PARASITISM OF *ANOPHELES* MOSQUITOES BY A *GASTROMERMIS* SP. (NEMATODA:MERMITHIDAE) IN SOUTHWESTERN LOUISIANA

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In 1967, a survey of the parasites and pathogens of the mosquitoes of southwestern Louisiana was conducted by personnel of the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory, Lake Charles, Louisiana. Larvae of *Anopheles crucians* Wiedemann from a single pool were the only ones found harboring this species of mermithid nematode which Dr. W. R. Nickle, Crops Research Division, ARS, Beltsville, Maryland, tentatively identified as an undescribed species of the genus *Gastromermis*. The only other record of *Gastromermis* parasitizing mosquitoes was reported by J. Coz in a WHO information document in 1966 from adult female *A. funestus* Giles in Africa.

Only a few reports have been made of mermithids in *Anopheles* in the Nearctic region. The first was that of Johnson (1903) who found six undescribed mermithid nematodes in the body cavities of larvae of unspecified *Anopheles* in New Jersey. Other reports were by Chapman *et al.* (1967a and 1967b) and Petersen *et al.* (1968) who found *Romanomermis* sp. in larvae of *A. crucians, A. quadrimaculatus* Say, and *A. punctipennis* (Say) in southwestern Louisiana. Finally, that of *Gastromermis* in *A. crucians* in Louisiana (Chapman *et al.,* 1970a and Chapman *et al.,* 1970b) included some general observations concerning the high level of parasitism by this mermithid in anopheline populations. A subsequent detailed study of the nematode was made to determine the life cycle and the value of this nematode as a possible biological control agent of mosquitoes.

INCIDENCE OF PARASITISM. Apparently, parasitism by *Gastromermis* sp. in populations of anopheline larvae in southwestern Louisiana is rare since only three of many hundreds of sites sampled yielded mosquitoes parasitized by this nematode. Also, at two of the three sites we were able to make only limited observations because the populations of *Anopheles* spp. were so low. However, one area (No. 2) produced enough larval *Anopheles* spp. during the winter of 1968–69 to permit meaningful sampling for parasite activity. This area was therefore surveyed once a week for 16 weeks (January to mid-May, 1969); the habitat dried up shortly thereafter preventing further sampling. During the survey, 247 of 563 *A. crucians* and 41 of 100 *A. quadrimaculatus* collected were found to be parasitized by *Gastromermis* sp. (Table 1).

Parasitism of *A. crucians* in the weekly collections ranged from 0 to 87 percent and appeared to increase progressively until the end of March; then with the onset of warm spring weather, parasite activity decreased. However, this reduced activity may not have resulted entirely from temperature. Several factors may have contributed: the reduction of anopheline breeding; the receding water levels in the habitats; and/or the high incidence of parasitism in February and March which may have resulted from larvae parasitized in the late fall and persisting in the environment because of the cold temperatures. Parasitism of *A. quadrimaculatus* remained high throughout the sampling period, but the trend to progressively increasing parasitism observed in *A. crucians* was not evident, probably due to the low numbers of *A. quadrima-*

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TABLE 1.—Survey of parasitism of *Anopheles crucians* and *Anopheles quadrimaculatus* by *Gastromermis* sp. in a single habitat from January to May, 1969.

Week	Number of larvae collected	Percentage parasitism	Mean number of nematodes per infected larva
		A. crucians	
1	28	25	1.3
2	48	8	....
3	31	13	15.0
4	23	16	5.6
5	77	45	6.3
7	43	58	....
8	80	69	....
9	52	77	....
10	61	82	6.3
12	25	52	....
14	35	0	0
15	13	23	1.0
16	47	38	2.4
		A. quadrimaculatus	
3	3	67	11.5
4	9	89	9.5
5	45	20	6.6
7	4	25	....
8	8	25	....
9	5	60	....
10	23	65	6.0
12	1	0	0
16	2	50	1.0

culatus collected. The percentage of parasitism appeared to vary considerably within a given area at a given time. During week 5 when water levels were low, the larvae were collected from several individual puddles within the site 2, and the collections from each were handled as independent populations. One puddle contained three (of 43) infected *A. crucians* and one (of 30) infected *A. quadrimaculatus;* another a few feet away had 33 (of 34) infected *A. crucians* and nine (of 15) infected *A. quadrimaculatus.* During week 8 when the whole area was heavily flooded, thus allowing a general mixing of populations, *A. crucians* larvae from the areas of the same two puddles were 70 and 63 percent infected, respectively.

The number of parasites was surprisingly high, and generally averaged six or more per host (1–32). It appears to us that some larvae were either more susceptible to attack or were exposed to higher localized concentrations of preparasitic nematodes. For example, 4 of 31 *A. crucians* in one collection (week 3) were parasitized, and all four infected larvae had multiple parasites (7–27 per larva). In another collection (week 9), 10 to 15 parasites per infected host were common, and several harbored as many as 32 nematodes though many others were not infected. When the *A. crucians* from the last collection (week 16) were separated by instar before examination, 25, 11, and 57 percent of the second-, third-, and fourth-instar larvae, respectively, were infected with an average of 1.6, 2.0, and 3.3 parasites per larva, respectively. The pooled collection of 47 larvae had 38 percent infected with a mean of 2.4 parasites per infected host. Thus, parasitism was occurring at the time of the last collection, and all stages of larval *A. crucians* are apparently parasitized by *Gastromermis* sp. This conclusion was substantiated by the great range in the size of the nematodes in fourth-instar larvae with multiple infections.

HOSTS. The *Gastromermis* sp. studied appears to be specific to larvae of anophelines since it has been found only in *A. crucians, A. quadrimaculatus,* and *A. punctipennis.* In the laboratory, equal numbers (50) of early instar larvae of *Culex territans* Walker, *C. peccator* Dyar and Knab, *C. salinarius* Coquillett, *C. tarsalis* Coquillett, *C. p. quinquefasciatus* Say, *Psorophora ferox* (Humboldt), *P. varipes* (Coquillett), *Aedes tormentor* Dyar and Knab, *A. triseriatus* (Say), *Anopheles bradleyi* King, and *A. barberi* Coquillett were exposed in separate containers to the preparasitic stage of *Gastromermis* sp. with 50 first-instar larvae of *A. quadrimaculatus* from a laboratory colony. After 5 days, none of the *Culex, Psorophora,* or *Aedes* larvae contained developing *Gastromermis* sp., but a high percentage of the *A. quadrimaculatus* in each container was infected with one or more parasites. The parasites also developed readily in larvae of *A. bradleyi* and *A. barberi.*

In another test, small groups (10) of first-instar larvae of the above mentioned species of *Psorophora, Culex* and *Aedes* were exposed to large concentrations of preparasitic *Gastromermis* sp. in 5 ml of water and examined after 24 hours. Only the controls, *A. quadrimaculatus,* contained nematodes, and all parasitized larvae had multiple infections. The preparasitics were apparently unable to enter the hemocoele of the culicine mosquitoes and we therefore believe that the resistance to *Gastromermis* by culicines is physical rather than physiological. To date, *Gastromermis* sp. has developed in larvae of of all species of *Anopheles* to which it has been exposed (5), which suggests that it will develop in most anopheline species.

Generally, species of mermithids appear to differ widely in their range of hosts. Some seem to be specific for one host: *Agamomermis culicis* Stiles in *Aedes sollicitans* (Petersen *et al.*, 1967); *Gastromermis* sp. in *Anopheles funestus* (by Coz, as noted); *Agamomermis* sp. in *Aedes vexans* (Meigen) (*Trpis et al.*, 1968). Others vary from a moderate to a wide range of hosts that spreads over several genera of mosquitoes (Tsai and Grundmann, 1969; Muspratt, 1945; Petersen *et al.*, 1968). As noted, the Louisiana *Gastromermis* sp. is the first report of a mermithid nematode being generically specific in mosquitoes. However, Iyengar (1927) reported on the life cycle of a mermithid from several species of *Anopheles* larvae in India; this also suggests that the nematode may be generically specific.

LIFE CYCLE. The life cycle of *Gastromermis* sp. is similar to that described by Iyengar (1927) for a mermithid parasite of *Anopheles* larvae in India. In our species, the eggs begin to develop soon after they are laid, are fully embryonated after 7 days at about 25° C. (ambient temperature), and begin hatching after about 7 more days. The preparasitic nematode then gains entry into the host by penetration of the cuticle. The process of entry and the subsequent development were described in detail by Iyengar (1927). In 6 to 8 days, when preparasitic development is complete and the host larvae are in the third or fourth instar, the parasite ruptures the cuticle of the host, usually in the anal region. Emergence occurs rapidly and is always fatal to the host. Nematodes have not been observed in the pupal or adult stages of their hosts.

In the laboratory, the postparasitic juveniles molt to adults, mate, and begin laying eggs in 9–12 days. The parasite is easily cultured since it is free living after it completes the parasitic stage, and maturation, mating, and oviposition take place readily in small containers of sand submerged in water. Once the preparasitic juveniles begin to hatch, the life of a culture can be extended by removing the free water and leaving the sand damp. Preparasitic juveniles can be obtained by flooding the container with chlorine-free water 24 hours before they are needed. Periodic floodings of cultures of this type have yielded large numbers of preparasitics for as long as 5 months.

EFFECTS OF HOST ON PARASITE. Welch (1960) and Petersen *et al.* (1968) reported some encapsulation of mermithids by host mosquitoes as a form of resistance. However, host resistance of this type to *Gastromermis* sp. has not been observed in either *A. crucians* or *A. quadrimaculatus.*

The size of the host and the number of parasites per host appear to have an effect on the sex of the mermithids. Similar observations were reported by Christie (1929), Petersen *et al.*, (1968), and Welch (1965). We found that as many as 13 parasites can survive and escape from a heavily infected host, but these parasites were small and predominantly male. Also, sampling of populations of *A. crucians* and *A. quadrimaculatus* from study areas 1 and 2 showed the same general ratio of sexes reported earlier for *Romanomermis* sp. (Petersen *et al.* 1968) (Table 2), that is, as the number of nematodes per

TABLE 2.—Sex ratio of *Gastromermis* sp. in larvae of *Anopheles crucians* and *A. quadrimaculatus*.

Number nematodes per infected larva	Area 1		Area 2		Total	
	Number nematodes	Percentage males	Number nematodes	Percentage males	Number nematodes	Percentage males
1	11	18	48	2	59	5
2	12	67	28	23	40	35
3	3	67	9	55	12	58
4	8	88	12	75	20	80
5	0	. . .	10	70	10	70
6	0	. . .	18	77	18	77
7 or more	10	100	13	85	23	91

infected mosquito larva increased, the ratio of males to females increased. Moreover, 5 percent of the nematodes from mosquitoes infected with one parasite were males compared with 91 percent when the host larvae contained seven or more parasites; larvae with more than two nematodes produced a majority of males. Tsai and Grundmann (1968) also reported that the sex ratios of *Reesimermis nielseni* Tsai and Grundmann fluctuated with the number of parasites per host. The worms were predominantly males when the burden was greater than four and almost exclusively female when it was less than four.

EFFECTS OF PARASITE ON HOST. Attack by the preparasitic juvenile appeared to have little or no effect on the host larva though numerous attacks sometimes proved fatal to early instar larvae. When early mortality did occur, it appeared to result from the very active nature of the parasite soon after entrance rather than from the wounds inflicted during entrance. The early instars of infected mosquito larvae appear normal, and the developing parasite can only be detected by dissection. However, as the parasite grows, it orients itself lengthwise in the haemocoele of the host and when fully developed can be readily observed from the ventral side of the host coiled back and forth several times with coils lying on both sides of the alimentary canal and ventral to it. The parasite prevents the development of fat body and the formation of leg rudiments and other preadult structures by robbing the host of sufficient nourishment or by liberation of a substance preventing pupal formation. Emergence of the parasite does not always kill the host immediately, but no surviving host has been observed to live more than 4 hours. Since infected mosquito larvae have not been observed to pupate, we believe that even if a parasite is occasionally carried through to the adult stage, it would occur under a very limited set of environmental conditions. As with *Romanomermis* sp. (Petersen *et al.*, 1968), this prevention of pupation by the nematode may account for the scarcity of the parasite throughout southwestern Louisiana.

Our observations lead us to believe that mermithid nematodes can be grouped into three categories by their effect on the pupation of the host: (1) those that rarely allow the host to pupate [Louisiana *Gastromermis* sp., *Romanomermis* sp. (Petersen *et al.*, 1968), *Mermis* sp. (Iyengar, 1927), *Hydromermis churchillensis* (Welch, 1960)]; (2) those that sometimes permit the host to pupate [*Romanomermis* sp. (Muspratt, 1965)]; and (3) those that do not develop until the host reaches the adult stage [*Romanomermis iyengari* Welch (Iyengar, 1927), *Agamomermis* sp. (Trpis *et al.*, 1968), *Gastromermis* sp. (Coz, as noted), and *Agamomermis culicis* (Petersen *et al.*, 1967)].

DISCUSSION. As other avenues of control besides insecticides become necessary, biological control agents such as viruses, bacteria, protozoans, fungi, and nematodes will command more attention. Welch (1962) discussed the potential value of nematodes as agents of insect control and stated that mermithids showed promise. Our observations also indicate that mermithid nematodes may be of value in certain situations. The *Gastromermis* sp. discussed here, is a species of prime interest because it is specific for anopheline mosquitoes, self limiting in its distribution, and easily cultured in the laboratory. However, work must be done to determine its full potential. In addition to the many factors mentioned by Petersen and Willis (1970), methods of mass rearing and of dispersal must be worked out before field testing can begin to evaluate the potential of this parasite for control.

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SOUTHEASTERN CONFERENCE ON MOSQUITO SUPPRESSION AND WILDLIFE MANAGEMENT

On October 22–23, the National Mosquito Control—Fish and Wildlife Coordination Committee will hold a joint meeting with the Florida Anti-Mosquito Association at the Cape Kennedy Hilton, Cape Canaveral, Florida. The Committee is sponsoring a Southeastern Conference on Mosquito Suppression and Wildlife Management. The first day will consist of a formal program with 8 speakers and the second day will be devoted to field trips to marshes in the Titusville area and to Tomoka Marsh. The proceedings of this conference will be available to the public early in 1971.