Bionomics of a mermithid parasitizing snow-pool *Aedes* spp. mosquitoes

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A mermithid nematode was isolated from all active stages of snow-pool *Aedes* spp. in northeastern New York. Invading nematodes initially entered the larval cephalic nervous tissue where limited development occurred, but subsequently migrated to the abdominal hemocoel of pupae and adults, where the rate of development increased sharply. Migration to the brain was a prerequisite for development in *Ae. stimulans*; nematodes not entering the brain elicited a host defense response. Most mermithids (64%) entering this host were encapsulated and killed before reaching the brain, but field infection rates were still high, with more than 60% of last-instar larvae successfully infected. Mermithids attacking *Ae. intrudens* and *Ae. provocans* seldom elicited an encapsulation response but nevertheless failed to complete parasitic development. Normal parasitic development with infrequent encapsulation was noted in *Ae. excrucians* and *Ae. fitchii*. Postparasites invariably emerged from adult stages, causing host death. Postparasites reared in the laboratory at 25°C molted, mated, and began oviposition within 1 month of emergence. Resulting preparasites initiated infections in four laboratory-reared species of mosquitoes but seldom completed parasitic development. The biological control potential of this mermithid is discussed.

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Un nématode mermithidé a été trouvé chez tous les stades actifs d'espèces d'*Aedes* habitant les mares de fonte des neiges, au Nord-Est de l'état de New York. Au départ, les nématodes envahissent le tissu nerveux céphalique de la larve où ils se développent quelque peu, puis ils migrent dans l'hémocèle de la pupe et de l'adulte où la vitesse de développement augmente brusquement. Chez *Ae. stimulans*, la migration vers le cerveau est indispensable au développement du nématode; les nématodes qui n'atteignent pas le cerveau suscitent une réaction de défense chez l'hôte. La plupart des mermithidés (64%) qui ont pénétré une larve de cette espèce ont été encapsulés et tués avant d'atteindre le cerveau; les taux d'infection en nature étaient élevés malgré tout, puisque 60% des larves de dernier stade étaient infectées. Les mermithidés provoquent rarement une réaction d'encapsulation chez *Ae. intrudens* ou *Ae. provocans*, mais n'arrivent cependant pas à compléter leur développement. Le développement du parasite semble se faire normalement chez *Ae. excrucians* et *Ae. fitchii* et l'encapsulation est rare. Les stades post-parasitaires émergent invariablement de l'adulte et causent la mort de l'hôte. Les individus post-parasitaires élevés en laboratoire à 25°C ont mué, se sont accouplés et ont pondu moins d'un mois après l'émergence. Les stades pré-parasitaires issus de ces élevages ont attaquée quatre espèces de moustiques élevées en laboratoire, mais ont rarement terminé leur développement. Le potentiel de ce mermithidé comme agent de contrôle biologique fait l'objet d'une discussion.

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Introduction

Parasitism of mosquitoes by mermithid nematodes has been widely reported. Petersen (1984) notes that mermithids have been isolated from at least 79 different mosquito species, mostly *Aedes* spp., although only 15 definitive species of mermithids have been described, 11 since 1970. Platzer (1981) separated these species into two groups: those maturing primarily in larval hosts, and those maturing primarily in adult hosts. Five mermithid species from four genera have been described from the latter group. The first observation of adult parasitism was apparently made in 1889 by John B. Smith (1904), who found mermithids in *Culex nemoralis* from Leipzig, Saxony. Smith (1904) later found a mermithid subsequently described as *Perutilimermis culicis* in New Jersey salt-marsh mosquitoes (Stiles 1903). Other initial reports were by Schakhov (1927) who isolated *Culicimermis schakhovii* from adults of *Ae. dorsalis* and *Ae. cantans* in Russia, and Steiner (1924) who described *Paramermis canadensis* from *Ae. vexans* in British Columbia. *Empidomermis cozii* (Poinar 1977) and *E. riouxi* (Doucet *et al.* 1979) were described from adult *Anopheles funestus* in West Africa and *Ae. detritus* in France respectively.

Little is known concerning the bionomics of most of the above mermithid species, particularly in regard to the early stages of parasitism. Two excellent studies have been conducted on as yet undescribed mermithids. Hagan and Hoopingarner (1968)[3] observed that a mermithid parasitizing *Ae. stimulans* adults in Michigan initiated infections in the brain tissue of early instar larvae but later changed its development site to the abdominal hemocoel of adult hosts, where growth accelerated. Harlos *et al.* (1980) noted a similar life cycle in a mermithid from *Ae. vexans* tentatively placed in the genus *Culicimermis* by Galloway and Brust (1976*b*).

A mosquito mermithid which also shows tissue specificity in entering the host brain during the early stages of parasitism and completing parasitic development in the adult abdomen was isolated from snow-pool *Aedes* spp. near Cambridge, New York, in 1980. This nematode has been placed in the genus

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[3]Hagan, N., and R. Hoopingarner. 1968. Mermithid nematode parasitism of *Aedes stimulans* (Walker) (Diptera: Culicidae) from Ingham County, Michigan. Unpublished document.

TABLE 1. Incidence of mermithid parasitism in field-collected and laboratory-reared mosquitoes[a]

	No. examined	Host		Mermithid	
		Penetration[b]	Infection[c]	Encapsulation[d]	Development
Field-collected:					
Aedes excrucians	80	+	+	+[f]	+
Ae. fitchii	5	+	+	−	+
Ae. intrudens	54	+	+	−	+[g]
Ae. provocans	94	+	+	+[f]	−
Ae. stimulans	668	+	+	+	+
Laboratory-reared:					
Ae. aegypti	64	+	+	+[f]	+
Ae. triseriatus	30	+	+	+[f]	+
Anopheles quadrimaculatus	54	+	+[e]	+[f]	−
Culex pipiens pipiens	15	+	+[e]	−	+[g]

[a]Includes all active host stages.
[b]Hosts containing one or more mermithids, regardless of viability.
[c]Hosts containing one or more viable (unencapsulated) mermithids.
[d]Hosts containing one or more encapsulated mermithids.
[e]A single positive observation.
[f]Infrequently observed and only in late instars.
[g]Limited development only.

Empidomermis and appears to be a new species; a taxonomic description is in preparation. Information on the bionomics of this parasite and its role in the natural regulation of its hosts is presented.

Materials and methods

Larval and pupal host stages were collected for dissection at biweekly intervals from a single large (ca. 1 ha) snow pool located on the J. Severson farm, 3 km north of Cambridge, New York, over a 6-week period from 25 March to 7 May 1980. Adult hosts were obtained by holding field-collected pupae in the laboratory at 20°C until eclosion. Following determination of stage and species, mosquitoes were decapitated, and the head and thoracic and abdominal segments were placed under separate coverslips for examination. The number, location, and development of invading nematodes were noted, as was the presence or absence of a host defense reaction. Water temperature over the collection period ranged from 1 to 22°C, with mean minimum and maximum temperatures of 6.7 and 17.8°C during weeks 3 through 6.

Adults hosts were held in screen-fitted plastic cups with water from the snow pool. Sucrose was provided as a food source and selected parasitized female hosts were blood-fed. Emerging postparasites were collected from the cup bottom daily and transferred to rearing dishes of sand overlaid with spring water. Nematodes were held at approximately 15°C for 3–4 weeks, and then separated for further rearing at 17.5, 20, or 25°C. Observations were made on survival, oviposition, and egg hatch. Preparasites hatched in the laboratory were used for infection experiments to further delineate host range. Challenges were conducted in 5-mL dishes with second-instar larvae of *Ae. aegypti*, *Ae. triseriatus*, *Anopheles quadrimaculatus*, and *Culex pipiens* at a parasite to host ratio of 3:1; *Ae. aegypti* was also tested at a ratio of 5:1. Larvae were exposed for 24 h at 20°C, then transferred to rearing trays until adult eclosion. Adults were maintained until death or nematode emergence.

Results and discussion

Dissections revealed mermithid parasitism in all active stages of snow-pool *Aedes* from the Severson farm site. Although all five species collected were susceptible to penetration (mermithid presence regardless of parasite viability) and infection (presence of viable mermithids), considerable differences were observed regarding their ultimate suitability as hosts, as only three closely related species (*Ae. stimulans*, *Ae. excrucians*, and *Ae. fitchii*) supported more than limited nematode growth (Table 1).

Mermithids penetrating *Ae. stimulans* larvae often caused infection for only short periods, since these nematodes were encapsulated and killed unless they soon migrated to the host head capsule. Here the parasites escaped the host defense reaction by entering into the cephalic nervous tissue. Migration to the head capsule without entry into the brain did not confer protection. Mermithids associated with the nervous tissue showed some growth, increasing two to four times in width, but principal nematode growth did not occur until after host pupation when the nematode reentered the hemocoel to complete development, increasing greatly in size but no longer eliciting a host response. The change in developmental site from the brain to the abdomen probably results from a lack of space within the head capsule to accommodate complete nematode growth. Nematodes developing within adult hosts were invariably found coiled within the abdominal hemocoel. Postparasite emergence was noted only from adults and took place through the abdominal wall or the anus. Mermithids invading *Ae. excrucians* and *Ae. fitchii* exhibited an identical behavior in temporarily migrating to the brain during the early stages of parasitism, but encapsulation was rarely observed (Table 1).

Entry of an invading mermithid into host nervous tissue to avoid encapsulation is an unusual but previously reported behavior. Poinar (1968) described infective-stage juveniles of *Filipjevimermis leipsandra*, parasites of *Diabrotica* beetle larvae, entering one of the ganglia of the host's central nervous system. Similarly, *Strelkovimermis singularis* establishes itself within the supraesophageal ganglion of chironomid larvae to avoid a defense reaction before reentering the hemocoel to complete development and emerge from adult midges (Strelkov 1964).

The form of resistance shown by *Ae. intrudens* and *Ae. provocans* is less clear than that of *Ae. stimulans* since no discernable host response was detected (Table 1); instead, parasitic development was completely or partially blocked. Entry into the brain was again an essential part of the life cycle, but here the parasites gradually became distorted and granular in appearance. Parasite growth was not observed in *Ae. provocans*, and the nematodes died without reentering the

TABLE 2. Incidence of mermithid infections in field-collected snow-pool *Aedes* spp. larvae

Host	% infection[a] per instar (no. infected/no. examined)			
	First instar	Second instar	Third instar	Fourth instar
Ae. excrucians	0 (0/9)	20.0 (1/5)	73.3 (11/15)	89.7 (26/29)
Ae. fitchii	—	—	—	100.0 (3/3)
Ae. intrudens	0 (0/3)	60.0 (3/5)	33.3 (2/6)	69.0 (20/29)
Ae. provocans	0 (0/15)	11.1 (1/9)	18.2 (2/11)	68.6 (24/35)
Ae. stimulans	1.5 (4/260)	15.7 (29/185)	47.3 (70/148)	60.9 (28/46)

[a]Host containing one or more viable (unencapsulated) mermithids.

TABLE 3. Weekly incidence of mermithid parasitism of *Aedes stimulans* larvae from March 25 to May 7, 1980

Collection week	No. of larvae examined	Larval penetration			Larval infection			Encapsulation[a]		
		No.	%	Mean no. per host	No.	%	Mean no. per host	No.	%	Mean no. per host
1	121[b]	0	0	—	0	0	—	—	—	—
2	121[c]	0	0	—	0	0	—	—	—	—
3	162[d]	52	32.1	1.2	22	13.6	1.1	34	21.0	1.2
4	133[e]	83	62.4	2.4	45	33.8	1.7	61	45.9	2.0
5	89[f]	85	95.5	5.0	55	61.8	2.5	79	88.8	3.6
6	14[g]	14	100.0	5.3	9	64.3	2.2	13	92.9	4.2

[a]Hosts containing one or more encapsulated mermithids.
[b]100% first instars.
[c]81% first and 19% second instars.
[d]19% first, 80% second, and 1% third instars.
[e]8% first, 24% second, 67% third, and 1% fourth instars.
[f]1% second, 64% third, and 35% fourth instars.
[g]100% fourth instars.

TABLE 4. Incidence of mermithid parasitism of *Aedes stimulans* larvae

Larval instar	No. of larvae examined	Larvae penetrated		Larvae infected		Encapsulation[a]	
		No.	%	No.	%	No.	%
1	260	4	1.5	4	1.5	0	0
2	186	71	38.2	29	15.6	49	26.3
3	148	114	77.0	70	47.3	97	65.5
4	46	45	97.8	28	60.9	41	89.1

[a]Hosts containing one or more encapsulated mermithids.

hemocoel. Those parasitizing *Ae. intrudens* did undergo slight growth, but again remained within the nervous tissue and died during the host pupal stage. The dead parasites continued to deteriorate, becoming increasingly difficult to detect in adult hosts. These two mosquito species are presumed to be unsuitable hosts because of a deficiency in essential nutrients or the presence of growth-inhibiting substances.

The rate of infection in the field varied with larval instar. Infections were initiated in first-instar larvae, although the infection rate was only 1.5% for *Ae. stimulans* and 1.4% overall (Table 2). This instar was present in large numbers during collection weeks 1 and 2, but parasitism was not detected until 8 April during the 3rd week (Table 3). Thus, the low infection rate recorded for this instar may be attributed to delayed egg hatch of the parasite rather than reduced first-instar susceptibility. The incidence of infection increased sharply in subsequent instars, ranging from 61% among fourth-instar *Ae. stimulans* larvae to 90 and 100% in *Ae. excrucians* and *Ae. fitchii* larvae respectively (Table 2). The moderate level of infection observed in *Ae. stimulans* reflects this species' ability to resist infection: 65% (497/763) of all penetrating mermithids were encapsulated. Consequently, although nearly all (98%) fourth-instar *Ae. stimulans* larvae had been penetrated, the incidence of successful infection reached only 61%, because 91% of these larvae encapsulated one or more mermithids (Table 4). Had no encapsulation response occurred, infection would have reached 98% instead of 61%. In contrast, infrequent mermithid encapsulation was observed in *Ae. excrucians* (1.75%), *Ae. fitchii* (0%), *Ae. intrudens* (0%), and *Ae. provocans* (1.0%), so penetration virtually always resulted in infection; however, as previously noted, infection did not result in normal mermithid development in some species (i.e., *Ae. intrudens* and *Ae. provocans*) because of stabilizing immunity (parasite inability to complete development following entry into a host). In short, infection and penetration are nearly synonymous in these four species, while in *Ae. stimulans* infection is equivalent to penetration minus encapsulation. Even so, parasitism is ultimately of no apparent

TABLE 5. The incidence of mermithids found penetrating, infecting, or encapsulated in *Aedes stimulans* larvae

Larval instar	Penetrating mermithids[a]			Infecting mermithids[b]			Encapsulated mermithids		
	No.	Range	Mean no. per host	No.	Range	Mean no. per host	No.	Range	Mean no. per host
1	4	1	1.0	4	1	1.0	0	—	—
2	94	1–4	1.3	34	1–2	1.2	58	1–3	1.2
3	403	1–19	3.5	138	1–8	2.0	261	1–10	2.7
4	262	1–14	5.8	81	1–9	2.9	178	1–14	4.3

[a]Mermithids found in a host, regardless of viability.
[b]Viable (unencapsulated) mermithids found associated with the host central nervous system; 10 mermithids found unencapsulated but not in the head capsule were deleted from computations.

consequence in *Ae. intrudens* and *Ae. provocans* as these hosts are not affected adversely. Successful parasitism is lethal, however, to *Ae. stimulans*, *Ae. excrucians*, and *Ae. fitchii*, and clearly parasitism here can exert significant pressure on populations of these hosts (Table 2).

A high incidence of multiple penetration, infection, and encapsulation was found in *Ae. stimulans* larvae (Tables 3, 5). As many as 19 mermithids were found to have penetrated a single third-instar larva with 8 causing successful infection and the remaining 11 being encapsulated. The sharp and progressive increase in the mean number of mermithids per host with each successive instar (Table 5) and week (Table 3), shows that the short-lived preparasites continued hatching and penetrating hosts over a 4-week period. Moreover, viable unencapsulated nematodes were occasionally (10 of 763 penetrating nematodes) found in the abdomen or thorax of second- through fourth-instar larvae, further substantiating that penetration had just occurred.

Parasitism exerted no obvious adverse effects on larval or pupal hosts, although infected pupae appeared somewhat sluggish. Infected adults were easily distinguished during the latter stages of parasitism by their greatly enlarged abdomen. Parasitized females showed repressed ovarian development and were not observed to oviposit. They died soon after postparasite emergence.

The assumption that the mermithid might be widely distributed because it exits from adult hosts was tested by examining late instar larvae collected from several area snow pools. *Aedes stimulans/excrucians* in three of six sites sampled contained mermithids morphologically and behaviorally similar to those originally isolated. These sites are up to 65 km from the original isolation site.

Efforts to rear the nematode were initiated with some of the more than 600 postparasites which emerged from male and female adult mosquitoes held in the laboratory. The ratio of emerging male to female postparasites was 2.9:1. This finding had been anticipated based on the extensive superparasitism observed (Tables 3, 5); it is well established that the degree of parasitism can influence mermithid sex determination (Petersen 1972, 1984; Platzer 1981), the ratio of male to female mermithids usually increasing as the number of parasites per host increases. Mosquitoes infected with a single parasite of *Romanomermis culicivorax*, for example, produce about 9% males compared with 100% in hosts with more than seven parasites (Petersen 1972). The mean number of mermithids infecting last instar *Ae. stimulans* larvae in the current study was 2.9 (Table 3). The skewed sex ratio characteristically produced from superparasitized hosts may serve a beneficial damping function in stabilizing parasite populations.

and began laying fertile eggs within 4 weeks of emergence. This temperature sharply accelerated the life cycle and may be the best for routine laboratory rearing, as three mermithid generations per year may be possible. Oviposition continued for 1–2 months. The eggs could be easily stored in moist sand at 4°C and hatched by flooding the cultures and raising the temperature to 10°C. Infective preparasites began emergence within 1–2 h under these conditions. This species clearly overwinters as embryonated eggs and hatches in the spring when the pools fill with snowmelt water and temperatures rise.

Laboratory-reared mosquito larvae were challenged with preparasites to further define the mermithid's host specificity and to attempt to find suitable laboratory hosts for nematode rearing (Table 1), since cultures of snow-pool mosquitoes are difficult to establish. Preparasites initiated infections in all four species exposed; however, *An. quadrimaculatus* and *C. pipiens* did not support nematode development. *Aedes aegypti* was moderately susceptible to infection, with 70% parasitism recorded from one test where the parasite to host ratio was 5:1, and 53% at a 3:1 ratio. Most adult hosts died before parasite emergence, even though these nematodes appeared almost fully developed. Poinar (1977) demonstrated that a blood meal was required for *E. cozii* to emerge as normal postparasites; however, host blood feeding did not improve our results. The best results were obtained with the tree-hole mosquito, *Ae. triseriatus*, where an infection rate of 73.3% (3:1 parasite to host ratio) was obtained. Here again, most (86%) parasitized hosts died before parasite development was complete, some as last instar larvae with well developed nematodes in the abdomen. Control mortality was less than 10%. Seven postparasites, all female, emerged from three hosts but none survived to the adult stage. Nearly all (97%) the parasitic stage nematodes which died with their hosts were also female, even though the mean infection rate was 2.1 parasites per host. An efficient laboratory host must be found if mass production for field release is desired. This may not be a simple task as our field and laboratory findings indicate that while this mermithid shows little host specificity in initiating parasitism, it is highly specific with regard to hosts in which it completes development. A similar conclusion was reached by Petersen *et al.* (1967) following their attempts to find an alternate host for *P. culicis*.

Observation of one preparasite exposed to a third-instar *Ae. aegypti* larva showed that complete penetration into the host hemocoel could occur in less than 5 min. After 10 min the nematode had migrated from the abdomen into the head capsule and soon began penetration into the nervous tissue.

The life cycle of this mermithid is similar to that of the undescribed mermithids reported by Hagan and Hoopingarner

Manitoba. All three mermithids are associated with the larval brain during the early stages of parasitism where they undergo limited development, but they subsequently migrate to the adult abdomen to complete parasitic development. Despite the similarity in life cycles, it seems unlikely that these two mermithids are conspecific with our isolate. The isolate of Harlos *et al.* (1980) is apparently in the genus *Culicimermis*, a group easily distinguished from *Empidomermis* on the basis of the vagina, which is elongate and nearly straight in *Culicimermis* but S-shaped and curved in *Empidomermis* (Poinar 1979). Less evident is the isolate of Hagan and Hoppingarner (1968, see footnote 3), since adult specimens of this nematode were not available for taxonomic examination. However, these investigators specifically noted no signs of "host resistance in the form of melanization [or] encapsulation" in *Ae. stimulans* larvae. Evidence of melanization and encapsulation in *Ae. stimulans* was too striking in our study to have been overlooked.

While it may be premature to speculate upon the biological control potential of this interesting but recently isolated parasite, the mermithid does possess several attractive attributes. First, it has excellent potential for dispersal from treatment pools to other mosquito habitats by way of infected hosts. This is a significant advantage over the extensively studied mosquito mermithid *R. culicivorax*, which emerges from larvae and therefore lacks a ready means of dispersal. Petersen *et al.* (1968) found *R. culicivorax* in only five of hundreds of mosquito pools sampled; by comparison, we recovered our isolate from four of seven pools. A second attribute is the nematode's capability of causing high rates of mortality among at least three species of snow-pool *Aedes* spp., which are annoying biters implicated as potential vectors of California group encephalitis viruses (Iverson *et al.* 1969) and canine filariasis (Yen 1938). No nematode has been developed for use against cold-water inhabiting mosquito species. *Romanomermis culicivorax* is ineffective at low water temperatures (Galloway and Brust 1976*a*), and mass production methods have not yet been developed for cold-water adapted mermithids like *Romanomermis nielseni*. Further studies are needed to determine the prospects for efficient *in vivo* production of our isolate. Also, like all mermithids, our isolate is adapted to the host environment, and consequently possesses potential for establishment and recycling, offering the prospect of long-term control. We have recovered parasitized *Ae. stimulans* larvae from the source snow-pool for 3 consecutive years. This is an important advantage over inundative agents like *Bacillus thuringiensis* or *B. sphaericus* that possess little or no potential for recycling in cold-water habitats. The future role of mermithids in vector control may be as inoculative agents in integrated pest management programs which exploit their potential for recycling.

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