

## *Heterorhabditis*, *Steinernema* and their bacterial symbionts — lethal pathogens of insects

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**Summary** – The entomopathogenic nematodes (EPN) *Heterorhabditis* and *Steinernema* together with their symbiont bacteria *Photorhabdus* and *Xenorhabdus*, respectively, are obligate and lethal parasites of insects. EPN can provide effective biological control of some important lepidopteran, dipteran and coleopteran pests of commercial crops and they are amenable to large-scale culture in liquid fermentors. They are unique among rhabditids in having a symbiotic relationship with an enteric bacterium species. The bacterial symbiont is required to kill the insect host and to digest the host tissues, thereby providing suitable nutrient conditions for nematode growth and development. This review describes the general biology of EPN and their symbionts and gives an overview of studies to date on EPN biodiversity, biogeography and phylogeny. The impetus for research in EPN and their symbionts has come about because of their biological control potential, with much of the focus in EPN research having been on applied aspects relating to pest control. However EPN and their symbionts are increasingly being viewed as exciting subjects for basic research in the areas of ecology, biodiversity, evolution, biochemistry, symbiosis and molecular genetics. Much progress has been made over the past 20 years in our understanding of the basic biology and genetics of EPN and their symbionts. We are now entering a new phase in which the tools of molecular genetics are being increasingly used to address a range of biological questions in EPN research. The knowledge gained from this endeavour should ensure that EPN will become even more effective biopesticides and should also ensure that EPN and their symbionts gain prominence as unique and intrinsically interesting biological systems.

**Résumé** – *Heterorhabditis*, *Steinernema* et leurs symbiotes bactériens — *Pathogènes mortels des insectes* – Les nématodes entomopathogènes (EPN) *Heterorhabditis* et *Steinernema*, avec leur bactéries symbiotes *Photorhabdus* et *Xenorhabdus*, respectivement, sont des parasites obligés et mortels des insectes. Les EPN peuvent servir à un contrôle biologique de quelques lépidoptères, diptères et coléoptères importants pour les cultures commerciales et ils sont élevables à grande échelle dans des fermenteurs liquides. Ils sont uniques chez les rhabditides par leur relation symbiotique avec une espèce de bactérie entérique. La bactérie symbiote est nécessaire pour tuer l'insecte hôte et pour digérer les tissus de l'hôte, permettant ainsi des conditions de nutrition favorables à la croissance et au développement du nématode. La présente revue décrit la biologie générale des EPN et de leur symbiotes et donne un état des études actuelles sur la biodiversité, la biogéographie et la phylogénie des EPN. L'impulsion donnée aux recherches sur les EPN et leur symbiotes provient de leur potentialités pour le contrôle biologique, une grande partie des recherches sur les EPN ayant trait à des aspects appliqués en relation avec ce contrôle des parasites. Cependant, les EPN et leur symbiotes bactériens sont de plus en plus considérés comme des sujets intéressants pour la recherche fondamentale dans les domaines de l'écologie, de la biodiversité, de l'évolution, de la biochimie, des processus symbiotiques et de la génétique moléculaire. De nombreux progrès ont été réalisés ces 20 dernières années dans la compréhension de la biologie et de la génétique des EPN et de leur symbiotes. Nous entrons actuellement dans une nouvelle phase où les moyens de la biologie moléculaire sont utilisés de manière croissante pour formuler une série de questions biologiques pour la recherche sur les EPN. Les connaissances résultant de ces efforts doivent conduire à vérifier que les EPN deviendront des biopesticides toujours plus efficaces et que les EPN et leur symbiotes prendront de l'importance en tant que systèmes biologiques uniques et intrinsèquement intéressants.

**Keywords** – biogeography, entomopathogenic nematode, habitat preference, *Photorhabdus*, phylogeny, symbiosis, *Xenorhabdus*.

There are many genera of nematodes that parasitise insects (reviewed by Poinar, 1979), however research on in-

sect parasitic nematodes is largely concentrated at present on two families of rhabditid nematodes: the Steinerner-

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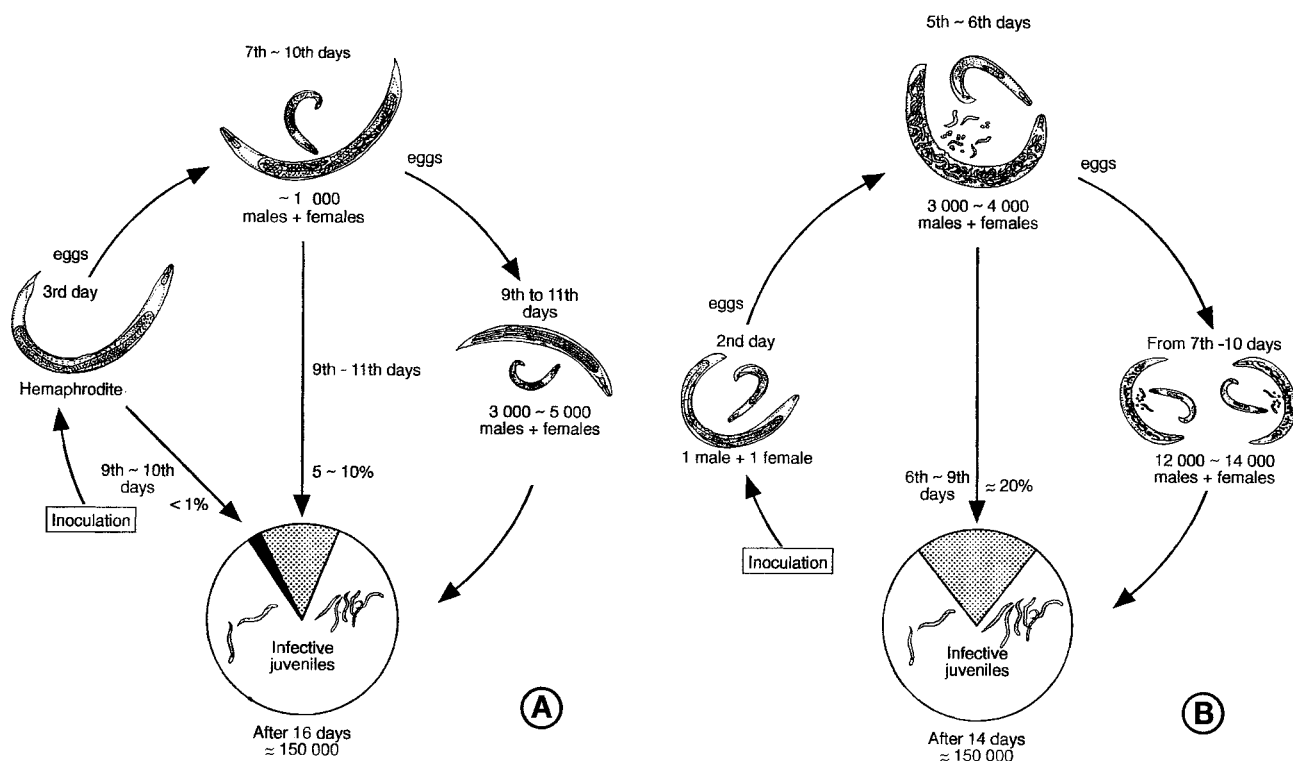
matidae Chitwood & Chitwood, 1937 and the Heterorhabditidae Poinar, 1976. These soil-dwelling nematodes are obligate and lethal parasites of insects and are usually referred to as entomopathogenic nematodes (EPN). EPN can provide effective biological control of some important lepidopteran, dipteran and coleopteran pests of commercial crops and they are amenable to large-scale culture in liquid fermentors. EPN are unique among rhabditids in having a symbiotic relationship with an enteric bacterium species. The bacterial symbiont is required to kill the insect host and to digest the host tissues, thereby providing suitable nutrient conditions for nematode growth and development. *Heterorhabditis* and *Steinernema* species have a global distribution (reviewed by Hominick *et al.*, 1996). Species in these genera exhibit differences in host range, infectivity, environmental tolerances and in suitability for commercial production and formulation and this has stimulated many surveys, seeking new strains and species of EPN for biocontrol applications. Thus the number of research publications on this group of nematodes has increased dramatically in recent years and a large number of laboratories world-wide are currently engaged in EPN research.

### General biology and life cycle

The third stage dauer juvenile (DJ) occurs free in the soil and its role is to seek out and infect an insect larva. *Steinernema* gains entry to the insect larva through natural openings (mouth, anus and spiracles). In addition to these modes of entry, *Heterorhabditis* also gains entry by abrading the intersegmental membranes of the insect using a dorsal tooth. Once in the haemocoel of the insect the DJ releases cells of a symbiont bacterium that it carries in its intestine. The insect haemolymph provides rich medium for the bacterial cells and these begin to grow, release toxins and exoenzymes and kill the insect. The insect dies rapidly, usually within 24–48 h. The nematodes resume development, moult to the J4 stage and reach adulthood within 2 (*S. carpocapsae*) or 3 (*H. bacteriophora*) days when cultured *in vivo* in larvae of the greater wax moth *Galleria mellonella* at 23°C (Wang & Bedding, 1996). Nematode reproduction continues over two to three generations until the nutrient status of the cadaver deteriorates whereupon adult development is suppressed and DJ accumulate. These non-feeding infective stages emerge into the soil where they may survive for several months in the absence of a suitable host.

In *Steinernema* reproduction is amphimictic. Steinerematid DJ mature to become either a male or a female and sex determination appears to be of the XX/XO type, typical of nematodes (Dix *et al.*, 1994). In *Heterorhabditis* by contrast, the DJ mature to give first generation hermaphrodite females, but these females give rise to a second generation of amphimictic males and females and to self fertile hermaphrodite females and DJ (Dix *et al.*, 1992; Strauch *et al.*, 1994). The male and female karyotypes of *Heterorhabditis* have not yet been determined, but available data indicate that sexual phenotype is environmentally determined. Wang and Bedding (1996) studied the dynamics of population development of *H. bacteriophora* and *S. carpocapsae* in larvae of *G. mellonella*, after injection into the insect haemocoel of one or two DJ, respectively. Under these conditions three adult generations were produced by both nematode species (Fig. 1). Individual *H. bacteriophora* hermaphrodites laid up to 1000 eggs which developed into second generation males and females, but the first generation hermaphrodites also retained about 500 eggs which developed into DJ *via endotokia matricida*. Second generation females also laid *ca* six to ten eggs which developed into another generation of adults, but they also retained another 30 eggs within the nematode body which developed into DJ *via endotokia matricida*. The third generation females did not oviposit and all of their eggs (*ca* 50 per female) developed *via endotokia matricida* into DJ. First and second generation *S. carpocapsae* were found to lay a larger proportion of their eggs than do *H. bacteriophora*, but all the eggs produced by third generation females developed *via endotokia matricida*. Unlike *H. bacteriophora*, the juvenile stages resulting from *endotokia matricida* in *S. carpocapsae* did not develop into dauer juveniles until they had exited from the body of the mother nematode.

In favourable nutritive conditions in liquid culture second generation *Heterorhabditis* DJ recover and develop to hermaphrodites (Strauch *et al.*, 1994; Johnigk & Ehlers, 1999). Strauch *et al.* (1994) have also shown that when J1 juveniles were starved for 24 h in Ringer solution 40% became hermaphrodites, 6.6% became amphimictic adults and 53% became DJ. Of the J1 that developed into hermaphrodites 90% had gone through a pre-dauer J2 stage, which was morphologically distinct from those J2 growing into amphimictic adults, and 10% were recovered DJ. These data clearly show the importance of nutritional signals in *Heterorhabditis* sex determination. The extent to which second generation DJ recover *in vivo* and resume development has not been determined, nor has the pro-



**Fig. 1.** Population dynamics of *A*: *Heterorhabditis bacteriophora* and *B*: *Steinernema carpocapsae* A24 in a larva of *Galleria mellonella* after injecting one or two DJ per insect respectively. The pie charts represent the number of DJ progeny recruited from each generation. (■): DJ progeny recruited from first generation females; (▨): DJ progeny recruited from second generation females; (□): DJ progeny recruited from third generation females (from Wang & Bedding, 1996).

portion of second generation juveniles which enter the J2 stage; however Dix *et al.* (1992) have shown that the early second generation adults which develop in *G. mellonella* are all amphimictic.

### The symbiotic association

Rhabditid parasites of both vertebrates and invertebrates are considered to have evolved from free living bacterial feeding nematodes (Adamson, 1986). Some rhabditid nematodes have an association with soil invertebrates for which Sudhaus and Schulte (1988) have introduced the term necromency. The DJ of necromenic nematodes enter their host by the body openings or are ingested by the host. The DJ remain quiescent in the host until it eventually dies and its body becomes invaded by saprophytic bacteria. Then the nematode DJ resumes development and growth and reproduction occurs based on the bacteria associated with the decaying cadaver. Sudhaus (1993) has suggested that *Heterorhabditis* and *Steinernema* most

probably evolved from necromenic nematodes which developed a symbiotic association with an entomopathogenic bacterium. Such a symbiosis specialised for parasitising animals has not been described so far for any other group of nematodes. However the nutritional interactions between EPN and their symbiont bacteria bear many similarities to the ectosymbioses between insects and filamentous fungi (Wilkinson & Hay, 1997). Symbionts associated with *Steinernema* are placed in the genus *Xenorhabdus* (Thomas & Poinar, 1979) while the bioluminescent symbionts associated with *Heterorhabditis* are placed in the genus *Photorhabdus* (Boemare *et al.*, 1993). Symbiont bacteria of both genera are motile and gram-negative and belong to the Enterobacteriaceae. Comparisons of 16S rDNA sequences show that species of *Photorhabdus* and *Xenorhabdus* form a phylogenetically coherent cluster that diverged early from the main line of radiation of the Enterobacteriaceae (Forst *et al.*, 1997).

When symbiont bacteria are released by the nematode into the insect haemolymph the bacterial cells begin to

grow and death of the insect ensues, either from toxæmia or from septicæmia, depending on the sensitivity of the insect and the symbiont strain (Forst *et al.*, 1997; Boemare & Givaudan, 1998). Some strains of *Xenorhabdus* and *Photorhabdus* are highly virulent: injection of less than ten cells of the bacterium into the haemocoel may be sufficient to kill a susceptible insect such as *G. mellonella* or *Manduca sexta* (Poinar & Thomas, 1967; Forst *et al.*, 1997, French-Constant & Bowen, 1999). When cultured in liquid medium, both genera of symbiont bacteria secrete highly virulent insecticidal toxins into the medium (Jarrett *et al.*, 1997; Bowen *et al.*, 1998). As the bacteria enter the stationary phase of their growth cycle they secrete lipase(s), protease(s) and several broad spectrum antibacterial and antifungal antibiotics (reviewed by Akhurst & Boemare, 1990; Forst & Nealon, 1996). The likely role for the degradative enzymes is to break down the insect tissues thereby providing a rich food supply for the developing nematode. The insect cadaver containing the rapidly expanding population of nematodes and bacteria retains its shape and does not putrefy, implying a role for the antibiotics produced by symbiont bacteria. However, Jaroz (1996) found relatively low levels of antibiotics in cadavers of *G. mellonella* infected with *S. carpocapsae* and he postulated that the lack of contamination of insect cadavers resulted from the ability of the symbiont bacteria to out-compete many of the normal gut microflora of the insect host.

Since the majority of EPN bacterial complexes are effective over a wide range of insect orders and the type of humoral and cellular defence reactions of the hosts varies significantly over that range (Akhurst, 1993), it is likely that both the nematodes and the bacteria utilise a variety of pathogenic strategies. Although nematode virulence strategies have received less attention than those of the bacterium, it is known that DJ of *S. carpocapsae* and *H. bacteriophora* release protease secretions which destroy the antibacterial factors of vaccinated *G. mellonella* larvae (Götz *et al.*, 1980; Simoes, 1998). The importance of the symbiotic interaction in the pathogenesis process is clearly seen in the *S. glaseri*/*X. poinarii* complex. When *G. mellonella* larvae were injected with either axenic *S. glaseri* or with 1150 cells of *X. poinarii*, the insect larvae survived. However co-injection of 115 *X. poinarii* cells and one *S. glaseri* DJ killed 75% of the insect larvae (Akhurst, 1986).

Both *Xenorhabdus* and *Photorhabdus* occur in two phenotypic forms. Phase I cells are larger than phase II cells and produce significantly greater amounts of exoenzymes,

toxins, antibiotics than phase II forms. However the nematode DJ package and transport only phase I cells. Phase I cells are stored in a special vesicle in the anterior of the intestine in steinernematids (Bird & Akhurst, 1983) while *Heterorhabditis* does not have a special vesicle but stores the cells of the symbiont in the anterior of the intestine (Endo & Nickle, 1991). The role of phase II cells in the symbiotic association is still unclear, as are the molecular mechanisms responsible for this phenomenon.

There are no reports of the isolation of *Xenorhabdus* and *Photorhabdus* from soil and it has been generally assumed that these bacteria cannot exist in the soil environment in the absence of their nematode associates. Morgan *et al.* (1997) released genetically marked strains of *X. nematophila* and *P. luminescens* into non-sterile soil microcosms and they found that the released cells declined to below detection limits within seven days. Although viable colony forming units could not be detected after 7 days, measurement of adenosine triphosphate (ATP) levels suggested that the cells may have entered into a dormant, non-culturable but viable phase. Bleakley and Chen (1999) reported that *P. luminescens* was able to survive and grow over a 30 day period when inoculated into sterilised soil to which nutrient amendments had been added.

## Taxonomic status

The family Steinernematidae Chitwood & Chitwood, 1937 is currently composed of two genera: *Steinernema* Travassos, 1927 and *Neosteinernema* Nguyen & Smart, 1994. The former genus with 25 species described and the latter with only one species: *N. longicurvicauda* (Table 1). The family Heterorhabditidae Poinar, comprises only one genus, *Heterorhabditis* Poinar, 1976 with *H. bacteriophora* as the type species and eight other species described (Table 2): however, the taxonomic status of some of these species has been questioned (Adams *et al.*, 1998).

EPN species have mainly been described using the Linnean and biological species concepts and morphological/morphometric criteria and cross-breeding tests have been the most frequently used tools for their identification (Poinar, 1990; Dix *et al.*, 1994; Nguyen & Smart, 1996; Kaya & Stock, 1997). Additionally, a number of molecular techniques, including isoenzyme patterns (Akhurst, 1987), total protein patterns (Poinar & Kozodoi, 1988; Joyce *et al.*, 1994a), RFLP analysis (Curran & Webster, 1989; Reid & Hominick, 1993; Joyce *et al.*, 1994b), RAPDs (Gardner *et al.*, 1994; Liu & Berry, 1995), satellite DNA (Grenier *et al.*, 1996), genomic DNA sequenc-

**Table 1.** *The genera and species of the family Steinernematidae.*


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Family Steinernematidae Chitwood & Chitwood, 1937  
= Neoplectanidae Sbolev, 1953

Type genus: *Steinernema* Travassos, 1927

Type species: *Steinernema kraussei* (Steiner, 1923) Travassos, 1927

Other species:

*S. abbasi* Elawad, Ahmad & Reid, 1997  
*S. arenarium* (Artyukhovskiy, 1967) Wouts, Mráček, Gerdin & Bedding, 1982  
*S. affine* (Bovien, 1937) Wouts, Mráček, Gerdin & Bedding, 1982  
*S. bicornutum* Tallosi, Peters & Ehlers, 1995  
*S. carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982  
*S. caudatum* Xu, Wang & Li, 1991  
*S. ceratophorum* Jian, Reid and Hunt, 1997  
*S. cubanum* Mráček, Hernandez & Boemare, 1994  
*S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982  
*S. glaseri* (Steiner, 1929) Wouts, Mráček, Gerdin & Bedding, 1982  
*S. intermedium* (Poinar, 1985) Mamiya, 1988  
*S. kariii* Waturu, Hunt & Reid, 1997  
*S. kushidai* Mamiya, 1988  
*S. longicaudum* Shen & Wang, 1992  
*S. monticolum* Stock, Choo & Kaya, 1992  
*S. neocurtillae* Nguyen & Smart, 1992  
*S. oregonense* Liu & Berry, 1996  
*S. puertoricense* Roman & Figueroa, 1994  
*S. rarum* (Doucet, 1986) Mamiya, 1988  
*S. riobrave* Cabanillas, Poinar & Raulston, 1994  
*S. ritteri* de Doucet & Doucet, 1992  
*S. scapterisci* Nguyen & Smart, 1992  
*S. siamkayai* Stock, Somsook & Kaya, 1998

Genus: *Neosteinerema* Nguyen & Smart, 1994

Type and only species: *Neosteinerema longicurvicauda* Nguyen & Smart, 1994

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ing (Liu *et al.*, 1997; Adams *et al.*, 1998), have been used, not only as diagnostic tools, but also to study phylogenetic affinities among EPN. Recently, Adams (1998) proposed that the most suitable species concept for use in nematology is an amalgamation of the phylogenetic and the evolutionary species concepts. Using *Heterorhabditis* taxa as an example, he delimited species of this genus based on this evolution-based approach.

**Table 2.** *The genera and species of the family Heterorhabditidae.*


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Family Heterorhabditidae Poinar, 1976

Type and only genus: *Heterorhabditis* Poinar, 1976

Genus *Heterorhabditis* Poinar, 1976  
= *Chromonema* Khan, Brooks & Hirschman, 1976

Type species: *Heterorhabditis bacteriophora* Poinar, 1976  
= *Chromonema heliothidis* Khan, Brooks & Hirschman, 1976  
= *H. heliothidis* (Khan, Brooks & Hirschman, 1976) Poinar, Thomas & Hess, 1977

Other species:

*H. argentinensis* Stock, 1993  
*H. brevicaudis* Liu, 1994  
*H. hawaiiensis* Gardner, Stock & Kaya, 1994  
*H. indica* Poinar, Karunakar & David, 1992  
*H. marelatus* Liu & Berry, 1996  
= *H. hepialius* Stock, Strong & Gardner, 1996  
*H. megidis* Poinar, Jackson & Klein, 1988  
*H. poinari* Kakulia & Mikaia, 1997  
*H. zealandica* Poinar, 1990

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## EPN biodiversity and biogeography

Hominick *et al.* (1996) provided a list with the geographic distribution of described EPN species at both continental and national level. Steinernematids have been recorded from all continents except Antarctica (Griffin *et al.*, 1990). Within the genus *Steinernema* two species, *Steinernema carpocapsae* and *Steinernema feltiae*, appear to have a global distribution (Hominick *et al.*, 1996). The other *Steinernema* species seem to have a more restricted geographic distribution and their occurrence has been recorded only at the continental or national level (Hominick *et al.*, 1996). However, as more surveys are performed, the known range of many species is expected to expand. For instance, *Steinernema kraussei* originally isolated in the Gegggen Mountains, Westphalia, Germany (Steiner, 1923) has subsequently been isolated from other locations in Germany (Mráček *et al.*, 1992; Mráček, 1994), and also from other European countries, such as the Czech Republic, (Mráček, 1977), the Netherlands (Hominick *et al.*, 1995), Switzerland (Steiner, 1994), the United Kingdom (Hominick *et al.*, 1995), and Spain (García del Pino & Palomo, 1996), suggesting this species has a Palearctic distribution. However, the known geographic range of this species has recently been expanded to include North America (Stock *et al.*, 1999b) thus indicating a Holarctic distribution. Similarly, *S. longicaudum*, originally isolated in China, has

recently been recovered in Korea and Western USA, indicating a wider geographic range for this species (Stock *et al.*, unpubl.).

The situation is somewhat different for heterorhabditids, particularly because fewer species have been described. For instance, *H. bacteriophora* is currently the widest geographically distributed heterorhabditid, found in all Americas, Southern and Central Europe, Australia and East Asia (China, Japan, Korea). *H. indica* also has a wide distribution, occurring in the tropics and subtropics, found in southern India, Sri Lanka, peninsular Malaysia, Indonesia, North Australia, the Caribbean region, Egypt, Kenya and in subtropical and warm temperate zones in Japan. In contrast, *H. zealandica*, and *H. marelatus* appear to be species with a more restricted geographic distribution having been found only, respectively, in New Zealand (Akhurst, 1987) and in Oregon and California, USA (Liu & Berry, 1996a; Stock *et al.*, 1997). The summary presented in Tables 1 and 2 indicates that the diversity of steinernematids is greater than that of heterorhabditids. This is also reflected in the DNA relatedness studies discussed in the next section.

Although a number of surveys have documented habitat preference of EPN, there are at present insufficient and contradictory data to test for correlations (Hominick *et al.*, 1996). However, several authors (Steiner, 1994; Hominick *et al.*, 1995; Stock *et al.*, 1999; Sturhan, 1999) have observed that some *Steinernema* species are associated with specific habitat types. For example, *S. feltiae*, *S. affine* and *S. intermedium* have been found mainly in grassland ecosystems (Boag *et al.*, 1992; Hominick *et al.*, 1995; Stock *et al.*, 1999). Other *Steinernema* species, by contrast, seem to have a wider habitat range. For instance, *S. kraussei* has been found in coniferous and deciduous forests (Mráček *et al.*, 1999; Steiner, 1994; Stock *et al.*, 2000) and also in grasslands (Sturhan, 1999). These habitat preferences may reflect not only the distribution of suitable insect hosts, but also physiological and behavioural needs that require specific niches (Kaya & Gaugler, 1993; Hominick *et al.*, 1996).

With respect to Heterorhabditidae, information on habitat specificity widely indicates that some species of this family are prevalent in coastal sandy soils (Griffin *et al.*, 1994; Yoshida *et al.*, 1998; Stock *et al.*, 1999). However, other surveys have indicated that *H. bacteriophora* can be found and is widely distributed in turf and weedy habitats (Stuart & Gaugler, 1994, Stock *et al.*, 1996). Additionally, Griffin *et al.* (1999) found that the Irish type of *Heterorhabditis*, which is restricted to the coastal regions

of Ireland and Britain, also occurs in grasslands of Central and Northern Europe.

In all these correlations and associations with habitat, it is important to bear in mind that factors such as sampling size, seasonality and spatial distribution should be taken into account when recording and interpreting data. Another critical aspect that needs to be carefully considered is the correct identification of the isolates which may require the combination of different methods (morphology, cross-hybridisation, molecular techniques) to avoid erroneous assumptions. Restriction digestion of DNA amplified by the polymerase chain reaction (PCR) from the rDNA ITS spacer region is a very convenient and reliable means of sorting new unidentified isolates into species groups. This technique is not difficult to set up in an ecology laboratory and diagnostic restriction profiles of several *Heterorhabditis* and *Steinernema* species have been published (Joyce *et al.*, 1994b; Reid *et al.*, 1997). Sufficient material for PCR amplification can be obtained from a single infective juvenile or young adult and it is not necessary to carry out a DNA extraction. A clear and comprehensive description of the protocols for the molecular characterisation of EPN via RFLP analysis of the rDNA ITS region is given in Hominick *et al.* (1997). Protocols for determining biological species by cross-breeding are given in Poinar (1967) and Akhurst and Bedding (1978) for *Steinernema* spp. and in Dix *et al.* (1992) for *Heterorhabditis* spp.

Five species of *Xenorhabdus* have been described (see Table 3). Three of these bacterial species are associated with a single species of *Steinernema* but *X. bovienii* is associated with four (Akhurst & Boemare, 1988; Fischer-Le Saux *et al.*, 1999a) and *X. poinarii* with two nematode species (Fischer-Le Saux *et al.*, 1999a). The genus *Photorhabdus* consists mostly of the bacterial symbionts of *Heterorhabditis* as well as some non-symbiotic clinical isolates from human wounds (Farmer *et al.*, 1989). Sequence analysis of the 16S rDNA gene of 40 strains of *P. luminescens* including four clinical samples, indicated that *P. luminescens* was a heterogeneous group and also showed that the clinical samples formed a closely related sub-cluster (Szallas *et al.*, 1997). Fisher-Le Saux *et al.* (1999b) have recently revised the taxonomy of the genus *Photorhabdus* and proposed the creation of two new species, *P. temperata* and *P. asymbiotica*, and, further, that *P. luminescens* be divided into three subspecies. An interesting question yet to be addressed is the frequency of co-speciation between the nematode hosts and their

**Table 3.** Described species of bacterial symbionts of entomopathogenic nematodes.

Genus: *Xenorhabdus* Thomas & Poinar, 1979

Type species: *Xenorhabdus nematophila* (Thomas & Poinar, 1979) Akhurst & Boemare, 1988

Other species:

*X. pionarii* Akhurst & Boemare, 1988

*X. bovienii* Akhurst & Boemare, 1988

*X. beddingii* Akhurst & Boemare, 1988

*X. japonica* Nishimura, Hagiwara, Suzuki & Yamanaka, 1994

Genus: *Photorhabdus* Boemare, Akhurst & Mourant, 1993

Type species: *Photorhabdus luminescens* (Thomas and Poinar, 1979) Boemare, Akhurst & Mourant, 1993

*P. luminescens luminescens* Fischer-Le Saux, Viillard, Brunel, Normand & Boemare, 1999

*P. luminescens akhurstii* Fischer-Le Saux, Viillard, Brunel, Normand & Boemare, 1999

*P. luminescens laumondii* Fischer-Le Saux, Viillard, Brunel, Normand & Boemare, 1999

*P. temperata temperata* Fischer-Le Saux, Viillard, Brunel, Normand & Boemare, 1999

Other species:

*P. temperata* Fischer-Le Saux, Viillard, Brunel, Normand & Boemare, 1999

*P. asymbiotica* Fischer-Le Saux, Viillard, Brunel, Normand & Boemare, 1999

symbiont bacteria and the extent of horizontal and vertical transfer of the symbiont among the nematode lineages.

### Phylogenetic studies of EPN

The evolutionary relationships of EPN were outlined for the first time by Poinar (1981) in his book *The natural history of nematodes*, where he speculated that Steinernematidae and Heterorhabditidae arose as two separate lineages, at roughly the same time in the mid-Palaeozoic, some 375 million years ago. He also indicated that similarities in their morphology, life cycles and bacterial symbiosis can be attributed to convergent evolution. Sudhaus (1993) also concluded that the similarities between *Heterorhabditis* and *Steinernema* are based on symplesiomorphic characters and convergence. Poinar (1993) suggested potential ancestors for both families based on a literature compilation of morphological, biological, physiological and distributional evidence. Examining similarities of the buccal capsule and male tail morphology, Poinar sug-

gested that heterorhabditids evolved from a 'Pellioiditis-like ancestor' in an arenicolous marine environment, and that steinernematids evolved from a 'proto-*Rhabditonema* ancestor' in a terrestrial environment.

Several approaches, both from the molecular and morphological perspectives, have been used to study the evolutionary relationships of EPN. Reid (1994) and Reid *et al.* (1997) studied phylogenetic relationships of Steinernematidae and Heterorhabditidae based on RFLP analysis of the rDNA repeat unit. For this study, 26 isolates representing 11 *Steinernema* and three *Heterorhabditis* species were considered. Additionally, two rhabditoids, *Caenorhabditis elegans* and *Phasmarhabditis* sp. were included for outgroup comparisons. The relationships between *Steinernema* species determined by restriction mapping, mirrored (in general terms) those for the morphological data. For example, *S. arenarium* and *S. glaseri*, two morphologically and biologically similar species, were clustered together. This analysis also showed a close relationship between *S. carpocapsae* and *S. scapterisci*, which was originally referred to as the Uruguay strain of *S. carpocapsae* (Nguyen & Smart, 1988). Reid *et al.* (1994) also showed that members of the family Heterorhabditidae were more closely related to one another than was the case with members of the Steinernematidae, the latter group being much more heterogeneous. This study also showed that the heterorhabditid and steinernematid genera investigated were more closely related to each other than to the two other rhabditoids used for outgroup comparisons.

Other approaches have included either combined analyses of morphological and RAPD fragments (Liu & Berry, 1996b), or nucleotide sequence analyses of various rDNA regions such as the 18S rDNA sequences (Liu *et al.*, 1997), the ITS-1 spacer region (Adams *et al.*, 1998) and also the ND4 sequences of mitochondrial DNA (Liu *et al.*, 1999). Liu *et al.* (1997) inferred phylogenetic relationships among both families of EPN using sequence data from part of the 18S rDNA gene. Seventeen isolates of EPN (12 described and five undescribed species) and six Rhabditidae taxa that included *Rhabditella axei*, *Rhabditis* spp. and four *Caenorhabditis* species. As in Reid's (1994) study, Liu *et al.* (1997) also found that steinernematids had more sequence divergence than heterorhabditids. This observation agreed with the documented morphological, biological and distributional evidence. Although the relationships of several *Steinernema* and *Heterorhabditis* species were not well supported in their cladistic analysis, final interpretation of their phylogenetic study indicated

that Steinernematidae and Heterorhabditidae are two independent monophyletic groups.

Phylogenetic relationships among currently recognised *Heterorhabditis* species were studied by Adams *et al.* (1998) based on 18S rDNA sequences. The relationships among taxa were well established, but lack of divergence within three lineages of sister taxa (*H. marelatus* + *H. hepialius*; *H. indica* + *H. hawaiiensis*; *H. bacteriophora* + *H. argentinensis*) suggested conspecificity. In support of this conclusion, a morphological re-examination has already led to synonymisation of *H. marelatus* and *H. hepialius* (Stock, 1997). The *Heterorhabditis* phylogeny of Liu *et al.* (1999) based on the ND4 mtDNA gene is broadly in agreement with that presented by Adams *et al.* (1998). The study of Adams *et al.* (1998) also indicated that the outgroup taxon *Pellioiditis* was more closely related to *Heterorhabditis* than to *Caenorhabditis* and *Steinernema*.

Blaxter *et al.* (1998) also investigated the phylogenetic relationships of EPN in their molecular framework of the phylum Nematoda. Based on the analysis of 18S rDNA sequences, they concluded heterorhabditids and steinernematids do not share a common ancestry. Their study indicated that *Heterorhabditis* was associated with Strongyloidea and *Steinernema* was more closely related to Panagrolaimidae and *Strongyloides*. An ongoing phylogenetic study (Stock *et al.*, unpubl.) based on a combined analysis of morphological and molecular characters (28S rDNA sequences) of 24 *Steinernema* and three *Heterorhabditis* species, suggests that the Steinernematidae constitute a paraphyletic group, and that members of the family Heterorhabditidae seem to have evolved within the Steinernematidae.

This summary of research on the phylogenetic relationships of EPN shows there is contradictory evidence on the relationships among these two families of EPN. While some authors indicated that heterorhabditids and steinernematids have evolved as two separate independent lineages (Liu *et al.*, 1997; Adams *et al.*, 1998; Blaxter *et al.*, 1998), others suggested these two families are either sister taxa (Reid, 1994), or have evolved together (Stock *et al.*, unpubl.). Incongruence between these studies may be attributed to many causes, including homoplasy, low resolving power of the techniques used, or use of tree building algorithms with different evolutionary assumptions. Therefore, rigorous examination of EPN species, with morphological and biological studies and sequencing of more genes, is encouraged to further assess robust phylogenetic relationships among this group of nematodes.

## Concluding remarks

The impetus for research in EPN and their symbionts has come about because of their biological control potential, so much of the focus in EPN research has been on applied aspects relating to pest control (see Gaugler & Kaya, 1990; Bedding *et al.* 1993 for reviews on these topics). However EPN and their symbionts are increasingly being viewed as an exciting subject for basic research in ecology, biodiversity, evolution, biochemistry and molecular genetics. The bacterial symbionts produce novel insecticidal toxins, antibiotics and exoenzymes, but many of these bacterial species and strains are still unexplored. The molecular interactions between EPN and their symbiont bacteria which enable the nematodes to package and transmit the bacteria are still largely unknown. EPN belong to the same family as *C. elegans* whose genome has been fully sequenced and annotated. Like *C. elegans*, their genome size is small (Grenier *et al.*, 1997). EPN also have the advantage that they can be grown *in vitro* on lipid agar plates and are extremely prolific. They are easy to isolate from soil by baiting with susceptible insect larvae thus facilitating studies in biogeography and habitat preference (Bedding & Akhurst, 1975). In the 10 years since the first international meeting on EPN at Asilomar (see proceedings edited by Gaugler and Kaya, 1990), much progress has been made in our understanding of the basic biology and genetics of EPN and their symbionts. We are now entering a new phase in which the tools of molecular genetics are being increasingly used to address a range of biological questions in EPN research. The knowledge gained from this endeavour should ensure that EPN will become even more effective biopesticides and should also ensure that EPN and their symbionts gain prominence as unique and intrinsically interesting biological systems.

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