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Effect of Exsheathment on Motility and Pathogenicity of Two Entomopathogenic Nematode Species

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Abstract: The effect of sheath loss on motility and pathogenicity of the entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapasae,* was examined using both naturally and chemically exsheathed (desheathed) infective juveniles. Exsheathed *S. carpocapsae* showed increased motility on agar compared to sheathed nematodes. The presence of a host increased motility threefold in all *S. carpocapsae* treatments. These results suggest that activation of *S. carpocapsae* host finding may result from sheath loss in addition to host stimuli. Desheathed *H. bacteriophora* were significantly less motile than the sheathed or exsheathed groups. The decreased motility may be due to adverse effects of the chemical treatment for desheathment. Sheath loss did not affect the pathogenicity of either species.

Key words: Heterorhabditis bacteriophora, host-finding behavior, nematode, sheath, *Steinernema carpocapsae.*

Steinernema carpocapsae and *Heterorhabditis bacteriophora* are two species of entomopathogenic nematodes used for biological control. These species retain the second-stage cuticle on the third-stage infective juvenile. Exsheathment (loss of the extra cuticle or sheath) is the first step in the infection process but can also occur during rearing and storage (4) or movement through the soil (18). As a result, exsheathed nematodes may be routinely used in laboratory and field studies.

The sheath can protect entomopathogenic nematodes against biotic (8,18) and abiotic (5) factors. If loss of the sheath increases susceptibility to detrimental factors, then selection pressure would promote sheath retention. Two behavioral strategies, with different degrees of motility, have been proposed for host finding by *H. bacteriophora* and *S. carpocapsae* infective juveniles (9). Ambushers adopt a sit-and-wait approach, remaining relatively sedentary, whereas hunters actively search for hosts. *Steinernema carpocapsae* loses its loose-fitting sheath when moving and may adopt the less active ambusher strategy (4). *Heterorhabditis bacteriophora* adopts an active hunter strategy (6) and retains its tight-fitting sheath while moving (4). However, Campbell and Gaugler (4) found that exsheathment in *H. bacteriophora* could result in reduced motility compared to sheathed nematodes, indicating a change in host finding behavior due to sheath loss.

Both *H. bacteriophora* and *S. carpocapsae* are associated with symbiotic bacteria (*Xenorhabdus* spp.) stored in the digestive tract of the infective juvenile (2). The symbiont contributes to their high virulence and broad host range. The sheath plugs the anal and oral openings of *H. bacteriophora* and *S. carpocapsae* (unpublished observation). Sheath loss, with the loss of these plugs, could affect the nematode–bacteria relationship and result in reduced pathogenicity. Westerman (19) compared the effect of storage on efficacy of *Heterorhabditis* sp. and noted that nematode "quality" was related to sheath retention.

The biological control potential of *H. bacteriophora* and *S. carpocapsae* depends on the ability of these nematodes to find and kill their hosts. Conditions associated with production and storage of these nematodes can influence sheath retention (4). Loss of the sheath may affect efficacy by altering nematode host finding or reducing pathogenicity. The purpose of this study was to evaluate the effect of sheath loss on the motility and pathogenicity of

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these two species of entomopathogenic nematodes.

Materials and Methods

Heterhorhabditis bacteriophora and *S. carpocapsae* were reared in larvae of the greater wax moth, *Galleria mellonella* (7). Infective juveniles were harvested 24–48 hours following emergence from the host. Nematodes were stored at 25 C for 1 week, then separated into sheathed, desheathed, and exsheathed groups (4). The sheathed group consisted of untreated nematodes. Desheathed nematodes were obtained by treating the nematodes with 1% sodium hypochlorite solution (NaOCl) for 5 minutes. Exsheathed nematodes were obtained using a filter paper barrier. A single sheet of 9-cm diameter (Fisher P8) filter paper was suspended over a Petri dish bottom (60 × 15 mm) containing deionized water, with the center of the paper contacting the water. Nematodes were applied to a 47-mm diameter, 5 μm membrane filter, and the membrane filter was inverted and placed on top of the filter paper. Nematodes that moved through the filter paper and into the water reservoir were exsheathed. All experiments were run in blocks, each block representing a replicate and consisting of each species/treatment combination.

Motility: The effect of sheath loss on nematode motility, measured by the distance moved from the point of application, was compared using two agar assays. The first assay was designed to compare the effect of sheath loss on the random motility of the nematodes in the absence of a host. A host was included in the second assay to compare the effect of sheath loss on nematode motility in the presence of host stimuli. In the no-host assay, 2% agar was poured into Petri dishes (150 × 15 mm) and cooled for 30 minutes. Sheathed, desheathed, or exsheathed nematodes (705 ± 107.9 nematodes per dish) were applied by brush to a 1-cm-d zone in the center of the dish, and the dishes were covered and incubated at 25 C for 1 hour. The dish was divided into two zones: the area within 2 cm of the point of application and the rest of the dish. The nematodes from these two zones were rinsed into Petri dishes and counted. There were five to eight replicates for each treatment group.

The motility of sheathed, desheathed, and exsheathed *S. carpocapsae* and sheathed and desheathed *H. bacteriophora* in the presence of a host was compared using the agar assay of Gaugler et al. (9). Due to insufficient quantities, no exsheathed *H. bacteriophora* were available for the host-present assay. Briefly, nematodes (1238.9 ± 103.8 nematodes per assay) were brushed into an application zone (1 × 4 cm) in the center of a 14 × 14 cm plate of agar. Two pipette tips were suspended over the agar surface at points equidistant (3 cm) to the application zone; one contained two last-instar *G. mellonella* larvae and the other served as a blank control. The plates were incubated for 1 hour at 25 C. Following incubation, nematodes were rinsed off the agar surface in three groups, those individuals: (i) moving towards the host (motile [+], (ii) remaining in the application zone (nonmotile), and (iii) moving away from the host (motile [−]). There were 8–11 replicates per treatment group.

To compare the trends of nematode motility in the different treatment–species combinations the following Motility Index was devised:

$$M = A - N,$$

where A = the percentage of motile nematodes (those that moved out of the application zone) and N = the percentage of nonmotile nematodes, or those infective juveniles that remained within the application zone. Thus, if all nematodes were nonmotile, M = (−100) and if all were motile M = (+100). The use of this index enables comparisons of the overall behavior of the nematode populations through the use of a single value.

Pathogenicity: Pathogenicity of desheathed, sheathed, and exsheathed *H. bacteriophora* and *S. carpocapsae* was compared on filter paper and in sand. The fil-

ter paper assay placed the nematode in close proximity to the host, thereby assuring host–parasite contact. In sand assays, host contact is not assured and host finding is required. The Median Lethal Dose (LD_{50}) for each species was determined, using a modification of the assay (14) described below, to be 6.63 (95% CI: 3.04–11.52) for *H. bacteriophora* and 0.78 (CI: 0.66–1.10) for *S. carpocapsae* on day 2 posttreatment. Nematode-induced host mortality did not alter significantly 2 or 3 days posttreatment (based on overlapping confidence intervals). Therefore, a time period of 2 days and doses of three *H. bacteriophora* and one *S. carpocapsae* per host were used in the pathogenicity comparisons. Both pathogenicity experiments included a no-nematode water control.

The first pathogenicity assay was a modification of that developed by Miller (14). Infective juveniles in 34.5 µl of deionized water were pipetted onto filter paper discs in multiwell tissue culture dishes. A single *G. mellonella* larva was added to each well, and the dishes were sealed and incubated at 25 C. Host mortality was recorded 2 days after treatment. There were four to six replicates per treatment consisting of ten *G. mellonella* each.

The effect of sheath loss on pathogenicity in sand was compared by burying a single *G. mellonella* larva in 3 g of sterilized sand with a moisture content of 10% by weight. Infective juveniles were added to the sand surface in 34.5 µl of deionized water. The dishes were sealed and incubated at 25 C. Host mortality was recorded after 2 days. There were four replicates consisting of 20 *G. mellonella* per treatment group.

Statistical analysis: Pathogenicity data were corrected for control mortality using Abbott's formula (1). Proportional data were normalized using an arcsin (square root) transformation prior to analysis. Pathogenicity and motility data were analyzed using General Linear Models Procedure (GLM) and Duncan's multiple-range test (16).

Results and Discussion

Motility: Although the two motility assays varied in the physical setup, the conditions and principles involved are the same. Therefore, comparisons of the behavioral trends of the populations in each assay will be made. In the no-host assay, the motility index of exsheathed *H. bacteriophora* was higher than the desheathed treatment ($P = 0.0001$) (Table 1). The motility index of the sheathed group increased significantly when a host was present, but no increase was seen in the desheathed group (Table 2). No difference was found between sheathed and desheathed in the percentage of nematodes moving toward the host

Table 1. Effect of sheath loss on the motility of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* infective juveniles in the absence of a host.

Species/treatment	Percentage of nematodes†		Motility‡ index
	Motile	Nonmotile	
H. bacteriophora			
Desheathed	75.6 ± 0.5 b	24.4 ± 0.5 a	51.1 ± 1.0 B
Sheathed	79.9 ± 3.0 ab	20.1 ± 3.0 ab	59.9 ± 5.9 AB
Exsheathed	85.8 ± 1.8 a	14.2 ± 1.8 b	71.6 ± 3.6 A
S. carpocapsae			
Desheathed	40.1 ± 5.0 b	59.9 ± 5.0 b	−19.8 ± 10.0 D
Sheathed	24.1 ± 3.8 c	75.9 ± 3.8 a	−51.7 ± 7.5 E
Exsheathed	59.0 ± 2.3 a	41.0 ± 2.3 c	17.9 ± 4.6 C

Values are the means of eight (*H. bacteriophora*) and five (*S. carpocapsae*) replicates. Means with the same lowercase letter are not significantly different within species and location ($P > 0.05$). Motility indices with the same uppercase letter are not significantly different ($P = 0.0001$).

† Percentage of nematodes recovered outside (motile) or within (nonmotile) the application zone.

‡ Motility index = (−1) (N) + (+1) (A−N).

TABLE 2. Effect of sheath loss on the motility of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* infective juveniles in the presence of a host.

Species and treatment	Percentage of nematodes†			Motility‡ index
	Motile (+)	Nonmotile	Motile (−)	
H. bacteriophora				
Desheathed	39.4 ± 5.2 a	34.2 ± 4.5 a	26.3 ± 3.0 b	31.5 ± 9.0 C
Sheathed	41.5 ± 5.5 a	9.1 ± 1.5 b	49.1 ± 6.9 a	81.2 ± 3.1 A
S. carpocapsae				
Desheathed	54.6 ± 2.4 ab	16.8 ± 1.8 ab	28.6 ± 1.6 a	66.3 ± 3.6 AB
Sheathed	51.0 ± 2.5 b	24.0 ± 2.8 a	25.0 ± 1.4 a	52.0 ± 5.6 B
Exsheathed	63.8 ± 4.5 a	15.2 ± 3.4 b	21.0 ± 3.1 a	69.6 ± 6.7 AB

Values represent the means (±SEM) of eight (*H. bacteriophora*) and 11 (*S. carpocapsae*) replicates. Means with the same lowercase letter are not significantly different within species and location ($P > 0.05$). Motility indices with the same uppercase letter are not significantly different ($P = 0.0001$).

† Percentage of nematodes recovered: outside the application zone and moving towards the most (motile +), moving away from the host (motile −), or recovered within the application zone (nonmotile).

‡ Motility index = (−1) (N) + (+1) (A−N).

(motile [+]), but more sheathed nematodes moved away (motile [−]) ($P = 0.0088$) (Table 2).

Exsheathed *S. carpocapsae* infective juveniles were more motile than the sheathed ones in the no-host assay, based on the motility indices ($P = 0.0001$) (Table 1). Host presence increased the motility of all *S. carpocapsae* treatment groups, with a threefold increase in the motility indices compared to those when no host was present ($P = 0.0001$) (Table 2). In addition, more exsheathed nematodes moved towards the host (motile [+]) than did sheathed nematodes ($P = 0.0209$) (Table 2).

The ambusher strategist takes a lie-and-wait approach, indicated by reduced motility, until "activated" by the presence of a potential host (9,12). We observed a threefold increase in motility of all *S. carpocapsae* treatment groups in the host-present assay compared to the no-host assay. Increased motility was also seen in sheathed *H. bacteriophora* in the host-present assay, suggesting that even the hunter strategist has an activated state of host finding.

In the absence of host cues, low motility index values were recorded for all *S. carpocapsae* treatments. However, the exsheathed and desheathed *S. carpocapsae* were significantly more motile than the sheathed. The infective stages of many parasitic organisms alter their behaviors in response to specific trigger cues (17). Sheath loss, in addition to host presence, may act as a trigger cue for *S. carpocapsae,* resulting in an increase of active host-finding behavior.

All *H. bacteriophora* groups had higher motility indexes than the *S. carpocapsae* groups in the no-host assay ($P = 0.0001$) (Table 1). However, no differences existed between the sheathed *H. bacteriophora* and exsheathed or desheathed *S. carpocapsae* groups when a host was present ($P = 0.0001$) (Table 2). Motility levels may be related directly to the host-finding strategy used by these two nematode species. The level of motility seen in sheathed *H. bacteriophora* demonstrates the active hunter strategy of host finding, whereas the lower motility of sheathed *S. carpocapsae* is indicative of the sedentary ambusher strategist. This agrees with previous studies of the host-finding behavior of these nematodes (3,6,9,10,15). The active strategy employed by *H. bacteriophora* increases the chance of encountering a host and escaping from unfavorable habitats (12). Exsheathment of *H. bacteriophora* did not alter this behavior. However, desheathed individuals, which had been chemically treated to remove the sheath, showed decreased motility compared to the sheathed and exsheathed juveniles. The potential negative effects of desheathment on ento-

mopathogenic nematodes have been noted in other studies (4,18). The negative effect observed here on *H. bacteriophora* motility raises further questions about the use of NaOCl for sheath removal of this species.

Pathogenicity: Sheath loss had no significant effect on the pathogenicity of either species of nematode. Desheathed *H. bacteriophora* caused lower host mortality than the sheathed and exsheathed treatments (69.5 ± 12.8% versus 86.4 ± 5.5% and 86.4 ± 5.3%), but the difference was not significant. No differences were found among the three *S. carpocapsae* treatment groups, with mortalities of 61.6 ± 11.6%, 72.6 ± 4.5%, and 61.6 ± 10.7% for desheathed, sheathed, and exsheathed, respectively.

No differences were found among the three *H. bacteriophora* treatments in the sand assay, with host mortalities of 74.6 ± 5.1%, 56.8 ± 6.4%, and 64.4 ± 6.6% for desheathed, sheathed, and exsheathed, respectively. However, differences in pathogenicity were seen between *S. carpocapsae* treatment groups in the sand assay: Desheathed *S. carpocapsae* caused higher *G. mellonella* mortality ($P = 0.0039$), with 39.4 ± 7.1% host mortality versus 9.2 ± 4.1% and 6.6 ± 4.8% for sheathed and exsheathed.

The increased host mortality caused by desheathed *S. carpocapsae* in sand suggests that the chemical treatment enhanced the nematode's host-finding ability. Some evidence exists for chemically induced activation of nematodes (11,13). Although no differences were found between the motility of the three *S. carpocapsae* groups in the agar assays, this assay recorded motility after only 1 hour, whereas the sand assay represented nematode motility after a 48-hour period. These results suggest that the chemically treated nematodes maintained a higher level of motility, resulting in an increased chance of finding and killing their host.

This study indicates that sheath loss does not reduce nematode pathogenicity, but may have a significant effect on nematode motility. Chemical desheathment significantly reduced *H. bacteriophora* motility. Conversely, sheath loss increased *S. carpocapsae* motility. Storage and rearing methods increase sheath loss (4). The effect that this may have on the biological control potential of these two species of nematode, particularly in the increased motility and possible increase in host finding by *S. carpocapsae,* deserves thorough examination.

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