

# Evolution of Host Search Strategies in Entomopathogenic Nematodes<sup>1</sup>

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**Abstract:** There is interspecific variation in infective juvenile behavior within the entomopathogenic nematode genus *Steinernema*. This variation is consistent with use of different foraging strategies along a continuum between ambush and cruise foraging. To address questions about the evolution of foraging strategy, behavioral and morphological characters were mapped onto a phylogeny of *Steinernema*. Three species, all in the same clade, were classified as ambushers based on standing bout duration and host-finding ability. One clade of six species were all cruisers based on both host-finding and lack of standing behavior. All species in the ambusher clade had a high rate of jumping, all species in the cruiser clade had no jumping, and most intermediate foragers exhibited some level of jumping. Response to volatile and contact host cues was variable, even within a foraging strategy. Infective juveniles in the ambusher clade were all in the smallest size category, species in the cruiser clade were in the largest size categories, and intermediate foragers tended to be more intermediate in size. We hypothesize that the ancestral *Steinernema* species was an intermediate forager and that ambush and cruise foraging both evolved at least once in the genus.

**Key words:** behavior, entomopathogen, evolution, foraging, host search, nematode, *Steinernema*.

Questions about behavior have been proposed to fall into one of four interrelated categories: causation, function, development, or evolutionary history (Tinbergen, 1963). Research in nematode behavior has focused primarily on causation (i.e., proximate mechanisms). Often, comparative studies of proximate mechanisms are used to make inferences about how organisms have become adapted to their environment. However, comparative studies have validity only if we understand the shared evolutionary history among the species being compared. Entomopathogenic nematode species in the genus *Steinernema* exhibit a great deal of variation in the behaviors exhibited by infective juveniles as they search for a host to infect. This behavioral variation is consistent with the use of different foraging strategies along a continuum between ambush and cruise foraging. The recent completion of a comprehensive phylogenetic reconstruction of the genus (Stock et al., 2001) provides a valuable tool to address questions about the evolution of host search behavior. Thus, the genus *Steinernema* provides a unique model system to address questions about the evolution of foraging strategy.

Foraging strategies can be classified into two broad categories, cruise (widely foraging) and ambush (sit-and-wait), that represent end points on a continuum of strategies (Huey and Pianka, 1981; Pianka, 1966; Schoener, 1971). Organisms are assigned to categories based on how foraging time is allocated to motionless

scanning and moving through the environment (Huey and Pianka, 1981; O'Brien et al., 1989). Cruise foragers allocate more of their foraging time to moving through the environment, scanning for resource-associated cues when moving or during short pauses, while ambush foragers allocate more time to long scanning pauses that are interrupted by shorter repositioning bouts. Foraging strategy influences the types of resources that an organism is likely to encounter. Cruise foragers are more effective than ambushers at finding sedentary and cryptic resources. Ambush foragers are more effective than cruise foragers at finding resources with high mobility. A suite of related characters can be associated with the adoption of a particular foraging strategy, forming what has been termed an adaptive syndrome (McLaughlin, 1989). As a result, understanding the foraging strategy that an organism uses can provide insight into other aspects of its biology.

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) are lethal endoparasites capable of infecting a broad range of insect species. These nematodes occur in soil and epigeal habitats and have been isolated from most regions of the world (Kaya and Gaugler, 1993). Natural populations of these nematodes can play an important role in ecosystems (Akhurst et al. 1992; Campbell et al., 1995; Strong et al., 1996) and have been used as biological control agents of a wide range of insect species in a wide range of crops (Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). There is only one free-living stage: the infective juvenile. Infective juveniles are a nonfeeding, non-developing, non-reproductive stage that bridges the gap between a depleted and a new host. Infective juveniles actively seek out insects to infect, so questions about the causation, function, development, and evolution of their host search behavior can be addressed. The majority of the described entomopathogenic species are in the genus *Steinernema*, and this genus also exhibits the greatest variation in infective juvenile behavior (Campbell and Kaya, 2002).

Adaptive syndromes have been developed for extreme ambush and cruise forager infective juveniles

Received for publication 8 July 2002.

<sup>1</sup> Paper delivered in a symposium on Evolution of Parasitism in Nematoda at the 40th Annual Meeting of The Society of Nematologists, 24–29 August 2001, Salt Lake City, UT.

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This paper was edited by B. C. Hyman.

based on detailed comparative studies of only a small number of species (Lewis, 2002). Ambush foragers have been demonstrated to be more effective at finding mobile insects, and cruise foragers are more effective at finding sedentary insects (Campbell and Gaugler, 1993, 1997). Standing and jumping behavior are correlated with being an ambush forager (Campbell and Gaugler, 1993, 1997; Campbell and Kaya, 1999a, 1999b; 2000). Cruise foragers are attracted to host volatile cues and ambush foragers are not (Lewis et al., 1993). Host contact cues cause cruise foragers to switch to a localized search, but ambush foragers continue ranging searches and are more attracted to host volatile cues (Lewis et al., 1992, 1995). Ambush foragers are associated with epigeal habitats and cruise foragers with deeper soil profiles (Campbell et al., 1996). Cruise foragers tend to be larger in size than ambush foragers (Campbell and Kaya, 2002). A study was undertaken to first assess the level of inter-specific variation in behavioral, morphological, and ecological traits and then develop some hypotheses about how foraging strategies and their associated adaptive syndromes evolved in *Steinernema* (Campbell et al., unpubl.). The results of that study are summarized here.

A hypothesis of the evolutionary history of *Steinernema* was developed based on molecular (regions of LSU rDNA and ITS) and morphological characters (Stock et al., 2001). A phylogenetic tree based on a strict consensus of 4 equally parsimonious trees inferred by maximum parsimony analysis is used here. *Cervidellus alutus* (Cephalobidae), *Pseudacrobeles variabilis* (Cephalobidae), and *Panagrellus redivivus* (Panagrolaimidae) were used as outgroups. Behavioral, ecological, and morphological characters were then mapped onto this phylogeny using MacClade 4 (Maddison and Maddison, 2000). We briefly summarize the results of mapping interspecific variation in the following characters: (i) impact of host mobility on nematode host-finding, (ii) expression of standing and jumping behavior, (iii) attracted to host volatile cues, (iv) response to host contact cues, and (v) infective juvenile size.

Species can be positioned along the continuum between ambush and cruise foraging based on (i) the duration of standing bouts and (ii) the difference in ability to locate insects that are mobile or sedentary. Species with long- and medium-duration standing bouts were classified as ambush foragers, species with short-duration standing bouts as intermediate foragers, and species with no standing bouts as cruise foragers (Campbell and Kaya, 2002). An index of host finding that ranges from -1.0 for extreme cruise to 1.0 for extreme ambush foragers also was used to classify the infective juveniles (Campbell and Gaugler, 1997). Three species were classified as ambush foragers (*S. carpocapsae*, *S. scapterisci*, and *S. siamkayai*) based on both approaches, and they were all in the same clade (Fig. 1). One clade of six species were all classified as cruise

foragers based on both host-finding and lack of standing behavior. The remaining species were cruise or intermediate foragers based on either of the two criteria.

Jumping rate varies considerably among species in *Steinernema* (Campbell and Kaya, 1999b), and jumping can be an important mechanism in facilitating contact with mobile insects (Campbell and Kaya, 1999a, 1999b; 2000). All species in the ambush forager clade had a high rate of jumping, and all species in the cruise forager clade had no jumping. Outside of these two clades, the expression of jumping was highly variable within and between clades, suggesting that increases in jumping rate and loss of jumping have evolved multiple times. There are some interesting exceptions to the general pattern. For example, *S. ceratophorum* had short-duration standing bouts and high rates of jumping (Campbell and Kaya, 2002), suggesting an ambusher to intermediate foraging strategy, but was classified as a cruise forager because it was more effective at finding sedentary hosts than mobile hosts.

The response to volatile and contact host cues was variable among the species tested, even within a foraging strategy (Campbell et al., unpubl.). Species with a strong response to insect host volatile cues from the waxworm *Galleria mellonella* were all, with one exception (*S. monticolum*), in the cruise forager group, but not all individuals in this group responded strongly. For example, *S. longicaudum* had a low level of attraction, and *S. karii* had a medium level of attraction. Ambush and intermediate foragers had low or medium levels of response to volatile cues, with the exception of *S. monticolum*. The response to host contact cues also was highly variable in all foraging categories. Within the cruise foraging clade, the response to host contact was a decrease, an increase, and no change in response to host cues. The interpretation of these behavioral responses may be complicated due to differences in host preference among the different species (Grewal et al., 1997).

Because infective juveniles are non-feeding and have a fixed amount of stored nutrients, we predicted that cruise foragers will be larger (i.e., store more lipids; Selvan et al., 1993) than ambush foragers to compensate for their more energetically expensive search strategy. There is considerable variation in size among species of *Steinernema* (Stock et al., 2001), and Campbell and Kaya (2002) noted that cruise foragers tended to have longer infective juveniles than ambush foragers. The three species of the ambusher clade were all in the smallest size category (<600  $\mu\text{m}$ ), and the species in the cruiser clade tended to be in the largest size categories (>801  $\mu\text{m}$ ). Intermediate foragers tended to be more intermediate in size, but infective juvenile size appears to be a highly plastic trait.

Mapping the traits discussed here onto the phylogeny of *Steinernema* enables us to make predictions about the evolution of foraging strategy in this genus. We hypothesize that the ancestral species was an interme-

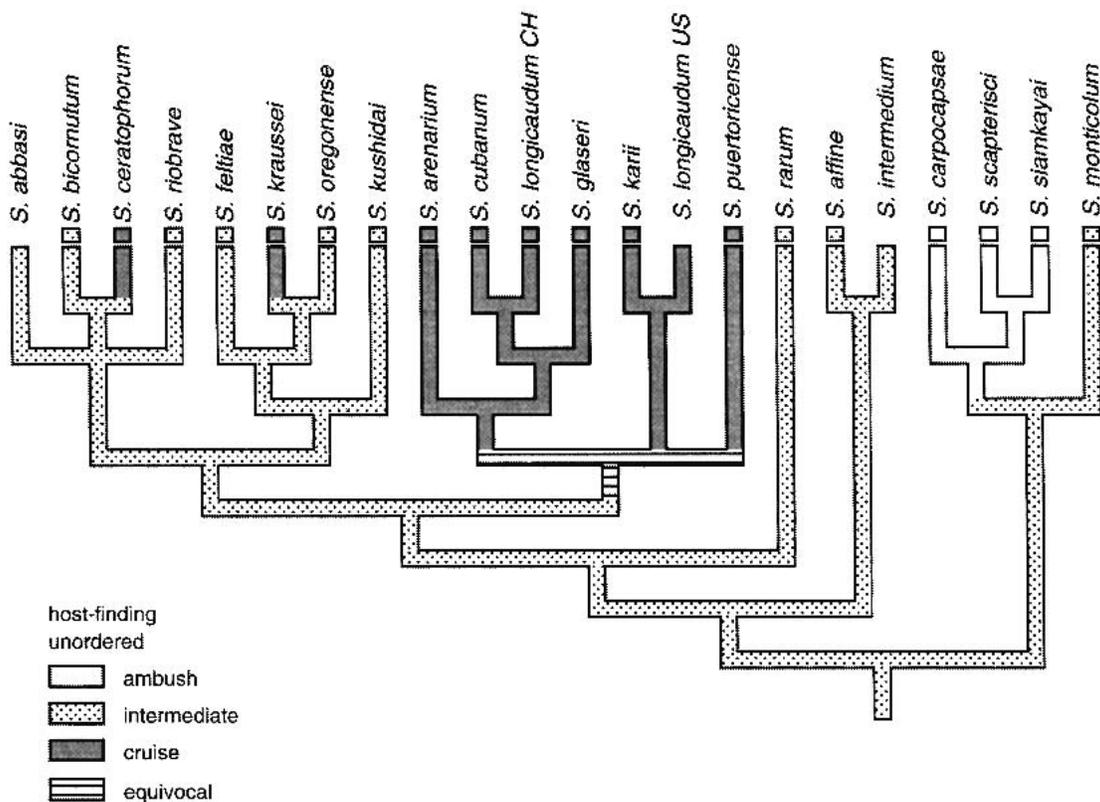


FIG. 1. Foraging strategy mapped onto a phylogeny of *Steinernema* (from Stock et al., 2001). Foraging strategy categories were based on ratio between number of infective juveniles finding a mobile host (unrestrained *Galleria mellonella* larva on the surface of a sand-filled 90-mm petri dish) minus the number finding a non-mobile host (*G. mellonella* larva buried beneath the surface of a sand-filled 90-mm petri dish) divided by the total number of nematodes finding both types of host (Campbell et al., unpubl.). Cruise foragers had ratios close to -1.0, intermediate foragers had ratios close to 0.0, and ambush foragers had ratios close to 1.0.

diate forager and that ambush and cruise foraging each evolved once in the genus. Unfortunately, because each foraging specialization occurs only in one clade (with the possible exceptions of two species, *S. ceratophorum* and *S. kraussei*), we do not have replicate clades to use for statistical tests about the assembly of traits associated with a given foraging strategy. Based on our results, we can hypothesize that the ancestral *Steinernema* had the following traits: low or no standing behavior, low frequency of jumping, medium size, low level of attraction to host volatiles, and no change to localized search after host contact.

Comparison of traits among species is a critical part of investigating adaptations, but it is important to consider the evolutionary relationships among the species being compared (Harvey and Pagel, 1991). Two species may share a trait due to common descent or because both species independently evolved the same response to similar selection pressure. The considerable variation in foraging strategy within the genus *Steinernema* led us to questions about how these strategies may have evolved and if the suites of traits associated with foraging strategy represent adaptations. Understanding the evolutionary history of a species does have some predictive value in terms of what type of foraging strategy a species uses, but there is considerable variability in

some of the traits assigned to different adaptive syndromes. The diversity in Steinernematidae has probably been under-sampled. As new species are described, hypotheses about the evolution of foraging strategy can be further tested and refined.

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