

## Perspectives on the Behavior of Entomopathogenic Nematodes from Dispersal to Reproduction: Traits Contributing to Nematode Fitness and Biocontrol Efficacy

CHRISTINE T. GRIFFIN

**Abstract:** The entomopathogenic nematodes (EPN) *Heterorhabditis* and *Steinernema* are widely used for the biological control of insect pests and are gaining importance as model organisms for studying parasitism and symbiosis. In this paper recent advances in the understanding of EPN behavior are reviewed. The “foraging strategy” paradigm (distinction between species with ambush and cruise strategies) as applied to EPN is being challenged and alternative paradigms proposed. Infection decisions are based on condition of the potential host, and it is becoming clear that already-infected and even long-dead hosts may be invaded, as well as healthy live hosts. The state of the infective juvenile (IJ) also influences infection, and evidence for a phased increase in infectivity of EPN species is mounting. The possibility of social behavior - adaptive interactions between IJs outside the host - is discussed. EPNs’ symbiotic bacteria (*Photorhabdus* and *Xenorhabdus*) are important for killing the host and rendering it suitable for nematode reproduction, but may reduce survival of IJs, resulting in a trade-off between survival and reproduction. The symbiont also contributes to defence of the cadaver by affecting food-choice decisions of insect and avian scavengers. I review EPN reproductive behavior (including sperm competition, copulation and evidence for attractive and organizational effects of pheromones), and consider the role of *endotokia matricida* as parental behavior exploited by the symbiont for transmission.

**Key words:** Behavior, biological control, ecology, *endotokia matricida*, entomopathogenic nematode, fitness trade-off, foraging strategy, *Heterorhabditis*, phased infectivity, *Photorhabdus*, reproduction, scavenging, sexual maturation, symbiosis, *Steinernema*, *Xenorhabdus*.

The entomopathogenic nematodes (EPN) *Heterorhabditis* and *Steinernema* have been used for several decades for the biological control of many important insect pests worldwide (Georgis et al., 2006). Major advances have been made in understanding the natural strategies of these organisms, which have contributed to improved success in their exploitation. With their short life cycle and ease of culture, EPN are ideal model organisms, and the study of their behavior increasingly addresses fundamental questions, focusing more on fitness of the nematodes themselves than on their success as biocontrol agents.

*Heterorhabditis* and *Steinernema* behave like parasites in the early part of their life cycle (Poinar, 1990). The free-living infective juvenile (IJ) is adapted for survival and host finding. It does not feed but utilizes stored reserves, and seeks out and enters live insect hosts. Once in the hemocoel the IJ releases cells of a bacterial symbiont (*Photorhabdus* spp. or *Xenorhabdus* spp., respectively) which multiply and kill the host. Thereafter, the nematodes are more akin to bacterial feeders, developing and reproducing on the bacteria and digested host tissues. *Heterorhabditis* and *Steinernema* are not closely related (Blaxter et al., 1998). It is believed that the entomopathogenic life style evolved independently in the two genera, and similarities in their life cycle and behavior are a result of convergent evolution (Poinar, 1993).

IJs’ responses to host insects (whether live, dead or infected), and to host habitats (including damaged or undamaged plants), are crucial to the nematodes’ own success as well as for the human user. Dispersal, host-finding and acceptance are key fitness traits that have

been shaped by evolution to fit the environment and hosts of the various species of EPN, though unfortunately, the natural habitat and host range of most species are poorly known. However, information on dispersal and host-finding derived from laboratory experiments in simplified conditions has contributed to the successful deployment of these nematodes as biological control agents, as well as allowing speculation as to their ecological niche. The behavioral ecology of EPN, mainly the IJ stage, was extensively reviewed by Lewis et al. (2006). It is not my intention to cover the same ground, but to indicate recent developments and some areas which were not covered in that review, including the behavior of adults which has received rather little attention compared to that of the IJ.

### FORAGING STRATEGIES REVISITED

Since the early 1990s, a series of papers has described the host-finding behavior of EPN IJs in terms of ambush and cruise foraging strategies (Grewal et al., 1994; Lewis et al., 1995; Campbell and Gaugler 1997; see Lewis et al. (2006) for a full review of the subject). According to this paradigm, cruise foragers tend to move actively through soil, and use distant volatile cues to assist in host-finding. Ambush foragers, in contrast, tend to remain near the soil surface where they lift their body into the air which facilitates attachment to passing insects. Most species, including all *Heterorhabditis* spp., are regarded as cruisers, a few such as *S. carpocapsae* are classed as ambushers, while others such as *S. feltiae* are said to employ an intermediate foraging strategy (Campbell and Gaugler, 1997). Foraging strategy has been used to make recommendations on which species to use in biological control programs. Cruise-foraging species are best suited to finding immobile hosts, including those deep in soil or in other cryptic habitats,

Received for publication January 4, 2012.  
Department of Biology, National University of Ireland, Maynooth, Maynooth, County Kildare, Ireland.  
Christine.griffin@nuim.ie  
This paper was edited by Nancy Kokalis-Burelle.

while ambush foragers are considered unsuitable for this task (Gaugler et al., 1997). However, an increasing number of field studies indicate that this distinction may be over-simplified. While there are clearly major differences in the habitual foraging mode of species, classification as an ambusher does not preclude success against distant cryptic insects. This is exemplified by the ability of *S. carpocapsae* to parasitize larvae and pupae of the large pine weevil (*Hylobius abietis*) which live under the bark of tree roots. *S. carpocapsae* applied at the soil surface parasitized these insects within the roots at depths of up to 40 cm in the soil (Dillon et al., 2006). In field trials, parasitization rates declined with depth of the insects in soil, but the rate of decline was similar for all EPN species tested, irrespective of whether they are considered to be ambush, cruise or intermediate foragers (Dillon et al., 2006). While in most trials *S. carpocapsae* was less effective than the classic cruise foraging species *Heterorhabditis downesi* (Dillon et al., 2006, 2007), it was sufficiently effective to be used against pine weevil on a semi-operational scale in the UK and Ireland as part of an integrated population suppression strategy (Georgis et al., 2006; Dillon and Griffin, 2008). Similarly, *S. carpocapsae* has shown promise against other subterranean root-dwelling weevils, with up to 95% control of flat-headed root borer *Capnodis tenebrionis* in roots of apricot trees (Martinez de Altube et al., 2008). In these examples, the presence of substantial plant roots may contribute to the success of *S. carpocapsae*, by providing a simplified routeway through the otherwise complex soil environment. Ambush foragers are reported to engage in "ranging search" on a smooth surface (Lewis et al., 1993). In the case of pine weevil larvae, laboratory simulations support the idea that roots facilitate the movement of *S. carpocapsae* through media (sand or sand/peat mix), and that this movement is enhanced by physical and/or chemical stimuli from weevils feeding on the roots (Ennis et al., 2010). While there is no doubt that species such as *S. carpocapsae* engage in behaviors such as standing and jumping that are suited for attaching to mobile hosts at the soil surface (Campbell and Gaugler, 1993; Campbell and Kaya, 2002), it is also clear that this should not preclude them from use against subterranean pests.

Kruitbos et al. (2010) have criticized the description of *S. carpocapsae* as an ambush forager, and propose instead that this species is a habitat specialist, adapted to organic media such as peat or leaf litter. They showed that IJs moved towards hosts more readily in peat than in sand, and suggested that the reason why *S. carpocapsae* typically remains near the surface is because it does not move well through the mineral soils or the pure sands frequently used in experiments. They further suggested that the body waving characteristic of *S. carpocapsae* IJs is an adaptation for bridging large pore spaces rather than attaching to passing insects at the soil surface. This intriguing suggestion requires further

investigation. Natural association of *S. carpocapsae* with organic soils (e.g. peat) or soil horizons (e.g. litter layers) would support this hypothesis, but I am not aware that any such association has been reported to date. In fact, in reiterating the belief that *S. carpocapsae* is a habitat specialist adapted to organic media, Wilson et al. (2012) cite a study by Powers et al. (2009) in which the entomopathogenic fauna of mineral and leaf litter components of a forest soil were distinct; but in that study it was *Heterorhabditis* species ("cruisers") that were found in the organic litter layer. Thus, while the ambush-cruise foraging strategy paradigm may be imperfect, it is premature to replace it with a habitat specialization paradigm to explain interspecific differences in IJ behavior.

#### INFECTION IN RESPONSE TO CUES FROM HEALTHY, INFECTED AND DEAD HOSTS

From the nematode perspective, entry into a host in which it cannot develop is a dead end, though it may fulfil the requirements of the biocontrol practitioner. Hosts of diverse species and developmental stage may be utilized, as well as already-infected and even dead hosts. At least some EPN species have a broad potential host range: in the laboratory *S. carpocapsae* infected over 250 species from 11 orders (Poinar, 1979), and the natural host range covers at least four insect orders (Peters, 1996).

IJs detect and respond to cues associated with living insects (feces, cuticle, etc) that assist them in assessing suitability of a host for entry (Grewal et al., 1993a; Lewis et al., 1996). The behavioral response of *S. carpocapsae* to various host species was correlated with the level of reproduction supported by the hosts (Lewis et al., 1996). This adaptive response is in contrast to responses to infected hosts: IJs will enter a host that is already occupied by conspecifics, even to the point of overcrowding (Lewis et al., 2006). This may depend on time since first occupation: Glazer (1997) found that *Steinernema* spp. were inhibited from entering a host that had been injected with conspecifics 6-9 hours previously, but Christen et al. (2007) found that while the number of *S. riobrave* IJs invading declined over time, they continued to enter for at least 72 hours since first infection of the host (by which time, presumably, the hosts were already dead). This behavior of IJs to invade hosts already harboring many competitors appears maladaptive, since overcrowding results in lower reproductive output per founder (Koppenhöfer and Kaya, 1995; Ryder and Griffin, 2002). Christen et al. (2007) suggest reasons why IJs behave in this apparently maladaptive manner: there may be few alternative hosts available, in which case it is better to invade a sub-optimal host than to reject it and die of starvation. Alternatively, they may have limited ability to assess the quality of the host, either because of lack of suitable

cues from the host or suitable sensory abilities of the IJs (Christen et al., 2007).

Recent studies have emphasized that IJs of most EPN species also enter hosts that have been killed by other causes (e.g. freezing) (San Blas and Gowen, 2008; Puza and Mracek, 2010). Species differed in the time after death that hosts were accepted; *Steinernema glaseri* invaded hosts killed up to 10 days previously while *Heterorhabditis indica* only accepted hosts that had been dead for three days (San Blas and Gowen, 2008). These authors recommend that EPN should be considered facultative scavengers rather than as obligate parasites. EPN can reproduce in host species that they cannot kill (Puza and Mracek, 2010), which may be important for the persistence of natural populations, as well as enhanced recycling of applied EPN, especially in integrated control programs where hosts killed by other biological or chemical agents may be available and suitable for development. For example, EPN can develop in hosts killed by several insecticides (Hara and Kaya, 1983; Koppenhöfer et al., 2003) and by granulosis virus (Kaya and Burlando, 1989).

It is unclear to what extent EPN in nature avoid insect hosts that are suboptimal for reproduction, or waste time and energy on hosts that they cannot even penetrate. EPN, especially species that infect a wide variety of healthy, infected and dead hosts, might be expected to use rather non-specific cues for penetration, like the cercariae of *Schistosoma japonica* whose low specificity in terms of chemical cues allows it to invade a broad spectrum of mammalian hosts (Haas, 2003).

#### INNATE INFECTION TENDENCY AND PHASED INFECTIVITY

The decision to invade an insect depends not only on external factors such as the cues from potential hosts, but also on the internal state of the IJ. In some species at least, it appears that the tendency to infect changes with time since emergence from the source cadaver—before the eventual decline in infectivity associated with ageing. There are two models of “phased infectivity”: Bohan and Hominick (1996, 1997) described fluctuations in infectivity of *Steinernema feltiae* and attributed it to a proportion of IJs switching between a non-infectious and an infectious state. Griffin (1996) described an increase in the infectivity of *H. megidis* in the initial weeks after emerging from the natal host, but as this was detectable only in a restrictive assay (with limited time available for invasion into the host) it was attributed to a gradual change in infection tendency of individual IJs, rather than a switch between states (Griffin, 1996; Dempsey and Griffin, 2002; Ryder and Griffin, 2003). Taking a modelling approach to the dynamics of infection, Fushing et al. (2008) distinguished between IJs in a population at a given time based on their sensitivity to risk: risk-takers invade readily, taking the risk of being overcome by the host defences,

while risk-averse individuals wait until the host has been invaded by others. For *S. feltiae*, a majority of individuals were risk averse, waiting until very few risk-prone individuals invaded (Fushing et al., 2008). This is reminiscent of the scenario described by Hay and Fenlon (1995), whose modified binomial model indicated that a sizeable proportion of *S. feltiae* infective juveniles “invade the host secondarily but cannot, or choose not to, initiate infection” (Hay and Fenlon, 1995). For *S. carpocapsae* the distinction into risk-prone and risk-averse individuals was seen only in response to certain hosts (Fushing et al., 2008), indicating a certain flexibility. Whether this tendency is also subject to change with time since emergence from the host has not been tested. However, these models may be useful in exploring phased infectivity. An increase in the proportion of risk takers in a population could correlate with an increase in the overall infectivity of a population.

From the nematode’s perspective, IJs that delay infectivity for some time during which they (or their competitors) migrate away from the natal host from which they have emerged en masse benefit by avoiding the overcrowding that otherwise might be expected in adjacent hosts (Dempsey and Griffin, 2002). Knowledge of the dynamic nature of EPN infectivity can also be of benefit to the biocontrol practitioner. For example, *H. megidis* IJs stored at 9°C for 4 weeks gave improved control of vine weevil larvae in potted plants (Fitters et al., 2001). Other species of EPN including *Steinernema* spp. also show an increase in infectivity with storage (Guy et al., 2009).

#### DO IJS BEHAVE AS SOCIAL ANIMALS?

Although IJs are studied in groups, their social behavior is not well known, but there are indications that they interact outside the host, whether in cooperation or competition.

Tens or even hundreds of thousands of IJs can emerge from an insect cadaver, from which they must then disperse in order to find new hosts to infect. Initially at least, they may be seen to travel together, whether on a two-dimensional substrate such as agar, or through soil (Rolston et al., 2006). Physical forces acting on the IJs undoubtedly contribute to this observed clumping, but El Borai et al. (2011) suggest that the IJs may be exhibiting herding or shoaling behavior additional to these forces. There are obvious advantages to moving in a group— for example, there may be a requirement for a critical mass of IJs in order to kill certain insects (Peters and Ehlers, 1997). Furthermore, each individual may benefit from the protection from natural enemies afforded by being in a group, through dilution and shielding effects (Hamilton, 1971). However, there are also disadvantages to migrating in a group – particularly since IJs emerging from a host may largely be close relatives - including competition

for host resources and inbreeding (Downes and Griffin, 1996). For IJs to make adaptive decisions to either remain with the group or go off alone, it would be helpful if they were able to sense the presence - and perhaps even the status- of their travelling companions, perhaps through chemical cues produced by IJs themselves as suggested by El Borai et al. (2011). In the presence of *S. glaseri*, *S. carpocapsae* IJs nictated more and moved towards the host, behavior that was seen as an adaptive response to the presence of a competitor (Wang and Ishibashi, 1999); this indicates that IJs may be able to recognize and respond adaptively to other IJs. In *Caenorhabditis elegans* there are strains where worms always remain solitary and others where they may form aggregations, depending on environmental conditions (De Bono and Bargmann, 1998). To form feeding aggregations, *C. elegans* individuals alter their behavior on physically encountering each other, though pheromones may also be involved in promoting aggregation (Boender et al., 2011).

Little is known about the variation between EPN species and strains in the tendency to move together as a group rather than as individuals, but such variation may in part underlie differences in relative success rate against different target insects. This is more likely to be a factor when EPN are applied in infected insect cadavers from which the IJs emerge in the field (Shapiro-Ilan et al., 2003), rather than when applied in aqueous suspension. Strains where IJs move as a herd may be better able to control insects that require a large inoculum potential to overcome their defences, such as *Tipula* spp. (Peters and Ehlers, 1997) while strains that tend to move as solitary individuals may be better against more susceptible and numerous hosts. Solitary IJ behavior is more likely to be prevalent as a natural strategy in *Heterorhabditis* spp., where each IJ can colonize a host, than in *Steinernema* which requires mating partners (discussed below). However, the trait may be relatively easy to manipulate: a simple mutation can transform a solitary strain of *C. elegans* into a social one (De Bono and Bargmann, 1998).

#### EFFECTS OF THE SYMBIONT ON NEMATODE BEHAVIOR AND FITNESS

Entomopathogenic nematodes are intimately associated with symbiotic bacteria (*Xenorhabdus* or *Photorhabdus*), on which they depend to a greater or lesser extent for death of the host and for nutrition (Han and Ehlers, 2000; Ciche et al., 2006). The bacterial symbiont also affects EPN behavior - indeed the behavior of the nematode can be considered an extended phenotype (Dawkins, 1982) affected by nematode and bacterial genes. Most of the effects on nematode behavior take place during the growth phase of the bacteria within the host. Thus, bacterial products signal recovery from the dauer-like IJ state (Aumann and Ehlers, 2001; Hirao

and Ehlers 2009) and affect the attractiveness or otherwise of the cadaver to late arriving nematodes (Grewal et al., 1997).

The bacterial symbiont can also affect the behavior of would-be scavengers of the insect cadaver. Bacterial products from both *Xenorhabdus* and *Photorhabdus* make the cadaver repellent to ants (Baur et al., 1998; Zhou et al., 2002). *Heterorhabditis bacteriophora*-infected cadavers were also protected from an avian predator, the European robin *Erithacus rubecula*, and this was attributed to the red color reinforced by unpalatable taste when cadavers were sampled (Fenton et al., 2011). The role of volatiles in deterring predation by robins could not be excluded (Fenton et al., 2011), and the fact that the birds did not need to bite cadavers to reject them implies that at least some deterrent factor is emitted through the cadaver cuticle. However, not all birds are equally fastidious in their feeding, and so it is unclear how effective this protection would be in natural conditions. When rooks (*Corvus frugilegus*) were given a choice of items - wax moth larvae infected with EPN (*H. downesi* or *S. feltiae*), freeze-killed wax-moths or bread in the size and shape of a wax-moth - they showed an initial prejudice against EPN-killed cadavers. In the first 2 mins there was a highly significant difference in consumption of the different items: rooks ate only 35% and 41% of the cadavers infected with *H. downesi* and *S. feltiae*, respectively, compared to 73% of the freeze-killed cadavers and 97% of bread pieces (Fogarty, 2007). However, protection was short-lived: within 30 mins, at least 75% of all food types had been consumed, and in 8 of the 13 trials conducted, all 10 items of each type had been consumed. In these experiments it is not clear what initially affected the rooks' preferences, but it could not be the red color as the *H. downesi*-infected wax moths were yellow and the *S. feltiae* ones were brownish. Moreover, a separate experiment showed that rooks were as likely to eat bread dyed bright red as normal white bread (Fogarty, 2007). Thus, while bacteria-induced coloration and taste or odors may protect against fastidious species such as robins, infected cadavers are likely to be eaten by birds with more eclectic taste such as rooks. Moreover, rooks and other birds that probe the soil for food are more likely than surface-feeding species such as robins to encounter EPN-killed cadavers. Feeding by birds and insect scavengers could potentially reduce the recycling potential of applied EPN, but represents a more serious threat when EPN are applied in cadavers. Appropriate formulation may be considered in areas where the risk of scavenging on applied cadavers is high (Del Valle et al., 2009).

Although the symbiotic bacteria are very important to the success of EPN, carrying them in the intestine also represents a cost to IJs; *S. carpocapsae* IJs without symbiont survived longer than those with them, and this was presumed to be due to the energetic cost of maintaining the bacteria (Mitani et al., 2004; Emelianoff et al., 2007).

Emelianoff et al. (2008) describe a trade-off between survival and reproduction, mediated by the bacterial symbiont: *S. carpocapsae* IJs with fewer bacteria survived longer than those with more, but had a lower reproductive rate on entry into a host (Emelianoff et al., 2008). This trade-off would be expected to respond to host availability, with low host availability favoring more prolonged survival at the expense of reproductive rate. Again, this may be exploited in biocontrol, especially in species such as *S. carpocapsae* that are not dependant on their symbiont to kill hosts (Ciche et al., 2006). If IJs without symbiont survive longer in the soil, they might be suitable for prophylactic application.

#### REPRODUCTIVE BEHAVIOR: MATING AND "PARENTING"

Reproductive behavior of EPN has received less attention than the behavior of IJs focussed on finding and infecting hosts. Nevertheless, an understanding of EPN reproductive biology is of relevance to the practical use of EPN including mass production, cross-breeding for species determination, and selective breeding, as well as being important to population biology. Since EPN reproduce within insect cadavers, it is the IJ that decides where reproduction is to take place, and its choice of host to invade also affects the pool of mating partners (and competitors) available to it when mature (Lewis et al., 2006). There is relatively little information on the reproductive behavior of the adult worms themselves, but sex pheromones, copulation behavior, sperm competition, sexual maturation and *endotokia matricida* have all received some attention.

The life histories of *Steinernema* and *Heterorhabditis* differ in one fundamental respect: every *Heterorhabditis* IJ develops into a self-fertile hermaphrodite while a *Steinernema* IJ develops into either a male or female (Poinar, 1990). This means that a heterorhabditid IJ can colonize a host on its own- possibly an adaptation to low characteristic abundance as a result of reproducing in small hosts and/or a tendency to disperse widely before infecting. At least one species of *Steinernema* (*S. hermaphroditum*) shows evidence of convergent evolution with *Heterorhabditis* in that most IJs develop into self-fertile hermaphrodites, possibly a response to similar ecological conditions (Griffin et al., 2001). Most *Steinernema* spp., however, require two or more IJs to invade and might thus be expected to have higher characteristic abundance and/or behavioral mechanisms to increase the probability of individuals of both sexes invading the same host (Downes and Griffin, 1996). This might be achieved if male *Steinernema* IJs disperse first and make hosts that they enter more attractive to females (the "male colonization" hypothesis of Grewal et al. 1993b), though later work suggests this may not be a widespread phenomenon (Stuart et al., 1998; Alsaiyah et al., 2009). What is the fate of a *Steinernema* IJ that invades alone? One IJ can easily kill

a permissive host such as the wax moth *Galleria mellonella*, and both male and female *S. feltiae* can survive alone in a wax moth they have killed for up to six weeks (Rolston et al., 2006) - much longer than would be expected in a normal, multiple-infected cadaver (Wang and Bedding, 1996). Adult nematodes, especially females, might be better able than IJs to render the cadaver they inhabit attractive to IJs of the opposite sex, through the production of pheromones (Neves et al., 1998), resulting in the recruitment of mating partners by a lone resident. However, neither the fecundity of these older nematodes nor the attractiveness of the cadavers they occupy has been tested.

Pheromones have been implicated in the sexual attraction of over 30 species of nematode, but relatively few genera (Lee, 2002). *Steinernema carpocapsae* females produce a pheromone that is attractive to males (Neves et al., 1998). Lewis et al. (2002) found that male *S. carpocapsae* were attracted to virgin females, but not to mated ones. This suggests that the pheromone is no longer secreted once the female has mated, and perhaps indicates that females do not mate repeatedly. This is supported by the apparent absence of mating plugs in this genus (Lewis et al., 2002; personal observations; S.P. Stock, University of Arizona, pers. comm.). There is evidence that diffusible substances originating from *Steinernema* females may have organizational effects on males, in addition to attraction: male *S. longicaudum* that developed alone did not mature sexually- they were incapable of fertilizing eggs and no sperm were visible in their reproductive tract, but exposure to conspecific females even when separated by a barrier allowed males to mature (Ebssa et al., 2008). This unusual female-dependent maturation can be seen as adaptive if, on the one hand *S. longicaudum* males sometimes develop alone in a cadaver, but are later joined by one or more females, and on the other hand if sperm are costly and subject to loss of quality if not used (Reinhardt, 2007). While sperm are typically regarded as cheap, the macrosperm of *S. longicaudum* represent 30-40% of its body volume, and production of the dual sperm (macrosperm and microsperm) (Yushin et al., 2007) typical of this species has unknown energetic costs. Thus, saving energy by delaying sperm production may facilitate survival until a mating partner eventually arrives.

The copulatory behavior of the two genera differs: in *Steinernema*, the male coils around the female at the vulval region during copulation (Strauch et al., 1994; Lewis et al., 2002), while in *Heterorhabditis* the male aligns his body approximately parallel to the female without coiling around her, making copulation in liquid culture impossible (Strauch et al., 1994). This failure to copulate in liquid has implications for mass production, as unproductive amphimictic adults (produced by the first generation hermaphrodites) compete for resources with the hermaphrodites and the IJs that develop in them (Ehlers, 2001).

Male animals typically compete for females, and this competition may continue after insemination as competition between sperm. Sperm competition is usually defined as the competition between the ejaculates of different males for the fertilization of a given set of ova (Parker, 1970), but may also occur between sperm produced by males and a hermaphrodite. Dix et al. (1994) showed that *H. bacteriophora* hermaphrodites preferentially use male sperm. A similar superiority of male over hermaphrodite sperm is seen in *C. elegans* (Ward and Carrel, 1979). This trait maximizes outcrossing after mating and may increase both genetic diversity and heterozygosity of offspring (LaMunyon and Ward, 1995). Male *Heterorhabditis* spp. deposit mating or copulatory plugs over the vulva (Dix et al., 1994; Strauch et al., 1994); such plugs are assumed to have evolved in response to male-male competition, in order to prevent a second male from mating (Barker, 1994). However, plugs are not always effective (Timmermeyer et al., 2010). Whether the copulatory plug prevents remating of *Heterorhabditis* spp. females or hermaphrodites has not been tested.

Following an initial period of egg-laying, both *Steinernema* and *Heterorhabditis* retain eggs which hatch in the uterus and consume the mother (Poinar, 1990). The term *endotokia matricida* that is frequently applied to this mode of development (Baliadi et al., 2004; Johnigk and Ehlers, 1999) implies control by the offspring over their fate. In *Heterorhabditis*, juveniles developing within the mother become exclusively IJs, while eggs laid into the cadaver may continue development to adult, so it is likely that the “sacrifice” of its soma to IJs is an adaptive response designed to optimize the use of a limited and ephemeral cadaver. There is evidence that the relative investment in intra-uterine IJs and laid eggs can be adjusted depending on food supply and number of competitors (Johnigk and Ehlers, 1999; Ryder and Griffin, 2002). In *Heterorhabditis*, only juveniles developing within the mother become colonized by bacteria, and Ciche et al. (2008) suggest that intra-uterine development is an adaptation for symbiont transmission. However, since intra-uterine hatching is also seen in many other species of nematode including *C. elegans* (Chen and Caswell-Chen, 2003), it is likely that this phenomenon has evolved to optimize female’s reproductive success in response to stressful or starvation conditions, and may be seen as a form of parental behavior, and that the symbiont later adapted its mode of transmission to the nematode’s reproductive biology.

#### CONCLUSIONS

Even within the small group of EPN species that have been studied, variation is seen in every trait. The observed variation in responses is compounded by differences in environmental and internal factors, including insect host, soil texture, age of IJ, the conditions under

which they developed, and the bacterial load carried by the IJs, making generalization difficult. It is challenging to understand the functional significance (either for EPN fitness or biocontrol utility) of the documented responses, since most behavioral studies are necessarily conducted in simplified conditions, far from the complexity of the natural environment. The impending sequencing of multiple EPN genomes opens up new scope for understanding the genetic basis and control of the behavior of these organisms, as exemplified by Dillman et al. (2011), with even further exciting challenges to understand the mutualistic association between animal and bacterial symbiont as holobiont, which can be regarded as the unit of selection (Feldhaar, 2011). As always, the biggest challenge will be to relate what is known in the laboratory to the real lives of EPN in soil, whether as natural populations or applied for biological control.

#### LITERATURE CITED

- Alsayyah, M. A. M., Ebssa, L., Zenner, A., O’Callaghan, K. M., and Griffin, C. T. 2009. Sex ratios and sex-biased infection behaviour in the entomopathogenic nematode genus *Steinernema*. *International Journal for Parasitology* 39:725–734.
- Aumann, J., and Ehlers, R. U. 2001. Physico-chemical properties and mode of action of a signal from the symbiotic bacterium *Photobacterium luminescens* inducing dauer juvenile recovery in the entomopathogenic nematode *Heterorhabditis bacteriophora*. *Nematology* 3:849–853.
- Baliadi, Y., Yoshiga, T., and Kondo, E. 2004. Infectivity and post-infection development of infective juveniles originating via endotokia matricida in entomopathogenic nematodes. *Applied Entomology and Zoology* 39:61–69.
- Barker, D. M. 1994. Copulatory plugs and paternity assurance in the nematode *Caenorhabditis elegans*. *Animal Behaviour* 48:147–156.
- Baur, M. E., Kaya, H. K., and Strong, D. R. 1998. Foraging ants as scavengers on entomopathogenic nematode-killed insects. *Biological Control* 12:231–236.
- Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., and Thomas, W. K. 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* 392:71–75.
- Boender, A. J., Roubos, E. W., and van der Velde, G. 2011. Together or alone? Foraging strategies in *Caenorhabditis elegans*. *Biological Reviews* 86:853–862.
- Bohan, D. A., and Hominick, W. M. 1996. Investigations on the presence of an infectious proportion amongst populations of *Steinernema feltiae* (Site 76 strain) infective stages. *Parasitology* 112:113–118.
- Bohan, D. A., and Hominick, W. M. 1997. Long term dynamics of infectiousness within the infective stage pool of the entomopathogenic nematode *Steinernema feltiae* (Site 76 strain) Filipjev. *Parasitology* 114:301–308.
- Campbell, J., and Gaugler, R. 1993. Nictation behavior and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* 126:154–169.
- Campbell, J. F., and Gaugler, R. 1997. Inter-specific variation in entomopathogenic nematode. Foraging strategy: dichotomy or variation along a continuum? *Fundamental and Applied Nematology* 20:393–398.
- Campbell, J. F., and Kaya, H. K. 2002. Variation in entomopathogenic nematode (Steinernematidae and Heterorhabditidae) infective-stage jumping behaviour. *Nematology* 4:471–482.

- Chen, J. J., and Caswell-Chen, E. P. 2003. Why *Caenorhabditis elegans* adults sacrifice their bodies to progeny. *Nematology* 5:641–645.
- Christen, J. M., Campbell, J. F., Lewis, E. E., Shapiro-Ilan, D. I., and Ramaswamy, S. B. 2007. Responses of the entomopathogenic nematode, *Steinernema riobrave* to its insect hosts, *Galleria mellonella* and *Tenebrio molitor*. *Parasitology* 134:889–898.
- Cliche, T. A., Darby, C., Ehlers, R. U., Forst, S., and Goodrich-Blair, H. 2006. Dangerous liaisons: The symbiosis of entomopathogenic nematodes and bacteria. *Biological Control* 38:22–46.
- Cliche, T. A., Kim, K. S., Kaufmann-Daszczuk, B., Nguyen, K. C. Q., and Hall, D. H. 2008. Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. *Applied and Environmental Microbiology* 74:2275–2287.
- Dawkins, R. 1982. *The extended phenotype*. Oxford: Oxford University Press.
- de Altube, M. D. M., Strauch, O., de Castro, G. F., and Pena, A. M. 2008. Control of the flat-headed root borer *Capnodis tenebrionis* (Linne) (Coleoptera: Buprestidae) with the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) in a chitosan formulation in apricot orchards. *BioControl* 53:531–539.
- de Bono, M., and Bargmann, C. I. 1998. Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell* 94:679–689.
- Del Valle, E. E., Dolinski, C., Barreto, E. L. S., and Souza, R. M. 2009. Effect of cadaver coatings on emergence and infectivity of the entomopathogenic nematode *Heterorhabditis baujardi* LPP7 (Rhabditida: Heterorhabditidae) and the removal of cadavers by ants. *Biological Control* 50:21–24.
- Dempsey, C. M., and Griffin, C. T. 2002. Phased activity in *Heterorhabditis megidis* infective juveniles. *Parasitology* 124:605–613.
- Dillman, A. R., Mortazavi, A., and Sternberg, P. W. 2011. Whole genomic sequencing of Steinernematids illuminates parasitism and mutualism in nematodes. *Journal of Nematology* 43:59 (Abstr.).
- Dillon, A. B., Ward, D., Downes, M. J., and Griffin, C. T. 2006. Suppression of the large pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes with different foraging strategies. *Biological Control* 38:217–226.
- Dillon, A. B., Downes, M. J., Ward, D., and Griffin, C. T. 2007. Optimizing application of entomopathogenic nematodes to manage large pine weevil, *Hylobius abietis* L. (Coleoptera : Curculionidae) populations developing in pine stumps, *Pinus sylvestris*. *Biological Control* 40:253–263.
- Dillon, A. B., Rolston, A. N., Meade, C. V., Downes, M. J., and Griffin, C. T. 2008. Establishment, persistence, and introgression of entomopathogenic nematodes in a forest ecosystem. *Ecological Applications* 18:735–747.
- Dillon, A. B., and Griffin, C. T. 2008. Controlling the large pine weevil, *Hylobius abietis*, using natural enemies. *COFORD Connects Series, Silviculture/Management* no. 15.
- Dix, I., Koltai, H., Glazer, I., and Burnell, A. M. 1994. Sperm competition in mated 1st generation hermaphrodite females of the HP-88 strain of *Heterorhabditis* (Nematoda, Heterorhabditidae) and progeny sex-ratios in mated and unmated females. *Fundamental and Applied Nematology* 17:17–27.
- Downes, M. J., and Griffin, C. T. 1996. Dispersal behaviour and transmission strategies of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema*. *Biocontrol Science and Technology* 6:347–356.
- Ebssa, L., Dix, I., and Griffin, C. T. 2008. Female presence is required for male sexual maturity in the nematode *Steinernema longicaudum*. *Current Biology* 18:R997–R998.
- Ehlers, R. U. 2001. Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology Biotechnology* 56:623–633.
- El-Borai, F. E., Campos-Herrera, R., Stuart, R. J., and Duncan, L. W. 2011. Substrate modulation, group effects and the behavioral responses of entomopathogenic nematodes to nematophagous fungi. *Journal of Invertebrate Pathology* 106:347–356.
- Emelianoff, V., Chapuis, E., Le Brun, N., Chiral, M., Moulia, C., and Ferdj, J. B. 2008. A survival-reproduction trade-off in entomopathogenic nematodes mediated by their bacterial symbionts. *Evolution* 62:932–942.
- Emelianoff, V., Sicard, M., Le Brun, N., Moulia, C., and Ferdj, J. B. 2007. Effect of bacterial symbionts *Xenorhabdus* on mortality of infective juveniles of two *Steinernema* species. *Parasitology Research* 100:657–659.
- Ennis, D. E., Dillon, A. B., and Griffin, C. T. 2010. Simulated roots and host feeding enhance infection of subterranean insects by the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Invertebrate Pathology* 103:140–143.
- Feldhaar, H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology* 36:533–543.
- Fenton, A., Magoolagan, L., Kennedy, Z., and Spencer, K. A. 2011. Parasite-induced warning coloration: a novel form of host manipulation. *Animal Behaviour* 81:417–422.
- Fitters, P. F. L., Dunne, R., and Griffin, C. T. 2001. Improved control of *Otiiorhynchus sulcatus* at 9° C by cold-stored *Heterorhabditis megidis* UK211. *Biocontrol Science and Technology* 11:483–492.
- Fogarty, L. 2007. Feeding preferences of predators with regard to nematode infected insect larvae. B.Sc. Thesis, National University of Ireland, Maynooth, Ireland.
- Fushing, H., Zhu, L., Shapiro-Ilan, D. I., Campbell, J. F., and Lewis, E. E. 2008. State-space based mass event-history model I: many decision-making agents with one target. *Annals of Applied Statistics* 2:1503–1522.
- Gaugler, R., Lewis, E., and Stuart, R. J. 1997. Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia* 109:483–489.
- Georgis, R., Koppenhöfer, A. M., and Lacey, L. A. 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control* 38:103–123.
- Glazer, I. 1997. Effects of infected insects on secondary invasion of steinernematid entomopathogenic nematodes. *Parasitology* 114:597–604.
- Grewal, P. S., Gaugler, R., and Selvan, S. 1993a. Host recognition by entomopathogenic nematodes: behavioral response to contact with host feces. *Journal of Chemical Ecology* 19:1219–1231.
- Grewal, P. S., Selvan, S., Lewis, E. E., and Gaugler, R. 1993b. Male insect-parasitic nematodes: a colonizing sex. *Experientia* 49:605–608.
- Grewal, P. S., Lewis, E. E., Gaugler, R., and Campbell, J. F. 1994. Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* 108:207–215.
- Grewal, P. S., Lewis, E. E., and Gaugler, R. 1997. Response of infective stage parasites (Nematoda: Steinernematidae) to volatile cues from infected hosts. *Journal of Chemical Ecology* 23:503–515.
- Griffin, C. T. 1996. Effects of prior storage conditions on the infectivity of *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Fundamental and Applied Nematology* 19:95–102.
- Griffin, C. T., O'Callaghan, K., and Dix, I. 2001. A self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes? *Parasitology* 122:181–186.
- Guy, A. G., Wright, D. J., Gaffney, M., and Griffin, C. T. 2009. Conditioning of *Steinernema kraussei* and *S. carpocapsae* through storage: enhancing their performance over wide temperature ranges. *IOBC/wprs Bulletin* 45:349–352.
- Haas, W. 2003. Parasitic worms: strategies of host finding, recognition and invasion. *Zoology* 106:349–364.
- Hamilton, W. D. 1971. Geometry for the selfish herd. *Journal of Theoretical Biology* 31:295–311.
- Han, R. C., and Ehlers, R. U. 2000. Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* under axenic in vivo conditions. *Journal of Invertebrate Pathology* 75:55–58.

- Hara, A. H., and Kaya, H. K. 1983. Development of the entomogenous nematode, *Neoplectana carpocapsae* (Rhabditida: Steinernematidae), in insecticide killed beet armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 76:423–426.
- Hay, D. B., and Fenlon, J. S. 1995. A modified binomial model that describes the infection dynamics of the entomopathogenic nematode *Steinernema feltiae* (Steinernematidae; Nematoda). *Parasitology* 111:627–633.
- Hirao, A., and Ehlers, R. U. 2009. Influence of cell density and phase variants of bacterial symbionts (*Xenorhabdus* spp.) on dauer juvenile recovery and development of biocontrol nematodes *Steinernema carpocapsae* and *S. feltiae* (Nematoda: Rhabditida). *Applied Microbiology and Biotechnology* 84:77–85.
- Johnigk, S. A., and Ehlers, R. U. 1999. Endotokia matricida in hermaphrodites of *Heterorhabditis* spp. and the effect of the food supply. *Nematology* 1:717–726.
- Kaya, H. K., and Burlando, T. M. 1989. Infectivity of *Steinernema feltiae* in fenamiphos-treated sand. *Journal of Nematology* 21:434–436.
- Koppenhöfer, A. M., Cowles, R. S., Cowles, E. A., Fuzy, E. M., and Kaya, H. K. 2003. Effect of neonicotinoid synergists on entomopathogenic nematode fitness. *Entomologia Experimentalis et Applicata* 106:7–18.
- Koppenhöfer, A. M., and Kaya, H. K. 1995. Density-dependent effects on *Steinernema glaseri* (Nematoda: Steinernematidae) within an insect host. *Journal of Parasitology* 81:797–799.
- Kruitbos, L. M., Heritage, S., Hapca, S., and Wilson, M. J. 2010. The influence of habitat quality on the foraging strategies of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis megidis*. *Parasitology* 137:303–309.
- Lamunyon, C. W., and Ward, S. 1995. Sperm precedence in a hermaphroditic nematode (*Caenorhabditis elegans*) is due to competitive superiority of male sperm. *Experientia* 51:817–823.
- Lee, D. L. 2002. Behaviour. Pp. 369–387 in D.L. Lee, ed. *The Biology of Nematodes*. London: Taylor and Francis.
- Lewis, E. E., Gaugler, R., and Harrison, R. 1993. Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 71:765–769.
- Lewis, E. E., Grewal, P. S., and Gaugler, R. 1995. Hierarchical order of host cues in parasite foraging strategies. *Parasitology* 119:207–213.
- Lewis, E. E., Ricci, M., and Gaugler, R. 1996. Host recognition behaviour predicts host suitability in the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Parasitology* 113:573–579.
- Lewis, E. E., Barbarosa, B., and Gaugler, R. 2002. Mating and sexual communication by *Steinernema carpocapsae* (Nematoda: Steinernematidae). *Journal of Nematology* 34:328–331.
- Lewis, E. E., Campbell, J., Griffin, C., Kaya, H., and Peters, A. 2006. Behavioral ecology of entomopathogenic nematodes. *Biological Control* 38:66–79.
- Mitani, D. K., Kaya, H. K., and Goodrich-Blair, H. 2004. Comparative study of the entomopathogenic nematode, *Steinernema carpocapsae*, reared on mutant and wild-type *Xenorhabdus nematophila*. *Biological Control* 29:382–391.
- Neves, J. M., Simoes, N., and Mota, M. 1998. Evidence for a sex pheromone in *Steinernema carpocapsae*. *Nematologica* 44:95–98.
- Parker, G. A. 1970. Sperm competition and its evolutionary consequences in insects. *Biological Reviews of the Cambridge Philosophical Society* 45:525–567.
- 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.
- Peters, A., and Ehlers, R. U. 1997. Encapsulation of the entomopathogenic nematode *Steinernema feltiae* in *Tipula oleracea*. *Journal of Invertebrate Pathology* 69:218–222.
- Poinar, G. O. 1979. *Nematodes for biological control of insects*. Boca Raton, Fla.: CRC Press.
- Poinar, G. O. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. Pp. 23–61 in R. Gaugler and H.K. Kaya, eds. *Entomopathogenic nematodes in biological control*. Boca Raton, Fla.: CRC Press.
- Poinar, G. O. 1993. Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. *Fundamental and Applied Nematology* 16:333–338.
- Powers, T. O., Neher, D. A., Mullin, P., Esquivel, A., Giblin-Davis, R. M., Kanzaki, N., Stock, S. P., Mora, M. M., and Uribe-Lorio, L. 2009. Tropical nematode diversity: vertical stratification of nematode communities in a Costa Rican humid rainforest. *Molecular Ecology* 18:985–996.
- Puza, V., and Mracek, Z. 2010. Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? *Journal of Invertebrate Pathology* 104:1–3.
- Reinhardt, K. 2007. Evolutionary consequences of sperm cell aging. *Quarterly Review of Biology* 82:375–393.
- Rolston, A. N., Griffin, C. T., and Downes, M. J. 2006. Emergence and dispersal patterns of two isolates of the entomopathogenic nematode *Steinernema feltiae*. *Journal of Nematology* 38:221–228.
- Ryder, J. J., and Griffin, C. T. 2002. Density dependent fecundity and infective juvenile production in the entomopathogenic nematode, *Heterorhabditis megidis*. *Parasitology* 125:83–92.
- Ryder, J. J., and Griffin, C. T. 2003. Phased infectivity in *Heterorhabditis megidis*: the effects of infection density in the parental host and filial generation. *International Journal for Parasitology* 33:1013–1018.
- San-Blas, E., and Gowen, S. R. 2008. Facultative scavenging as a survival strategy of entomopathogenic nematodes. *International Journal for Parasitology* 38:85–91.
- Shapiro-Ilan, D. I., Lewis, E. E., and Tedders, W. L. 2003. Superior efficacy observed in entomopathogenic nematodes applied in infected-host cadavers compared with application in aqueous suspension. *Journal of Invertebrate Pathology* 83:270–272.
- Strauch, O., Stoessel, S., and Ehlers, R.-U. 1994. Culture conditions define automictic or amphimictic reproduction in entomopathogenic rhabditid nematodes of the genus *Heterorhabditis*. *Fundamental and Applied Nematology* 17:573–582.
- Stuart, R. J., Abu Hatab, M., and Gaugler, R. 1998. Sex ratio and the infection process in entomopathogenic nematodes: Are males the colonizing sex? *Journal of Invertebrate Pathology* 72:288–295.
- Timmermeyer, N., Gerlach, T., Guempel, C., Knoche, J., Pfann, J. F., Schliessmann, D., and Michaels, N. K. 2010. The function of copulatory plugs in *Caenorhabditis remanei*: hints for female benefits. *Frontiers in Zoology* 7:28.
- Wang, J., and Bedding, R. A. 1996. Population development of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in larvae of *Galleria mellonella*. *Fundamental and Applied Nematology* 19:363–367.
- Wang, X. D., and Ishibashi, N. 1999. Infection of the entomopathogenic nematode, *Steinernema carpocapsae*, as affected by the presence of *Steinernema glaseri*. *Journal of Nematology* 31:207–211.
- Wilson, M. J., Ehlers, R. U., and Glazer, I. 2012. Entomopathogenic nematode foraging strategies – is *Steinernema carpocapsae* an ambush forager? *Nematology*, in press doi.org/10.1163/156854111X617428.
- Yushin, V. V., Yoshida, M., and Spiridonov, S. E. 2007. Riders on the sperm: sperm dimorphism and spermatozeugmata in nematodes from the genus *Steinernema* (Rhabditida: Steinernematidae). *Nematology* 9:61–75.
- Zhou, X. S., Kaya, H. K., Heungens, K., and Goodrich-Blair, H. 2002. Response of ants to a deterrent factor(s) produced by the symbiotic bacteria of entomopathogenic nematodes. *Applied and Environmental Microbiology* 68:6202–6209.