Fundam. appl. Nematol., 1994, **17** (3), 245-253

Characterisation of the entomopathogenic nematode *Heterorhabditis* (Nematoda : Heterorhabditidae) from Ireland and Britain by molecular and cross-breeding techniques, and the occurrence of the genus in these islands

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Accepted for publication 2 September 1993.

Summary – Soil surveys were conducted in Ireland and Britain for *Heterorhabditis* nematodes. Soil samples taken from sandy locations were baited with *Galleria mellonella* larvae. *Heterorhabditis* was detected at 18/169 sites in Ireland, 2/51 sites in the North of Scotland, and 9/20 sites in the South of Wales. All of the positive sites were coastal; the genus was not detected at any of the 40 inland sites sampled. All of the 76 isolates recovered in these surveys were identified, using isoelectric focusing, DNA restriction and cross-breeding methods, as belonging to the Irish Group of *Heterorhabditis*. No member of the North-West European Group of *Heterorhabditis* was recovered. However, an isolate recovered by other workers and originating in the south of England was identified as belonging to that group. Members of the Irish Group did not generally interbreed with members of the NWE Group from either England or continental Europe, though fertile infective juveniles resulted in a minority (3/15) of intergroup crosses.

Résumé – ***Caractérisation des nématodes entomopathogènes du genre* Heterorhabditis *(Nematoda : Heterorhabditidae) d'Irlande et de Grande-Bretagne par des techniques moléculaires et d'hybridation, et présence de ce genre dans ces îles*** – Des examens de sol ont été effectués en Irlande et en Grande-Bretagne pour rechercher les nématodes du genre *Heterorhabditis*. Des échantillons des sols prélevés sur des sites sablonneux furent pourvus de larves de *Galleria mellonella* comme appâts. Des *Heterorhabditis* furent collectés sur 18 sites sur 169 en Irlande, 2 sur 51 dans le nord de l'Écosse et 9 sur 20 dans le sud du Pays de Galles. Tous les sites positifs sont situés sur le littoral; aucun *Heterorhabditis* n'a été détecté dans les échantillons prélevés dans 40 sites situés à l'intérieur des terres. La totalité des 76 isolats recueillis au cours de ces analyses ont été identifiés comme appartenant au groupe irlandais d'*Heterorhabditis*, à l'aide de méthodes de concentration isoélectrique, de restriction de l'ADN et d'hybridation. Aucun *Heterorhabditis* appartenant au groupe de l'Europe du nord-ouest n'a été recueilli. Cependant, un isolat recueilli par d'autres chercheurs et provenant du sud de l'Angleterre a été identifié comme appartenant à ce groupe. Les membres du groupe irlandais ne se croisent généralement pas avec les membres du groupe de l'Europe du nord-ouest, bien que des juvéniles infestants fertiles aient été produits dans un nombre limité (3/15) de croisements entre ces groupes.

Keywords : *Heterorhabditis*, entomopathogenic nematode, distribution, characterisation, cross-breeding, isoelectric focusing, DNA restriction.

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae have considerable potential for biological control of insect pests (Gaugler & Kaya, 1990). The non-feeding infective juvenile, carrying cells of the symbiotic bacteria *Xenorhabdus* spp. in its gut, migrates through soil, enters susceptible insects and releases the symbiont into the haemocoel. Proliferation of the bacteria leads to death of the insect within days, followed by nematode growth and reproduction. Surveys for entomopathogenic nematodes have been conducted in many parts of the world, including Australia (Akhurst & Bedding, 1986), the United States (Akhurst & Brooks, 1984; Hara *et al.*, 1991) and Europe (Mráček, 1980; Burman *et al.*, 1986; Deseö *et al.*, 1984; Blackshaw, 1988; Vänninen *et al.*, 1989; Hominick & Briscoe, 1990; Griffin *et al.*, 1991), both for the purposes of recovering potentially useful isolates and of gaining an insight into the ecology of the nematodes. A greater understanding of the factors governing the natural occurrence and abundance of the nematodes is of importance in formulating a rational approach to their utilisation as bio-control organisms (Kaya, 1990).

The results of extensive surveys for entomopathogenic nematodes in Britain and Ireland indicated that, while steinernematids were common (Blackshaw, 1988; Hominick & Briscoe, 1990; Griffin *et al.*, 1991; Boag *et al.*, 1992), heterorhabditids were rare, being recovered from only one site on each island (Hominick & Briscoe, 1990; Griffin *et al.*, 1991). On the basis of these findings, together with the apparent absence of heterorhabditids in Finland and Sweden (Burman *et al.*, 1986; Vänninen *et al.*, 1989) and the fact that members of the family are reported to have higher temperature requirements than steinernematids (Molyneux, 1986), we suggested that in these islands heterorhabditids are at the northern edge of their range, limited by temperature (Griffin *et al.*, 1991).

ISSN 1164-5571/94/03 / $ 4.00 /

Both the Irish and British isolates were recovered from sandy soils close to the coast (Griffin *et al.*, 1991; Hominick, pers. comm.). We subsequently tested soils from inland and coastal sandy areas for the presence of *Heterorhabditis* using the *Galleria* baiting method of Bedding and Akhurst (1975). The results of these surveys in Ireland, the north of Scotland and the south of Wales are presented here.

Smits *et al.* (1991), using DNA restriction fragment length polymorphisms, showed that three types of *Heterorhabditis* occur in Europe : the *H. bacteriophora* group (including isolates from Italy, Spain and Darmstadt, Germany); the North-West European Group (including isolates from the Netherlands, northern Germany and northern Poland), and the Irish group, then consisting of only three isolates from Ireland. No isolates from Britain were included in that study. We subsequently presented evidence, from cross-breeding studies and isoelectric focusing techniques, to show that the Irish Group should be considered as a separate species, closely related to the NWE Group (Dix *et al.*, 1992; Joyce *et al.*, 1994). Isoelectric focusing (IEF) was found to be a sensitive technique for distinguishing between the Irish and NWE Groups of *Heterorhabditis,* and both it and cross-breeding experiments led to similar conclusions (Joyce *et al.*, 1994). We used this technique for the identification of the *Heterorhabditis* isolates recovered in the surveys, together with a number of other isolates from North-West Europe. We also report the outcome of further crossbreeding experiments between members of the two groups.

Material and methods

Areas with sandy soils were identified from maps (including the Soil Map of Ireland and satellite maps) and by local inspection. Sites were at least 1 km apart. One or several samples were taken from a site, depending on its area. Samples consisted of approximately 40 pooled subsamples taken to a depth of about 10 cm with a soil corer of 1.2 cm diameter. In one area, the South-West of Ireland, additional samples were taken at most (31/38) sites using a small spade. These samples were comprised of up to ten subsamples of approximately 3 × 2 × 10 cm (length × breadth × depth).

Samples were transported to the laboratory and were baited with *Galleria mellonella* larvae (Bedding & Akhurst, 1975) in screw topped glass jars of 500 ml capacity. There were five bait insects per jar. Baited soils were incubated at 15-20 °C. At intervals, the jars were inspected in the dark for bioluminescence. The presence of luminescent cadavers was used as an indication that the insects had been parasitised by *Heterorhabditis* spp., whose bacterial symbiont *(Xenorhabdus luminescens)* normally emits light (Khan & Brooks, 1977; Thomas & Poinar, 1979). Insects were recovered from the soils after 7 (20 °C) – 10 (15 °C) days, or when luminescent cadavers were detected. Cadavers were incubated on damp paper and transferred to modified White traps for emergence of infective juveniles (IJs). Nematodes originating from individual bait *Galleria* were regarded as separate isolates. Emerging nematodes were used to infect fresh *Galleria* larvae for identification and establishment of cultures.

A representative selection of soil samples from which *Heterorhabditis* was recovered were analysed for pH and organic carbon by the Teagasc Soil Laboratory, Johnstown Castle, Ireland. Determination of organic carbon was by the Walkley Black Method (Walkley & Black, 1934).

Identification

Source and maintenance of nematode and bacterial isolates.

In addition to isolates obtained in the course of the survey, *Heterorhabditis* isolates UK211 and UK462 were obtained from Dr W. Hominick, Imperial College, London. Dutch isolates HL81, HF85 and HW79 were obtained from Ir Paula Westerman, Friesland College of Agriculture, The Netherlands; HSH1 (from Germany) and HSIE (from Poland) were obtained from Dr P. H. Smits, Institute for Plant Protection Wageningen, The Netherlands, and *H. megidis* (Poinar *et al.*, 1987) (from Ohio, USA) from Dr Ray Akhurst, CSIRO Division of Entomology, Adelaide, Australia.

All isolates were cultured routinely in last instar larvae of *G. mellonella.* Infective juveniles were harvested in modified White traps. Symbiotic bacteria *(Xenorhabdus luminescens)* were isolated from the intestine of surface sterilized infective juveniles as described by Akhurst (1980) and the primary forms of the bacteria were maintained on NBTA plates (Akhurst, 1986).

Isoelectric focusing

Protein extracts of IJs were prepared and the proteins were focused on agarose IEF gels in the pH ranges 3-10 and 4.0-6.5, as described by Joyce *et al.* (1993).

DNA isolation, restriction and electrophoresis

DNA was extracted from *ca.* 1 g (wet weight) of infective juveniles as described by Smits *et al.* (1991). Total genomic DNA was digested with EcoR1 (Boehringer) for 3 h at 37 °C, in the buffer supplied by the manufacturer. Digested DNA (1.2 μg/lane) was mixed with loading dye (0.25 % bromophenol blue, 0.25 % xylene cyanol, 40 % w/v sucrose in water) and then run on a 2 % agarose gel at 60 V for 3 h in TBE (1 M Trizma base, 0.9 M boric acid, 10 mM EDTA, pH 8.0). Gels were then stained with 5 μg/ml ethidium bromide for 20 min before being photographed under UV light. Molecular weight markers were supplied by Gibco.

Species determination by crossbreeding Heterorhabditis *isolates*

Crosses were carried out on lipid agar plates and in insect larvae, as described by Dix *et al.* (1992). Second generation males and females were dissected from *G.*

mellonella larvae which had been incubated at 20-25 °C for 6-7 days post infection with the appropriate nematode isolate. The cadavers were dissected in Ringer's solution. Only females which had immature gonads where mature oocytes were not visible were used for the cross-breeding experiments. At least 25 females were tested for each cross between two isolates. In addition, the following controls were set up for each cross, using similar numbers of animals : *a)* virginity/self fertility test, where some of the collected females were incubated without males, and *(b)* self cross, where females were mated with males of the same isolate.

Crosses on lipid agar plates : Lipid agar plates (5 cm diam.) were prepared by the method of Dunphy and Webster (1989). The plates were inoculated with the primary form of the bacterial symbiont from the female parent and incubated at 30 °C for 48 h before addition of the nematodes to be crossed. For each test cross, five virgin females and five males of the appropriate strains were placed on each of five lipid agar plates. The plates were incubated at 25 °C and three days later, an additional five males was added to each plate. One week later, half of the females were removed from plates where no progeny was observed and the extent of egg development was recorded. The remaining nematodes were examined two weeks after the cross was set up. Where progeny of a cross were fertile, IJs were collected and transferred to lipid agar to establish a hybrid line.

Crosses in G. mellonella *cadavers :* For each cross five females and five males were injected into each of ten *G. mellonella* cadavers, which had been prepared by injecting with *X. luminescens* bacteria three days previously. They were incubated at 20 °C in a Petri dish lined with damp filter paper. Results of the crosses were assessed by dissection and by observing the emergence of infective juveniles in White traps.

Results

Recovery of *Heterorhabditis* from soil samples

Heterorhabditis was recovered from Ireland, Scotland and Wales (Table 1; Fig. 1). In Ireland, it was detected at 18 (10.5 %) of the 169 sites sampled. Isolates were recovered from 2 (3.9 %) of the 51 sites sampled in Scotland, and at 9 (45 %) of the 20 sites on the south coast of Wales. It was present at all times of the year at which sampling was carried out (Table 1).

Heterorhabditis was not detected at any of the 40 inland sites sampled, but was recovered from 29 (or 14.5 %) of the 200 coastal sites (Table 1). The difference in the frequency of recovery between coastal and inland sites was significant (Chi-square test, $P < 0.02$). Most of the sites at which *Heterorhabditis* was detected were within a few hundred metres of the sea; one Welsh site was 1.5 km from the coast, but was part of an extensive coastal dune system. The sites were either unmanaged dune systems or grassland, including permanent unimproved pasture and areas used for recreation (caravan parks, golf links). Only sands or sandy loam soils were sampled. The organic matter content of eight representative samples from which *Heterorhabditis* was recovered ranged from 3 to 7 %, and the pH from 4.6 to 8.1 (Table 2).

Table 1. *Number of sites sampled in regions of Ireland, Scotland and Wales, sampling dates and number of sites at which* Heterorhabditis *was recovered.*

Region	Date sampled	Coastal sites		Inland sites*	
		Number of sites sampled	Number of sites with *Heterorhabditis*	Number of sites sampled	Number of sites with *Heterorhabditis*
Ireland					
Midlands	Nov. 88	0		18	0
South east	Oct./ Nov. 88	23	1	10	0
South east	Oct. 89	3	0		
West	Jan. 89	2	1	3	0
South west	Jun. 89	38	5	0	
East	Nov. 89	10	3	0	
North west	Jun. 90	55	8	0	
West	Jul. 92	0		7	0
Scotland					
North	Sep. 90	11	1	0	
North east	Nov. 90	38	1	2	0
Wales					
South	Apr. 91	20	9	0	

* Sites at least 5 km from the sea.

Table 2. *Location, habitat, site code, pH and organic matter of eight soils from which* Heterorhabditis *sp. was recovered.*

Location	Habitat	Code	pH	% organic matter
Curracloe, Co. Wexford	Grassland	K122	4.6	3.5
Kilmore Quay, Co. Wexford	Inland edge of dunes	M145	5.3	6.9
Inchydonny, Co. Cork	Grassland	M208	7.4	3.3
Portmarnock, Co. Dublin	Grassland	M215	5.6	7.2
Magilligan, Co. Derry	Dunes	M245	8.1	3.5
Dooagh, Co. Donegal	Inland edge of dunes	M262	7.8	4.8
Maghery, Co. Donegal	Grassland	M362	7.8	5.0
Fraserburgh, Aberdeenshire	Dunes	S159	7.9	3.3

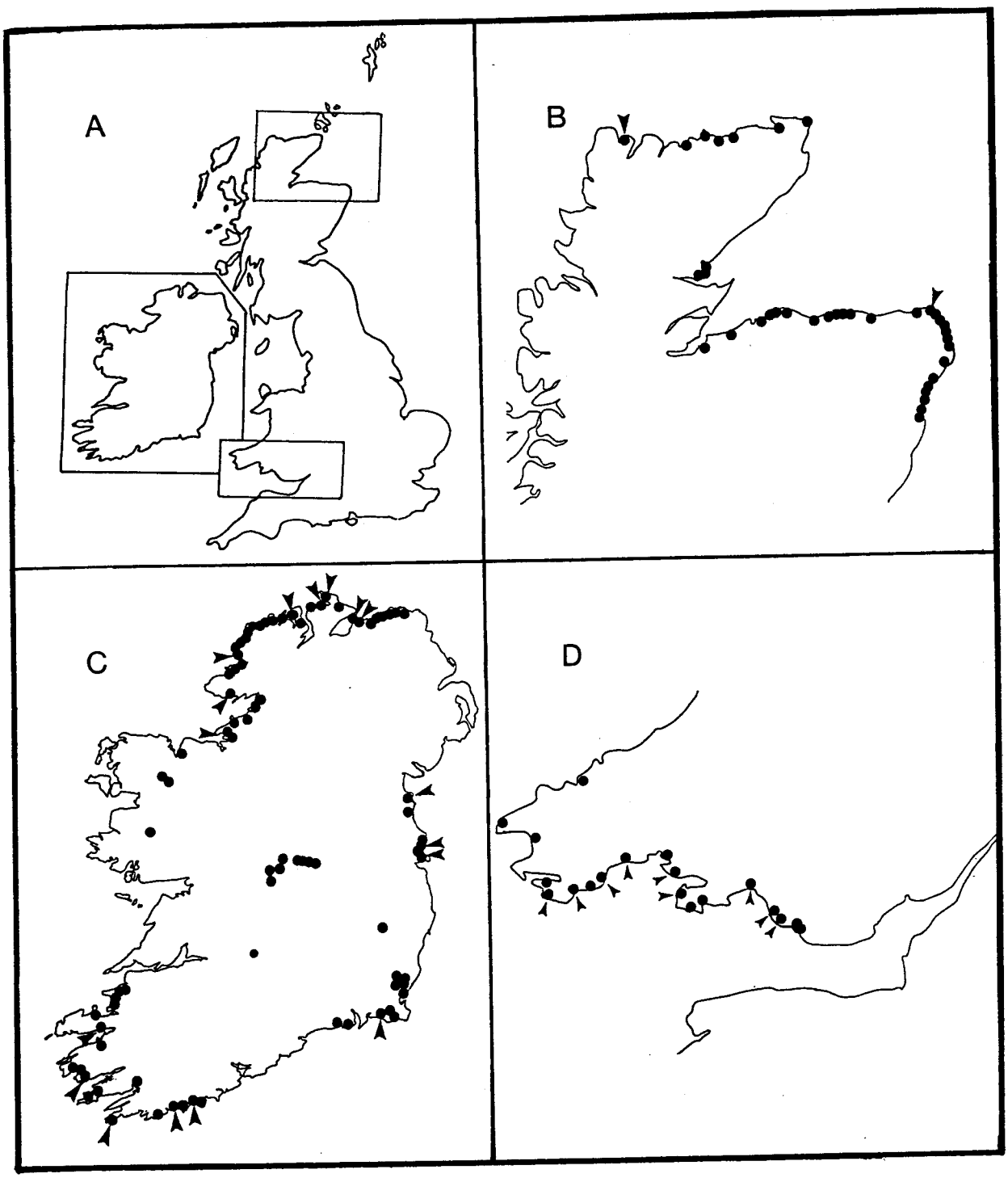

Fig. 1. *A : Areas where surveys for* Heterorhabditis *were conducted; B, C, D : Locations in Scotland, Ireland and Wales, respectively, where samples were taken (circles) and where* Heterorhabditis *was detected (arrows).*

Insects infected with the nematodes recovered in our survey rarely turned the deep red colour characteristic of other *Heterorhabditis* (Poinar, 1975; Poinar *et al.*, 1987). Instead, they characteristically turned either (1) yellow-orange or (2) colourless at time of death, deepening to green-purple, or (3) red. In Ireland, nematodes from only one (Killybegs) of the eighteen sites turned cadavers red. The majority of the Irish isolates were of types (1) and (2), as were the Welsh isolates. The isolates from the two Scottish sites were of types (1) and (3), respectively.

Altogether, 76 *Heterorhabditis* isolates were recovered : 48 from Ireland, 12 from Scotland and 16 from Wales.

IDENTIFICATION OF *HETERORHABDITIS* ISOLATES

IEF gel electrophoresis

When run on IEF gels in the pH range from 3 to 10, all of the isolates recovered in these surveys, from Ireland, Wales and Scotland exhibited the Irish type profile as described by Joyce *et al.* (1994), with three bands unique to the group at the pH range 4.9 to 5.1. Representative isolates from Ireland (lanes 1-4), Scotland (lanes 5, 6) and Wales (lanes 7, 8) are shown in Figure 2. Of the *Heterorhabditis* isolated in England, UK462 exhibited the Irish profile (Fig. 2, lane 9), while UK211 (lane 11) exhibited the profile typical of the NWE Group. Four continental members of this group (lanes 10 & 12-14) are included in Figure 2 for comparison. Gels run in the pH range from 4-6.5 confirmed these findings (Fig. 3).

EcoR1 digests of total genomic DNA

Restriction of genomic DNA with EcoR1 confirmed the results obtained using IEF. The gel presented in Figure 4 shows the EcoR1 digestion patterns of selected isolates from Ireland, Scotland, Wales and England (UK462), all of which share the restriction pattern characteristic of the Irish species with a diagnostic band visible at *ca.* 180 base pairs. Also included in Figure 4 are digests of the NWE isolates HL81 (Netherlands), HSH1 (North Germany) and UK211 (England), all of which have EcoR1 digestion patterns identical to *H. megidis* (lane 10) with the characteristic band at *ca.* 350 bp originally described by Smits *et al.* (1991).

Cross-breeding experiments

Cross-breeding experiments on lipid agar plates confirmed the results of the IEF and EcoR1 restriction fragment analysis. Isolates from Ireland, Wales and Scotland resulted in fertile progeny when crossed with each other (Table 3) but did not hybridise with isolates exhibiting the NWE Group IEF profile. Crosses between isolates of the latter group also resulted in fertile progeny. The crosses confirm that UK462 belongs to the Irish group of isolates and UK211 to the NWE Group (Table 3). Crosses in *G. mellonella* cadavers gave broadly similar results. Fertile progeny resulted from crosses between 21 pairs of isolates exhibiting the Irish type IEF profile, and from crosses between 12 pairs of North-West European isolates. Crosses in cadavers between 12 of 15 pairs of isolates of mixed IEF group did not yield infective juveniles, but limited cross-hybridisation was detected between the other three pairs tested (Table 4). Females of the Irish type M217 hybridised with male UK211 (NWE), small numbers of fertile IJs emerging

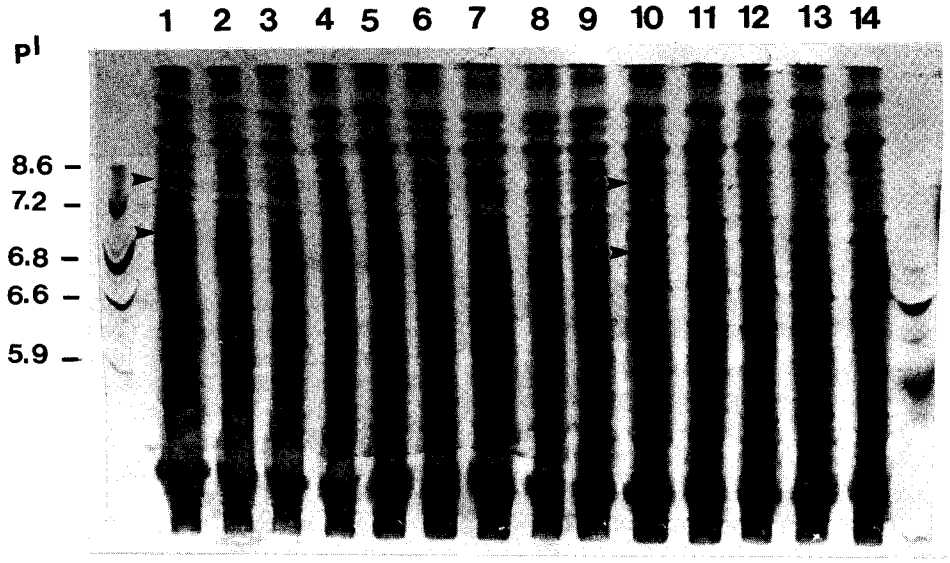

Fig. 2. *Isoelectric focusing (range pH 3-10) electropherograms of soluble proteins from Irish, British and North-West European isolates of* Heterorhabditis. *1 : M244, 2 : M145, 3 : M170, 4 : K122, 5 : S29, 6 : S159, 7 : W50, 8 : W9, 9 : UK462, 10 : HSH1, 11 : UK211, 12 : HSie, 13 : HL81, 14 : HF85. Arrows identify the presence of unique polypeptides.*

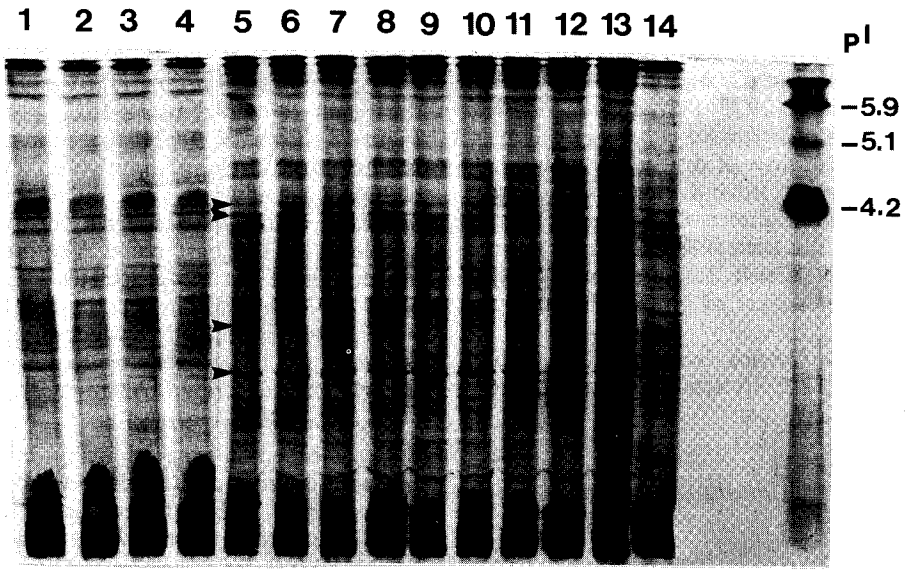

Fig. 3. *Isoelectric focusing (range pH 4-6.5) electropherograms of soluble proteins from Irish, British and North-West European isolates of* Heterorhabditis. *1 : HF85, 2 : HL81, 3 : HSie, 4 : UK211, 5 : UK462, 6 : W9, 7 : W50, 8 : S159, 9 : S29, 10 : K122, 11 : M170, 12 : M145, 13 : M244, 14 : M385. Arrows identify the presence of unique polypeptides.*

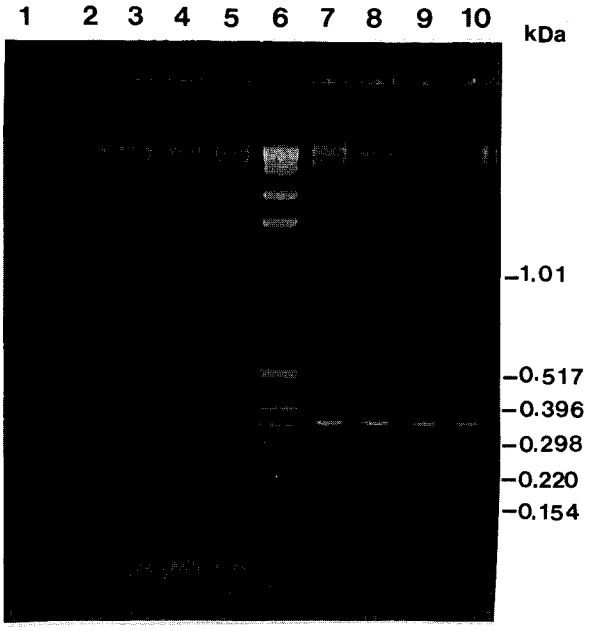

Fig. 4. *EcoR1 digestion of genomic DNA of* H. megidis *and of Irish, British and North-West European isolates of* Heterorhabditis. *1 : K122, 2 : W9, 3 : S159, 4 : S29, 5 : UK462, 6 : mol. wt. markers, 7 : UK211, 8 : HL81, 9 : HSH1, 10 :* H. megidis.

from five out of six cadavers, but the reciprocal cross was not successful. The cross was repeated on lipid agar plates with the same outcome. In the two other cases in which limited fertility was detected in inter-group crosses in cadavers, IJs were not produced when the same crosses were repeated on lipid agar plates.

Table 3. *Results of cross-breeding experiments between* Heterorhabditis *isolates from Ireland (K122), Wales (W24 & W50), Scotland (S29), England (UK211 & UK462) and the Netherlands (HL81).*

Female / Male	K122	W24	W50	S29	UK462	UK211	HL81
K122	+	+	+	+	+	–	–
W24	+	+	+	+	+	–	–
W50	+	+	+	+	+	–	–
S29	+	+	+	+	+	–	–
UK462	+	+	+	+	+	–	–
UK211	–	–	–	–	–	+	+
HL81	–	–	–	–	–	+	+

\+ Fertile infective juveniles.
– No infective juveniles.

Discussion

From the results of these surveys, it appears that *Heterorhabditis* is of widespread occurrence in the islands of Britain and Ireland but, in contrast to *Steinernema* (Blackshaw, 1988; Hominick & Briscoe, 1990; Griffin *et al.*, 1991; Boag *et al.*, 1992), is restricted to very sandy soils around the coast. Hara *et al.* (1991) noted a similar distribution of *Heterorhabditis* in the Hawaiian islands.

Mobility and survival of entomopathogenic nematodes are favoured in soils with a high sand content (Molyneux & Bedding, 1984; Kung *et al.*, 1990). Soils with a high clay content have many small pores which physically restrict nematode movement (Wallace, 1958), and have poor aeration properties, which can

Table 4. *Results of cross-breeding experiments between N.W. European (NWE) and Irish (Ir) Type* Heterorhabditis *where limited cross fertility was detected.*

Isolate		Outcome	
Female parent	Male parent	Crossed in *G. mellonella*	Crossed on lipid agar plates
M217 (Ir)	UK211	+	+
UK211 (NWE)	M217	–	– a
K122 (Ir)	HF85	+	– c
HF85 (NWE)	K122	NT	– b
S29 (Ir)	HSH1	–	– c
HSH1 (NWE)	S29	+	– b

\+ Fertile IJs.
– No IJs.
a Embryonal lethal.
b Larval lethal.
c Sterile F1 progeny.
NT Not tested.

result in inefficient utilisation of stored food reserves (Kung *et al.,* 1990). The association of *Heterorhabditis* with sandy soils in these islands may reflect an intolerance by the indigenous nematodes of low oxygen content; if so, then this would suggest that heterorhabditids from the region have a much lower tolerance of poor oxygenation than steinernematids, as the latter are found in a wider range of soil types in these islands (Blackshaw, 1988; Hominick & Briscoe, 1990; Griffin *et al.,* 1991). Sandy substrates without shading vegetation represent thermally favourable habitats which may be especially important for species at the limits of their range (Ford, 1982).

Hominick (1990) suggests that populations of entomopathogenic nematodes may become extinct at certain sites, followed by re-establishment from neighbouring sites. If this is a common phenomenon, then sites that are in relatively close proximity to other suitable sites from which immigration may occur will be more likely to maintain populations of these nematodes. This is the situation on the coastal sandy fringe. Inland, suitably sandy locations are scattered and may not be well located for replenishment following local extinctions. However, the apparent absence of *Heterorhabditis* from sandy soils in inland locations might be due to edaphic factors, or to mis-matches between features of their life history and the life histories of available hosts.

No attempt was made to identify the natural hosts of *Heterorhabditis* during this survey. The invertebrate fauna of the dunes and dune grasslands of these islands includes many potential hosts for these nematodes, including lepidoptera and coleoptera. Several species of large scarabaeids and curculionids, including *Otiorhynchus* species are common in the dunes (Hincks, 1951; Welch, 1989; Speight, 1991). High populations of the scarabaeid *Phyllopertha horticolla* were observed at several sampling sites in the south-west and north-west of Ireland, including sites positive for *Heterorhabditis.* These areas were visited during the insect's flight period in June; the species may also have been present undetected in other localities which were surveyed at other times of the year. In the Netherlands, *Heterorhabditis* has been recovered from naturally parasitised larvae of both *Otiorhynchus* sp. (Simons, 1981) and *P. horticolla* (Smits, 1992).

Our sampling was designed primarily to test whether heterorhabditids are more prevalent in these islands than had been indicated by previous surveys, and to yield new strains of the genus, rather than to compare prevalance in different regions. Sampling in different regions was carried out at different times of the year. Some tentative conclusions can be drawn, however, by comparison of areas where sampling was carried out at the same time of the year. In Ireland, there is no evidence for a reduction in prevalence of the genus from south to north; sampling was carried out in June both in the southwest and northwest of Ireland, and *Heterorhabditis* was recovered from 13 % and 15 % of sites respectively. On the other hand, *Heterorhabditis* may be less prevalent in northern Britain; only one of the 40 sites sampled in the north-east of Scotland in November yielded *Heterorhabditis* compared with 30 % of the sites in the east of Ireland, also sampled in November.

The only species of *Heterorhabditis* isolated in Ireland to date are members of the Irish Group; this also appears to be the dominant type in the regions of Britain in which we have sampled.

We have previously reported that the Irish Group is reproductively isolated from *H. megidis* and the North-West European Group (Dix *et al.,* 1992; Joyce *et al.,* 1994). The additional crossbreeding experiments which we describe here provide further support for the recognition of the Irish Group as a distinct species. Of 34 different crosses between the Irish and North-West European groups, only three yielded fertile infective juveniles. The corresponding reciprocal crosses, however, gave rise to fertile progeny in only one combination.

There are many documented examples of hybrid progeny being obtained from distinct animal species that are reproductively isolated in nature. One of the mechanisms responsible for reproductive isolation is hybrid inviability resulting from negative interactions between the zygotic genotype and maternal factors. Within the genus *Drosophila,* such maternal effects are common (Orr, 1989), such that the outcome of an interspecific cross can depend on which species is used as the female parent. Baird *et al.* (1992) investigated the extent of reproductive isolation between four species of *Caenorhabditis* and found that only one interspecific cross yielded progeny which survived to adulthood. In the

reciprocal cross, the progeny arrested as embryos. This is an analogous situation to that reported here for *Heterorhabditis*.

The ability of closely related species of entomopathogenic nematodes to interbreed and produce fertile progeny in at least some parental combinations may be important in allowing combination of desirable traits from different species to produce a nematode with improved potential for biocontrol. However, following interspecific crosses " hybrid breakdown " can sometimes occur in the F2 or backcross generations (Dobzhansky, 1970).

The Irish type has not yet been detected on mainland Europe; the yellow and green colours characteristic of cadavers parasitised by these nematodes may make them less likely to be detected than the red colours associated with most other *Heterorhabditis*.

Alternatively, the Irish type may be restricted to these islands, though there are few examples of animal species peculiar to these islands. The only representative in these islands of the North-West European Group, the dominant *Heterorhabditis* along the northern coast of continental Europe, was isolated in the south of England (Hominick, pers. comm.). Many plants and animals are restricted to the south-east of England, which is warmer and drier than the rest of the island (Tansley, 1965). The occurrence of this type at a single site may point towards a recent introduction from continental Europe, though more extensive sampling northwards from that site may reveal a more extensive range in Britain.

Acknowledgements

The authors are grateful to Dr W. M. Hominick for nematodes and for permission to refer to unpublished data, and to Mr L. Rogers of the British Museum for confirming the identification of *P. horticolla*. We gratefully acknowledge the technical assistance of Ms Rita Dempsey. Part of the work was carried out with the generous support of the EC (ECLAIR Contract AGRE 0002).

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