundance of animal and plant species. *Nature*, *London* **296**, 245–248.

ANDERSON, R. M. & MAY, R. M. (1978). Regulation and stability of host–parasite population interactions. I. Regulatory processes. *Journal of Animal Ecology* **47**, 219–247.

ANDERSON, R. M. & MAY, R. M. (1985). Helminth infections of humans – mathematical models, population dynamics, and control. *Advances in Parasitology* **24**, 1–101.

ANDERSON, R. M. & MAY, R. M. (1991). *Infectious Diseases of Humans : Dynamics and Control*. Oxford University Press, Oxford.

BOHAN, D. A. (1995). *Modelling* Steinernema feltiae *Infection Biology*. Ph.D. thesis. University of London.

BOHAN, D. A. & HOMINICK, W. M. (1995). Examination of the *Steinernema feltiae* (Site 76 strain) infection interaction with the *Galleria mellonella* host, using an infection model. *Parasitology* **111**, 617–625.

BOHAN, D. A. & HOMINICK, W. M. (1996). Investigations on the presence of an infectious proportion amongst populations of *Steinernema feltiae* (Site 76 strain) infective stages. *Parasitology* **112**, 113–118.

BOHAN, D. A. & HOMINICK, W. M. (1997*a*). Long-term dynamics of infectiousness within the infective-stage pool of the entomopathogenic nematode *Steinernema feltiae* (Site 76 strain) Filipjev. *Parasitology* **114**, 301–308.

BOHAN, D. A. & HOMINICK, W. M. (1997*b*). Sex and the dynamics of infection in the entomopathogenic nematode *Steinernema feltiae* Filipjev. *Journal of Helminthology* **71**, 197–201.

CURRAN, J. (1990). Efficacy of entomopathogenic nematodes in field soils. *Proceedings and Abstracts of the Vth International Colloquium on Invertebrate Pathology and Microbial Control*. Adelaide, Australia, 1990, pp. 224–227.

DUNPHY, G. B. & THURSTON, G. S. (1990). Insect immunity. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. K.), pp. 301–323. CRC Press, Boca Raton, USA.

FAIRBAIRN, J. P., FENTON, A., NORMAN, R. A. & HUDSON, P. J. (2000). Re-assessing the infection strategies of the entomopathogenic nematode *Steinernema feltiae* (Rhabditidae; Steinernematidae). *Parasitology* (in the Press).

FAN, X. (1989). *The Bionomics of a British Steinernematid*. Ph.D. thesis. University of London.

FODOR, A., VECSERI, G. & FARKAS, T. (1990). *Caenorhabditis elegans* as a model for the study of entomopathogenic nematodes. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. K.), pp. 249–270. CRC Press, Boca Raton, USA.

GREGORY, R. D., KEYMER, A. E. & CLARKE, J. R. (1990). Genetic, sex and exposure – the ecology of *Heligmosomoides polygyrus* (Nematoda) in the wood mouse. *Journal of Animal Ecology* **59**, 363–378.

GRENFELL, B. T., DIETZ, K. & ROBERTS, M. G. (1995). Modelling the immuno-epidemiology of macroparasites in wildlife host populations. In *Ecology of Infectious Diseases in Natural Populations* (ed. Grenfell, B. T. & Dobson, A. P.), pp. 362–383. Cambridge University Press, Cambridge.

GREWAL, P. S., GAUGLER, R. & LEWIS, E. E. (1993*a*). Host recognition behavior by entomopathogenic nematodes during contact with insect gut contents. *Journal of Parasitology* **79**, 495–503.

GREWAL, P. S., GAUGLER, R. & SELVAN, S. (1993*b*). Host recognition by entomopathogenic nematodes: behavioral response to contact with host feces. *Journal of Chemical Ecology* **19**, 1219–1231.

HUDSON, P. J. & DOBSON, A. P. (1997). Transmission dynamics and host–parasite interactions of *Trichostrongylus tenuis* in red grouse. *Journal of Parasitology* **83**, 194–202.

HUDSON, P. J., DOBSON, A. P. & NEWBORN, D. (1998). Prevention of population cycles by parasite removal. *Science* **282**, 2256–2258.

KENNINGTON, J. L. & HELGASON, R. V. (1980). *Algorithms for Network Programming*. Wiley, New York.

KEYMER, A. E. & SLATER, A. (1990). Animal models and epidemiology. In *Hookworm Disease – Current Status and New Directions* (ed. Schad, G. A. & Warren, K. S.), pp. 129–144. Taylor & Francis, London.

KOPPENHÖFER, A. M., CAMPBELL, J. F., KAYA, H. K. & GAUGLER, R. (1998). Estimation of entomopathogenic nematode population density in soil by correlation between bait insect mortality and nematode penetration. *Fundamental and Applied Nematology* **21**, 95–102.

LEWIS, E. E., GAUGLER, R. & HARRISON, R. (1992). Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology* **105**, 309–315.

LEWIS, E. E., GAUGLER, R. & HARRISON, R. (1993). Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. *Canadian Journal of Zoology* **71**, 765–769.

MAY, R. M. & ANDERSON, R. M. (1978). Regulation and stability of host–parasite population interactions. II. Destabilising processes. *Journal of Animal Ecology* **47**, 249–267.

PANNATIER, Y. (1996). *VARIOWIN: Software for Spatial Data Analysis in 2D*. Springer-Verlag, New York.

PENNYCUICK, L. (1971). Frequency distributions of parasites in a population of three-spined sticklebacks, *Glasterosteus aculeatus* L., with particular reference to the negative binomial distribution. *Parasitology* **63**, 389–406.

PERRY, J. N. (1995*a*). Spatial analysis by distance indices. *Journal of Animal Ecology* **64**, 303–314.

PERRY, J. N. (1995*b*). Spatial aspects of animal and plant distribution in patchy farmland habitats. In *Ecology and Integrated Farming Systems* (ed. Glen, D. M., Greaves, M. P. & Anderson, H. M.), pp. 221–242. Wiley, Chichester.

POINAR, G. O. Jr (1979). *Nematodes for Biological Control of Insects*. CRC Press, Boca Raton.

POINAR, G. O. Jr (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. K.), pp. 23–61. CRC Press, Boca Raton.

POINAR, G. O. Jr & LEUTENNEGGER, R. (1968). Anatomy of the infective and third-stage juveniles of *Neoaplectana carpocapsae* Weiser (Steinernematidae: Nematoda). *Journal of Parasitology* **54**, 340–350.

POPIEL, I., GROVE, D. L. & FRIEDMAN, M. J. (1989). Infective juvenile formation in the insect parasitic nematode *Steinernema feltiae*. *Parasitology* **99**, 77–81.

REID, A. P., HOMINICK, W. M. & BRISCOE, B. R. (1997). Molecular taxonomy and phylogeny of entomopathogenic nematode species (Rhabditida: Steinernematidae) by RFLP analysis of the ITS region of the ribosomal DNA repeat unit. *Systematic Parasitology* **37**, 187–193.

SCHMID, W. D. & ROBINSON, E. J. (1972). The pattern of a host–parasite distribution. *Journal of Parasitology* **58**, 907–910.

SMITH, G. & GUERRORO, J. (1993). Mathematical models for the population biology of *Ostertagia ostertagi* and the significance of aggregated parasite distributions. *Veterinary Parasitology* (Special Issue) **46**, 243–257.

SPIRIDONOV, S. E. & VORONOV, D. A. (1995). Small scale distribution of *Steinernema feltiae* juveniles in cultivated soil. In *Ecology and Transmission Strategies of Entomopathogenic Nematodes* (ed. Griffen, C. T., Gwynn, R. L. & Masson, J. P.), pp. 36–41. COST 819, Publication of the EU, Luxembourg.