THE BIONOMICS OF A MERMITHID NEMATODE OF LARVAL MOSQUITOES IN SOUTHWESTERN LOUISIANA [1]

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Nematodes of the family Mermithidae have been recorded from 23 North American mosquitoes. Jenkins (1964) reported 16 mosquito hosts of mermithid parasites, and Chapman *et al.* (1967) reported 7 additional mosquito hosts in Louisiana. However, only Welch (1960) and Petersen *et al.* (1967) described the life cycle and bionomics of these parasites in North America.

During surveys conducted in southwestern Louisiana to discover parasites and pathogens that might be used in the biological control of mosquitoes, the authors found a mermithid nematode, apparently an undescribed species of the genus *Romanomermis,* parasitizing the larvae of several species of mosquitoes. A review of the available literature revealed that only one species of *Romanomermis* was described as parasitizing mosquitoes, *Romanomermis iyengari* Welch from Bangalore, India. A second species, probably an undescribed species of *Romanomermis,* was found by Muspratt (1965) in Zambia, Africa. When further investigation showed that the *Romanomermis* nematode found by the authors had a wide host range and parasitized large percentages of some populations of mosquitoes, we investigated its life cycle and bionomics.

INCIDENCE OF PARASITISM. Although we examined collections of mosquito larvae made through all seasons for more than 3 years from hundreds of locations in several parishes in Louisiana, nematodes were found in mosquitoes in only 5 ponds. This sparse distribution may be related to

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the life cycle of the parasite: it is completed within a single pond, and the host, seldom if ever, develops enough to emerge from that pond. These 5 fresh water ponds were all in semiopen areas of piney woods, and periodic samples of the water never showed a salinity exceeding 96 ppm total soluble salts.

Table 1 shows that we found 13 species of mosquitoes serving as hosts of the Louisiana *Romanomermis* sp. in the field. *Aedes atlanticus, Aedes mitchellae, P. ciliata, P. discolor,* and *Culex restuans* are newly recorded hosts. Larvae of *Aedes vexans* that were infected were also collected, but this host was recorded previously from Pennsylvania by Stabler (1952).

Several of the 5 pools that produced larvae infected with the nematode were

TABLE 1.—Mosquito species that are natural hosts of *Romanomermis* sp., that acquired the nematode when exposed to it in the field, or that were infected in the laboratory, (+=infected; −=uninfected).

Mosquito host	Natural field infection	Field infection in plastic containers	Laboratory infection
Aedes			
aegypti (L.)	−	−	+
atlanticus Dyar and Knab	+	−	−
canadensis (Theobald)	−	−	+
mitchellae (Dyar)	+	−	−
sierrensis (Ludlow)	−	−	+
sollicitans (Walker)	−	−	+
taeniorhynchus (Wiedemann)	−	−	+
thibaulti Dyar and Knab	−	−	+
tormentor Dyar and Knab	−	−	+
triseriatus (Say)	−	−	+
vexans (Meigen)	+	+	+
Anopheles			
barberi Coquillett	−	−	+
bradleyi King	−	+	−
crucians (Wiedemann)	+	−	−
punctipennis (Say)	+	−	−
quadrimaculatus Say	+	+	+
Culex			
erraticus (Dyar and Knab)	+	−	−
peccator Dyar and Knab	−	−	+
p. quinquefasciatus Say	−	−	+
restuans Theobald	+	−	+
salinarius Coquillett	−	−	+
Culiseta			
inornata (Williston)	−	+	+
melanura (Coquillett)	−	−	+
Orthopodomyia signifera (Coquillett)	−	−	+
Psorophora			
ciliata F.	+	−	−
confinnis (Lynch-Arribalzaga)	+	+	+
cyanescens (Coquillett)	−	+	−
discolor (Coquillett)	+	−	−
ferox (Humboldt)	−	−	+
varipes (Coquillett)	−	−	+
Uranotaenia			
lowii Theobald	+	−	−
sapphirina (Osten-Saken)	+	−	+
Total 32	13	6	22

ecologically distinct. One (Haymark 1) was a fairly permanent pool that never was observed to dry up though the water level receded during dry spells. This pool produced nematodes in larvae of *Anopheles crucians, Anopheles punctipennis, Anopheles quadrimaculatus, Culex erraticus, Culex restuans, Aedes vexans, P. confinnis, U. lowii,* and *U. sapphirina.* However, the level of parasitism, especially in *Anopheles* spp. was variable (0–30 percent). *Uranotaenia sapphirina* was the most common species, and the mean level of parasitism in 10 positive collections during 1967 was 31 percent. When parasitism was negligible or absent, the eggs of the nematode and the mosquito were probably not hatching at the same time.

Other pools fluctuated drastically during the hot summer; the total areas would be flooded after a rain and would then often dry to small pockets of water before the next rain. During long periods of drought, the ponds dried out completely. Chloe 1 produced infected larvae of *Aedes atlanticus, Aedes mitchellae, Anopheles crucians, Anopheles punctipennis, Anopheles quadrimaculatus, P. ciliata, P. confinnis, P. discolor, U. sapphirina,* and *U. lowii;* the most abundant species were *P. confinnis* and *U. sapphirina.* From April through June 1967, the mean level of infection in larvae of *P. confinnis* (7 collections) and *U. sapphirina* (8 collections) in Chloe 1 was 74 percent. In a single population of both (collections made at different times) 93 percent were infected.

In 1967, Chloe 1 dried out for several weeks during July and September, but light rains produced some breeding of *P. confinnis,* and a low level of parasitism was observed after each such flooding. However, the transient rains were insufficient, and the mosquito larvae became stranded at an early instar. A final flooding of the pool in September produced a 44 percent level of infection in a substantial brood of *P. confinnis.*

Thus, the highest levels of parasitism occurred when the water level of a pool fluctuated, but the pool did not dry up completely. When water levels receded, larvae of the permanent water species (*Anopheles* and *Uranotaenia* spp.) maintained the parasite, then, when the water level was raised, the first instar larvae of the temporary water species (*Aedes* and *Psorophora* spp.) bore the brunt of the parasitism. Since our subsequent laboratory observations indicated that mature nematode eggs hatched at flooding, the first instar larvae of floodwater mosquitoes and the preparasitic nematodes were present almost simultaneously in the field. Other nematode eggs that were immature at the time of flooding continued to mature, eventually hatched, and were responsible for the invasion of the permanent water breeding mosquitoes.

In the field, the most heavily parasitized hosts were larvae of *P. confinnis* and *U. sapphirina* and then *Aedes atlanticus* and *Anopheles crucians.* Larvae of *Culex territans* Walker were abundant throughout the year in the 5 infected pools, but no infected larvae were seen. However, *Culex territans* is a host of a nematode since we recently examined a fourth instar larva (Chapel Hill, N.C.; H. A. Bond, collector) that possessed a large juvenile nematode (genus unknown).

The host range of the nematode was also checked by exposing first instar larvae from our laboratory colonies or native females for several days in small plastic containers in the pools infected with the nematode. These plastic containers (4.5 in. diameter and 8.5 in. tall) were fitted with 80-mesh screen on the bottom and along the lower sides. Exposed fourth instar larvae were checked for infections. Table 1 shows that larvae of *Aedes vexans, Anopheles bradleyi, Anopheles quadrimaculatus, Culiseta inornata, P. confinnis,* and *P. cyanescens* became infected though the level of infection in all containers was generally low and never exceeded 40 percent.

Also, variable numbers of early instar larvae (usually first instar) of many species were exposed in the laboratory to variable numbers of preparasitic nema-

TABLE 2.—Multiple parasitism of larvae of *Psorophora confinnis* and *Uranotaenia sapphirina* exposed to natural infections in the field.

No. collections	Date	Location	No. parasites per infected larva								
			0	1	2	3	4	5	6	7	8+
			P. confinnis								
1	4/20	Chloe 1	28	7	15	21	14	12	6	5	1
			U. sapphirina								
6	4/12–5/17	Haymark 1	..	24	15	6	5	0	0	1	0
7	4/25–5/19	Chloe 1	..	46	48	19	0	1	0	0	0

todes to determine their potential as hosts of the Louisiana *Romanomermis*. Table 1 shows that the nematode developed to maturity in 22 species and that *Aedes aegypti, Culex p. quinquefasciatus, Culiseta inornata,* and *P. confinnis* were the most attractive hosts.

The degree of multiple infection and its effect on the parasitized larvae were investigated by a special, exposure sampling of Haymark 1 and Chloe 1 in April and May 1967. Table 2 summarizes the findings and indicates a much higher incidence of multiple infections than would be expected considering the number of single infections, especially in *P. confinnis*. Apparently, once a mosquito larva became parasitized, it was more susceptible to additional attacks, or certain areas within the pool had a greater number of preparasitic nematodes. Welch (1960) reached a similar conclusion in studies of multiple infections in *Aedes communis* (DeGeer).

The number of parasites in a host is thought to influence the sex of mermithid nematodes (Christie 1929 and Welch 1965). Ratios of males and females were therefore determined for the *Romanomermis* sp. in *P. confinnis* and *U. sapphirina* (Table 3). The 81 parasitized larvae of *P. confinnis* examined all contained one or more juvenile female parasites and 58 percent of the 299 parasites were females. However, many larvae of *U. sapphirina* had only male nematodes, and only 1 of the 165 contained more than 1 female (it contained 2 males and 2 females). Thus 64 percent and 81 percent of the nematodes emerging from *U. sapphirina* collected from Haymark and Chloe, respectively, were males. These data indicate that the host species may influence the sex ratios of the Louisiana nematode, a factor that may be important if attempts are made to establish it permanently in a new host.

TABLE 3.—Sex ratios of the Louisiana *Romanomermis* sp. in multiple infections of *Psorophora confinnis* and *Uranotaenia sapphirina*

No. nematodes per infected larvae	*P. confinnis* Chloe 1		*U. sapphirina* Chloe 1		*U. sapphirina* Haymark 1	
	No. nematodes	% Males	No. nematodes	% Males	No. nematodes	% Males
1	7	0	47	62	23	39
2	30	20	110	83	32	72
3	63	33	48	92	18	89
4	56	45	0	..	8	75
5	60	50	5	100	0	..
6	36	55	0	..	0	..
7 or more	35	69	0	..	7	100

Emergence of the postparasitic nematode from *P. confinnis* occurred first from larvae containing the highest number of nematodes. Within 5 days, the average numbers emerging per individual per day were: 6.7, 5.0, 3.7, 2.8, and 2.4, respectively. Male nematodes predominated the first day of escape, but this ratio gradually reversed until females predominated. This reversal was expected since the most heavily infected mosquitoes contained more males.

LABORATORY CULTURE OF *Romanomermis* sp. *Romanomermis* sp. collected from larvae in the infested ponds were able to complete adult development in the laboratory, either in water or when they were placed on moist soil or sand. Eggs laid in containers filled with water hatched as they matured which did not permit control of the hatching. However, the hatching of eggs laid on moist sand or soil could be controlled by flooding at the desired time. The following procedure was therefore adopted to maintain a laboratory culture of the *Romanomermis* sp.

About 30–40 postparasitic juveniles were placed in round plastic pill boxes (3 cm. in diameter) containing about 5–7 mm. of sand moistened with distilled water. Tight fitting lids were then placed on the containers, and the containers were held at ambient temperatures for at least 6–8 weeks. After this time, preparasitic juveniles could be obtained whenever desired by flooding the contents of the container.

Muspratt (1965) described a more complex procedure for the culturing of a similar nematode. He gradually desiccated the culture container over a period of several months. However, he also found that sand which had been dry for some time was not entirely satisfactory. Our studies indicated that both soil with low salinity and sand were satisfactory, especially when the soil or sand was sterilized. Sand is presently being used in our cultures because moisture levels are easier to adjust, and turbidity is no problem when the samples are flooded.

LIFE CYCLE. The life cycle of the Louisiana *Romanomermis* sp. resembles that of the mermithids described by Iyengar (1927) and Welch (1960) for the mermithids they found parasitizing mosquito larvae. In our laboratory cultures, the preparasitic juveniles were active soon after hatching and apparently swam about randomly until they came in contact with a suitable host apparently through accidental collisions. Cuticular penetration by the parasite was the main, if not the only, mode of entry of the nematode into the haemocoele of the host, as reported by Iyengar (1927) and Welch (1960). Complete penetration of the host occurred in less than 7 minutes in first instar larvae of *P. confinnis*. The preparasitic juveniles were about 1150μ long but varied in size depending on the size of the nematode laying the eggs. They did not appear to be capable of penetration after about 2 days, and most of them became sluggish then. If they did not find a suitable host, they died within 3–4 days. This short lifespan of the preparasitic stage appears to be characteristic of many mermithids (Welch 1960, Phelps and DeFoliart 1964, and Petersen *et al.* 1967).

The parasitic stage lasted about 8 days at ambient temperatures of 24–26.5° C., and the nematode usually escaped by rupturing the cuticle of the host in the thoracic region though some escaped from other body regions and from natural openings. Nematodes were seen escaping from third and fourth instar mosquito larvae but not from the pupal or adult stages. As noted, multiple infections were common and as many as 12 parasites were observed escaping from a single host. In the laboratory, the postparasitic juveniles molted to adults, mated, and laid eggs within two weeks (11–13 days).

The eggs of the Louisiana species of *Romanomermis* were transparent, nearly spherical, and measure about 80 μ in diameter. Egg development, which was easily followed through the transparent egg covering, began soon after oviposition.

The eggs took at least 7 days to mature and hatch when they were flooded.

The life cycle may be completed in about 4 weeks in the laboratory, and adults lived for long periods in moist soil. In the laboratory, active, gravid females were observed more than 6 months after their escape from the host.

EFFECTS OF THE PARASITE ON THE HOST. The Louisiana *Romanomermis* sp. developed to the postparasitic stage in the thorax of its host (except *Anopheles* spp.), and could easily be seen in the late stages of parasitism coiled within the somewhat enlarged thorax. In *Anopheles* spp., the nematodes oriented themselves lengthwise through the ventral portion of the thorax and abdomen, which made their detection more difficult than in hosts of other genera. The nematodes apparently robbed the host of nourishment and thus prevented the development of fat body, leg rudiments, and other preadult structures. When the parasite emerged, the host died immediately, probably because of the loss of its body fluids.

When mosquitoes in the laboratory colony were fed insufficient food, the parasitized larvae were much smaller than uninfected larvae in the same containers. However, infected and uninfected larvae from the same field collections were similar in size and appearance except for the swelling of the thorax.

Muspratt (1965) found that high temperatures or food rich in vitamins enabled culicine larvae to pupate despite the presence of mermithid nematodes. However, among thousands of infected mosquitoes of many species observed in our laboratory, only two larvae of *P. confinnis* and two of *P. howardii* Coquillet were observed to pupate while they harbored the nematode. (All 4 pupae were heavily parasitized since each possessed 12–25 nematodes.) Attempts were therefore made to infect various larval instars of *Culex p. quinquefasciatus* to see whether larvae infected at later stages could become adults while they were parasitized. Parasitism was accomplished in all four larval instars, but no infected larvae pupated; larvae infected at a late stage remained in the fourth instar until the nematode completed its development. This retardation of pupation indicates that substances may be produced by the parasites that prevent formation of pupal and adult structures. Thus, if parasitism by this *Romanomermis* sp. is successfully carried through to adults, it must happen rarely. Perhaps this rarity is one reason the nematode is not more widely distributed in mosquito larvae in southwestern Louisiana.

EFFECTS OF THE HOST UPON THE NEMATODE. Of the 32 species of mosquitoes either naturally or artificially infected with the Louisiana *Romanomermis* sp., 6 had varying degrees of host resistance; *Aedes triseriatus, Culex salinarius, Culex territans, Culiseta melanura, P. discolor,* and *P. ferox.* All except *Culex salinarius* appeared to develop their resistance early. However, parasites were able to complete development occasionally in all species except *Culex territans.* The mechanism of host resistance appeared to be similar to that of *Aedes communis* to the mermithid *Hydromermis churchillensis* Welch (Welch, 1960).

The size and species of the host mosquito directly affected the size of the emerging parasites. *Uranotaenia* spp. and *Anopheles barberi* generally produced postparasitic juveniles that were about half as large as those produced by larger species. Smaller nematodes also resulted from multiple infestations. Also, as noted, host species may have an effect on the sex ratio of the escaping nematodes (see Incidence of Parasitism).

SUMMARY. Eggs of the *Romanomermis* sp. found in 5 ponds in southwestern Louisiana matured in about 7 days; the ensuing preparasitic stage penetrated mosquito larvae within a day or two. The parasitic stage lasted about 8 days in the body of a mosquito larva; then the mature postparasitic nematode escaped from and killed the host larva. The postparasitic nematode molted to an adult, mated and laid eggs in slightly less than 2 weeks.

The life cycle was completed in about 4 weeks in the laboratory.

The parasite always killed the mosquito larvae before it emerged as an adult. When fourth instar mosquito larvae were infected, the larvae remained in that stage until the nematode completed its development.

Thirty-two species of mosquitoes were infected either naturally or artificially with the nematode. *Aedes atlanticus, Aedes mitchellae, P. ciliata, P. discolor,* and *Culex restuans* are newly recorded hosts and bring to 13 the number of natural hosts.

Our field observations showed that the highest levels of parasitism occurred in both temporary and permanent water mosquitoes when the water level of a pool fluctuated but the pool never completely dried up. The most heavily parasitized larval hosts in the field were *P. confinnis* and *U. sapphirina, Aedes atlanticus,* and *Anopheles crucians* in that order. Larvae of *Aedes aegypti, Culex p. quinquefasciatus, Culiseta inornata,* and *P. confinnis* were the most attractive hosts of the 22 species tested in the laboratory.

Romanomermis sp. was maintained in the laboratory by placing 30–40 postparasitic juveniles on moistened, sterilized sand in small plastic pill boxes. When the contents of the pill boxes were flooded after 6–8 weeks, numerous preparasitic juveniles were produced.

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