Ultrastructural Characteristics of the Infective Juveniles of Steinernema spp. (Rhabditida: Steinernematidae) with Reference to Their Motility and Survival¹

Eizo Kondo and Nobuyoshi Ishibashi

Laboratory of Nematology and Entomology, Department of Horticultural Science, Saga University, Saga 840, Japan

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The ultrastructure of infective juveniles of Steinernema feltiae, S. bibionis and S. glaseri was compared under SEM and TEM to obtain structural evidence for differences in their motility and survival abilities, both of which are crucial to infectivity under natural conditions. The arrangements of the labial/cephalic papillae and amphidial apertures of these nematodes were similar, however, the amphidial aperture with its connecting large nerve bundle was more conspicuous in S. feltiae. The lipid droplets were deposited deep in the cells of the lateral chord and intestine of which microvilli had degenerated. The arrangement of thick and thin myofilaments was clear in somatic muscle cells which always contained many glycogen granules and mitochondria with developed cristae. For all nematodes examined, the cuticle consisted of external cortical (ECL), internal cortical (ICL) and basal layers (BL); the median layer was not clearly differentiated from ICL. The three layers were also recognized in the ensheathing cuticle. The osmiophilic ECL and striated BL were developed to the greatest extent in S. feltiae which also showed the highest nictitating activity and leaping behavior. These structural features may explain the differences in motility and survival ability in the infective juveniles of the three species of steinernematid nematodes.

INTRODUCTION

The steinernematid nematodes have been used as biocontrol agents against various insect pests (Poinar, 1979). In a series of inoculation experiments, the common cutworm, Spodoptera litura, was infected by Steinernema feltiae, S. bibionis and S. glaseri. However, the infectivity of these nematodes was not only considerably different from each other (Kondo and Ishibashi, 1986a) but was also greatly affected by environmental factors such as temperature and moisture (Poinar, 1979; Kondo and Ishibashi, 1985). To establish an infection, an applied nematode should actively move to invade a host (Poinar, 1979). Besides various biological studies on the nematode infectivity, the mode of nematode infection was also studied under a scanning electron microscope (Kondo and Ishibashi, 1987). To obtain detailed information on the structure and function, a transmission electron microscope (TEM) was extensively used on nematodes of different ecological habitats. On entomogenous steinernematid nematodes, how-

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ever, TEM has not yet been used to any great extent, except for studies conducted by Poinar (1967, 1968). The present electron microscopic study on the infective juveniles of steinernematid nematodes was conducted to investigate the structural rationale of their high motility and survival ability, both of which are important for the nematode to infect insect pests in a fluctuating environment.

MATERIALS AND METHODS

Nematodes. Infective juveniles (J_{118}) of S. feltiae, S. bibionis and S. glaseri were used; the J_{118} used were recovered from the cadavers of G. mellonella larvae 14 days after inoculation with ca. 1,000 J_{118}.

Scanning electron microscopy (SEM). The surface fine structure of the head portion of J_{118} of each species was observed for comparison. The method employed to prepare nematodes for SEM observation was that developed for soft biological materials (Kelley et al., 1973) with modifications. The nematode was pre-fixed in 2% OsO_4 in 0.1 M phosphate buffer (pH 7.2) for two days at 5°C, thoroughly rinsed with distilled water, and treated with a saturated water solution of thiocarbohydrazid for 45 min at room temperature. After being rinsed with distilled water, the materials were post-fixed in 1% OsO_4 for 30 min at room temperature and then dehydrated through a graded ethanol series. The dehydrated materials were kept in pure ethanol for more than two days, critical-point dried, mounted on specimen stubs using a conductive tape, coated with gold for 2 min by ion-sputter, and observed under JSM F-15 operated at 15 kV.

Transmission electron microscopy. The J_{118} of the three nematode species were fixed in buffered 3% glutaraldehyde for 48 hr at 5°C. To improve the fixation, the tail tips of nematodes were cut with a surgical knife and fixed for an additional 2 hr in the new cold fixative. After thoroughly rinsing in the cold phosphate buffer, the specimens were post-fixed by 1% OsO_4 for 2 hr at room temperature. The fixed materials were dehydrated in a graded ethanol series and finally embedded in Spurr's low viscosity epoxy resin. To improve the resin infiltration, the materials were placed under gentle vacuum for 1 hr before polymerization at 70°C for 8 hr. Using an LKB ultramicrotome, ultrathin longitudinal, cross and oblique sections were made, then stained in a saturated water solution of uranyl acetate for 20 min, and finally counter-stained in lead citrate for 5 min at 25°C. Observation was made with a JEM-2000FX electron microscope operated at 80 kV. The thickness of cuticle layers and the distance between the striations of the basal layer were measured on the cross section. For numerical comparison of the layers, eight to ten individuals were used for each nematode species.

RESULTS

Head surface morphology of infective juveniles
The head surface structure of the ensheathed J_{118} differed among nematode species (Fig. 1), although structural variations were individually recognized in a given species. The non-functional oral aperture was nearly closed in all three species. The lip surrounding the oral aperture was almost flat or slightly elevated (Fig. 1). The crescent shaped opening of the amphid aperture was more conspicuous in S. feltiae (Fig. 1A) than in the other two nematode species. In all three species, numbers of labial and cephalic
papillae were six and four, respectively. In *S. feltiae*, the cephalic papillae were more clearly recognized than the labial ones.

**Internal structure of infective juveniles**

Morphologically, the nerve system was most conspicuous in the *J*" of all steinernematid nematodes examined. In the cross section posterior to the amphid aperture, a large nerve bundle ran in the lateral chord (Fig. 2A). At the mid body portion, there were larger nerve bundles located in the deeper portion of the lateral chordal cells (Fig. 2C), and in the ventral chord a large nerve bundle was also observed (Fig. 2F).

The digestive tract of *J*" was largely degenerated. In the anterior body portion, the triradiated intestinal lumen of the esophagus was compressed; the proximal region of the lumen was often constricted (Fig. 2A). In the mid body portion, the functional intestinal lumen with microvilli was not observed in *S. feltiae* and *S. glaseri* (Fig. 2B). In *S. bibionis*, partially degenerated microvilli projected into the intestinal lumen which were surrounded by many mitochondria (Fig. 3).

The lipid droplets deposited deep in the intestinal cells of all nematode species; most of the droplets were nearly spherical but some were fused with each other (Figs. 2C-D). They were commonly associated with clusters of glycogen granules, especially in the peripheral portion of the intestinal cell. Besides the lipid droplets and glycogen granule clusters, several electron dense protein balls were found in the intestinal and chordal cells (Fig. 2C).

The somatic muscle cell of all infective juveniles was platymyarian and meromyarian; the number of somatic cells between the chords was six in the anterior and mid body portions. The thick and thin myofilaments were clearly arranged in these somatic cells. The non-contractile portion of the muscle cell contained many mitochondria with well developed cristae. These mitochondria were always associated with many glycogen granule clusters (Figs. 2D–G).

The average body length of the *J*" used was 529 µm for *S. feltiae*, 685 µm for *S. bibionis*, and 1,325 µm for *S. glaseri*. Cuticle thickness was generally proportionally
related to the body size: about 300 nm in *S. feltiae*, about 400 nm in *S. bibionis*, and about 790 nm in *S. glaseri* (Table 1). In all species, the cuticle consisted of three layers; the external cortical, internal cortical and basal layers (Fig. 4). The external cortical layer was a highly osmiophilic membrane. The percent thickness of this layer was the greatest in *S. feltiae*, followed by *S. bibionis* and *S. glaseri* (Table 1). Except for a deeper portion of such cuticular invaginations as excretory pore and amphid aperture, this layer covered the whole nematode body. However, it was thin in the area of lateral field involutions (Figs. 4D–E) and between the cuticle annulations (Fig. 4B).

The internal cortical layer was not as clearly differentiated from the median layer as in the infective stage juveniles of tylenchid nematodes. This layer was almost evenly electron stained, except for the deeply stained internal cortical layer around the
Fig. 3. Cross section of intestinal cells of *S. bibionis* infective juvenile. A: densely packed lumen of intestine (LI) containing many mitochondria (Mt), lipid droplets (Li) and glycogen granules (G). Nu, nucleus. B: Lumen of intestine with partially degenerated microvilli.

Table 1. Numerical comparison of cuticle thickness and the ratio of cuticle layers among the infective juveniles of steinernematid nematodes

<table>
<thead>
<tr>
<th>Nematodes</th>
<th>Thickness of cuticle (nm)a</th>
<th>% cuticle layersb</th>
<th>Distance between BL striations (nm)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. feltiae</td>
<td>298</td>
<td>ECL 8.5 ICL+ML 36.8 ML 54.7</td>
<td>21.8</td>
</tr>
<tr>
<td>S. bibionis</td>
<td>403</td>
<td>6.1 ICL+ML 43.4 ML 50.5</td>
<td>20.2</td>
</tr>
<tr>
<td>S. glaseri</td>
<td>788</td>
<td>5.3 ICL+ML 55.3 ML 39.4</td>
<td>22.4</td>
</tr>
</tbody>
</table>

a Thickness of cuticle at the mid body portion of infective juveniles.
b Percentage of the thickness of the external cortical layer (ECL), internal cortical layer (ICL) plus median layer (ML), and striated basal layer (BL).
c Distance between striations of basal layer in the longitudinal section of the cuticle.

involvement of lateral field (Fig. 4D).

The most outstanding structural characteristic of the infective juvenile cuticle was the high proportion of the striated basal layer (Figs. 4A–C). The percentage of this layer was higher in *S. feltiae* and *S. bibionis* than in *S. glaseri* (Table 1). The oblique section of the cuticle indicated a regular arrangement of cross-linked rods in the basal layer (Fig. 4C). The striations gradually diminished toward the lateral field (Fig. 4E) and completely disappeared under the lateral field (Fig. 4D). No clear boundary was recognized between the basal layer and rather thick underlying hypodermis (Figs. 4A–C, E).

The body of infective juveniles was enclosed within a sheath which originated from the cuticle completely shed by the preceding 2nd stage juvenile; the sheath consisted of the external cortical, internal cortical and basal layers (Fig. 4D).

**DISCUSSION**

Infective juveniles of steinernematid and heterorhabditid nematodes are enclosed
within a second stage cuticle, and have a higher survival ability and motility than the parasitic nematodes (Poinar, 1979). The present electron microscopic studies on *S. feltiae*, *S. bibionis* and *S. glaseri* revealed the structural characteristics which are considered to be largely responsible for this persistence and motility.

The infective juveniles have to survive without feeding under natural conditions because they do not have functional feeding and digestive organs, as was clearly observed under SEM and TEM. Accordingly, the period of their survival depends entirely on the amount of substances stored in their body; to successfully infect an insect, the applied nematode is required to survive for a desired time by consuming these stored substances. The chief reserves of food in free-living nematodes, in plant-parasitic nematodes, and in the free-living stages of many animal-parasitic nematodes are lipids, although glycogen is also a main food reserve in most adult animal-parasitic nematodes (Lee and Atkinson, 1976). This storage lipid locates mostly in the hypodermis, especially in the lateral chords, in the non-contractile part of the muscle cells, in the intestine, and in the ovaries. In the infective juveniles of plant-parasitic nematodes, small, spherical lipid droplets are present in the intestinal cells (Dropkin and Acedo, 1974; Kissel et al., 1974).
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The present study clearly showed that large lipid droplets were deposited deep in the intestinal and lateral chordal cells of the infective juveniles of the three species of steinernematid nematodes.

In most adult animal-parasitic nematodes, the other main food reserves are glycogen which is used prior to lipids as an energy source (Lee and Atkinson, 1976). The mobilization of lipid droplets to glycogen granules has been observed under TEM in some plant parasitic nematodes; for instance, the lipid droplets are mobilized to glycogen granules and consumed during the development of the dispersal 4th stage juvenile of the pine wood nematode, Bursaphelenchus xylophilus (Kondo and Ishibashi, 1978). Similarly, the lipid droplets of infective juveniles of steinernematid nematodes examined in the present study were partly mobilized and decreased during storage at 25°C under a moist condition (unpublished data). The oxygen consumption of S. feltiae infective juveniles is temperature dependent (Burman and Pye, 1980). Therefore, the temperature should be carefully considered in evaluating the nematode infectivity, because the movement and survival of nematodes primarily depend on the amount of remaining stored energy.

Structure of the cuticle is also very important for the movement and survival of nematodes. Generally, the cuticle of tylenchid and rhabditid nematodes consists of the cortical, median and basal layers (Bird, 1971; Kondo, 1984). The outermost part of the cuticle is composed of three membranes: a thin osmiophilic membrane which mainly consists of lipoprotein, a moderately osmiophilic middle membrane, and a highly osmiophilic membrane (Bird, 1971). The outermost osmiophilic membrane clearly plays a role in decreasing water loss from the nematode body and the desiccation survival of nematodes is closely related to the thickness of this membrane (Kondo and Ishibashi, 1978). In the case of the steinernematid nematodes used in this study, the thickness of the external cortical layer was about 25 nm for S. feltiae and S. bibionis and about 42 nm for S. glaseri, the cuticle of the latter also being thicker than that of the former two species. Including the external cortical layer, the total thickness of the 3rd stage cuticle was related to the resistance to drying and glutaraldehyde treatments: these treatments less affected the infective juveniles than the parasitic ones which have thinner cuticle. The ratio of the cuticle thickness to the body diameter was 1: 46 for the parasitic and 1: 27 for the infective juveniles of S. feltiae (unpublished data).

The basal layer has a striation which originates from the cross-linkage of protein and gives a structural integrity to the cuticle (Bird, 1971). This layer occasionally changes during nematode development; the layer of the root-knot nematode, Meloidogyne javanica, disappears two days after infection in the roots, although the infective juveniles and male adults retain the distinct layer (Bird, 1968). In B. xylophilus, the basal layer is greatly developed in the dispersal 4th stage larvae (Kondo and Ishibashi, 1978) which actively move from the pupal chamber wall to the adult of the insect vector, Monochamus spp. and then to wounded tissues on healthy pine trees. These results may indicate that the striated basal layer is important for nematode mobility. Actually, the ratio of the basal layer was greater in infective than in parasitic juveniles in all three species of steinernematid nematodes examined.

As was indicated in our previous study (Kondo and Ishibashi, 1986 b), the three species of steinernematid nematodes showed considerably different behavior on the soil surface: S. feltiae actively nictated and frequently leaped, S. bibionis did so for a shorter time than S. feltiae, and S. glaseri mostly crawled without nictating. The present
TEM observation showed a positive relation between this nictating activity and the ratio of the basal layer against total cuticle thickness; the ratio was the highest in *S. feltiae* which showed the most active nictating and leaping behavior.

Although the nictating behavior is considered to relate to the host-finding behavior in *S. feltiae* (Poinar, 1979), no direct evidence of this has yet been obtained. Among nematode sensory organs, the amphid aperture is considered to be a main receptor accepting the stimuli emanating from the hosts. Poinar (1968) assumed that the highly developed amphids and somatic muscles are important in locating new hosts. The present TEM observations supported his assumption because of the greatly developed nerve bundles in the three species of steinernematid nematodes, although they showed considerably different infectivity on *S. litura* larvae (Kondo and Ishibashi, 1986 a). The SEM observations on the head region of the infective juveniles also showed difference among nematode species. Although the observed structure did not really indicate the sensory organs of the infective juveniles but the rudiments of the preceding stage, the nematode infectivity on *S. litura* larvae was superficially related to the degree of amphid development; the amphid and its connecting nerve bundle in infective juveniles were more conspicuous in *S. feltiae* than in *S. bibionis* and *S. glaseri* which have weaker infectivity than *S. feltiae* on the common cutworm (Kondo and Ishibashi, 1986 a). For a precise clarification of the host-finding mechanism, detailed neurophysiological and ultrastructural studies must be conducted in future.

REFERENCES


KONDO, E. and N. ISHIBASHI (1987) Histological and SEM observations on the invasion and succeeding
Ultrastructure of *Steinernema* spp.


