Parasitic Development of *Filipjevimermis leipsandra* Poinar and Welch (Mermithidae) in *Diabrotica u. undecimpunctata* (Chrysomelidae)

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Introduction

In July 1967 Dr. F. P. Cuthbert sent the author some living mermithids which had developed in larvae of *Diabrotica balteata* and were subsequently described as a new species, *Filipjevimermis leipsandra* Poinar and Welch (in press). The life history of this species will be discussed by Cuthbert (in press).

F. leipsandra is unusual in several respects. Males are very rare and not necessary for the propagation of the species. Another interesting character of this nematode is its association with the central nervous system of the host during the early stages of parasitism.

After penetrating through the cuticle and entering the hemocoel of the host larva, the infective stage nematode seeks out and enters one of the ganglia of the central nervous system. The protocerebral lobes and subesophageal ganglion are most frequently attacked, but other ganglia may also be entered. The nematodes do not initiate development without first entering a ganglion, and those which remain in the hemolymph are encapsulated and eventually killed.

Further studies on the host range of this nematode and selection of ganglia will be presented by the author and Dr. Götz, a visitor in this laboratory for three months during the autumn of 1967.

The present paper discusses the parasitic development of *F. leipsandra* and its association to the central nervous system of *D. u. undecimpunctata*.

Materials and Methods

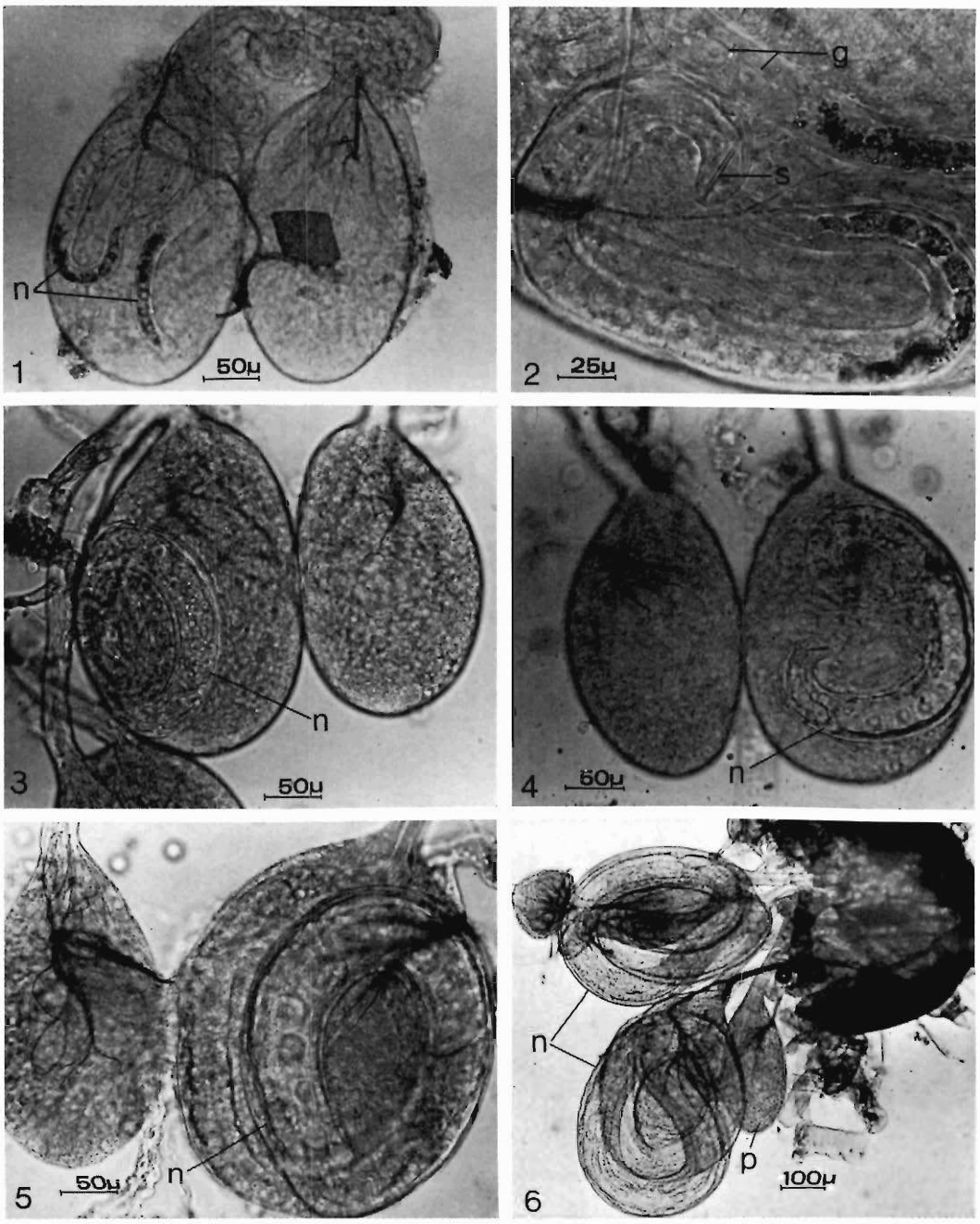
Adult mermithids oviposited in dishes of water. After the eggs hatched, the infective juveniles were held at 12 C for future infection experiments. Since *D. balteata* does not occur in Northern California, a local species, *D. undecimpunctata undecimpunctata* was successfully infected and served as a host for the nematode. Comparisons of this host with *D. balteata* showed that the parasitic development was similar in both insects. *Diabrotica u. undecimpunctata* was grown on corn seedlings using a modification of the method presented by Rimando et al. (1965). Larval development ranged from 18–25 days, with three instars.

One-day-old *Diabrotica* larvae were placed between two layers of moist filter paper in a small petri dish for laboratory infections. Infective stage nematodes were placed directly on the inner side of both pieces of filter paper. The edges of the paper were held together with a ring and the infection chamber left at 70 C for 80 minutes. The insects were then removed and kept on roots of germinating corn seedlings during the remainder of the experiment.

At regular intervals over a 4-week period, an infected host was dissected in Ringer's solution and nematode development noted. All observations, including photographs and drawings, were made by the author with fresh material lightly stained with cotton blue.

Results

The juvenile of *F. leipsandra* molted once within the egg before hatching. After hatching, the preparasitic stages were extremely active and entered the host by direct penetration through the cuticle. Observations indicated that both glandular secretions and stylet action aided penetration. When contact with the host was made, the nematode forced its head against the cuticle and began a succession of stylet movements. Occasionally, a quick movement of the host brushed off the nematode and viscous material could be seen coming from the mouth of the latter. In one instance after entering the hemocoel, the nematode migrated to the head end of the host and explored the periphery of the subesophageal ganglion for several seconds. It then forced its head through the neural lamella and perineurium and slowly entered the gan-



Figures 1–6. 1. Two juveniles of *F. leipsandra* 2 days after infection in one of the protocerebral lobes of a first instar *Diabrotica* larva. n = nematode. 2. Close-up of nematodes in figure 1 showing the gland cells or stichocytes (g) of 1 specimen and the stylet(s) of another. 3. *F. leipsandra* in a protocerebral lobe of a first instar *Diabrotica* larva 4 days after infection. n = nematode. 4. *F. leipsandra*

glion, following the inner contour of the neural lamella and eventually coming to rest in the neuropile. It appears that the cells and fibers in the ganglion are not broken, but just pushed aside as the nematode enters. Once inside the ganglion, the parasite remains still, only altering its position through subsequent growth.

The nematode may remain within the host from 12 to 22 days, depending on the state of the host and number of parasites present. After 22 days, the parasite may reach a size of 4.70 cm and a width of 0.26 mm. This is an enormous change from the average length (0.54 mm) and width (0.018 mm) of the infective juvenile.

The size of the parasite after 24 hours in the host did not differ significantly from that of the infective stage (Fig. 7). The well-developed stylet leads directly into the pharyngeal tube which runs the length of the pharynx. The anterior portion of the pharynx is narrow and consists of a cuticle lined tube surrounded by a layer of nucleated sheath tissue. The prominent nerve ring encircles this anterior portion of the pharynx, which gradually widens to its full width at the level of the atrophied gland reservoirs. These latter paired oval shaped bodies are what remains of the two long gland reservoirs of the infective juvenile.

In the infective stage, these reservoirs are filled with globules which are probably enzymes used in softening the host cuticle during penetration. The basal two-thirds of the pharynx, including the stichosome (Steiner, 1933) contains 16 gland cells or stichocytes. These cells are arranged in two rows forming the two subdorsal portions of the pharynx. The pharynx is attached to the intestine by a thin layer of connective tissue, however, this is only a physical, not a functional union and does not persist for long. The intestine proper is filled with globules, but a lumen in the anterior portion contains large crystals of unknown origin. These crystals are very distinct in the early parasitic stages, but gradually disappear and are absent in the postparasitic juvenile. The cellular structure of the gut is still obscure, but a distinct rectum and anus are present. The gonad rudiment lies on the ventral side of the body just behind the junction of the pharynx and intestine.

Figure 1 shows two nematodes in one of the protocerebral lobes of the host 2 days after infection. Although still no significant increase in length, the width was now 0.025 mm and the stichocytes were prominent (Fig. 2). Little noticeable change occurred after 3 days in the host except for an increase in width (0.034 mm). After 4 days, however, a significant increase in length (0.712 mm) as well as width (0.053 mm) produced a noticeable enlargement of the infested protocerebral lobe of the host (Fig. 3).

After 5 days within the protocerebral lobe of a first instar host, the nematode reached a length of 0.741 mm (Fig. 4, 8). The stylet and pharyngeal tube were distinct and the stichocytes increased greatly in size. Remnants of the paired gland reservoirs were still visible. Movement of the crystals within the intestinal lumen suggested that the lumen was filled with liquid. The cellular structure of the intestine proper is now evident and the rectum and anus are still distinct. Cells of the hypodermal cords are prominent. Increase in growth of the parasite after 6 days (L = 1.36 mm, W = 0.08 mm) forced it against the neural lamella of the protocerebral lobe in a second instar host (Fig. 5).

Most of the parasites broke out of the ganglion proper on the 7th day, but were still contained by the neural lamella which protected them from direct contact with the hemolymph (Fig. 9). The length increased greatly to 2.39 mm and the width to 0.11 mm. The structure of the stylet was obscure and the stichocytes began to elongate. The junction between the pharynx and intestine was severed and the gut grew forward. The gonad rudi-

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in a protocerebral lobe of a first instar *Diabrotica* larva 5 days after infection. n = nematode. 5. Greatly enlarged protocerebral lobe of a second instar *Diabrotica* larva containing a developing juvenile of *F. leipsandra* 6 days after infection. n = nematode. 6. Two specimens of *F. leipsandra* breaking out of a protocerebral lobe and the subesophageal ganglion, respectively, of a second instar *Diabrotica* larva 8 days after infection. Note that both specimens are still contained by the neural lamella which surrounds the ganglia. n = nematode; p = uninfected protocerebral lobe.

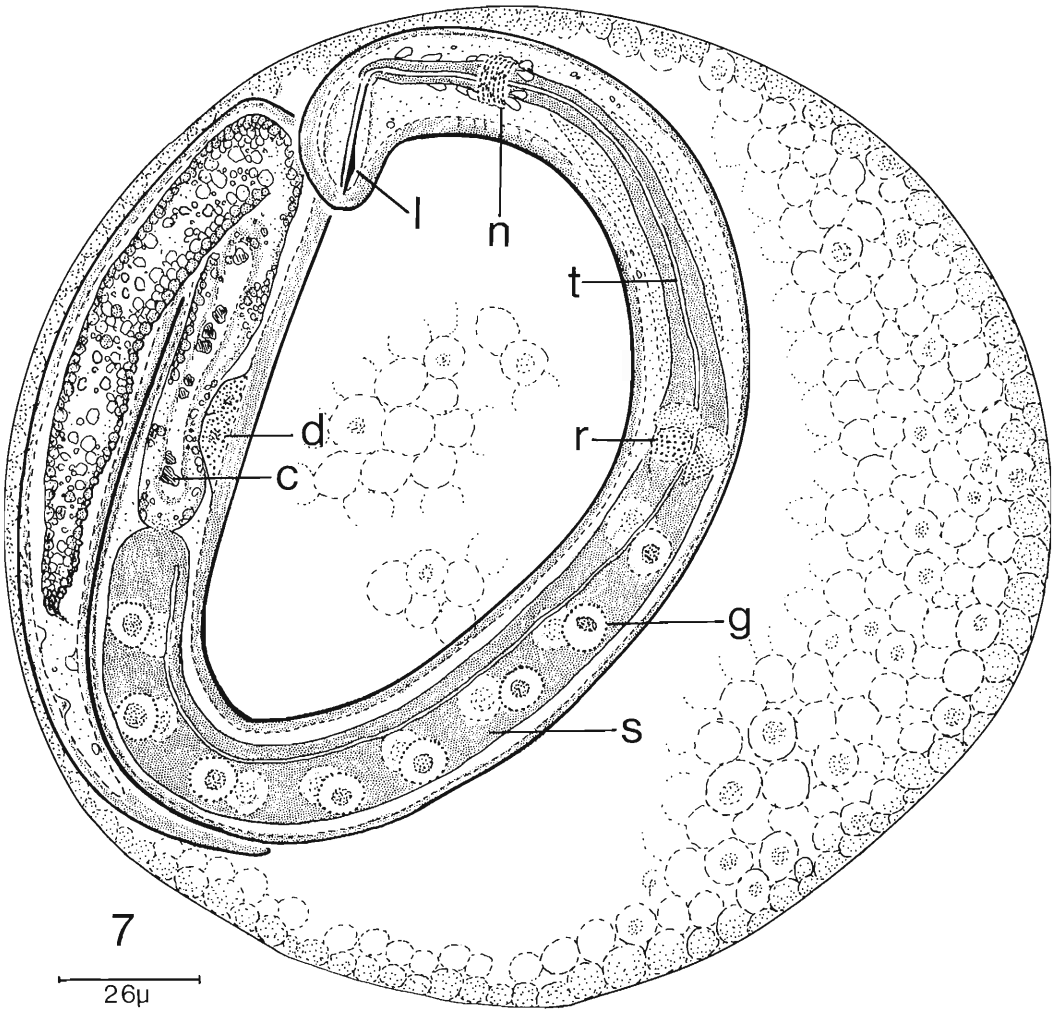

Figure 7. ***F. leipsandra*** **in the first thoracic ganglion of a first instar** ***Diabrotica*** **1 day after infection. s = stichosomal portion of pharynx; g = gland cell or stichocyte; r = gland reservoir; t = pharyngeal tube; n = nerve ring; l = stylet; d = gonad; c = crystals in anterior lumen of intestine.**

ment was still an undifferentiated mass of cells. After 8 days in the ganglia of a second stage host, the parasite reached a length of 2.7 mm (Fig. 6). The neural lamella continued to stretch, and on the 9th day still contained the parasite, which had reached a length of 5.00 mm and a width of 0.11 mm. The pharynx had now assumed a distinct tripartate structure with the ventral portion containing the pharyngeal tube, and the two subdorsal portions each with a row of eight large stichocytes. The intestine reached its definitive location in relation to the rest of the body and the gonad rudiment began to differentiate into vaginal and ovarial portions (Fig. 10).

The nematode remained within the pouch for two more days while the neural lamella stretched up to 3 mm in length. The membrane broke on the 12th day after infection and the parasites moved directly into the host hemocoel. The period the nematode remained inside the ganglia proper and the membrane,

respectively, varied depending on the rate of infection and age of the host. Growth in length continued at a phenomenal rate and on the 14th day, the nematode was 34.6 mm long and 0.20 mm wide.

When the nematode emerged from the host, in this case on the 22nd day, it had reached a length of 47.0 mm and a width of 0.26 mm. Although the intake of nourishment probably ceased even before the nematode made its exit from the host, sexual differentiation continued up to the postparasitic molts and ovarial development continued until the nematode expired.

Discussion

The parasitic development of *Filipjevimermis leipsandra*, as with mermithids in general, results in an extreme increase in length rather than width as is found in other entomogenous nematodes (Poinar, 1965). In one case, the length and width increased 87 and 14 fold respectively over a 22-day period in the host. No sign of a molt was noted during the parasitic period. One molt occurred within the egg just before hatching and the postparasitic juvenile molted twice before oviposition. The sensory papillae were obscure during parasitic development, although the nerve ring and associated cells were distinct at all stages.

An increase in length did not occur until the 4th day after infection. This may be typical of mermithid development since Christie (1936) also remarked that with *Agamermis decaudata* in *Melanoplus femur-rubrum*, there was little change in length of the parasite during the first three days in the host. However, internal development began immediately after entering the host. This was especially evident with the stichocytes of the pharynx.

Although Hyman (1951) considered the pharynx and stichosome as separate structures in the Mermithidae, it is obvious that they are part of the same organ and the stichosome is regarded here as a highly specialized part of the pharynx.

During parasitic development, the basal portion of the pharynx differentiates into three longitudinal divisions. The ventral part contains the remainder of the pharyngeal tube which ends blindly at the base of the organ. The two subdorsal portions each contain a row of eight gland cells or stichocytes and they may jointly be regarded as the stichosome (Steiner, 1933). The stichocytes are large clear cells with granular nuclei and are most prominent during the early stages of parasitism. They probably play an important role in the nutrition of the nematode, but their exact nature is unknown. In later parasitic and postparasitic stages the stichosome tissue breaks down and the posterior part of the pharynx consists of isolated stichocytes attached to the slender pharyngeal tube.

The number of stichocytes in *F. leipsandra* was constantly 16 which may be basic for mermithids since Christie (1936) also found 16 in developing juveniles of *Agamermis decaudata* and Götz (1964) recorded 16 in juveniles of *Gastromermis rosea*. However, other workers have found variable numbers; Johnson (1955) recorded from 15–17 in *Hydromermis contorta* depending on the age of the juvenile, and Couturier (1963) found up to 10 in *Tunicamermis melolonthae*. A bulblike enlargement of the pharynx as Christie (1936) reported in juveniles of *A. decaudata* was lacking in *F. leipsandra*.

The intestine, or trophosome as it is called in its modified form in mermithids, appears initially as a tube filled with globular inclusions. Intestinal cells become distinct by the 5th day and the intestine rapidly begins to elongate by the 7th day. The significance of the lumen in the anterior portion of the trophosome with its crystal contents is unknown.

A minute, nonfunctional anus and rectum are present throughout most of the parasitic development, but only a vestigial anus remains in the postparasitic juvenile. The cells forming the hypodermal chords are distinct during all stages of development and some appear to be binucleate or in process of division. Although cells of the gonad rudiment begin dividing early in the development, sexual differentiation was completed only after the parasites left the host. No sign of an excretory pore was seen during this study.

Most nematodes which develop exclusively in invertebrates are not known to invade specific tissues of the host, in contrast to the filarioid and spiruroid nematodes which utilize invertebrates as intermediate hosts. However, even in the latter two groups, a record of

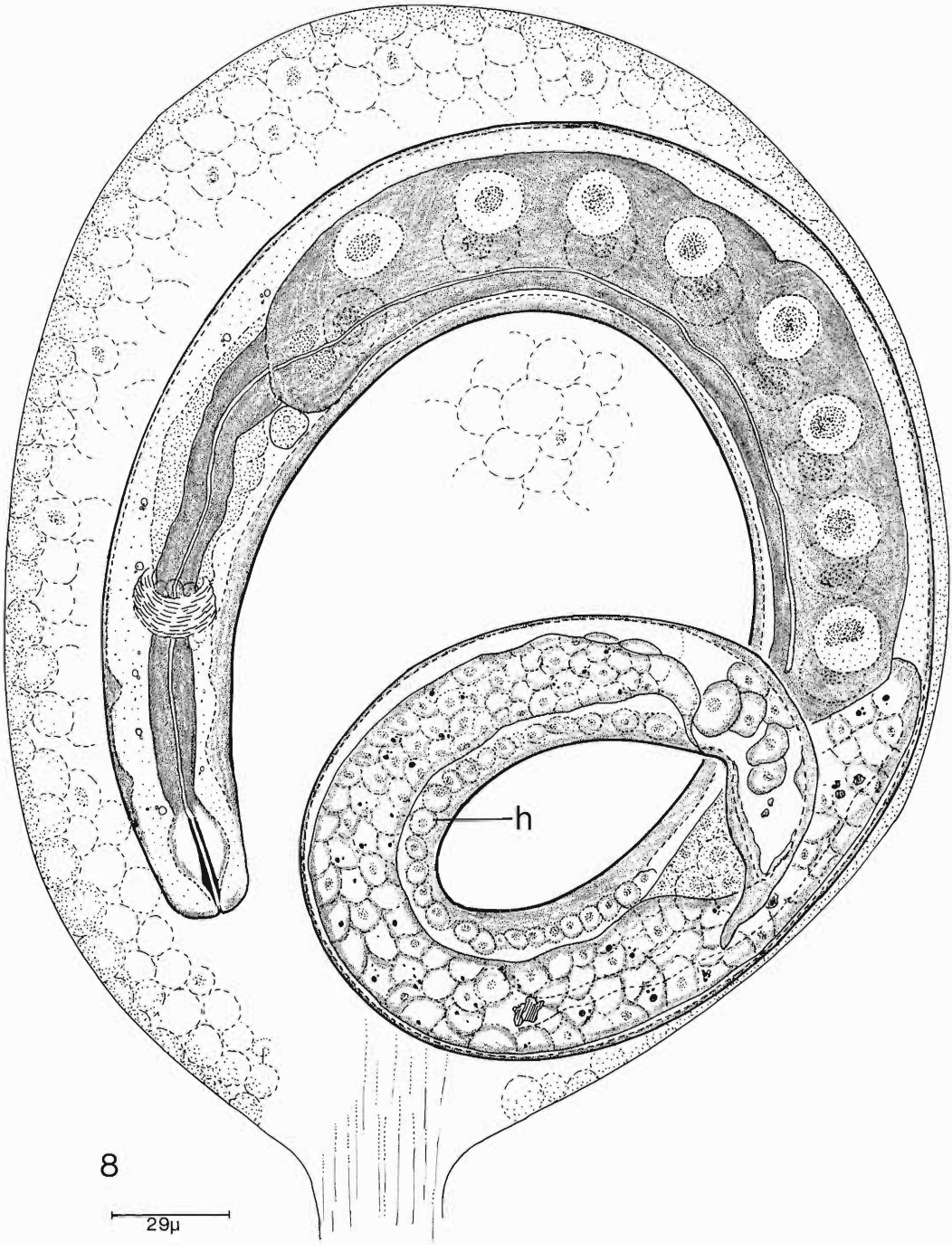

Figure 8. Developing juvenile of *F. leipsandra* 5 days after infection in a protocerebral lobe of a first instar *Diabrotica* larva. h = hypodermal cell.

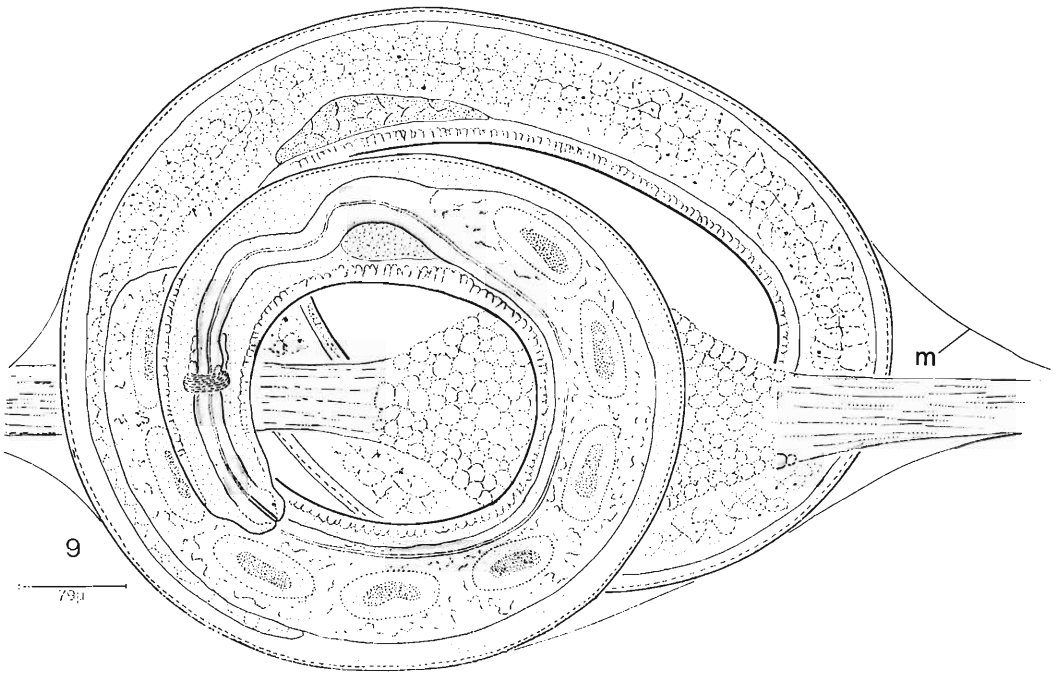

Figure 9. Developing juvenile of *F. leipsandra* which has just broken out of an abdominal ganglion of a second instar *Diabrotica* larva 7 days after infection. It is retained by the thin neural lamella (m).

development in the nervous system of the host could not be found, although Lavoipierre (1958) mentions once finding a juvenile of *Loa loa* partly embedded in the brain of the adult fly, *Chrysops silicea*.

Thus, it was surprising to discover a mermithid which showed an affinity to the ganglia of an insect host. Still, the affinity of mermithid nematodes to specific tissues of insects may be more widespread than imagined since Hagan and Hoopingarner (in press) found the early stages of an undetermined mermithid in the brain lobes of larvae of *Aedes stimulans*.

One of the most obvious reasons for *F. leipsandra* to enter the ganglia of *Diabrotica* would be to escape encapsulation and subsequent death. It is interesting that the parasite is not attacked by blood cells when it breaks out of the ganglion and neural lamella 9–12 days after infection. Whether the nematode is now too large or has acquired some attribute which makes it "acceptable" to the host is not known. A similar situation was reported by Strickland (1930) working with the tachinid *Gonia* and noctuid larvae. Upon entering the body cavity, the parasites were encapsulated unless they entered the supraesophageal ganglion of the host. After remaining there for a few days, they re-entered the body cavity and were not attacked by blood cells. Other mermithids occurred in a sheath within their hosts, but whether this protected them from an encapsulation reaction is not known (Rennie, 1925; Couturier, 1963).

The "normal" host of an entomogenous nematode is usually considered one in which the association supposedly has been of such long standing that the parasite is now "accepted" with a minimum of host reaction (Poinar, in press). The behavior of *F. leipsandra* could represent an intermediate step in adapting to a parasitic development free in the hemolymph of *Diabrotica*, or it could represent the end of a specialized line of selection, enabling the parasite to avoid the defense reaction of various hosts.

If the latter is true, and artificial infection studies with various hosts suggest this, then

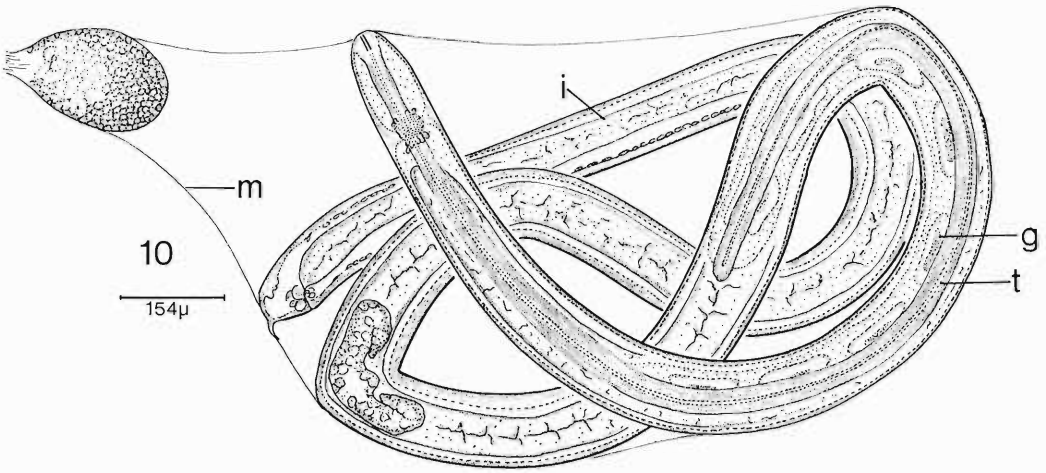

Figure 10. ***F. leipsandra*** **9 days in the host and held within the neural lamella (m) of the protocerebral lobe of a second instar** ***Diabrotica*** **larva. i = intestine or trophosome; g = gland cell or stichocyte; t = pharyngeal tube.**

the nematode may not be hindered by the defense reaction of new "unusual" hosts and would be able to expand its host range. In fact, *Diabrotica* may be a relatively "recent" host since it could easily destroy the parasites by encapsulation if they did not enter the ganglia. Of course, the possibility that the ganglia furnish developing juveniles of *F. leipsandra* some subtle nutritional factor necessary for development is still open for investigation.

Summary

First instar larvae of *Diabrotica u. undecimpunctata* were infected in the laboratory with juveniles of the mermithid nematode, *Filipjevimermis leipsandra*. The infective stage nematodes penetrated directly into the hemocoel and then entered one of the ganglia of the host. The protocerebral lobes and subesophageal ganglion were most frequently attacked. The parasites developed initially within the ganglia, then after outgrowing the latter, were retained by the neural lamella, which stretched a considerable distance before finally breaking and liberating the nematodes in the host hemolymph.

The growth and internal development of the parasite were studied by dissecting infected hosts at regular intervals. Parasitic development lasted from 12 to 22 days, a relatively short period for such a tremendous increase in length of the nematode.

Acknowledgments

The author expresses his sincere thanks to Dr. Cuthbert for providing the original supply of nematodes, to Mr. R. A. Corey of the Shell Development Company, Modesto, California, for his help in rearing methods of *Diabrotica* and to Mr. Carl Reiner who patiently maintained colonies of *D. u. undecimpunctata* for experimental use at the University. Grateful appreciation is also extended to Mr. Gerard Thomas who assisted in technical aspects of this study.

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A Revision of the Genus *Rotylenchulus* Linford and Oliveira, 1940 (Nematoda: Tylenchidae)[1]

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Species of the genus *Rotylenchulus* have been one of the most misidentified groups of all the tylenchs. As evidence, nematodes belonging to this genus have been described in at least four different genera. Also this genus has been variously assigned to three families: Tylenchidae (Linford and Oliveira, 1940; Thorne, 1949, 1961; Allen and Sher, 1967); Heteroderidae (Chitwood and Chitwood, 1950; Skarbilovich, 1960); Hoplolaimidae (Hopper and Cairns, 1959; Goodey, 1963; Husain and Khan, 1967) and five different subfamilies: Nacobbinae (Hopper and Cairns, 1959; Goodey, 1963); Pratylenchinae (Thorne, 1949, 1961; Baker, 1962); Tylenchulinae (Skarbilovich, 1960); Hoplolaiminae (Loof and Oostenbrink, 1962); Rotylenchulinae (Husain and Khan, 1967; Allen and Sher, 1967).

The genus *Rotylenchulus* was proposed by Linford and Oliveira in 1940 when they described *R. reniformis*. Yokoo and Tanaka (1954) described *Tetylenchus nicotiana* from Japan which was subsequently transferred to the genus *Rotylenchulus* by Baker (1962).

Three other species (*Helicotylenchus elisensis* Carvalho, 1957, 1959; *Spirotylenchus queirozi* Lordello and Cesnik, 1958, and *Helicotylenchus parvus* Williams, 1960) were transferred to the genus *Rotylenchulus* by Sher (1961). In 1960 Das proposed a new genus, *Leiperotylenchus*, which he considered to be closely related to *Tylenchus* and *Ditylenchus*. However, the position of the dorsal gland orifice and characters of male tail indicated a close relationship with *Rotylenchulus*. Indeed, Loof and Oostenbrink (1962) transferred *Leiperotylenchus leiperi* to the genus *Rotylenchulus*. Goodey (1963) synonymized *elisensis*, *parvus*, *leiperi* and *queirozi* with *R. reniformis*. Husain and Khan (1965) described

[1] A part of the thesis submitted by senior author in partial fulfillment of the requirements for the Ph.D. degree, University of California, Davis.