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Mermithid parasitism (Nematoda: Mermithidae) in *Ochlerotatus cantans* (Meigen) (Diptera: Culicidae) in Denmark

Boy Overgaard Nielsen
Department of Zoology, Institute of Biological Sciences, University of Aarhus,
Building 135, Universitetsparken, DK-8000 Århus C, Denmark.
Email: boy.overgaard.nielsen@biology.au.dk

Abstract

An unidentified mermithid species parasitizing *Ochlerotatus cantans* is presented, being the first mosquito-parasitic mermithid recorded from Denmark. The parasitic development is initiated in the larval host, continues in the pupa and is completed in the abdominal haemocoel of the adult, the mean body length of the parasite increasing from about 1 mm to 12-13 mm. From 1 to 5 mermithid worms were found per adult host. Up to 87% of male and female mosquitoes hatched in the laboratory or swept in the herbaceous layer surrounding woodland pools were parasitized. The prevalence of the parasite varied between pools.

Introduction

At least 25 species of mermithid nematodes parasitize mosquitoes (Blackmore, 1994). Infection is always initiated in the larval host; in some species of mosquito-parasitic mermithids the parasitic phase of development is completed primarily in the larval stages of the host, while other species mature primarily in adult mosquitoes (Platzer, 1981). Mosquito-parasitic mermithids have frequently been recorded from various parts of the world, but in northern Europe mermithid parasitism of mosquitoes is apparently relatively rare (Blackmore, 1994). From Fennoscandia only three cases of mosquito populations parasitized by unidentified mermithids have been reported, *viz.* from Finland (larvae of *Culex* sp.), Norway (larvae of *Ochlerotatus hexodontus* (Dyar)) and Sweden (adult mosquitoes, primarily *Ochlerotatus communis* (De Geer)) (Levander, 1904; Nielsen *et al.*, 1981; Blackmore, 1994). However, during studies on the reproductive biology of Danish woodland mosquitoes several adult *Ochlerotatus cantans* were found to be parasitized by a mermithid nematode. This is the first observation of mosquitoes parasitized by mermithids in Denmark. This paper deals with parasitic association, prevalence and development of the mermithid.

Materials and methods

Sampling was conducted in Lisbjerg-Trige Forest (56°10'N, 10°12'E), 10 km NW of Århus, eastern Jutland, Denmark. In early May 1984-1988 and in 1998-1999 mosquito larvae and pupae were collected by dipping at various sites in about thirty woodland pools. From mid-May to early September 1983-1988 and 1998-2000 mosquitoes attracted to man were hand-netted (using a standardized sampling procedure). Further specimens were collected by sweep-netting the vegetation surrounding pools.

Larvae, pupae and adults were dissected under a stereo microscope and checked for parasitism; number and location of mermithids present were recorded, and length and width of the worms were measured with an ocular micrometer. The reproductive status of each parasitized female mosquito was assessed according to Christophers (1911).

Mosquito larvae and pupae were reared in 500ml beakers kept in outdoor cages placed in the shade. After eclosion some adult *Oc. cantans* were dissected, others were transferred to screen-fitted cups and maintained on a 5% sucrose solution supplied in a tube closed with a cotton plug. Emerging post-parasitic mermithids were collected from the cup bottom daily, transferred to dishes with boiled sand soaked in boiled tap water and maintained at 15°C. After 3-4 weeks the dishes were examined; unfortunately, no mermithids completed the final moult to the adult stage and therefore could not be identified to species.

Results

The prevalence of mermithid parasites in adult mosquitoes

The proportions of parasitized male and female *Oc. cantans* hatched in the laboratory 1984-1987 were within the ranges 51-87% and 47-86% respectively (Table 1). No emerged *Oc. communis* (n = 90 males, 100 females), *Oc. punctor* (Kirby) (n = 40 males, 44 females), *Oc. cataphylla* (Dyar) (n = 68 males, 72 females) and *Oc. annulipes* (Meigen) (n = 32 males, 38 females) were parasitized.

Table 1. Prevalence of mermithid parasites in *Ochlerotatus cantans* collected as larvae in two temporary pools in a Danish forest (May 1984-1987) and reared to adults.

	Dissected (n)		Parasitized (n)		Parasitized (%)	
	males	females	males	females	males	females
Pool 1						
1986	126	60	64	28	50.8	46.7
Pool 28						
1984	11	36	7	31	63.6	86.1
1985	62	80	48	52	77.4	65.0
1986	90	73	48	35	53.3	47.9
1987	39	22	34	18	87.2	81.8

In May-June of 1984-1986 *Oc. communis* (n = 104 females), *Oc. punctor* (n = 66 females), *Oc. cataphylla* (n = 11 males, 41 females), *Oc. annulipes* (n = 34 females) and *Oc. cantans* (n = 363 males, 416 females) swept in the herbaceous layer surrounding two pools in the forest were dissected; only the latter species was parasitized (Table 2). In the three years the proportions of male and female *Oc. cantans* parasitized were within the ranges 35-86% and 25-80% respectively. A total of 24 females attracted to man in early June 1983-1986 were all parasitized.

Proportions of *Oc. cantans* parasitized by mermithid nematodes varied between pools. In mid-May 1987 mosquito pupae were collected from 27 pools and *Oc. cantans* (males, females) hatching were dissected and checked for mermithids. Mosquitoes from four pools were parasitized: pool 1, n = 142, 36.6%; pool 10, n = 120, 11.7%; pool 12, n = 92, 8.7%; pool 28, n = 146, 73.9%. In May 1999 the parasite was found in 15.1% of males (n = 167) and 20.9% of females (n = 178) hatched from pupae collected in pool 12.

In parasitized adult *Oc. cantans* 1984-1987 (n = 726; see Tables 1 & 2) 1-5 mermithid nematodes were found per host. No difference in parasite load of male and female mosquitoes was found; 40.9% of the hosts were parasitized by 1 worm, 27.3% by 2 worms, 27.3% by 3 worms, 2.3% by 4 worms and further 2.3% by 5 worms. The parasites were generally found in the abdominal haemocoel of the mosquitoes, however, in about 7% of the cases the thorax was also partly occupied by mermithids. No encapsulation of parasites was observed either in aquatic stages of the host or in adult mosquitoes. The ovaries of parasitized female mosquitoes were strongly suppressed (biological castration); no ovarian development beyond Christophers' stage II was observed.

Table 2. Prevalence of mermithid parasites in *Ochlerotatus cantans* swept in the herbaceous layer surrounding two temporary pools in a Danish forest (late May-early June 1984-1986).

	Dissected (n)		Parasitized (n)		Parasitized (%)	
	males	females	males	females	males	females
Pool 1						
1984	83	70	44	56	53.0	80.0
1985	48	37	23	13	47.9	35.1
1986	41	46	16	15	39.0	32.6
Pool 28						
1984	36	88	31	53	86.1	60.2
1985	36	82	24	21	66.7	25.6
1986	119	93	42	23	35.3	24.7

The development of the mermithid in the host

Small mermithids were found coiled up in the posterior part of the head capsule of fourth instar *Oc. cantans* larvae (but apparently not in brain tissue), while significantly larger ones occurred in the thorax and the abdominal haemocoel of the larval host (Table 3). The mean length of the worms was expected to decrease with increasing number of parasites per host; however, the results achieved were inconsistent. Thus the body length of worms from single and multiple infections were pooled and mean body lengths calculated. The body length of the mermithids increased significantly through the stages of host development to the post-parasitic mode of life. The largest increase in mermithid body length was observed between stages parasitizing larval and pupal hosts respectively. The largest body length and width, *viz.* 19.9 mm and 0.3 mm respectively, were found in a post-parasitic mermithid.

In the laboratory the post-parasitic emergence of mermithids started within the first week after the eclosion of the adult mosquitoes, taking place through the intersegmental membranes of the abdomen or the anus of the host. After the emergence of the parasite the host died. No emergence from larval or pupal hosts was observed. In the field the proportion of adult *Oc. cantans* parasitized declined rapidly after eclosion. On May 24, May 27 and June 7 (1987) 70-77%, 35-42% and 0% of males and 73-89%, 25-53% and 0-1% of females swept in the vegetation at two temporary pools (pool 1, pool 28) were parasitized (n = 178 males, 231 females). In 1999 the first parasitized *Oc. cantans* were recorded on May 18 (n = 51, 15.7% parasitized), while the last infected hosts were observed on June 2 (n = 250, 2.0% parasitized).

Table 3. Mermithids parasitizing *Oc. cantans* from pool 28, Lisbjerg-Trige Forest, May 1986. Mean body lengths (mm ±SD) of nematodes found in larval body sections, pupae and adults (at eclosion) and in the early post-parasitic stage. The mean body lengths (a-f) were compared by a t-test: a:b, t = 3.934***; a:c, t = 6.443***; b:c, t = 0.862 NS; bc:d, t = 17.144***; d:e, t = 2.893**; e:f, t = 8.264*** (** $p<0.005$, *** $p<0.001$, NS $p>0.05$).

	In mosquito larvae			In pupae	In adults	Post-parasitic mermithids
	Head	Thorax	Abdomen			
n	23	11	24	46	30	21
body length	0.98^a ±0.304	1.47^b ±0.415	1.59^c ±0.342	6.96^d ±1.837	8.16^e ±1.663	12.45^f ±2.035

Discussion

The unidentified mermithid was observed only in *Oc. cantans*, which is the predominant mosquito species in the woodland site (Nielsen, unpublished). The parasitic development of the mermithid is apparently initiated in the head capsule of the larval host and is completed in the abdominal haemocoel of the adult mosquito. Mermithids that complete their parasitic development in the adult host only are usually specific for a single host (Petersen, 1984, 1985). Furthermore, the migration of parasites within the host has been observed only in species that emerge from adult hosts (Blackmore, 1994). The life cycle of the mermithid is similar to that of other species parasitizing adult mosquitoes, including *Culicimermis schakhovii* Rubtsov & Isaeva which parasitizes *Oc. cantans* and other *Ochlerotatus* spp. in Russia (Schakhov, 1927; Petersen *et al.*, 1967; Rubtsov & Isaeva, 1975; Isaeva, 1977; Harlos *et al.*, 1980; Gaugler *et al.*, 1984). However, it is unknown whether *C. schakhovii* and the Danish species are conspecific.

Mermithid parasitism was only observed early in the mosquito season, *viz.* in May-early June. Post-parasitic emergence was observed in female as well as male hosts. In some mermithid species parasitizing adult female mosquitoes a host bloodmeal seems obligate for successful development of the parasite, in other species blood feeding is no prerequisite of success (Poinar, 1977; Blackmore, 1994). In this study a few infected female mosquitoes were collected at human bait. In the field blood feeding of *Oc. cantans* is apparently not initiated until about 3 weeks after hatching (Service, 1977). If blood feeding of the mosquito was obligate for successful parasitic development of the mermithid, the parasite would not be expected to leave a female host shortly after eclosion and infection of male mosquitoes would be highly inappropriate. Furthermore, a male mosquito is not expected to return to a temporary pool after eclosion, thus a parasitized male dying away from forest wetland would be a 'dead-end' host. Infected male *Oc. cantans* were swept in the vegetation surrounding temporary pools; it is unknown whether emerged parasitized males simply stay near the emergence site.

Parasite prevalence varied between pools. High parasite levels resulted in extensive infection of adult *Oc. cantans.* Since the host species emerged from practically all pools in the forest (Nielsen, unpublished), it is surprising that a mermithid completing its parasitic development in mobile adult mosquitoes is restricted to a few ponds, but presumably, the flight range of *Oc. cantans* in woodland is limited. In Southern England the majority of marked *Oc. cantans* were recaptured within a distance of 25 m from the point of release at a pool (Service, 1977). Moreover, the flight range of parasitized mosquitoes may be reduced. However, physical or biological conditions of the pools may also operate, *e.g.* the character of the bottom sediment, the seasonal fluctuation of water level or the density of host larvae. For instance, restoration of temporary waters in Lisbjerg-Trige Forest, including extensive dredging of a pool (No. 28) with a high level of parasitism, resulted in high, stable water levels and local extinction of *Oc. cantans* and thus of its mermithid parasite.

Mermithid parasites may have been sufficiently numerous in some of the pools to check local population growth of *Oc. cantans*. However, the patchy distribution of the parasite and the low mean infection rate of *Oc. cantans* emerging from the pools imply that mermithid parasitism is unlikely to reduce mosquito nuisance in the forest.

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