Biocontrol Science and Technology (1996) 6, 317–331

CARFAX

Entomopathogenic Nematodes: Biodiversity, Geographical Distribution and the Convention on Biological Diversity

WILLIAM M. HOMINICK, ALEXANDER P. REID, DAVID A. BOHAN AND BERNARD R. BRISCOE

CABI International Institute of Parasitology, 395A Hatfield Road, St Albans, Herts AL4 0XU, UK

This paper addresses three major issues. Firstly, molecular taxonomy and its application to elucidate the biodiversity and biogeography of entomopathogenic nematodes is considered. Accurate identification is fundamental for understanding biodiversity, and because these nematodes are morphologically conservative, molecular techniques will provide the insights necessary to develop a robust, morphologically based taxonomy. Secondly, a review of the knowledge on their biogeography and habitat specificity, including a consideration of the limitations to the available data is given. Much of the information is presented in two tables which summarize the distributions of recognized species at continental and national levels. Thirdly, this paper provides a brief consideration of the Convention on Biological Diversity and its implications for future work with entomopathogenic nematodes and biological control.

Keywords: Steinernema, Heterorhabditis, *biodiversity, geographical distribution, molecular taxonomy, habitat specificity*

INTRODUCTION

Knowledge of the biodiversity and geographical distribution of entomopathogenic nematodes is in its infancy, yet these are key issues for regulatory authorities. Even though a number of surveys have been carried out, covering parts of every continent, most of the world remains unexplored. In many of these surveys, major questions regarding the identification of the nematodes isolated and the methods used to recover them cannot be resolved. To add to the uncertainty, the Convention on Biological Diversity, which came into effect in December 1993, has legally binding regulations regarding ownership of organisms, rights to collect them and rights to involve third parties. This will undoubtedly impinge on future work, but in an unknown way because the mechanisms for dealing with the Convention are not yet in place.

This paper is structured to deal with three major topics:

(1) molecular taxonomy and its application to elucidate the biodiversity and biogeography of entomopathogenic nematodes;
(2) a review of current knowledge of the biogeography and habitat specificity of these nematodes;
(3) a brief consideration of the Convention on Biological Diversity and its implications for future work.

0958–3157/96/030317–15 $7.50 ©1996 Journals Oxford Ltd

MOLECULAR TAXONOMY

Accurate identification is fundamental to understanding the geographical distribution and habitat specificity of any organism. Thus, while the results of a large number of surveys are reported in the literature, those in which the entomopathogenic nematodes were identified only as *Steinernema* sp. or *Heterorhabditis* sp. are useless for biogeography and habitat information. The only conclusions that can be drawn from such studies are that entomopathogenic nematodes are widespread. However, accurate identification of the nematodes is not a trivial task. It is both labour intensive and time consuming, and these nematodes are morphologically conservative. We would argue that this is a case where molecular techniques must lead traditional morphological methods. That is, distinctions based on molecular characterization will elucidate species and groupings which can then be studied for morphological characters that distinguish them from each other. We do not advocate the replacement of traditional methods by molecular ones, but we do believe that molecular techniques will provide the insights necessary to develop a robust, morphologically based taxonomy.

A number of molecular techniques have been used for entomopathogenic nematode identification, including isoenzyme patterns (Akhurst, 1987), total protein patterns (Poinar & Kozodoi, 1988), immunological techniques (Jackson, 1965) and restriction fragment length polymorphism (RFLP) detection within total genomic DNA (Curran & Webster, 1989; Smits *et al*., 1991; Reid & Hominick, 1993). However, these techniques all dealt with relatively small numbers of samples. To identify large numbers of samples, a simple and, most importantly, reliable test is required. The RFLP analysis of polymerase chain reaction (PCR) amplified products from specific regions of the genome satisfies these criteria, and is a powerful taxonomic tool that can be used for the identification of single nematodes. Regions of taxonomic importance include the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) repeat unit and the region of the mitochondrial genome which separates the cytochrome oxidase subunit II (COII) and the 16S genes. Both of these regions have been widely used for nematode identification (Vrain *et al*., 1992; Powers & Harris, 1993; Curran & Driver, 1994; Joyce *et al*., 1994a,b; Reid, 1994), and will make major contributions to the identification of entomopathogenic species.

It is now abundantly clear that entomopathogenic nematodes can exist as strains which differ biologically (e.g. Hominick & Reid, 1990; Glazer *et al*., 1993; Gouge & Hague, 1995). Thus, the next stage is the development of techniques for the identification of strains of entomopathogenic nematodes. Curran and Driver (1994) have recently shown intra-specific variation in heterorhabditids, and Reid and Hominick (1993) have shown that *S. feltiae* exists as two RFLP types. To this end, it may be possible to use the random amplification of polymorphic DNA (RAPD) technique (Welsh & McClelland, 1990; Williams *et al*., 1990). Unlike the PCR procedure to amplify portions of the ITS and mitochondrial DNA the RAPD procedure obviates the need for prior sequence information by using a single 10-mer oligonucleotide of arbitrary sequence. When two such primer sequences are present in the target genomic DNA, in the correct orientation and on opposite strands within a readily amplifiable distance (usually less than 3 kilobases), a discrete product is formed. In many cases, individual RAPD primers will detect a number of such products from a genomic DNA sample (typically between 5–10 bands in the size range 30–3000 base pairs) which yields a 'fingerprint' for the sample. However, of all the PCR methods available, the RAPD reaction is the most sensitive to changes in the reaction conditions such as DNA concentration, buffer conditions (especially the $MgCl_2$ concentration), the number of amplification cycles and the accuracy of cycling of the PCR machine. The potential for artefactual results is therefore high, and a large number of different primer sequences must be screened to ensure that only those that give clear, unequivocal and consistent results are used and to ensure, as far as possible, that the reaction conditions from one run to the next are identical. Despite these potential problems, application of the RAPD technique to plant-parasitic nematodes has yielded polymorphisms that can be used for strain identification (Fargette *et al*., 1994; Opperman *et al*., 1994). There is no reason to suppose that strains of entomopathogenic species will not be characterized as well, although initial work on the use of RAPDs for

entomopathogenic nematode identification has largely centred on species identification (Gardner *et al.*, 1994; Berry & Liu, 1995). The ability to characterize strains would be useful in order to monitor the outcome of releases of non-indigenous strains. In some cases, such as in population biology studies, individual nematodes need to be identified to strain level. If so, it will be necessary to develop techniques further because individuals cannot be reliably identified with the RAPD technique, at least for the present time.

GEOGRAPHICAL DISTRIBUTION

The term 'geographical distribution' is meaningless without an attendant scale. Geographical distribution can be viewed at the global, continental, national and local field level (macrodistribution), and then on a scale related to the distributions of individuals in the soil (microdistribution). In this section, we consider macrodistribution to the national level. In the next section it is considered at the field level, which. can also be discussed as habitat specificity. Microdistribution is beyond the scope of this paper, though it is a fundamental consideration for population biology, gene flow and biological control programmes.

In discussing geographical distribution, the fundamental assumptions are that species were originally correctly identified and that sampling has been adequate (see Walther *et al.*, 1995). The question of identification has been discussed and it is necessary to consider, though briefly, the adequacy of sampling for entomopathogenic nematodes.

Most surveys have used a bioassay based on the '*Galleria* trap' (Bedding & Akhurst, 1975), which recovers nematodes which are infective to *Galleria* at the time of sampling but which leaves the identity of the natural host unknown. What about species which may not infect *Galleria?* Also, infectivity may vary temporally, so that a negative assay at one place may not prove to be negative if a sample is taken from the same site at another time (e.g. Hominick & Briscoe, 1990a) or the same sample may test negative in one bioassay but positive in a later one (Hominick & Briscoe, 1990a,b; Stuart & Gaugler, 1994). Passive soil extraction methods, such as centrifugation, differential settling or Whitehead trays, recover many more nematodes and species (see Ehlers & Peters, 1995), and hence are to be preferred for geographical studies. However, the methods are very labour intensive, so fewer samples can be processed (Curran & Heng, 1992). Furthermore, spatial patchiness in populations (Fan, 1989; Boag *et al.*, 1992; Stuart & Gaugler, 1994; J. M. Mason & W. M. Hominick, personal communication, 1993) has implications for sampling, whether techniques use active or passive methods of extraction. Also, the information available may reflect the distribution of scientists working on particular groups or species rather than the distribution of the organisms. Finally, the increasing use of these nematodes for biological control may result in the establishment of exotic species, and hence a wider distribution than would occur naturally. For example, *S. scapterisci* was thought to be a strain of *S. carpocapsae* when it was isolated in Uruguay. It was released into Florida, where it has now become established and can be dispersed by infected mole crickets (see Parkman & Smart, 1996). When all these limitations are considered, the reader should be cautious in drawing conclusions from what follows.

Global Distributions

In the broadest sense, entomopathogenic nematodes are widespread. The only continent where they have not been found is Antarctica (Griffin *et al.*, 1990). This conclusion arises from the results of a number of surveys (most of them cited in this review) but, for reasons outlined in the taxonomy section, more precise statements are difficult because identification frequently stopped at the generic level. Also, some literature suggests that steinernematids dominate in cooler, temperate soils while heterorhabditids dominate in tropical conditions, but this broad generalization has recently been called into question (Amarasinghe *et al.*, 1994). Table 1 summarizes the information on the geographical distribution by continent for the entomopathogenic species described up to the time of writing. Table 2 is an attempt to narrow the available information down to a national level, assuming that the authors identified the nematodes

accurately. Readers should also consult the review by Peters (1996), which summarizes all available information from naturally infected hosts and which provides valuable biogeographical, as well as host specificity, information.

Within the steinernematids, two species appear to have a global distribution, namely *S. feltiae* and *S. carpocapsae* (Tables 1 and 2). In fact, *S. feltiae* is widespread in Tasmania and other states of south-eastern Australia, and is found in habitats such as pastures, roadsides, forests and gardens, as well as in national parks where human influences on the environment are minimal (R. A. Bedding, personal communication, 1995). This species also dominates in Europe, particularly in fields and roadside verges (see below). Such a wide global distribution suggests that *S. feltiae* is an efficient disperser, possibly through man's activities in moving soil and/or by infected insects. Alternatively, this is an ancient species which was present before continents began breaking up and drifting. The other steinernematid species appear to have a more restricted distribution and are recorded at continental or national levels. For example, *S. affinis* and *S. kraussei* appear to be restricted to Europe (Hominick *et al*., 1995), while *S. rara* and *S. ritteri* appear to be restricted to South America.

The picture is somewhat different for the heterorhabditids. To start with, fewer heterorhabditids have been described and fewer *Heterorhabditis* isolates have been identified to the species level because they are morphologically conservative and positive identification requires rare expertise, DNA fingerprinting or cross-breeding techniques (Dix *et al*., 1992). Nevertheless, the data that are available yield entirely different results when compared with the steinernematids. One species in particular, *H. indicus*, appears to be found throughout the tropics. Identification based on RFLPs of the ITS region of the rDNA repeat have shown this species to be present in India, Sri Lanka, Japan, northern Australia, Cuba and the Caribbean (Joyce *et al*., 1994a; A. P. Reid, unpublished). Another widely distributed heterorhabditid is *H. bacteriophora*, found in North and South America (Poinar, 1990), southern Europe (Smits *et al*., 1991; De Doucet & Gabarra, 1994), Australia (Poinar, 1990) and China (Li & Wang, 1989). In comparison, *H. hawaiiensis* has so far only been found in Hawaii (Gardner *et al*., 1994) and therefore appears to be more localized, but this may simply reflect its recent discovery.

The identification and distribution of *H. megidis* raise some intriguing questions. This species was originally isolated from Ohio in the US and has two very closely related RFLP types in Europe. One was designated the 'NW European species' (Smits *et al*., 1991; Joyce *et al*., 1994a) and is found throughout the Netherlands and Germany and has also been isolated once from the UK (Hominick *et al*., 1995). The other was designated the 'Irish species' and is found only in Ireland and the UK. However, both of these are extremely closely related at the DNA level to the original Ohio *H. megidis* (Curran & Driver, 1994; Reid, 1994). To further complicate matters, we have recently identified the Ohio type *H. megidis* from Japanese soils (M. Yoshida & A. P. Reid, unpublished). Questions such as whether we are dealing with different species or strains, their relatedness and their origin and evolution remain to be answered before the geographical distribution of this complex is resolved.

Examination of Tables 1 and 2 and comparison of Table 1 in Curran and Driver (1994) with Table 1 in Reid (1994) reveal that many more steinernematids than heterorhabditids have been described. This may simply be an artefact, reflecting the practical difficulty of distinguishing heterorhabditid species. Alternatively, it may be a real phenomenon, reflecting the fact that the biodiversity of steinernematids is greater than that of heterorhabditids. Recent work in our laboratory in collaboration with M. Yoshida of The National Institute of Agro-Environmental Sciences, Tsukuba, Japan supports such an hypothesis. Of 93 samples collected from Japan and identified by molecular techniques, eight distinct *Steinernema* genetic types were identified. Steinernematids made up 73% of the collection and only one of these had been described (*S. kushidai*); the other seven were all new to science. One of the species dominated and made up 45% of the identified samples. In contrast, only two *Heterorhabditis* species were isolated, *H. indicus* and *H. megidis*, though they made up 15 and 12% of the collection respectively. RFLP studies of the ITS regions showed that the Japanese *H. indicus* is identical to our Sri Lankan (HSL 43) isolate and the D1 population from Australia. The Japanese *H. megidis*

TABLE 1. Recorded distribution of described species of entomopathogenic nematodes of the genera Steinernema, Neosteinernema and Heterorhabditis in different continents

Species[a]	*Europe and Russia*	*North America*	*South America*	*Africa and India*	*Australia and New Zealand*	*Japan and China*	*Others*
S. affinis (Bovien, 1937)	+						
S. anomali (Kozodoi, 1984)	+						
S. bicornutum, Tallosi et al., 1995	+						
S. carpocapsae (Weiser, 1955)	+	+	+		+		
S. caudatum, Xu et al., 1991						+	
S. cubana, Mráček et al., 1994							+
S feltiae (Filipjev, 1934)	+	+	+		+		+
S. glaseri (Steiner, 1929)	+	+	+			+	
S. intermedia (Poinar, 1985)		+					
S. kraussei (Steiner, 1923)	+						
S. kushidai, Mamiya, 1988						+	
S. longicaudum, Shen, 1992					+	+	
S. neocurtillis, Nguyen & Smart, 1992		+					
S. rara (De Doucet, 1986)			+				
S. riobravis Cabanillas et al., 1994		+					
S. ritteri, De Doucet & De Doucet, 1990			+				
S. scapterisci, Nguyen & Smart, 1990			+				
S. serratum, Lui, 1992						+	
N. longicurvicauda, Nguyen & Smart, 1994		+					
H. argentinensis, Stock, 1993b			+				
H. bacteriophora, Poinar, 1976	+	+			+	+	+
H. hawaiiensis, Gardner et al., 1994							+
H. indicus, Poinar et al., 1992				+	+	+	+
H. megidis, Poinar et al., 1987	+	+				+	
H. zealandica (Poinar, 1990)					+		

[a]*Current names of nematodes are given; author citations in parentheses refer to papers where previous names were used.*

TABLE 2. Recorded distribution of described species of entomopathogenic nematodes of the genera *Steinernema*, *Neosteinernema* and *Heterorhabditis* in different countries

Species	Country	Reference
S. affinis	Belgium	A. P. Reid (unpublished)
	Denmark	Poinar (1988)
	Ireland	Griffn *et al.* (1991)
	Germany	Ehlers *et al.* (1991)
	Holland	Hominick *et al.* (1995)
	Switzerland	Steiner (1994)
	UK	Hominick *et al.* (1995)
S. anomali	Russia	Kodozoi (1984)
S. bicornutum	Serbia	Tallosi *et al.* (1995)
S. carpocapsae	Australia	Poinar (1986)
	Brazil	A. P. Reid (unpublished)
	Canada	Poinar (1986)
	Czech Republic	Poinar (1986)
	France	Poinar (1986)
	Italy	Ehlers *et al.* (1991)
	Korea	Choo *et al.* (1996)
	Mexico	Poinar (1986)
	Poland	Poinar (1986)
	Russia	Poinar (1986)
	Sweden	Poinar (1986)
	USA	Poinar (1986)
S. caudatum	China	Xu *et al.* (1991)
S. cubana	Cuba	Mráček *et al.* (1994)
S. feltiae	Argentina	Stock (1993a)
	Australia	Poinar (1986)
	Belgium	J. S. Miduturi & A. P. Reid (unpublished)
	Denmark	Poinar & Lindhardt (1971)
	Ireland	Griffin *et al.* (1991)
	Estonia	A. P. Reid (unpublished)
	Finland	Vänninen *et al.* (1989)
	France	Poinar (1986)
	Greece	A. P. Reid (unpublished)
	Hawaii	A. P. Reid (unpublished)
	Holland	Hominick *et al.* (1995)
	Hungary	Mráček & Jensen (1988)
	New Zealand	Poinar (1986)
	Norway	Haukeland (1993)
	Poland	Sandner & Bednarek (1987)
	Russia	A. P. Reid (unpublished)
	Slovakia	Mráček *et al.* (1982)
	Sweden	Burman *et al.* (1986)
	Switzerland	Steiner (1994)
	Turkey	A. P. Reid (unpublished)
	UK	Hominick *et al.* (1995)
	USA	Poinar (1992)
S. glaseri	Brazil	Pizano *et al.* (1985)
	China	Li & Wang (1989)
	Spain	De Doucet & Gabarra (1994)
	USA	Poinar (1986)
S. intermedia	USA	Poinar (1985)
	Switzerland	Steiner (1994)

TABLE 2. *Continued*

S. kraussei	Czech Republic	Mráček (1980)
	Germany	Mráček *et al.* (1992)
	Germany	Mráček (1994)
	Holland	Hominick *et al.* (1995)
	Russia	A. P. Reid (unpublished)
	Switzerland	Steiner (1994)
	UK	Hominick *et al.* (1995)
S. kushidai	Japan	Mamiya (1988)
S. longicaudum	Australia	R. A. Bedding (personal communication, 1995)
	China	Shen (1992)
S. neocurtillis	USA	Nguyen & Smart (1992)
S. rara	Argentina	De Doucet (1986)
S. riobravis	USA	Cabanillas *et al.* (1994)
S. ritteri	Argentina	De Doucet & De Doucet (1990)
S. scapterisci	Argentina	Stock (1992)
	Uruguay	Nguyen & Smart (1990)
S. serratum	China	Lui (1992)
N. longicurvicauda	USA	Nguyen & Smart (1994)
H. argentinensis	Argentina	Stock (1993b)
H. bacteriophora	Australia	Akhurst (1987)
	China	Akhurst (1987)
	Israel	Glazer *et al.* (1993)
	Italy	Akhurst (1987)
	Korea	Choo *et al.* (1995)
	Spain	De Doucet & Gabarra (1994)
	USA	Poinar & Georgis (1990)
H. hawaiiensis	Hawaii	Gardner *et al.* (1994)
H. indicus	Australia	Akhurst (1987)
	India	Poinar *et al.* (1992)
	Japan	A. P. Reid (unpublished)
	Sri Lanka	Amarasinghe *et al.* (1994)
	Trinidad	A. P. Reid (unpublished)
H. megidis	Belgium	Miduturi & A. P. Reid (unpublished)
	Canada	Mráček & Webster (1993)
	Ireland	Joyce *et al.* (1994a)
	Holland	Hominick *et al.* (1995)
	Japan	A. P. Reid (unpublished)
	UK	Hominick *et al.* (1995)
	USA	Poinar *et al.* (1987)
H. zealandica	New Zealand	Akhurst (1987)

yielded an identical pattern to that from the original isolate from Ohio (which displays minor, but distinct, RFLPs when compared with the NW European and Irish types). These differences in the biogeography of the two families could be an effect of the first-generation hermaphroditic reproductive strategy of the heterorhabditids, which could lead to the production of 'clones' after infection by single nematodes, while the obligate sexual life cycle of the steinernematids may favour greater genetic diversity and speciation. This hypothesis is supported by molecular data. A phylogenetic tree constructed from restriction enzyme site data of the rDNA repeat unit suggests that heterorhabditid species are much more closely related to one another than

are steinernematid species (Reid, 1994). Similarly, despite testing a large number of geographically separated isolates, Curran and Driver (1994) found that the heterorhabditids fell into a few species groups and that more work was required to determine whether these groups represented specific or subspecific differences. Hence, many more steinernematid than heterorhabditid species may exist on a global scale and these may show a greater biological diversity, with attendant diverse characters useful for particular biological control programmes.

European Studies

In documenting geographical distribution, the prevalence of species (i.e. whether particular species are common or rare) is an important consideration. For regulatory purposes, it is probably different to contemplate release of a strain of a species which is indigenous and dominant compared with releasing a strain of a species which is rare. While the question of prevalence cannot be addressed on a global basis because of the scarcity of surveys and hence small sample sizes, it can start to be addressed within Europe. As a result of the European Union Cooperation in the Field of Science and Technical Research (COST) Action 812 (Burnell *et al*., 1994) and its successor COST 819 on entomopathogenic nematodes, European collaboration is extensive and a number of surveys exist, both published and unpublished. Thus, Europe is the most intensively surveyed region of the globe for entomopathogenic nematodes and is the region for which most quantitative data is available.

A great deal of variation in the prevalence of entomopathogenic nematodes has been recorded in European soils and steinernematids predominate compared with heterorhabditids. Hominick *et al*. (1995) used a sensitive homologous rDNA probe cloned from a steinernematid to provide unequivocal identification of entomopathogenic nematodes isolated during surveys of the UK and the Netherlands. The prevalences of steinernematids that they reported (37–49%) are the highest documented so far in northern Europe and are at least partly explained by their method. In this, the soil in a negative assay was exposed to *Galleria* larvae for a second time because nematodes frequently do not infect in the first bioassay (Hominick & Briscoe, 1990a,b). Other northern European surveys varied widely in the prevalence of steinernematids, ranging from 5.8% in Finland (Vänninen *et al*., 1989) to 10.4% in the Republic of Ireland (Griffin *et al*., 1991), 18.3% in Norway (Haukeland, 1993), 25% in Sweden (Burman *et al*., 1986), 26.5% in the Swiss Alps (Steiner, 1994) and 36.8% in Czechoslovakia (Mráček, 1980). Boag *et al*. (1992) found that only 2.2% of 1014 sites in Scotland tested positive, and felt that this low recovery was a reflection of the cold climate in that country. Such a conclusion is supported by data from a recent survey in western Canada (Mráček & Webster, 1993). Blackshaw (1988) reported a prevalence of 3.8% in Northern Ireland, although the majority of the sample sites contained clay type soils which consistently yield low numbers of insect-parasitic nematodes (Hominick & Briscoe, 1990a). It thus appears that the climate and soils of England, Wales and the Netherlands provide excellent conditions for steinernematid populations in particular.

Heterorhabditids are rare in most European surveys. This contrasts with a recent quantitative survey in New Jersey, USA, where heterorhabditids, probably *H. bacteriophora*, predominated over steinernematids (Stuart & Gaugler, 1994). Hominick and Briscoe (1990a) and Steiner (1994) each found only one site positive for heterorhabditids. In their survey of the Republic of Ireland, Griffin *et al*. (1991) also found only one heterorhabditid, which was isolated from a coastal, sandy site. A subsequent survey of coastal areas in Ireland, Scotland and Wales (Griffin *et al*., 1994) showed that heterorhabditids are much more common than previously indicated, with prevalences as high as 45%, but only when this particular habitat type is sampled. The second UK survey of Hominick *et al*. (1995) targeted sandy, coastal soils within 1 km of the sea and yielded two heterorhabditid isolates, at a prevalence higher by an order of magnitude compared with their first inland survey. Heterorhabditids are frequently found in sites adjacent to the sea (Hara *et al*., 1991; Poinar, 1993; Amarasinghe *et al*., 1994; Griffin *et al*., 1994), an association that is intriguing and unexplained. A recent study on spiders living on barren islands in the Gulf of California (Polis & Hurd, 1995) may be relevant in the explanation. Washed-up marine detritus provides a rich source of nutrients in the form of kelp and other algae and

carcasses of animals, which support large populations of insects which then support large populations of spiders. Entomopathogenic nematodes could flourish equally well under these conditions. However, a longer distance from the sea does not preclude the occurrence of heterorhabditids, as Hominick *et al.* (1995) found that 13 of the 100 sites they sampled in the Netherlands yielded heterorhabditids even though most of the sites were inland. The results of Stuart and Gaugler (1994) from New Jersey are similar.

When the nematodes from northern European surveys have been identified, they have proved to be mostly *S. feltiae*. This agrees with Hominick *et al.* (1995) who found that this species was the most common in the UK and the Netherlands. In addition to the standard RFLP type of *S. feltiae*, their surveys yielded a further RFLP variant of this species found only in the UK, and six other species/RFLP types, one of which was *S. affinis*, one *S. kraussei* (designated B1; see Reid & Hominick, 1993; Hominick *et al.*, 1995) and some of them new to science. Steiner (1994) surveyed the Swiss Alps and found *S. affinis, S. intermedia, S. feltiae, S. kraussei* and an isolate close to *S. intermedia* but with a distinctive RFLP type. He also reported that *S. kraussei* predominated in the alpine environment while *S. feltiae* predominated in the lower Alps. Although *S. carpocapsae* has been found in British soils (Georgis & Hague, 1981, 1988), the extensive surveys reported above have never positively identified this species, so it must be rare in northern European localities.

Habitat Range

The literature on habitat preference for steinernematids is, by and large, contradictory. This is mainly due to the need for large sample sizes and accurate identification to make a meaningful assessment of potential habitat preferences. Most surveys yield insufficient data to test for correlations. However, Hominick *et al.* (1995) demonstrated that at least some steinernematid species/strains show a distinct habitat preference, with four out of five species (*S. feltiae, S. kraussei, S. affinis* and *Steinernema* sp. B3) found in sufficient numbers and displaying significant associations with specific habitat types. Their paper should be consulted for details, but *S. kraussei* (B1) was most frequently found in woodland, *S. feltiae* and *S. affinis* were found in fields and verges and B3 was exclusive to woodlands. Another species (*Steinernema* sp. C1) also appeared to show a habitat preference for roadside verges when data from a survey by Gwynn (1993) were combined with that of Hominick *et al.* (1995). Steiner (1994) found no relationship between vegetation and the presence of *S. kraussei*, but did conclude that *S. feltiae, S. affinis* and *S. intermedia* were typical of grassland ecosystems. These habitat preferences may reflect the distribution of suitable hosts and lend support to the growing opinion that these nematodes are more restricted in their natural host range than laboratory infections suggest (see Kaya & Gaugler, 1993). In addition, the various species will be physiologically and behaviourally adapted so that their specific niche requirements are satisfied only in particular habitats. Thus, Steiner (1994) reported that *S. kraussei* dominated in soils with relatively low pH values, while *S. intermedia* and *S. feltiae* avoided extremes of pH and *S. affinis* was confined to a pH near neutrality.

Information from other European surveys is more equivocal. Griffin *et al.* (1991) found *S. feltiae* in woodland, tilled fields, pasture and roadside verges, while *S. affinis* was absent from woodland. However, the prevalences were low and statistical analyses showed no significant associations between nematode occurrence and habitat. Boag *et al.* (1992) found *S. feltiae* mostly in pasture and absent from heathland, but again prevalences of less than 5% made it difficult to draw firm conclusions.

So far, information on habitat specificity for heterorhabditids is dominated by the correlation between members of this family and coastal sandy soils (Griffin *et al.*, 1994). This observation has led to the hypothesis that heterorhabditids evolved from marine nematodes and steinernematids from terrestrial nematodes (Poinar, 1993). The validity of such a broad generalization awaits further studies, but DNA analyses and other molecular techniques have the potential to verify the possibility. One other relevant study on habitat preference of heterorhabditids is that of Stuart and Gaugler (1994). In their New Jersey survey, they found that heterorhabditids were

equally abundant in turf and weedy habitats, but were absent from closed-canopy forest. This contradicted a previous survey and pointed to the difficulties of making valid comparisons between surveys because of the influence of many confounding and uncontrolled factors. The association with the sea remains the most robust correlation between habitat and the presence of heterorhabditids.

In all these correlations and associations with habitats it is prevalence that is being assessed and definitive statements should be made cautiously. A much more critical assessment requires measurement of population sizes and the spatial variability of the nematodes. As discussed earlier, there is a sampling problem in that the distribution of entomopathogenic nematodes is highly aggregated at very low spatial scales. Hence, small sample sizes and chance can together produce misleading results. Using spatially structured sampling and large sample sizes J. M. Mason (unpublished) and S. Spirodonov (unpublished) both found that some species of steinernematids do not progress beyond certain habitat boundaries. J. M. Mason (unpublished) found that the mean densities of certain species changed from grassland to woodland sites. In Spirodonov's survey (unpublished), particular species stopped at the canopy boundary of a woodland site and were also associated with specific strata of soil. In any case, habitat specificity should not be surprising as all organisms have niche requirements that will be satisfied only in particular habitats.

It should now be apparent that large sample sizes and accurate identifications are necessary before detailed questions regarding geographical and habitat specificity can be answered. The collection of such results requires a large amount of effort which is beyond the capability of most individuals and laboratories. However, if all the data collected globally were put into a single database, the possibility for gaining valuable insights into the distribution of entomopathogenic nematodes becomes realistic. Therefore, we have established an entomopathogenic nematode database, based on our own extensive surveys and collaborations. Questionnaires are available from us for individuals who would like to add their records to ours, with confidentiality guaranteed if requested. We emphasise that we can only use data from sites where the species of nematode was determined, even if some doubt remains. Filling in a questionnaire for each sample will be laborious, as will entering the information into the database, but we feel that the effort will be worthwhile. For example, reliable information on habitat specificity could be useful for biological control programmes. There may be little point in attempting to control an insect pest in pasture with a nematode species found exclusively in woodland. The information will also prove invaluable for regulatory purposes.

CONVENTION ON BIOLOGICAL DIVERSITY

The United Nations Conference on the Environment and Development (the Earth Summit) was held in Rio de Janeiro in June 1992 and produced two international treaties—the Convention on Biological Diversity and the Convention on Climate Change. The Convention on Biological Diversity was signed by 153 countries and the European Union, and came into effect in December 1993. It has now been ratified by over 100 countries and is a legally binding document. While it is beyond the scope of this paper to consider the Convention in detail, accurate identification of organisms and an understanding of their biogeography are fundamental for its implementation, and it is important to be aware of the obligations that the Convention on Biological Diversity imposes. The International Institute of Biological Control (IIBC) has produced a document (International Institute of Biological Control, 1994) to explain biological control within the context of the Convention. It provides the basis for some of the following discussion.

The objectives of the Convention are the conservation of biodiversity, the sustainable use of its components and the equitable sharing of benefits arising from the utilization of genetic resources. Biological control requires continued, responsible exchange of natural enemies between countries, as well as *in situ* conservation of natural enemies and their habitats. These

requirements are embodied in various articles of the Convention, and therefore the future of biological control is intimately linked to the Convention on Biological Diversity.

The Convention has an important role to play in ensuring continuing, global access to biological diversity for control of alien pests. In Article 15.2, parties to the Convention are asked to "Endeavour to create conditions to facilitate access to genetic resources for environmentally sound uses by other Contracting Parties". Biological control agents are part of these genetic resources and the Convention stipulates that their collection should be carried out by mutual agreement between parties and that the party providing these agents should do so in a context of prior informed consent. In practice, this will mean close cooperation between countries providing and receiving biological control agents, and it places an even greater responsibility on intergovernmental institutions such as IIBC and Food and Agriculture Organization (FAO), which assist countries in this process.

The Convention has been signed into law, but in many cases the funds and official mechanisms to implement and regulate the Articles do not exist. For example, in the context of this paper, much of the world has not been surveyed for entomopathogenic nematodes, yet habitats are continuously under threat. Loss of habitats means loss of biodiversity and loss of potentially useful species. The Convention requires contracting parties to take action to improve *in situ* conservation of genetic resources through a broad programme of habitat conservation (Article 8). Few countries have submitted national plans for protecting their biodiversity and, for developing countries, the question of who will pay is not resolved. It is consistent with the Convention on Biological Diversity that material should not be collected from another country without permission from that country's appropriate authority. This assumes that an appropriate authority exists, which may not always be the case. Similarly, for material sent outside a country, it is implied that the sender has the permission of the relevant authorities to do so. Also, the receiver of the material should not make it available to third parties without permission. However, for collaborative scientific research, in which the biology of the organisms is studied to facilitate their use in biological control programmes, the conditions may be perceived as being different, and hence third parties could become involved provided they agreed to abide by the Convention on Biological Diversity.

It is clear that the Convention is highly relevant for work on entomopathogenic nematodes and for the practice of biological control and that the issues are complicated. It is also clear that national and international agreements and regulations will be a long time in developing. It is not practical to stop all international work or to restrict work to national projects. In our opinion, the way forward is to be aware of the legal obligations of the Convention, and to operate within its spirit on a case-by-case basis, until agreements and the legal machinery necessary to assure the implementation of the Convention are in place.

ACKNOWLEDGEMENTS

The authors are indebted to a large number of colleagues who provided specimens from their collections and unpublished data and information, making their network truly international. Some of this work was supported by contracts from the Agricultural Genetics Company and by grants from the Agriculture and Food Research Council (now Biotechnology and Biological Sciences Research Council).

REFERENCES

AKHURST, R.J. (1987) Use of starch gel electrophoresis in the taxonomy of the genus *Heterorhabditis* (Nematoda: Heterorhabditidae). *Nematologica* **33,** 1–9.

AMARASINGHE, L.D., HOMINICK, W.M., BRISCOE, B.R. & REID, A.P. (1994) Occurrence and distribution of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) in Sri Lanka. *Journal of Helminthology* **68,** 277–286.

BEDDING, R.A. & AKHURST, R.J. (1975) A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* **21,** 109–110.

BERRY, R.E. & LIU, J. (1996) Phylogenetic analysis of the genus *Steinernema* by morphological characters and random amplified polymorphic DNA fragments. *Fundamental and Applied Nematology* (in press).

BLACKSHAW, R.P. (1988) A survey of insect parasitic nematodes in Northern Ireland. *Annals of Applied Biology* **113,** 561–565.

BOAG, B., NEILSON, R. & GORDON, S.C. (1992) Distribution and prevalence of the entomopathogenic nematode *Steinernema feltiae* in Scotland. *Annals of Applied Biology* **121,** 355–360.

BOVIEN, P. (1937) *Some Types of Association between Nematodes and Insects*. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, Copenhagen, Denmark, **101,** pp. 1–114.

BURMAN, M., ABRAHAMSSON, K., ASCARD, J., SJÖBERG, A. & ERIKSSON, B. (1986) Distribution of insect parasitic nematodes in Sweden, in *Proceedings of the Fourth International Colloquium of Invertebrate Pathology* (SAMSON, R.A., VLAK, J.M. & PETERS, D., Eds) Society of Invertebrate Pathology, Wageningen, the Netherlands, p. 312.

BURNELL, A.M., EHLERS, R.-U. & MASSON, J.P. (Eds) (1994) *COST 812 Biotechnology: Genetics of Entomopathogenic Nematode–Bacterium Complexes, Proceedings of Symposium and Workshop,* St Patrick's College, Maynooth, Co. Kildare, Ireland. European Commission, DG XII, Luxembourg.

CABANILLAS, H.E., POINAR, G.O., JR. & RAUSTON, J.R. (1994) *Steinernema riobravis* sp. nov. (Rhabditida: Steinernematidae) from Texas. *Fundamental and Applied Nematology* **17,** 123–131.

CHOO, H.Y., KAYA, H.K. & STOCK, S.P. (1996) Isolation of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Korea. *Japanese Journal of Nematology* (in press).

CURRAN, J. & DRIVER, F (1994) Molecular taxonomy of *Heterorhabditis*, in *COST 812 Biotechnology: Genetics of Entomopathogenic Nematode–Bacterium Complexes, Proceedings of Symposium and Workshop,* St Patrick's College, Maynooth, Co. Kildare, Ireland (BURNELL, A.M., EHLERS, R.U. & MASSON, J.P., Eds) European Commission, DG XII, Luxembourg, pp. 41–48.

CURRAN, J. & HENG, J. (1992) Comparison of three methods for estimating the number of entomopathogenic nematodes present in soil samples. *Journal of Nematology* **24,** 170–176.

CURRAN, J. & WEBSTER, J.M. (1989) Genotypic analysis of *Heterorhabditis* isolates from North Carolina, USA. *Journal of Nematology* **21,** 140–147.

DIX, I., BURNELL, A.M., GRIFFIN, C.T., JOYCE, S.A., NUGENT, M.J. & DOWNES, M.J. (1992) The identification of biological species in the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) by breeding second generation amphimictic adults. *Parasitology* **104,** 509–518.

DE DOUCET, M.M.A. (1986) A new species of *Neoaplectana* Steiner 1929 (Nematoda: Steinernematidae) from Cordoba, Argentina. *Revue de Nematology* **9,** 317–323.

DE DOUCET, M.M.A. & DOUCET, M.E. (1990) *Steinernema ritteri* n. sp. (Nematoda: Steinernematidae) with a key to the species of the genus. *Nematologica* **36,** 257–265.

DE DOUCET, M.M.A. & GABARRA, R. (1994) On the occurrence of *Steinernema glaseri* (Steiner, 1929) (Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Heterorhabditidae) in Catalogne, Spain. *Fundamental and Applied Nematology* **17,** 441–443.

EHLERS, R.-U. & PETERS, A. (1995) Entomopathogenic nematodes in biological control: feasibility, perspectives and possible risks, in *Biological Control: Benefits and Risks* (HOKKANEN, H.M.T. & LYNCH, J.M., Eds) Cambridge University Press, Cambridge, pp. 113–136.

EHLERS, R.-U., DESEÖ, K.V. & STACKEBRANDT, E. (1991) Identification of *Steinernema* spp. (Nematoda) and their symbiotic bacteria *Xenorhabdus* spp. from Italian and German soils. *Nematologica* **37,** 360–366.

FAN, X. (1989) Bionomics of British strains of entomopathogenic nematodes (Steinernematidae). PhD thesis, Imperial College, University of London, UK.

FARGETTE, M., BLOK, V.C., PHILLIPS, M.S. & TRUDGILL, D.L. (1994) Genetic variation in tropical *Meloidogyne* species, in *Advances in Molecular Plant Nematology* (LAMBERTI, F., DE GIORGI, C. & BIRD. D. MCK., Eds) Plenum Press, New York, NY, pp. 91–96.

FILIPJEV, I.N. (1934) Micellanea Nematologica 1. Eine neue Art der Gattung Neoaplectana Steiner nebst Bemerkungen über die systematische Stellung der letzteren. *Magazine de Parasitologie de l'Institut Zoologique de l'Academie de l'USSR* **4,** 229–239.

GARDNER, S.L., STOCK, S.P. & KAYA, H.K. (1994) A new species of *Heterorhabditis* from the Hawaiian islands. *Journal of Parasitology* **80,** 100–106.

GEORGIS, R. & HAGUE, N.G.M. (1981) A neoaplectanid nematode in the larch sawfly *Cephalcia lariciphila* (Hymenoptera: Pamphiliidae). *Annals of Applied Biology* **99,** 171–177.

GEORGIS, R. & HAGUE, N.G.M. (1988) Field evaluation of *Steinernema feltiae* against the web-spinning larch sawfly *Cephalcia lariciphila. Journal of Nematology* **20,** 213–320.

GLAZER, I., LIRAN, N., POINAR, G.O. JR. & SMITS, P.H. (1993) Identification and biological activity of newly isolated heterorhabditid populations from Israel. *Fundamental and Applied Nematology* **16,** 467–472.

GOUGE, D.H. & HAGUE, N.G.M. (1995) The susceptibility of sciarid flies to entomopathogenic nematodes. *Journal of Helminthology* **69,** 313–318.

GRIFFIN, C.T., DOWNES, M.J. & BLOCK, W. (1990) Tests of Antarctic soils for insect parasitic nematodes. *Antarctic Science* **2,** 221–222.

GRIFFIN, C.T., MOORE, J.F. & DOWNES, M.J. (1991) Occurrence of insect-parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. *Nematalogica* **37,** 92–100.

GRIFFIN, C.T., JOYCE, S.A., DIX, I., BURNELL, A.M. & DOWNES, M.J. (1994) 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. *Fundamental and Applied Nematology* **17,** 245–254.

GWYNN, R.L. (1993) Development of cold active nematodes for insect control. PhD thesis, University of Reading, UK.

HARA, A.H., GAUGLER, R., KAYA, H.K. & LEBECK, L.M. (1991) Natural populations of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) from the Hawaiian islands. *Environmental Entomology* **20,** 211–216.

HAUKELAND, S. (1993) Entomopathogenic nematodes found in Norway. *Norwegian Journal of Agricultural Sciences* **7,** 17–27.

HOMINICK, W.M. & BRISCOE, B.R. (1990a) Survey of 15 sites over 28 months for entomopathogenic nematodes (Rhabditida: Steinernematidae). *Parasitology* **100,** 289–294.

HOMINICK, W.M. & BRISCOE, B.R. (1990b) Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. *Parasitology* **100,** 295–302.

HOMINICK, W.M. & REID, A.P. (1990) Perspectives on entomopathogenic nematology, in *Entomopathogenic Nematodes in Biological Control* (GAUGLER, R. & KAYA, H.K., Eds) CRC Press, Boca Raton, FL, 327–345.

HOMINICK, W.M., REID, A.P. & BRISCOE, B.R. (1995) Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes isolated during soil surveys of the UK and the Netherlands. *Journal of Helminthology* **69,** 27–32.

INTERNATIONAL INSTITUTE OF BIOLOGICAL CONTROL (1994) *Using Biodiversity to Protect Biodiversity. Biological Control, Conservation and the Biodiversity Convention*. CAB International, Wallingford, p. 14.

JACKSON, G.J. (1965) Differentiation of three species of *Neoaplectana* (Nematoda: Rhabditida), grown axenically. *Parasitology* **55,** 571–578.

JOYCE, S.A., BURNELL, A.M. & POWERS, T.O. (1994a) Characterisation of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. *Journal of Nematology* **26,** 260–270.

JOYCE, S.A., REID, A., DRIVER, F. & CURRAN, J. (1994b) Application of polymerase chain reaction (PCR) methods to the identification of entomopathogenic nematodes, in *COST 812 Biotechnology: Genetics of Entomopathogenic Nematode–Bacterium complexes, Proceedings of Symposium and Workshop,* St Patrick's College, Maynooth, Co. Kildare, Ireland (BURNELL, A.M., EHLERS, R.-U. & MASSON, J.P., Eds) European Commission, DG XII, Luxembourg, pp. 178–187.

KAYA, H.K. & GAUGLER, R. (1993) Entomopathogenic nematodes. *Annual Review of Entomology* **38,** 181–206.

KOZODOI, E.M. (1984) A new entomophagous nematode *Neoaplectana anomali* sp. n. (Rhabditida: Steinernematidae) and its biology. *Zoologicheskii Zhurnal* **63,** 1605–1909.

LI, X.F. & WANG, G.H. (1989) Preliminary investigation on the distribution of Steinernematidae and Heterorhabditidae in Fujian, Guangdong and Hainan. *Chinese Journal of Zoology* **24,** 1–4.

LUI, J. (1992) Taxonomic study of the genus: *Steinernema* Travassos and *Heterorhabditis* Poinar, in *Proceedings of the XIX International Congress of Entomology*, Beijing, China, p. 318.

MAMIYA, Y. (1988) *Steinernema kushidai* n. sp. (Nematoda: Steinernematidae) associated with scarabaeid beetle larvae from Shizuoka, Japan. *Applied Entomology and Zoology* **23,** 313–320.

MRÁČEK, Z. (1980) The use of '*Galleria* traps' for obtaining nematode parasites of insects in Czechoslovakia (Lepidoptera: Nematoda, Steinernematidae). *Acta Entomologica Bohemoslovaca* **77,** 378–382.

MRÁČEK, Z. (1994) *Steinernema kraussei* (Steiner 1923) (Nematoda: Rhabditida: Steinernematidae): redescription of its topotype from Westphalia. *Folia Parasitologica* **41,** 59–61.

MRÁČEK, Z. & JENSEN, G. (1988) First report of emomogenous nematodes of the families *Steinernematidae* and *Heterorhabditidae* from Hungary. *Acta Phytopathologica et Entomologica Hungarica* **23,** 153–156.

MRÁČEK, Z. & WEBSTER, J.M. (1993) Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in western Canada. *Journal of Nematology* **25,** 710–717.

MRÁČEK, Z., GUT, J. & GERDIN, S. (1982) *Neoaplectana bibionis* Bovien 1937, an obligate parasite of insects isolated from forest soil in Czechoslovakia. *Folia Parasitologica* **29,** 139–145.

MRÁČEK, Z., HERNANDEZ, E.A. & BOEMARE, N.E. (1994) *Steinernema cubana* sp. n. (Nematoda: Rhabditida: Steinernematidae) and the preliminary characterisation of its associated bacterium. *Journal of Invertebrate Pathology* **64,** 123–129.

MRÁČEK, Z., WEISER, J., BURES, M. & KAHOUNOVA, L. (1992) *Steinernema kraussei* (Steiner, 1923) (Nematoda: Rhabditida)—rediscovery of its type locality in Germany. *Folia Parasitologica* **39,** 181–199.

NGUYEN, K.B. & SMART, G.C., JR. (1990) *Steinernema scapterisci* n. sp. (Rhabditida: Steinernematidae). *Journal of Nematology* **23,** 187–199.

NGUYEN, K.B. & SMART, G.C., JR. (1992) *Steinernema neocurtillis* n. sp. (Rhabditida: Steinernematidae) and a key to the genus *Steinernema. Journal of Nematology* **24,** 463–477.

NGUYEN, K.B. & SMART, G.C., JR. (1994) *Neosteinernema longicurvicauda* n. gen., n. sp. (Rhabditia: Steinernematidae), a parasite of the termite *Reticulitermes flavipes* (Koller). *Journal of Nematology* **26,** 162–174.

OPPERMAN, C.H., DONG, K. & CHANG, S. (1994) Genetic analysis of the soybean–*Heterodera glycines* interaction, in *Advances in Molecular Plant Nematology* (LAMBERTI, F., DE GIORGI, C. & BIRD. D. MCK., Eds) Plenum Press, New York, NY, pp. 65–67.

PARKMAN, J.P. & SMART, G.C. (1996) Entomopathogenic nematodes, a case study: introduction of *Steinernema scapterisci* in Florida. *Biocontrol Science and Technology* **6,** 413–419.

PETERS, A. (1996) The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Science and Technology* **6,** 389–402.

PIZANO, M.A., AGUILERA, M.M., MONTEIRO, A.R. & FERROY, L.C. (1985) Incidenciade *Neoaplectana glaseri* Steiner, 1929 (Nematoda: Steinernematidae) parasitando ovo de *Mygdolus frynus* (Westwood 1863) (Col: Cerambycidae), in *Proceedings of the Ninth Meeting of the Society of Brasilian Nematology*, Piracicaba, Brazil, pp. 9–10.

POINAR, G.O., JR (1976) Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen. n. sp. (Rhabditida: Heterorhabditidae N. Fam.). *Nematologica* **21,** 463–470.

POINAR, G.O., JR (1985) *Neoaplectana intermedia* n. sp. (Steinernematidae: Nematoda) from South Carolina. *Revue de Nematologie* **8,** 321–327.

POINAR, G.O., JR (1986) Recognition of *Neoaplectana* species (Steinernematidae: Rhabditida). *Proceedings of the Helminthological Society of Washington* **53,** 121–129.

POINAR, G.O., JR (1988) Redescription of *Neoaplectana affinis* Bovien (Rhabditida: Steinernematidae). *Revue de Nematologie* **11,** 143–147.

POINAR, G.O., JR (1990) Taxonomy and biology of Steinernematidae and Heterorhabditidae, in *Entomopathogenic Nematodes in Biological Control* (GAUGLER, R. & KAYA, H.K., Eds), CRC Press, Boca Raton, FL, pp. 23–61.

POINAR, G.O., JR (1992) *Steinernema feltiae* (Steinernematidae: Rhabditidae) parasitizing adult fungus gnats (Mycetophilidae: Diptera) in California. *Fundamental and Applied Nematology* **15,** 427–430.

POINAR, G.O., JR (1993) Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema. Fundamental and Applied Nematology* **16,** 332–338.

POINAR, G.O., JR & GEORGIS, R. (1990) Description and field application of the HP88 strain of *Heterorhabditis bacteriophora. Revue de Nematologie* **13,** 387–393.

POINAR, G.O., JR & KOZODOI, E.M. (1988) *Neoaplectana glaseri* and *N. anomali:* sibling species or parallelism? *Revue de Nematologie* **11,** 13–19.

POINAR, G.O., JR & LINDHARDT, K. (1971) The re-isolation of *Neoaplectana bibionis* Bovien (Nematoda) from Danish Bibionids (Diptera) and their possible use as biological control agents. *Entomologica Scandinavica* **2,** 301–303.

POINAR, G.O., JR, JACKSON, T. & KLEIN, M. (1987) *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida), parasitic in the Japanese beetle, *Popillia japonica* (Scarabidae: Coleoptera), in Ohio. *Proceedings of the Helminthological Society of Washington* **53,** 53–59.

POINAR, G.O., JR, KARUNAKAR, G.K. & DAVID, H. (1992) *Heterorhabditis indicus* n. sp. (Rhabditida: Nematoda) from India: separation of *Heterorhabditis* spp. by infective juveniles. *Fundamental and Applied Nematology* **15,** 467–472.

POLIS, G.A. & HURD, S.D. (1995) Extraordinarily high spider densities on islands: flow of energy from marine to terrestrial food webs and the absence of predation. *Proceedings of the National Academy of Sciences* **92,** 4382–4386.

POWERS, T.O., & HARRIS, T.S. (1993) A polymerase chain reaction method for the identification of five major *Meliodogyne* species. *Journal of Nematology* **25,** 1–6.

REID, A.P. (1994) Molecular taxonomy of *Steinernema*, in *COST 812 Biotechnology: Genetics of Entomopathogenic Nematode–Bacterium Complexes, Proceedings of Symposium and Workshop,* St Patrick's College, Mamooth, Co. Kildare, Ireland (BURNELL, A.M., EHLERS, R.-U. & MASSON, J.P., Eds) European Commission, DG XII, Luxembourg, pp. 49–58.

REID, A.P. & HOMINICK, W.M. (1993) Cloning of the rDNA repeat unit from a British entomopathogenic nematode (Steinernematidae) and its potential for species identification. *Parasitology* **107,** 529–536.

SANDNER, H. & BEDNAREK, A. (1987) Entomophilic nematodes in the Swietokrzyski National Park. *Fragmenta Faunistica* **31,** 3–9.

SHEN, C.P. (1992) Description of a entomopathogenic nematode, *Steinernema longicaudum* sp. nov. and its application, in *Proceedings of the 19th International Congress of Entomology*, Beijing, China, pp. 220–231.

SMITS, P.H., GROENEN, J.T.M. & DERAAY, G. (1991) Characterisation of *Heterorhabditis* isolates using DNA restriction fragment length polymorphisms. *Revue de Nematologie* **14,** 445–453.

STEINER, G. (1923) *Aplectana kraussei* n. sp., eine in der Blattwespe *Lyda* sp. parasitierende Nemodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektions, Krankheiten und Hygiene, Abteilung II* **59,** 14–18.

STEINER, G. (1929) *Neoaplectana glaseri* n. g. n. sp. (Oxyuridae), a new nemic parasite of the Japanese beetle (*Popillia japonica*, NEWM). *Journal of the Washington Academy of Science* **19,** 436–440.

STEINER, W.A. (1994) Distribution of entomopathogenic nematodes in the Swiss Alps, in *COST 812 Biotechnology: Genetics of Entomopathogenic Nematode–Bacterium Complexes, Proceedings of Symposium and Workshop,* St Patrick's College, Maynooth, Co. Kildare, Ireland (BURNELL, A.M., EHLERS, R.-U. & MASSON, J.P., Eds) European Commission, DG XII, Luxembourg, p. 218.

STOCK, S.P. (1992) Presence of *Steinernema scapterisci* Nguyen & Smart parasitizing the mole cricket *Scapteriscus borellii* in Argentina. *Nematologia Mediterranea* **20,** 163–165.

STOCK, S.P. (1993a) Description of an Argentinian strain of *Steinernema feltiae* (Filipjev, 1934) (Nematoda: Steinernematidae). *Nematologia Mediterranea* **21,** 279–283.

STOCK, S.P. (1993b) A new species of the genus *Heterorhabditis* Poinar, 1976 (Nematoda: Heterorhabditidae) parasitizing *Graphognathus* sp. larvae (Coleoptera: Curculionidae) from Argentina. *Research and Reviews in Parasitology* **53**, 103–107.

STUART, R.J. & GAUGLER, R. (1994) Patchiness in populations of entomopathogenic nematodes. *Journal of Invertebrate Pathology* **64,** 39–45.

TALLOSI, B., PETERS, A. & EHLERS, R.-U. (1995) *Steinernema bicornutum* sp. n. (Rhabditida: Nematoda) from Vojvodina, Yugoslavia. *Russian Journal of Nematology,* **3,** 71–80.

VÄNNINEN, I., HUSBERG, G.-B. & HOKKANEN, M.T. (1989) Occurrence of entomopathogenic fungi and entomoparasitic nematodes in cultivated soils in Finland. *Acta Entomologica Fennica* **53,** 65–71.

VRAIN, T.C., WAKARCHUK, D.A., LEVESQUE, A.C. & HAMILTON, R.I. (1992) Intraspecific rDNA restriction fragment length polymorphisms in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15,** 563–574.

WALTHER, B.A., COTGREAVE, P., PRICE, R.D., GREGORY, R.D. & CLAYTON, D.H. (1995) Sampling effort and parasite richness. *Parasitology Today* **11,** 306–310.

WEISER, J. (1955) *Neoaplectana carpocapsae* n. sp. (Anguillulata, Steinernematidae) novy cizopasnik housenik obalece jablecného, *Carpocapsa pomonella* L. *Vestnik Ceskoslovenské Zoologické Spolecnosti* **19,** 44–52.

WELSH, J. & MCCLELLAND, M. (1990) Fingerprinting genomes with PCR using arbitrary primers. *Nucleic Acids Research* **18,** 7213 –7218.

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFOLSKI, J.A. & TINGEY, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18,** 6351–6535.

XU, Z., WANG, G. & LI, X. (1991) A new species of the genus *Steinernema* (Rhabditida: Steinernematidae). *Zoological Research* **12,** 17–20.