MERMITHID NEMATODE PARASITISM OF <u>AEDES STIMULANS</u> (WALKER) (DIPTERA: CULICIDAE) FROM INGHAM COUNTY, MICHIGAN

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ABSTRACT

MERMITHID NEMATODE PARASITISM OF AEDES STIMULANS (WALKER) (DIPTERA: CULICIDAE) FROM INGHAM COUNTY, MICHIGAN

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A study on mermithid nematode parasitism of *Aedes stimulans* (Walker) was conducted and infestation rates were compiled essentially through dissection of the mosquito host in all stages of growth. Observations were made of the developmental pattern of the nematode within its host and culturing of the nematode was attempted. Histological sections of the juvenile mermithid in the head of the fourth instar larva of the mosquito were obtained.

In the prime collecting area, approximately 55% of the mosquitoes were parasitized but in individual pools the percentage rose to 78%. The distribution of the mermithid nematode was found to be discontinuous and up to seven nematodes infested a single host.

A minimum length of time for parasitic existence prior to emergence coincided with the life cycle of the mosquito host up to the adult stage. Emergence of the parasite invariably caused the death of the host. Eight weeks after emergence the nematodes were still undergoing moulting. Gonads had not fully formed at this stage but the beginning of spicules could be observed in the males.

Ectocommensal protozoa were noted on both parasitized and non-parasitized mosquitoes.

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By

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INTRODUCTION

In the summer of 1964, a high incidence of an unidentified mermithid nematode parasite was reported in *Aedes stimulans* (Walker) from vernal ponds in Ingham County, Michigan. Additional collections were made the following spring which confirmed the 1964 report. A high percentage of mortality was observed in these parasitized mosquitoes. Dr. H. E. Welch to whom the specimens were sent for identification reported that the parasites were larval mermithids, which lacked characters for positive identification, but were close to larval specimens of *Hydromermis sp.*, a mermithid reported from several species and genera of mosquitoes.

These reports stimulated interest in nematode distribution within the range of their host, and particularly in their role as natural agents controlling mosquito populations. The primary objective of this investigation, therefore, was to compile information on the occurrence of mermithid nematode parasites in certain mosquitoes in Ingham County, Michigan, to follow the developmental cycle of the nematode within the mosquito and outside it, and to attempt in culturing the nematode to adulthood to determine its taxonomy.

These studies started in the summer of 1965, with a survey of the potential mosquito breeding grounds in the county, followed by collection and dissection of immature mosquitoes in the area to record any incidence of nematode parasitism in them. The investigation continued until the end of summer, 1966.

REVIEW OF THE LITERATURE

Hyman (1951) frequently places the Mermithoidea under Enoploidea and generally regards them as related to the Dorylaimoidea. The mermithoids are smooth filiform worms often of considerable length (50 cm., but usually shorter) that are parasitic in juvenile stages in terrestrial or fresh-water invertebrate hosts, usually insects, but also crustaceans, spiders, and snails whereas the adults lead a free existence, often of some duration in soil or fresh water. The superfamily embraces two families: Tetradonematidae and Mermithidae. The taxonomy of the latter rests mainly on Russian nematologists, most notably Filipjev and Schuurmans Stekhoven (1941) but due to the difficulties of the taxonomy of these worms only a few contributions were made in the last twenty years (Welch, 1963).

Polozhetsev and Artyukhovskii reviewed the genera, Hydromermis (1960) and Paramermis (1959). Artyukhovskii revised Pseudomermis (1960) and Amphidomermis (1963), erecting in the latter work two new genera, Spiculimermis and Melolonthimermis. Ipateva (1963) described a new species of Filipjevimermis. Kir'yanova *et al*. (1959) reviewed Kirgistan mermithids and erected the genus Pologenzevimermis. Welch and Rubstov (1964) studied a species complex in Gastromermis. Coman (1961) published a monograph on the mermithids of Roumania, and listed eight genera and 29 species of which two genera, Romanomermis and Quadrimermis and eleven species were new.

In North America, Johnson (1963) reported Octomyomermis, a new mermithid from chironomids. Poinar and Gyrisco (1962) named a new Hexamermis, and Poinar (1964) described a new and unusual genus and species from chironomids. Welch (1960 a, b, c,) named a new Hydromermis and discussed taxonomic problems of Hydromermis contorta (Linstow, 1889) Hagmeier, 1912. He named a new species of Gastromermis, of Isomermis, and of Mesomermis from simuliids (1962) and new Hexamermis from Australia (1963). A new Romanomermis from mosquitoes in India was also described (1964).

The occurrence of mermithid nematode parasites in mosquito larvae was reviewed by Jenkins and West (1954), who reported finding high infestation by the worm (subsequently described as Hydromermis churchillensis by Welch, 1960) in larvae of Aedes communis (De Geer) in northern Canada, with light incidence in two other species of Aedes, namely A.nearticus Dyar and A.nigripes (Zett). In 1956 Laird reviewed world records and later Welch (1960) summarized mermithid parasitism in North American species of mosquitoes. Frohne (1953, 1955) also reported the presence of mermithids in Aedes communis, and added several species to the list of known mosquito hosts given by preceeding authors.

The earliest record of mermithid infestation in mosquitoes dates back to 1898 when Ross observed parasitism in Culex fatigans from India. According to Muspratt (1945) nematodes other than Filaria, parasitic in mosquitoes have been recorded from the United States, Canada, Europe, Russia, India, Ceylon, Sumatra, and Africa. These mermithids were Limnomermis Daday, Paramermis Linst, Mermis Duj. and Agamomermis Stiles. Agamormermis is an artificial collective group for larvae which cannot be identified.

At Leipzig, Germany, Agamomermis sp. were collected in the abdominal cavity of larvae pupae, and adults of Culex nemoralis by Stiles (1903). Infestation was thought to have occurred in water. The infested insects were very sluggish in movement and many of them died from the effects of the parasite. The ovaries of parasitized females were underdeveloped and during the years when the nematodes were most common the mosquitoes were less numerous.

Smith (1904) collected in New Jersey, large numbers of female Aedes sollicitans (Walker) which were infested with Agamomermis culicis Stiles (1903) from late June to late September, 1903. Up to 50 percent of the specimens collected contained worms. The peak of infestation occurred in late July and into August. The ovaries of A. sollicitans did not develop when the female was parasitized and the nematode was considered to be a material check on the species.

In French Guiana, nematodes were found in pairs, one large and one small worm in the body cavity of Aedes aegypti (L.) The larvae developed normally but just before pupation the worms left the host at the posterior end of the body by perforating the membrane surrounding the anus. The larger worm emerged first followed by the smaller and both died several hours later. The mosquito larvae died as a result of injuries caused by the emerging worms. These observations by Gendre (1909) also mention that no parasitized adults were found.

The life cycle of mermithids that destroy mosquito larvae was studied in India by Iyengar (1927). He found that the minute pre-parasitic worms swim in water and probably penetrate the cuticle of mosquito larvae. The smallest nematodes seen in the mosquito larval haemocoele were 650 to 700 microns in length and 115 to 165 microns in width. The parasitized mosquito larvae were found to remain as fourth instar longer than normal larvae. The larvae died on emergence of the nematodes. Muspratt (1964) has shown that an undetermined mermithid, subsequently referred to as Romanomermis Coman, will readily parasitize mosquito larvae of the Culex pipiens complex.

Hearle (1926) reported that as many as 80 percent of Aedes vexans Meig. mosquitoes in the Fraser Valley, British Columbia in 1920, contained a nematode parasite, Paramermis canadensis Steiner (1924), but only about 20 percent in 1921. Hearle observed the effect of the parasite to be a retardation of the development of the ovaries, since no parasitized females contained well-developed eggs. His measurement of the largest worm was 1.25 in. in length, with from one to six nematodes in a single mosquito. Hearle's (1929) further observation of mermithid parasitism of mosquitoes was that of an adult female Aedes flavescens (Miller) in Canada infested with a single small nematode, and a single female _Aedes_ _aldrichii_ D. and K.

These earlier observations of parasitism of mosquitoes by mermithids have been confirmed more recently by Smith (1961) and notably by Welch (1960). The literature gives detailed account of the structure of those members of the family that have been described. According to Welch (1963) members of the Mermithoidea are generally identified by the degenerate musculature of the oesophagus, the very long oesophagus, the presence of numerous oesophageal cells along its length and the development of the intestine as a food storage organ. Hyman (1951) gives a more detailed description of the superfamily and emphasizes the absence of a buccal capsule and the direct opening of the mouth into the pharynx. She describes the pharynx as long and tenuous, reaching half or more the body length of some mermithids, and consists of cuticular tube embedded in a thin cytoplasmic layer. Posteriorly they are attached to a varying number of variously arranged stichocytes, four in the family Tetradonematidae and usually more than four in the Mermithidae. The didelphic female system is of the opposite type. The males, much smaller than the females, are usually diorchic with one or two specules and with numerous genital papillae on their posterior ends. Reproduction is either bisexual or parthenogenic.

Most authors agree on the subdivision of the Mermithoidea into two families, the Tetradonematidae and the Mermithidae. According to Filipjev _et_ _al_. (1941), Hyman (1951) and Welch

(1963, 1965) only three of the Tetradonematids have been named, though Rubstov (1963) found new unnamed species in all stages of simuliids. The Tetradonematids are generally regarded as primitive mermithoids which remain and become adults in the haemocoele of their hosts whereas the mermithids emerge and become free-living adults.

The life cycle of mermithid parasites varies for different species. Welch (1963) states that it commences when the second stage juvenile armed with an odontostyle penetrates the host cuticle and enters the body cavity. The nematodes grow, fill the host body cavity, then emerge to begin a free-living existence. Maturation, fertilization, and oviposition require varying periods according to the specific mermithid. According to Anderson and Defoliart (1962), in aquatic forms such as _Gastromermis_ sp. and _Isomermis_ sp., the above sequence of events may take several months, or take up to a year and half in terrestrial forms such as _Agamermis decaudata_ parasitic in grasshopers (Cobb, Steiner, and Christie 1923, Christie, 1929, 1936). Welch (1963) states the case in _Mermis nigrescens_ Dujardin, a parasite of Orthoptera and Dermaptera, in which the life cycle is altered to passive host infection through the consumption of elaborately tasseled eggs deposited on grass blades. Certain species, however, require rather short life cycles. Musspratt (1963) refers to some species parasitizing chironomids which require only 24 hours or even less. Welch (1960) found that the subarctic mosquito parasite _Hydromermis churchillensis_ requires 10-15 days to attain sexual maturity after emergence from the host while in two blackfly mermithids it varies from 5 to 12 days.

Most records in the literature give only larval hosts, and Stabler (1952) concluded that the presence of the worm prevented pupation. Apparently the worms can carry over from larval through pupal to adult hosts. (This occurred frequently in the Michigan observations). According to Welch (1960) this assists, in the culicids, in the distribution of the parasite but in simuliids it also counteracts the tendency of the river current to carry the free living stages downstream.

Many records of percentage parasitism may be found in the literature. Welch (1963) advises that despite the sampling problems involved in their derivation, and the care that must be exercised in their use, they are indicative of interactions. Rates range from 0 to 100%; their magnitude usually shows an increase with host development or age, or else with the degree of suitability of the physical environment of the nematode such as the moisture content of the habitat. Correlations with host abundance are given in a few cases. Records of mermithid parasitism of mosquitoes is rather fragmentary. Most of the data is on parasitism of simuliids, grasshoppers and chironomids. Krall (1959) recorded 50 percent parasitism of adults of Chironomus plumosus L. in a lake in Estonia. Johnson (1963) reported 20 percent of the larvae of this same insect parasitized in Minnesota. The numerous data on mermithid parasitism of simuliids were analyzed by Weiser (1963) and Welch (1960, 1963). A detailed study by Phelps and DeFoliart (1964) on parasitism of Simulium vittatum Zetterstedt by three mermithids in Wisconsin showed 50 percent parasitism of larvae and 37 to 63 percent of adults. The authors considered that mermithids were a limiting factor on simuliid populations and that eradication had occurred twice. Rubstov (1963) suggested that simuliid eradication by mermithids had occurred in certain Soviet Rivers. He recorded an average of 17 percent and range of 0 to 99 percent in the Leningrad region. Shipitsina (1963) gave parasitism rates of adult simuliid flies from the Krasnoyarsk region of 1 to 11 percent.

High rates of mermithid parasitism of mosquitoes have been recorded in pools by various authors who have pointed out that their high rates were rare, as most breeding sites are without nematodes. These and his own observations led Laird (1956) to generalize that wherever mermithid parasitism was noted, its incidence was high. Welch (1960) does not support this view. The parasitism given by these authors seems to correspond only to the maximums of their ranges. Welch (1960) gives more realistic figures of infestation rates of Aedes communis (DeG.) by Hydromermis churchillensis n.sp. in three areas at Churchill, Manitoba. The average infestation per pool for each area where infestation occurred was almost the same each year and was in the order of 5.1, 0.2 - 1.4, and 1.1 - 9.8 percent. The corresponding range of infestation for the three areas were 0 - 32, 0 - 20, and 0 - 82 percent, respectively.

Welch (1965) states that percentages of parasitism given in the literature are true measures of insect mortality resulting from mermithid infection as mermithids kill their hosts upon emergence. Many authors agree that mermithids damage their hosts only during the later stages of their parasitic development when their growth is rapid, and their relatively large size compared to that of the host.

Instances of multiple parasitism are often recorded. Welch (1959, 1960) and other authors have analyzed frequency distribution of the number of parasites per host and showed that departures from a chance distribution were involved in all the reported cases. This, Welch (1963, 1965) suggests, shows a discontinuous or contagious distribution of nematodes within the range of their host. Such discontinuous parasite distributions are apparent not only within, but also between populations of the same host. Gendre's (1909) observation of high but localized parasitism of mosquito larvae by mermithids was duplicated in Canada by Welch (1960) who found neighboring pools to have infested and non-infested populations of larvae. In a number of observed cases high parasite numbers per host usually results in the emergence of males; and low numbers, in female mermithids.

There are few observations on the factors that cause emergence from the host. According to Welch (1960) emergence of mermithids is correlated with special developmental events, such as pupation or adult emergence of the host. Very little is known about the causative factor of mermithid emergence from the adult host.

Insects generally show little outward evidence of the presence of nematodes and dissections are usually necessary to reveal nematode parasites. The literature gives an account of anomalies due to external effects of mermithid parasitism of insects other than mosquitoes. These involve the shortening of wing in ants and locusts, and the malformation of elytra in weevils (Poinar and Gyrisco, 1962). Various authors have reported formation of intercastes and intersexes in ants, chironomids, culicoides and simuliids. Discoloration and cuticular transparency have also been noted in mermithid infested weevil larvae.

Internal effects of mermithid parasitism of Aedes communis (DeG.) larvae was reported by Welch (1960) who noted a reduction in fat body tissue and also in thoracic musculature. Other authors agree that probably because of the large size of mermithids, their damage to genital and intestinal organs is greater than that caused by other nematodes.

Host reaction of mosquito larvae to nematode infestation by means of melanization and encapsulation was probably first recorded by Welch (1960) from observations in first and second instar larvae. Brug (1932) summarized the records in adult mosquitoes. Welch and Bronskill (1962) discovered the melanization and encapsulation of a Neoaplectanid nematode by larvae of several species of mosquitoes. Aedes stimulans is reported to react similarly to an unidentified rhabditoid nematode.

The culture of nematodes has been reviewed in considerable detail by Dougherty (1960). The literature cites instances of many entomophilic nematodes that were cultured xenically. A sand culture of a mermithid nematode of mosquitoes by Muspratt (1964) illustrates a most convenient way of culturing this group of nematodes.

METHODS AND MATERIALS

Studies and observations were commenced in the summer of 1965. The season proved to be rather dry, especially in the first half, and consequently many of the potential breeding grounds for mosquitoes were virtually dry, particularly puddles and vernal ponds which serve as good sources for mosquito larvae.

A survey, covering nearly 144 sq. miles of the north western portion of Ingham County, was conducted to map out the potential mosquito breeding habitats. This included essentially four townships of the county, namely, Lansing, Meridian, Delhi and Alaiedon.

Collecting Equipment and Techniques. Much of the equipment and procedure used in searching for the immature stages of mosquitoes are not completely original. Some of these have been mentioned in previous publications and are considered to be standard methods of collecting mosquitoes. The basic equipment comprised metal laddles, white enamel trays and large glass containers with fine mesh wire or cotton lids which were used in transporting the mosquito infested pond water to the laboratory. In the absence of dippers large metal beakers served as good substitutes.

The collecting of immature stages rather than adults was preferred in view of the ease of capture. Where larvae or pupae were present in ponds or streams there was no difficulty in locating them and effort was made to examine every available water holding in the survey area. A majority of the ponds that were examined yielded no immature mosquitoes and it was not until mid-July that mosquitoes were encountered in the field as a result of a few scattered rains. In addition, some tree holes in the area were examined for mosquitoes. The problems involved in collecting immature stages of tree hole breeding mosquitoes include the dark color of most tree hole water, the small amount of water involved, and the fact that most tree holes are less accessible than the breeding habitats of most other species. Where tree holes were found to contain water it was difficult or impossible to determine under field conditions whether or not specimens were present in a given collection. Generally tree hole water was poured into white enamel trays for examination in the field, but young instars or eggs might be present and not discovered. All water in a tree hole was routinely taken and the hole flushed with pond water.

When collecting with specimens in the car, care was taken to have good ventilation, and if possible to park in the shade to keep the specimens cool. This account so far applies primarily to routine collecting when rainfall was sufficient to deposit water in many dry ponds and tree holes. At the end of the days collecting, each collection was labelled giving its location and date and taken to the laboratory for further examination.

Laboratory Procedure - (Nematode investigations in the immature mosquitoes.) In the laboratory, collections of larvae in the large glass transporting jar were transferred into white enamel pans from which they were easily picked and fixed. First to third instar larvae were generally left until they reached fourth instar stage when they showed sufficient characters for positive identification. The fourth instar larvae were quickly killed and fixed in FAA solution (made up of 15-20 ml. of ethyl alcohol, 1 ml. of acetic acid, 6 ml. formalin and 40 ml. distilled water). This fixative has the property of keeping nematodes in a fairly good state, free from being brittle, and has a clearing effect on the entire mosquito larva.

Following fixation larvae were identified to species under low power stereoscopic microscope after which they were dissected without much delay to look for mermithid nematodes.

Recovery of mosquito larvae from sod samples. In view of the existing drought conditions of the summer of 1965, with its attendant drying of local ponds and the consequent reduction of mosquito breeding in the field, attempt was made to recover mosquito larvae from the potential breeding grounds by flooding sod samples from these areas. Sod samples from a previously recorded *Aedes* breeding habitat were collected in plastic bags and taken to the laboratory for flooding. This selected area located in Delhi township of Ingham County, is adjacent to the Sycamore Creek on the east bank. (This is the primary collecting area referred to elsewhere in this paper as Willoughby No. 1). The area is essentially level and wooded and is the site of vernal ponds which appear just after the annual spring thaw.

Beginning in August 3, 1965, 32 sod samples of 1 ft. square each were cut to a depth of 2-4 inches from eight different sites, with four samples from each site. Each sample was put into a plastic bag and kept in the shade until ready for transfer to the laboratory.
For flooding, a sample was transferred to an enamel or plastic pan and sufficient water was used to fully cover the sod and any plant material. Four sod samples were flooded each day for 12 days. After a flooding period, the water was poured off carefully to look for larvae.

In these flooding exercises some attention was given to some suitable egg hatching stimulus. The literature on egg hatching stimuli has been reviewed by Burgess (1959 after Shannon and Putnam, 1934; Garnet and Haynes, 1944; Gjullin _et. al._, 1941; Borg and Horsfall, 1953; Horsfall, 1956; Horsfall _et. al._, 1958; Barr and Al-Azawi, 1958). The hatch-promoting stimuli have been found to be essentially distilled water and distilled water with low oxygen tension. Both types of distilled water were used for flooding. Dissolved oxygen was removed from distilled water by boiling for thirty minutes, then cooling it to 28-29°C. for 30 minutes in the absence of air. This has been proven to reduce the amount of dissolved oxygen in the distilled water from its normal value of 7-8 p.p.m. to less than 2 p.p.m. by weight. (After Burgess, 1959).

The results from flooding sod samples of eight different sides in four replications yielded only 25 larvae of _Aedes vexans_ (Meigen) from one sample. The possible reasons for this astonishingly low yield will be discussed later. These larvae were free from any nematode parasites (Table 1.).

Attempt at recovering mermithids from the soil. The 1965 season investigations embraced processing of soil from habitats where mermithid nematode and mosquito relationships had been previously reported. Soil samples from "Willoughby 1" were obtained as previously described and passed through 25, 60, 100 and 200 mesh sieves and finally centrifuged in sugar solution. This technique leaves all nematodes in every stage of growth in the supernatant fluid. This method of investigation yielded some plant parasitic nematodes, but no mermithids.

INVESTIGATIONS IN THE 1966 SEASON

With the onset of the spring season, the search for _Aedes_ mosquitoes was most actively resumed. Attention was paid to previously recorded _Aedes_ breeding places in the four townships of the county previously mentioned. Three areas were selected for collecting and were designated Willoughby No.I, Willoughby No.II, and Holt. Willoughby Nos. I and II are situated on the east bank of the Sycamore Creek and might be regarded as similar habitats except that the two are separated by a road. The third location in Holt, about three miles away from the first two, was chosen to see any differences due to distance that might exist in the incidence of parasitism of the mosquito populations.

When the winter snow had sufficiently melted for ground pools to form, the search for mosquito larvae was commenced. Larvae were collected from the three sites and taken to the laboratory every five days starting on April 21, 1966. The regularity of sampling was broken owing to the unusually cold spells that occurred intermittently during the spring season. Collecting was therefore limited to good weather conditions as well as the unavailability of working material in the laboratory. The woodland nature of the collecting sites is shown in Figure 1.

Aedes Stimulans and Its Bionomics. The species that was found to occur in tremendous numbers in woodland pools and

Figure 1

HABITAT OF <u>AEDES STIMULANS</u>

Woodland pool, near the Sycamore Creek in Ingham County, Michigan. This spring pool contains an enormous population of <u>Aedes stimulans</u>. It dries up in summer.

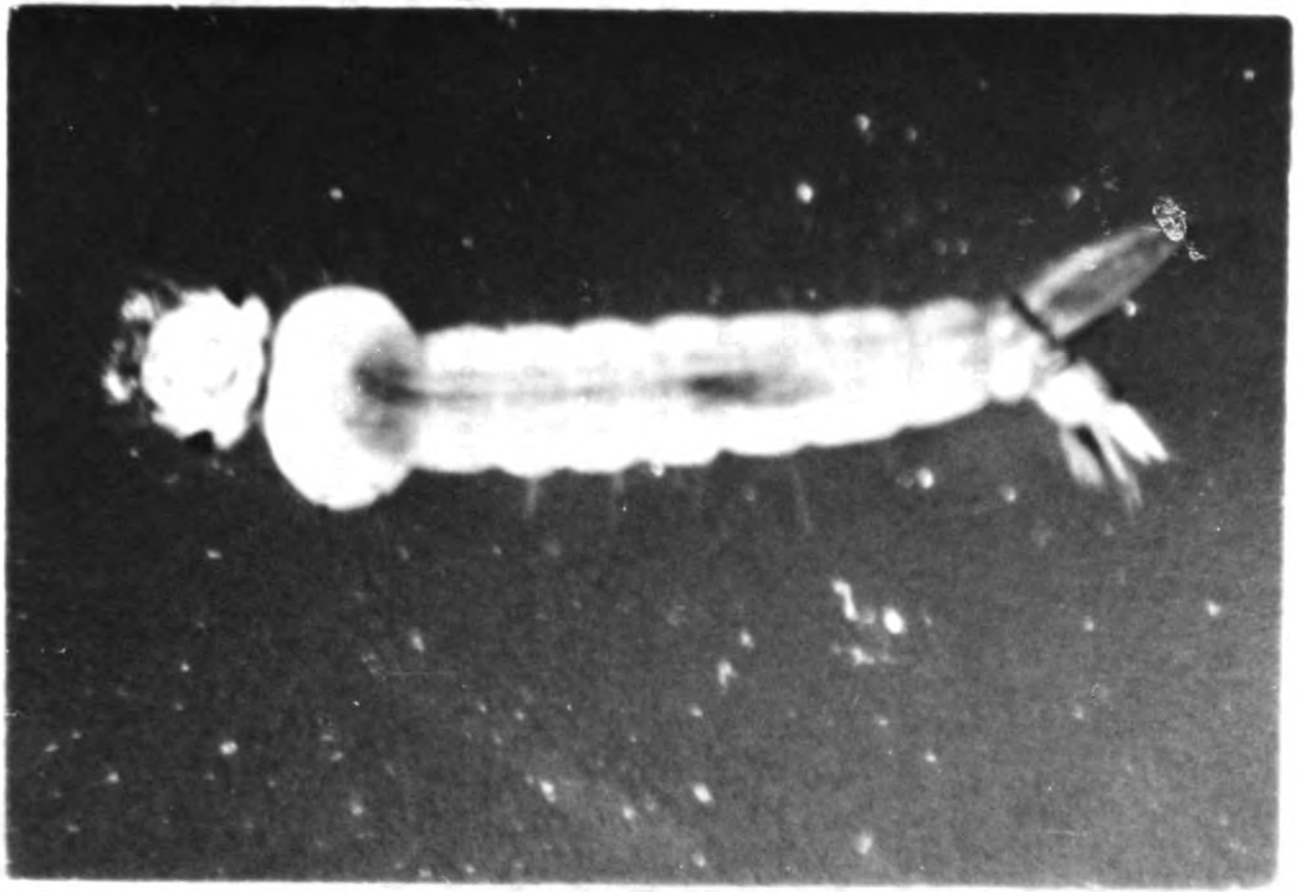
stump holes in all the three sites throughout the spring is *Aedes stimulans* (Walker). Ross (1947) reports that the larvae of this species (Fig. 2) occur in cold water of woodland ground pools flooded by melted snows, develop slowly, but are usually fully grown by the time the first warm weather of May occurs. Horsfall stated that a survey of 50 snow pools showed 58 percent of the larvae to be this species in Canada (Horsfall, 1955 after Twinn, 1931).

The range of *Aedes stimulans* is widespread throughout the northeastern states, across the north central states, through Canada, and into the Yukon (Ross, 1947). The species is reported throughout the state of Michigan. It has one generation per year (Horsfall, 1955 after Twinn, 1931; Owen, 1937).

The pupal stage requires 3-4 days when the temperature range is 20°-25° (Horsfall, 1955 after Jordan, 1902). The females seem to stay near their larval sites, where they attack all sorts of animals that come near. This is evidenced by the ferocity with which they attacked to bite during the collecting period. Horsfall (1955) reports that the males feed on the flowers of smilax.

A.stimulans in captivity is found to be reluctant to oviposit on moist cellucotton where conditions are favorable for obtaining eggs of other species like *A.vexans* or *Psorophora sp.* Like *A.trivittatus*, most eggs have been obtained only when caged females were trapped on the surface of the water, and eggs were extruded before they died. (Horsfall,1955).

Figure 2



FOURTH INSTAR LARVA OF _A_. _STIMULANS_

A mermithid nematode parasite occurs in the head of the larva

Magnification -- 15x

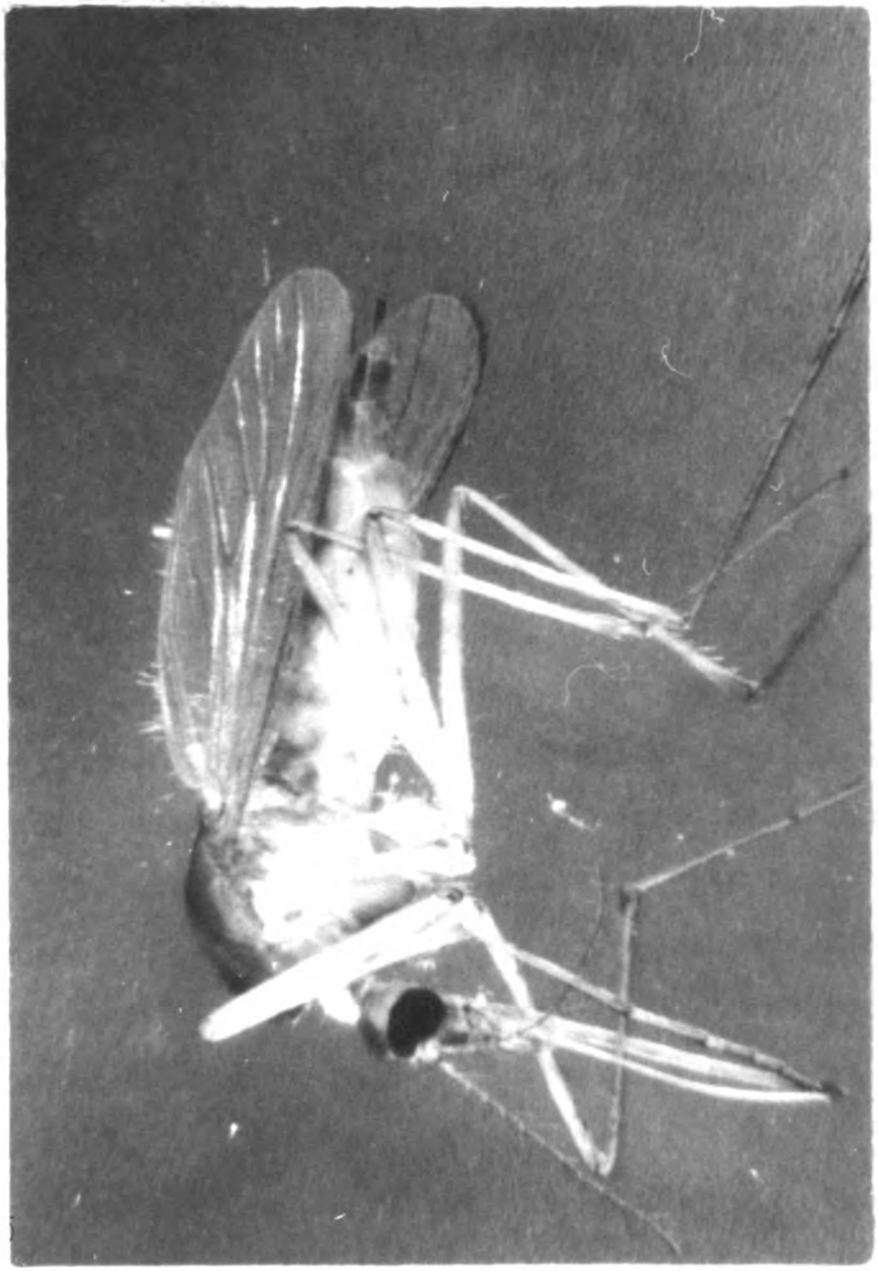
By late June and early July adults (Fig. 3) could still be found in the field in Ingham County, Michigan, but reports state that stragglers are still present in early September (Horsfall, 1955 after Twinn, 1931).

Studies on Mermithid Parasitism of *Aedes Stimulans*. Sampling of ponds by the last week of April, 1966, had clearly revealed that the predominant mosquito species in the snow pools was *Aedes stimulans* (Walker). At this time most of the larvae had reached the third instar stage although second instar and at times first instar larvae could be found in some pools.

The method of investigation for the presence of the nematode parasite in the mosquito was essentially by dissection, since the larvae showed practically no outward evidence of the presence of the parasite. Larvae were held in pond water with a few dead leaves for food until ready for dissection. Since the aim of the study was to note the incidence of parasitism as well as recovering the nematode for culturing purposes, larvae and pupae were dissected in physiological saline without resorting to the use of any fixative that might kill the nematode.

Dissections were done under low power binocular microscope and any nematodes recovered were held in physiological saline in separate specimen tubes and labelled according to the stage of mosquito which they parasitized. The nematodes began to show signs of death within an hour, and were subsequently transferred from the saline into F.A.A. fixative

Figure 3



ADULT FEMALE <u>AEDES</u> <u>STIMULANS</u>

Magnification -- 15x

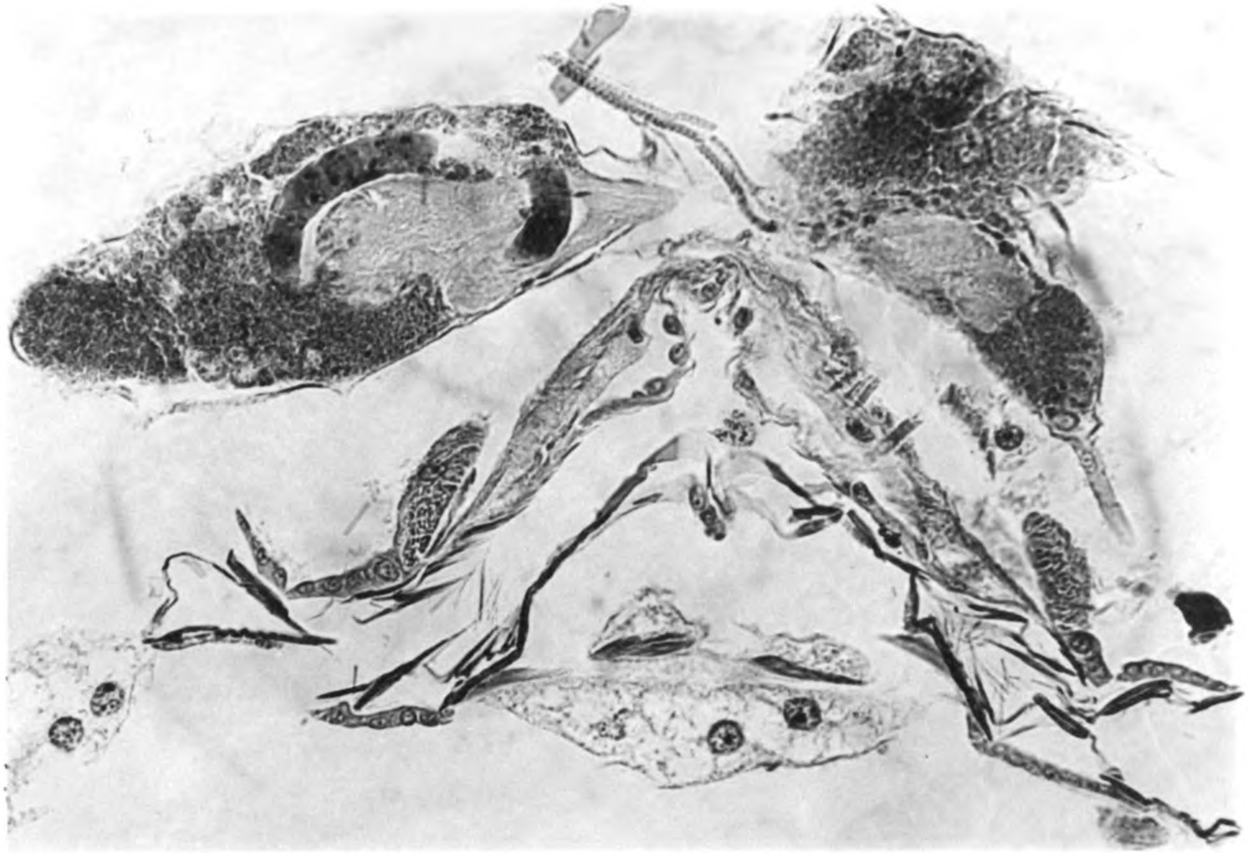
and ultimately processed for slide preparations. Adult mosquitoes were likewise dissected upon emergence from pupae.

These dissections revealed that <u>Aedes stimulans</u> was infested with a mermithid nematode, obviously a juvenile stage, as early as the second instar larva of the mosquito. Very few first instar larvae that were dissected showed any infestation. The nematode was seen in the second, third, and fourth instar larvae of the mosquito in the region of the head below the eyes, in location within the brain lobes and at times in the posterior end of the head. An attempt was made to document the definitive positions of the nematode in the larval head by sectioning. Whole larvae were fixed in methanol, chloroform and propionic acid (6:3:2) for forty-eight hours and later dehydrated in two changes of tetrahydrofuran for six hours. This was followed by a modified double embedding technique (French, 1964, after Salthouse, 1958) involving one, two and three percent solutions of parloidin in tetrahydrofuran. The larvae were then rinsed for ten minutes in tetrahydrofuran and finally embedded at 60°C in paraffin wax for not more than two hours.

Ten micron sections were cut and stained with Mallory's triple stain - McFarlane's modification (Guyer, 1953). A sagittal section of a fourth instar <u>A.stimulans</u> showing the definitive nematode in the head appears in Figures 4, 5, 6, 7, 8 and 9.

Permanent mounts of the different stages of the nematode were prepared by a rapid method of transferring nematodes

Figure 4

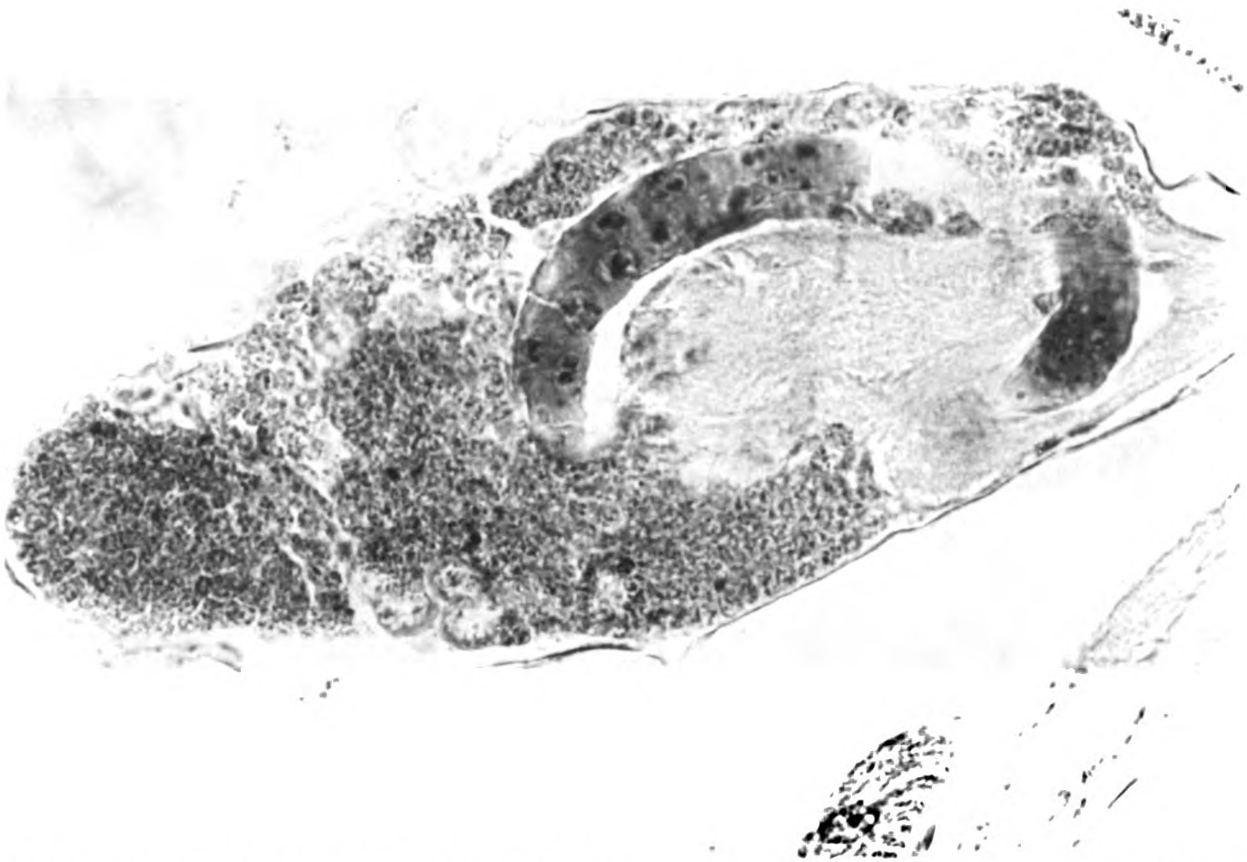


SAGITTAL SECTION OF THE HEAD OF A FOURTH INSTAR LARVA OF <u>AEDES STIMULANS</u>

Left protocerebral lobe of the brain shows the presence of an early juvenile stage of a mermithid nematode, bent as a result of the parasite's irregular looping movement. Below the brain lobes is the foregut opening with its chitinous lining.

Stain -- Mallory's triple stain (McFarlane's modification)
Magnification -- 2[illegible]5x

Figure 5



SAGITTAL SECTION THROUGH THE BRAIN OF A FOURTH INSTAR LARVA OF AEDES STIMULANS

The definitive position of the juvenile nematode in the left protocerebral lobe of the brain. A stichosome of large darkly-staining stichocyte cells appears on the anterior (left) half of the worm.

Stain - Mallory's triple stain (McFarlane's modification) Magnification - 540x

Figure 6

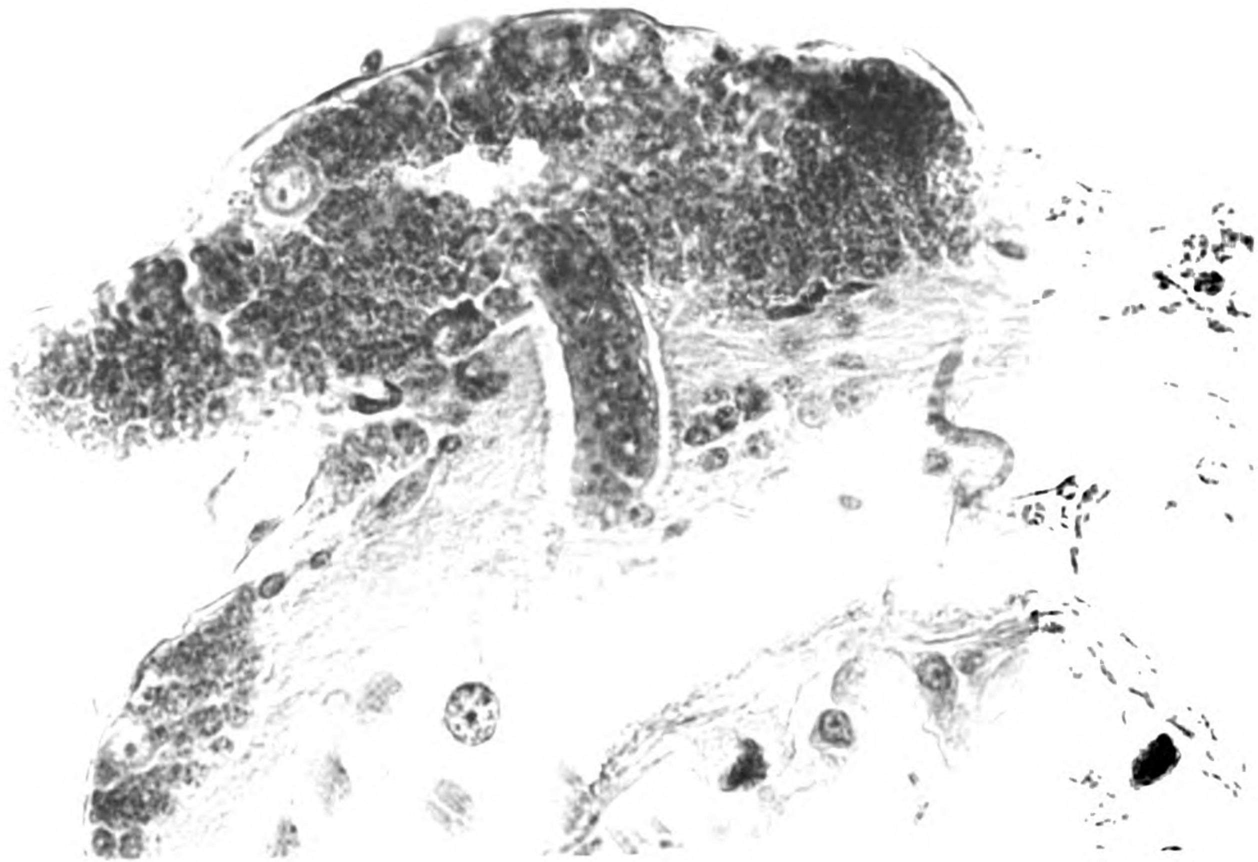
SAGITTAL SECTION OF THE HEAD OF A FOURTH INSTAR LARVA OF <u>AEDES STIMULANS</u> TO SHOW MULTIPLE PARASITISM

A case of multiple parasitism with three worms. The left brain lobe shows a sagittal section of one worm. The right brain lobe shows a sagittal section of the second worm and a transverse section of the third.

Stain -- Mallory's triple stain (McFarlane's modification)

Magnification -- 540x

Figure 7



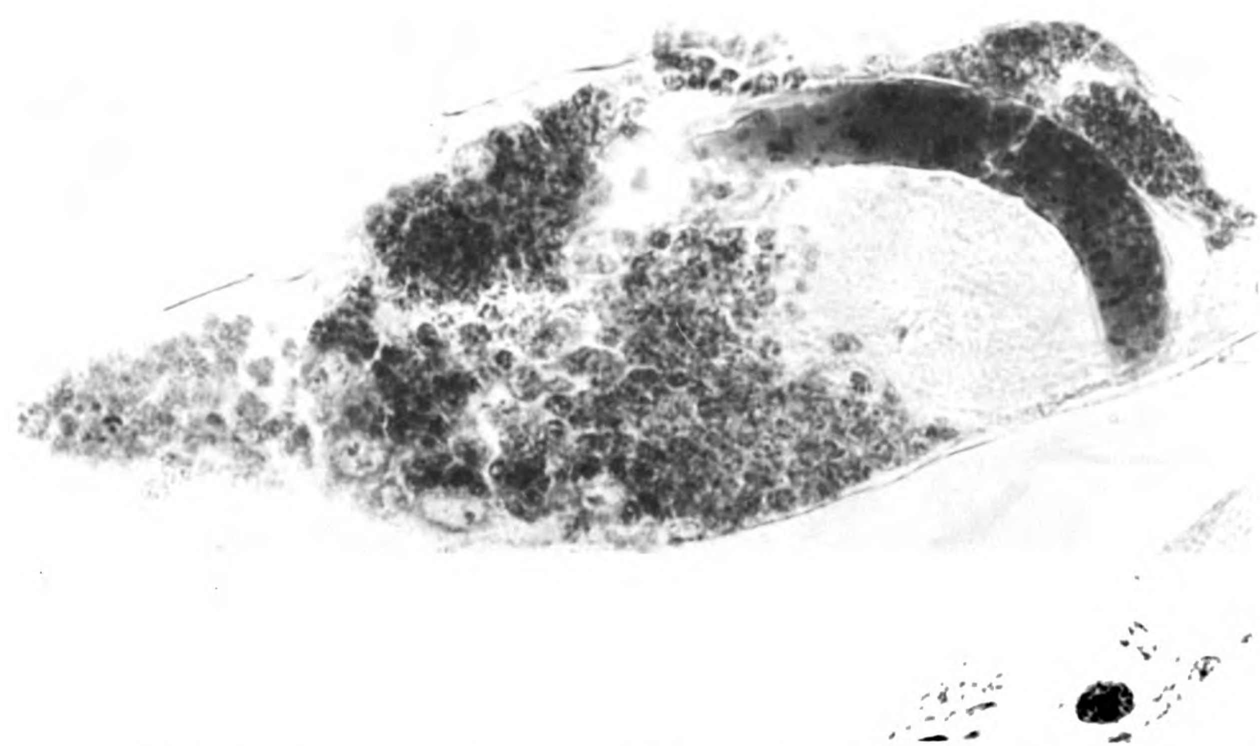
SAGITTAL SECTION OF A JUVENILE MERMITHID NEMATODE WITHIN THE LEFT BRAIN LOBE OF A FOURTH INSTAR LARVA OF <u>AEDES STIMULANS</u>

Stain -- Mallory's triple stain (McFarlane's modification)

Magnification -- 540x

THESIS

Figure 3



SAGITTAL SECTION OF A JUVENILE MERMITHID NEMATODE WITHIN THE BRAIN LOBE OF A FOURTH INSTAR LARVA OF AEDES STIMULANS

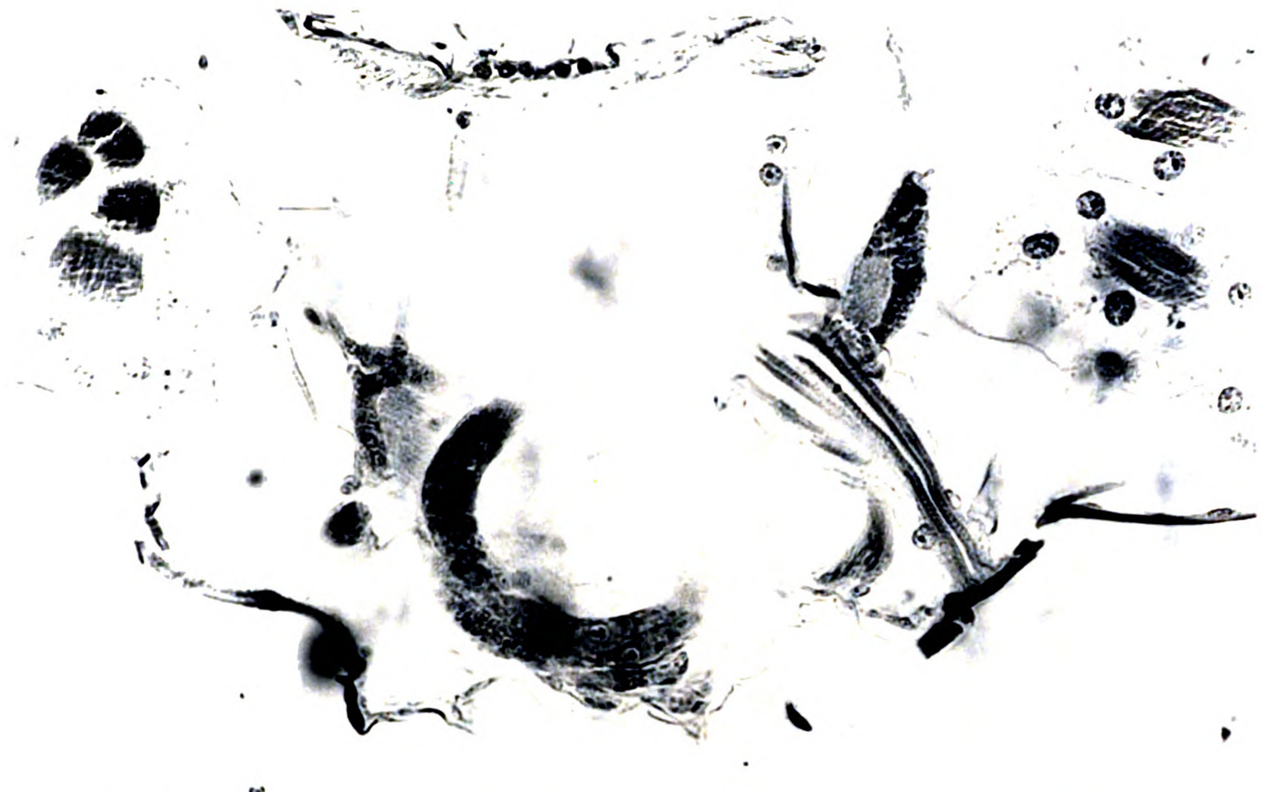
Nematode occupies position in brain similar to that shown in preceding figures.

Stain -- Mallory's triple stain (McFarlane's modification)

Magnification -- 540x

THESIS

Figure 9



SAGITTAL SECTION THROUGH THE HEAD (POSTERIOR HALF) OF A FOURTH INSTAR LARVA OF AEDES STIMULANS.

Juvenile nematode is seen at the posterior end of the host's head. This stage of development just precedes the transition stage of development within the adjacent thorax.

The larger darkly staining stichocyte cells in one half and the smaller granular cells of the trophosome in the other half of the body are clearly seen.

Stain -- Mallory's triple stain (McFarlane's modification) Magnification - 265x

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from fixative to anhydrous glycerine (Seinhorst, 1959). The procedure for processing the nematodes through 96 percent ethanol to glycerine was by an initial transfer of the nematodes from F.A.A. fixative (overnight) to a syracuse watchglass with 0.5 ml. of a mixture (of 96% ethanol - 20 parts; glycerine - 1 part; distilled water - 79 parts). This was designated Seinhorst Solution 1. The container with the nematodes was placed in a closed desiccator containing an excess of 96% ethanol and kept at 35-40°C for at least 12 hours in an oven. The 20% ethanol attracts ethanol from the saturated atmosphere and the water evaporates and is attracted by the 96% ethanol in the dessicator (Seinhorst, 1959). The syracuse watchglass container was then filled with a solution of 5 parts of glycerine and 95 parts of 96% ethanol (Seinhorst Solution II) and placed in a partly closed petri dish. This was kept in an oven at 40°C for at least 3 hours until all ethanol had evaporated. The containers were then placed in a desiccator with calcium chloride, or the nematodes were mounted immediately in anhydrous glycerine.

Mounted specimens of the different stages of the nematode were sent to Dr. H.E. Welch of University of Manitoba, Winnipeg, Canada, for identification.

<u>Culturing of the Immature Nematodes</u>. Culturing of the nematodes was conducted in two ways - one under conditions closely simulating those prevailing in the natural habitats and the other by a modified Muspratt (1964) sand culture.

Two terraria were set up using rectangular glass containers of 12" x 7½" x 9½" dimensions. Soil was collected from

one of the mosquito breeding sites (Willoughby No. 1) in a manner previously described and sifted through 25-mesh sieve to remove twigs, dead leaves, annelids and other material that would interfere with later processing of the soil to recover the cultured nematodes. Pond water was used to incorporate as much microflora and fauna of the habitat in the sifted soil. This was evidenced by the presence of Daphnia, some copepods and algae in the seived material. The water-ladden sieved soil was left for at least two days to sediment and compact before introducing into the terraria containers to form a slant. Pond water was poured into the terraria to cover the lower slopes of the soil, leaving the upper slopes uncovered and dry. This was designed to give the nematodes a choice in the range of moisture in the growing medium.

About 100-200 pupae of A.stimulans from infested pools were put into each of the terraria and covered with plastic gauze. The terraria were kept at temperatures of 24.5°C - 27°C for six weeks. Adult mosquitoes emerged from the pupae and died in the terraria. It was thought that any nematodes infesting the mosquitoes would emerge to start the post-parasitic free living phase of their life cycle in the soil. Periodic examination showed no nematode emergence from the dying mosquitoes. The adult mosquitoes died over water surface as well as the dry soils of the upper slopes.

After six weeks the terraria soil was processed through 25-, 60-, and 100-mesh sieves. No nematodes were recovered.

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This erratic result will be discussed later.

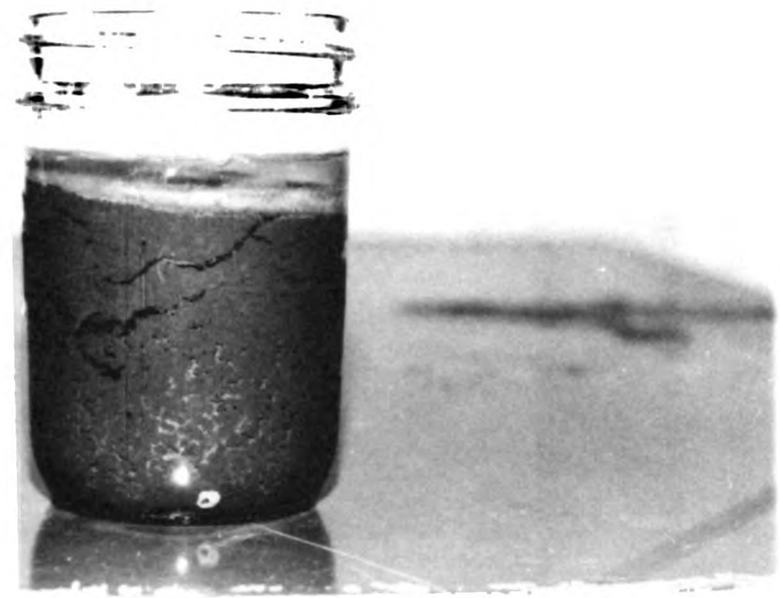
Muspratt's sand culture technique. Four glass jars each of 2.5 inches diameter and 4 inches height were used. Into these were put 3 inches of soil (as used in the terraria), to form a substrate over which tap water was poured to form a top layer of 0.5 inch.

Immature nematodes were obtained by dropping A. stimulans adults in tap water in a large glass dish. The nematodes emerged within ten minutes to one hour and could be seen looping in the water. 30-40 worms were transferred into each of the rearing jars and within fifteen minutes they had borrowed into the soil. A day later entangled masses of worm (each made up of about 10 worms) were seen to form white knots at the bottom of the culture jars. The worms borrowed into the soil a distance of 3 inches to form the knot which is believed by some authors to consist of male and female worms. (Fig. 10).

The cultures were kept at room temperature. Periodically water was added to keep the soil from drying completely. This was a departure from the original Muspratt technique which calls for withdrawal of the water in the top layer followed by partial drying of the sand by packing the space above with absorbent cloth and screwing on a lid. In a control jar with no worms this resulted in desiccation and caking of the soil.

After six and eight weeks soils from two cultures were sieved through 25-mesh sieve to catch the worms. A microscope

Figure 10



A SOIL CULTURE OF MERMITHID NEMATODES

Worms resting in a coiled position at the bottom of a culture jar. Worms have formed a whitish knot after burrowing through three inches depth of soil.

examination revealed that these cultured worms had developed some anatomical and morphological features not shown by freshly emerged worms. It was not known whether at that time the worms had reached the adult stage. But molting had definitely occurred since emergence from the adult mosquito as revealed by cast cuticle and worms undergoing molting near the top soil of the cultures.

Specimens of these cultured nematodes were also sent to Dr. Welch for identification.

RESULTS

Local Distribution of Mosquitoes. Out of the many streams, ponds and tree holes that were examined throughout the summer of 1965 only three habitats yielded mosquitoes. Three species of culicine mosquitoes were detected in these searches, namely, *Culex Pipiens* L. complex, *Culex territans* Walker and *Culex tarsalis* Coquillett with *C. pipiens* being the most predominant. A fourth species, *Aedes vexans* (Meigen), was recovered from a flooded sod sample. Most of the *Culex pipiens* were collected in the vicinity of Lake Lansing where larvae were found extremely abundant in margins of a polluted stream which appeared to be a sewage effluent. Irwin (1942) has noted that foul ground water as well as shaded sphagnum bogs is often the source of *C. pipiens* larvae in Michigan. Other reporters agree on the affinity of this species of mosquito to polluted ground water. Artificial containers, like discarded utensils that were examined, yielded ample supply of larvae and pupae. When in artificial containers and static ground pools, the sites were protected from long exposure of intense sunlight. When present in moving ground water, as margins of streams or drains, larvae were seen in direct sun only in those portions where movement prevents high surface temperatures. Ordinarily thick populations of larvae were found especially when they were beneath canopy shade and when protected from the wind.

Culex territans Walker was collected mainly from the vicinity of Lake Dobie in Alaiedon township of the County, and Culex tarsalis Coquillett from a pond in Delhi township.

The result of dissections of these four species of mosquitoes in the 1965 season shows that none were infested with mermithid parasites (Table 1). Small snails collected from the same stream as Culex pipiens L. complex were also examined for nematodes but none were found.

Bionomics of the Parasite. Observational data obtained from dissection of immature A. stimulans have shown infestation to begin at least with the second instar larva of the mosquito. In this and subsequent larval instars, the nematode was confined to the head, coiled below the eye and in the case of multiple parasitism, below the two eyes, within the brain lobes or superimposed on them, and in the posterior end of the head adjacent to the thorax.

The early parasitic juvenile worm as seen in the third instar mosquito larva was slender but tapered gradually in the posterior half to a thin pointed terminus. It had a maximum width of 0.036 mm. and a length of about 0.6 mm. The shape of the worm was retained throughout the life stages of the mosquito host but growth was in proportion to that of the host.

The parasitic juvenile worm, from a fourth instar mosquito larva, measured approximately 0.06 mm. in maximum width and 1.59 mm. in length. Sexes were indistinguishable at this stage due to lack of gonad development.

The late parasitic stage of the worm, as it appeared in

TABLE I

Result of a preliminary survey to determine, by dissection, infestation of mosquitoes in the northwestern part of Ingham County, Michigan. Summer, 1965

*Mosquito Species	No. dissected.	Nematode Infestation
Culex pipiens L.	185	None
Culex territans Walker	134	"
Culex tarsalis Coquillett	61	"
**Aedex vexans (Meigen)	25	"

* Only larval specimens were used.

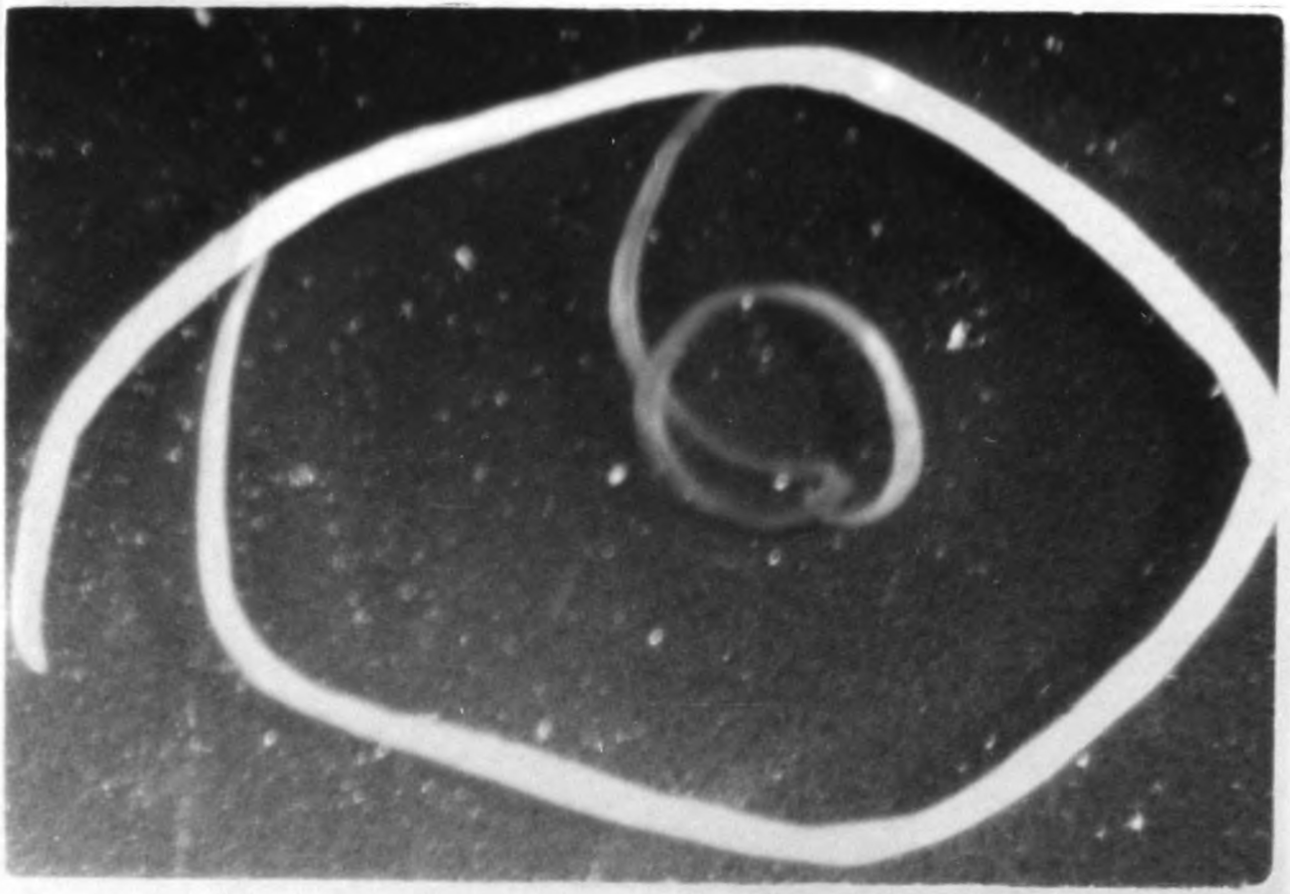
** Recovered from egg hatch in flooded sod sample.

the late pupa or adult mosquito, did not show any marked differentiation from the newly emerged free-living, post parasitic, juvenile stage. It measured 0.102 - 0.258 mm. in width and 14.2 - 25.9 mm. in length. Gonads could not be seen at this stage, but sexes were often separated by the larger size of the female (Fig. 11). The terminus is drawn out into a slender filament. A trophosome extends throughout the body.

No free-living preparasitic and postparasitic stages of the worm were recovered from any of the soil samples processed. Nematode eggs or early parasitic stages of the worm were never found in the gut of the mosquito. Any worms found were always in the haemocoele of the host. The means of infestation was probably not passive, that is, by consumption of nematode eggs or swallowing of the preparasitic worms. If infestation was active as has been reported in some cases of mermithid parasitism of mosquitoes then penetration was most likely to occur in the head region of the mosquito larva where the mermithid was first seen. Further investigation is needed to answer this question.

Nematodes were never observed emerging from any larval instar or pupa of the mosquito. Any emergence that occurred was seen only in the adult mosquito. They emerged head first usually through the intersegmental joints of the host's abdomen, at either the anterior, or more often the posterior end (Figs. 12 & 13). One instance of emergence through the anus was also observed but none were seen emerging through

Figure 11

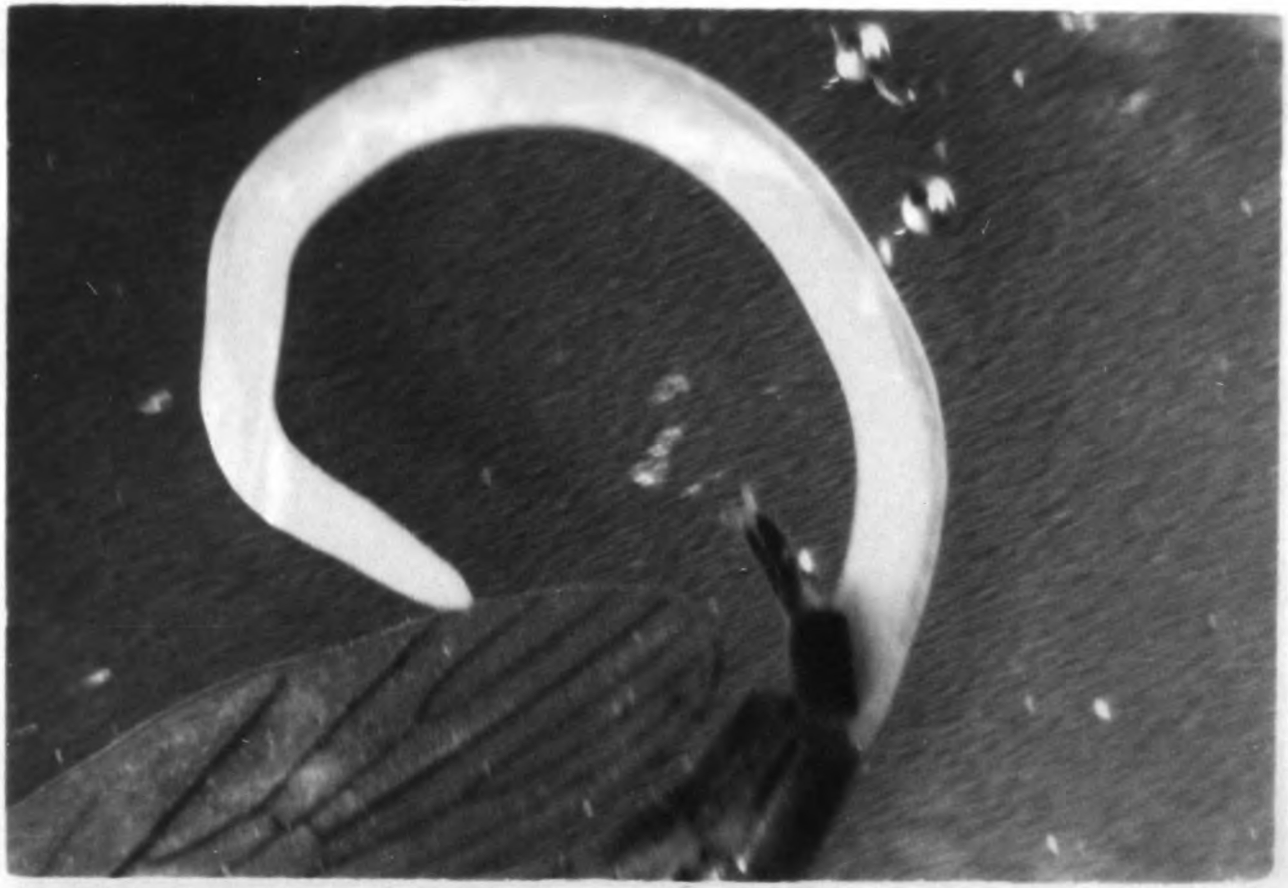


POST-PARASITIC, JUVENILE MERMITHIDS

Sexes are determined at this stage by the larger size of the female. The male is artificially encircled by the female.

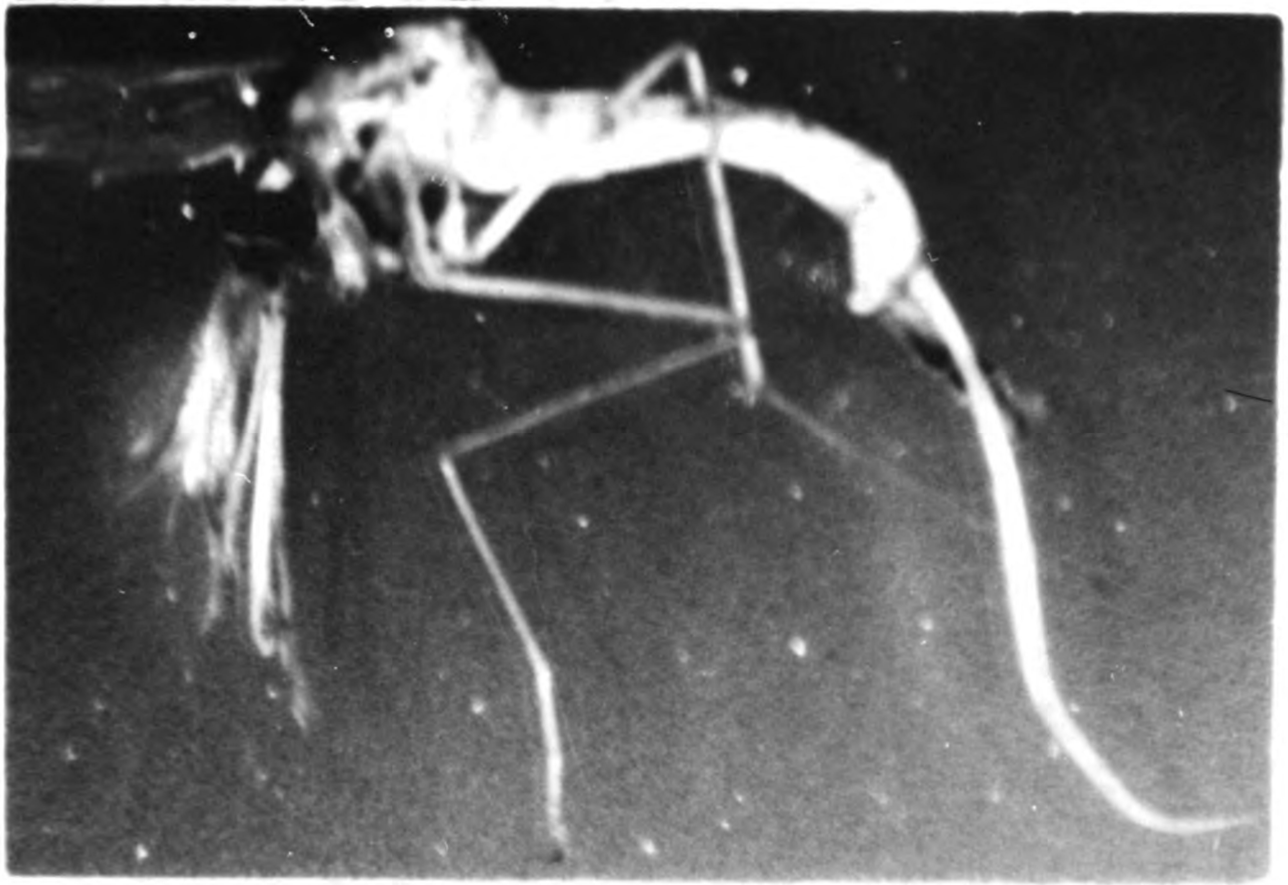
Magnification -- 15x.

Figure 12



A FEMALE JUVENILE MERMITHID EMERGING FROM THE POSTERIOR REGION OF THE ABDOMEN OF ADULT _A_. _STIMULANS_ Magnification -- 30 x

Figure 13



A MALE JUVENILE MERMITHID EMERGING FROM INTERSEGMENTAL FOLD AT THE POSTERIOR REGION OF THE ABDOMEN OF A MALE <u>A. STIMULANS</u>.

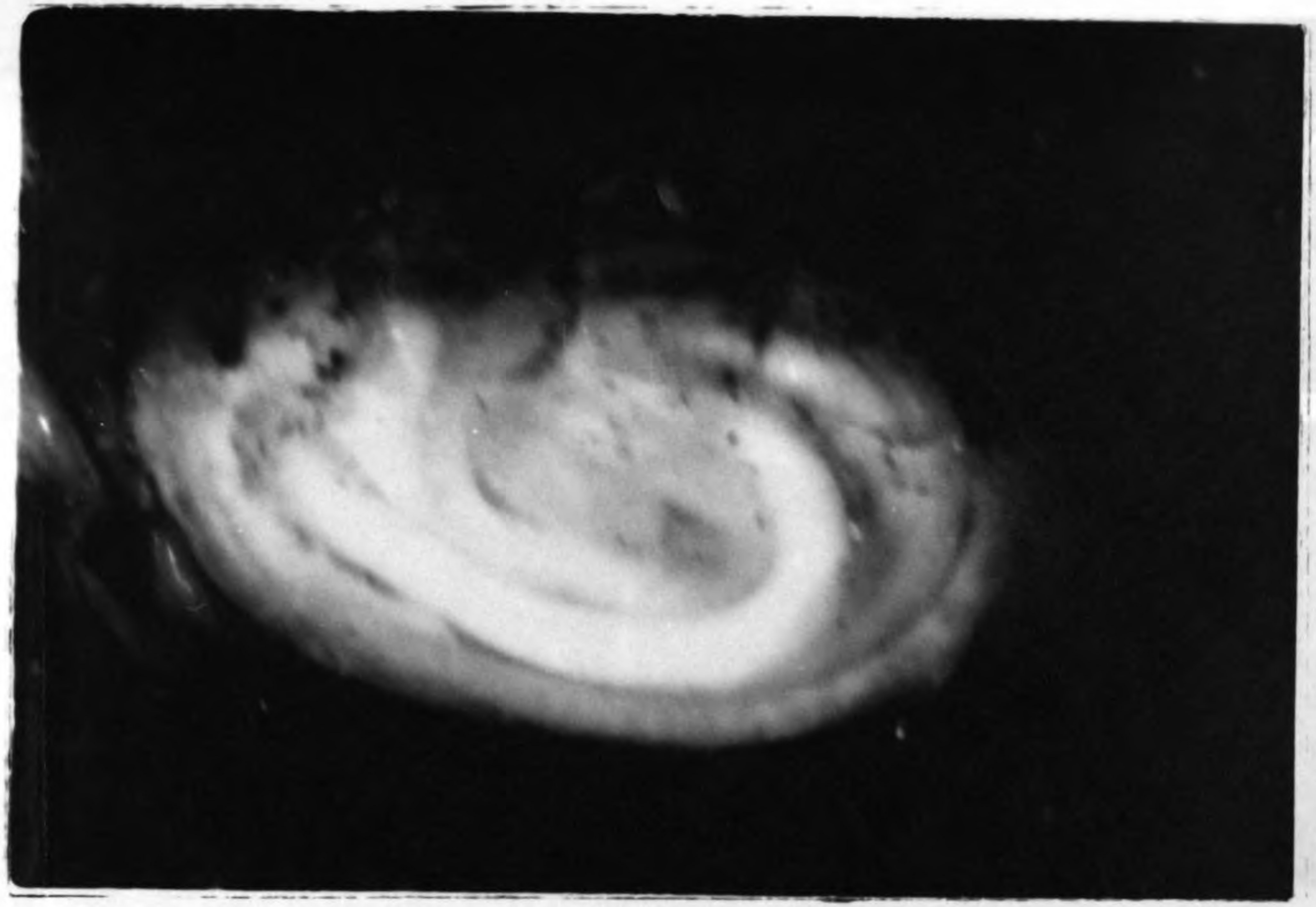
Magnification -- 15x

the siphon as reported in the parasitism of Aedes communis (De.G.) by Hydromermis churchillensis (Welch, 1960).

Growth of the nematode within the mosquito host as revealed by dissection of the various stages of the mosquito, showed a distinctive pattern. With growth and maturation of the mosquito, the nematode showed corresponding growth, migrating from the head where it was first seen in the larval instars to the abdominal haemocoele where it was seen irregularly coiled or doubled back several times (Fig. 14). After emergence the worm was seen to be definitely longer than the adult mosquito it had parasitized (Fig. 15). Evidence in support of this migratory tendency of the parasite was afforded by the observation of nematodes within the thorax of fourth instar larval mosquito, in two dissections. In all other cases of nematode infestation in the larva, it was always seen in the head, and when it occurred in the pupa, it was seen mostly in the abdomen or both the abdomen and the posterior part of the cephalothorax. Nematode infestation of the adult mosquito was invariably within the abdominal haemocoele. This pattern of growth also showed a marked departure from that of most reported cases where infestation was never seen beyond the larval stage of the mosquito, and usually resulted in preventing pupation, and the killing of the host larva after emergence of the worm.

The life cycle of this nematode has not been fully worked out. Postparasitic stages that emerged from adult mosquitoes have been cultured for nearly three months. After six weeks

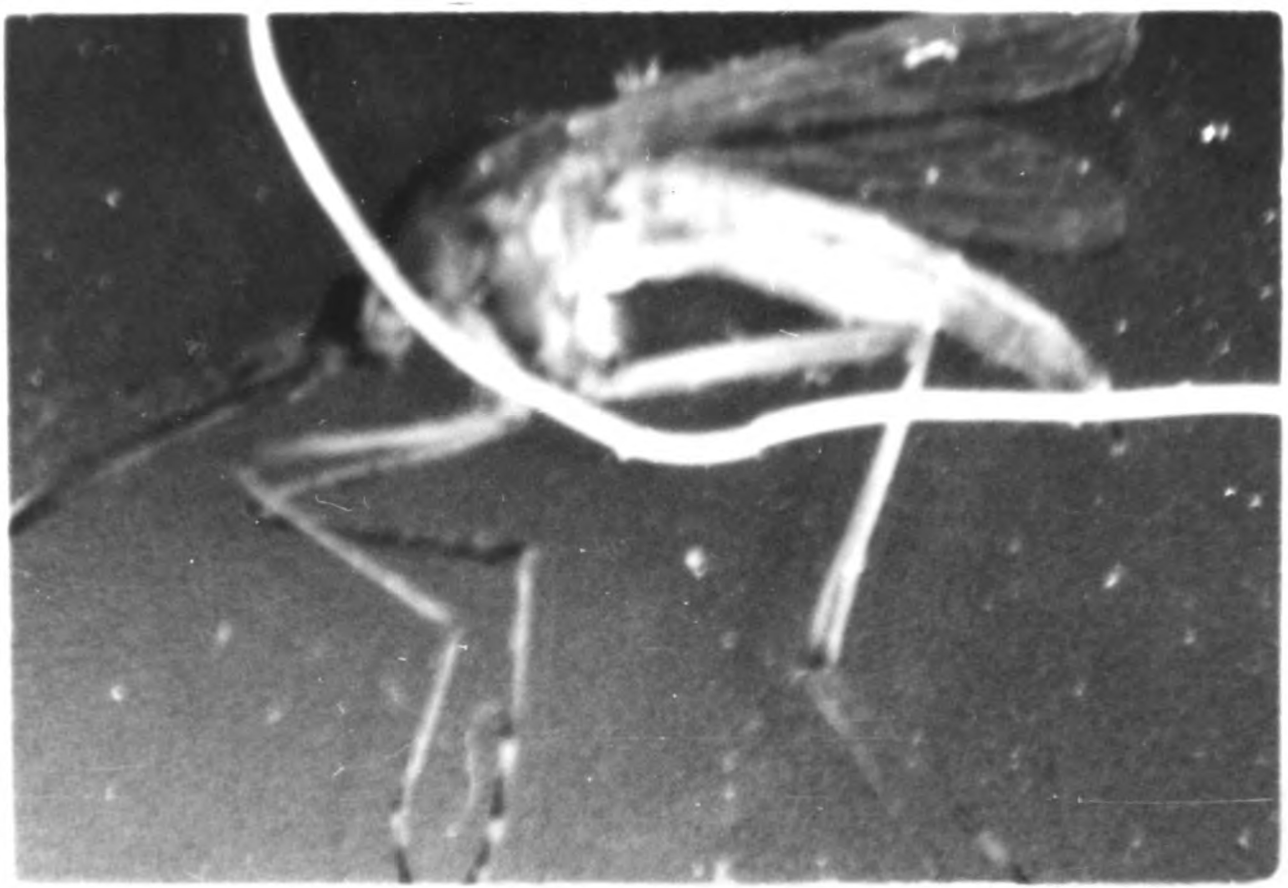
Figure 14



ADULT A.STIMULANS WITH DISTENDED ABDOMEN SHOWING THE POSITION OF IRREGULARLY COILED MERMITHID PARASITE IN SITU.

Worm is a juvenile stage. Magnification -- 15x

Figure 15



RELATIVE SIZES OF A MERMITHID NEMATODE PARASITE AND ITS MOSQUITO HOST.

A post-parasitic nematode artificially placed on adult _A. stimulans_ to compare their lengths. Only part of the nematode is shown.

Magnification -- 15x

of culturing the worms had developed features characterizing adults. Soil cultures that were processed after six and eight weeks did not reveal any nematode eggs and it was assumed that at that time oviposition had probably not occurred. Cultures that were processed after fourteen weeks did not show any nematodes which indicated that the nematodes that were alive at the 12-week stage had decomposed at the end of the 14-week period.

Like Welch's (1960) *Hydromermis churchillensis*, it is assumed that the namatode overwinters in the egg stage but this calls for an intensive field search for the egg during the winter. Limited field observations made in the fall and winter did not show any eggs. The chances of the infective juvenile to survive the winter is practically non-existent as *A. stimulans* has only one generation a year and no other hosts could be seen in sufficient numbers when *A. stimulans* was most prevalent.

Effect of the Parasite on the Host. A noticeable effect of the worm on the mosquito was the prolongation of larval and pupal life. Pupae which normally took 3 - 4 days to mature took 5 - 7 days in some infested cases. Infested larvae, especially in the fourth instar, were more sluggish in their movement. Welch (1960) observed that uninfested larvae broke the water surface with their siphons twice as much as infested larvae. In the adult mosquito, infestation sometimes caused a swollen abdomen and after emergence of the worm the mosquito died.

Presence of Ectocommensals in the Host. A dense mass of epizootic protozoans, *Vorticella*-type (Peritricha, Protozoa), and algae covered most of the *A.stimulans* larvae examined. As it was impossible to tell outwardly if larvae were infested, it was at first assumed that the ectocommensal ciliate associated with both infested and non-infested larvae. Dissections showed this to be true. Jenkins and West (1954) reported that ectocommensal growth on *Aedes communis* (De.G.) occurred in smaller quantities in uninfested larvae. The reverse was true in Welch's (1960) observation on the same species of mosquito.

Culex pipiens complex which were examined in the summer of 1965 were also covered with a similar vorticellid ectocommensal.

Effect of Host on the Nematode. Host resistance in the form of melanization, encapsulation and expulsion of the encapsulated nematode as seen in the first and second instar larvae of *A. communis* (Welch, 1960) was not observed in *A. stimulans* in Michigan.

Incidence of Parasitism. The rate of infestation was determined by dissecting samples of larvae, pupae and adults from the three selected areas. In all, eight pools were examined-five in the Willoughby No. I site, two in Willoughby No. II, and one in Holt. The results are shown in Table II. Varying degree of infestation was noted in all the pools examined.

TABLE II.

Percentage parasitism of *Aedes stimulans* (Walker) by a mermithid nematode in three areas of Ingham County, Michigan.

	Willoughby I	Willoughby II	Holt
Number of larvae and pupae examined.	436	225	121
No. of pools inspected.	5	2	1
Range of infestation.	5.8-78.1	4.0-24.0	-
Average percent in infestation per pool.	54.6	14	9.2

In dissections of adult mosquitoes, infestation was observed in both males and females. There appeared, however, to be differences in the rate of infestation in the sexes of the host. Of the 162 adult mosquitoes from Willoughby No. I dissected 76.5 percent of the infestation occurred in the female.

Multiple parasitism was a common feature in many of the observed cases of infestation. The maximum number of parasites found in one host was seven. Table III gives a frequency distribution of the mermithid nematode parasite in 436 hosts. The two highest instances of multiple parasitism, with six and seven nematodes per host, were all recorded in the female mosquito.

A statistical analysis involving a test of significance in a Poisson distribution was used to analyze the frequency distribution data on the multiple infestation of 436 mosquitoes. The distribution showed a significant departure at the 5% level from a Poisson distribution which suggested that once a host was attacked it was more susceptible to a second attack. This indicated that exposure or susceptibility to mermithid parasitism was uneven within the mosquito population. Welch (1960a) arrived at the same conclusions in his studies on the parasitism of <u>Aedes</u> <u>communis</u> (DeG.) by <u>Hydromermis</u> <u>churchillensis</u> n.sp. Other workers have confirmed such discontinuous parasite distribution within other insect populations.

TABLE III.

Frequency distribution of mermithid nematode parasites in Aedes stimulans (Walker) from one location - Willoughby I, Ingham County, Michigan.

	No. of nematodes per host*								Total no. of hosts examined.
	0	1	2	3	4	5	6	7	
Frequency	233	119	47	25	9	0	2	1	436

*Includes all stages of the mosquito.

Attempt At Identifying the Nematode. On examining the specimens of the different stages of the nematode, including the 'six-week' culture, Dr. Welch was unable to identify the worms, but pointed out that they were obviously of acquatic genera, possibly of Mesomermis sp. The young stages showed a resemblance in outline to those that Rubstov and Welch showed for Gastromermis from Leningrad (Welch, personal communication).

The 'six-week' and 'eight-week' nematodes had developed various structures, but it was difficult to make a determination. Their oesophagus was more wavy and the stichosome was breaking down. This normally accompanies the maturing of the gonads. It was possible to observe the amphids and their pores which resembled those of acquatic genera. The trophosome was little changed and in certain worms, the immature males, it was possible to observe the beginning of the spicule or spicules. It was also obvious that the nematodes had moulted as evidenced by the wrinkled cuticle along the sides of the worms.

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DISCUSSION

The preliminary survey in the 1965 season to determine nematode infestation of mosquitoes in Ingham County had shown that nematodes did not occur in this county in the species examined, namely the Culex pipiens L. complex, Culex territans Walker and Culex tarsalis Coquillett. Although Muspratt (1964) succeeded in infecting in the laboratory, mosquitoes of the Culex pipiens complex, (C. pipiens pipiens, C.p. fatigans and C. terrentium), with Romanomermis Coman, these mosquitoes are seldom the natural hosts of the mermithid.

The negative results obtained from processing soils to recover mermithids seemed to leave only one reasonable conclusion - that if mermithids have not been recovered through dissection of mosquitoes, there was very little hope that they would be found in examination of soils of the mosquito breeding habitats.

The low yield of mosquito larvae from the flooded sod samples could be attributed to a number of factors. There could have been a possible absence of eggs in most of the 32 random samples of soil flooded. Only one sample yielded 25 larvae of Aedes vexans (Meigen) which showed no mermithid infestation. The literature contains references to "broods" but contains little reliable information on generations of A. vexans, since knowledge of hatching of eggs is inaccurate. Horsfall (1955) states that the number of generations of this species is one or more according to the extent of summer rainfall necessary for inundating oviposition sites. Owen (1937) states that one generation occurs in Minnesota. There are also numerous references to apparent erratic hatching in nature but there is much agreement on the necessary sequence of events for hatching namely, (1) a minimum lapse of time for the embryos to mature, (2) an interval of drying prior to flooding, and (3) submergence by water at a favorable temperature. Any of these factors especially that of favorable temperature could have interfered with hatching since flooding of sod was done only at room temperature.

The one generation per year life cycle of A. stimulans was confirmed in the 1966 season by field observations. At the time of the drying of the original snow pools in which larvae bred, only adult mosquitoes were seen in the field. Subsequent pools resulting from the summer rains never contained larvae of A.stimulans.

Survival of The Mermithid Parasite. There was very little success to retain the mermithid parasites obtained from dissections for culturing, by holding them in physiological saline. That worms dissected from all stages of the mosquito died within an hour when held in saline or put on top of moist soil, points to the fact that the exposure of the worms to free living existence was premature. There appears to be a minimum length of time for parasitic existence before emergence. Whenever worms were prematurely dissected out of adult mosquitoes they died within the hour, but dissected

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worms from adult mosquitoes did not appear morphologically different from worms that emerged naturally from the host, yet only naturally emerged worms survived to be used in cultures, some of which showed live worms after almost three months. The importance of a minimum parasitic life is well illustrated in the above observations.

Failure of the nematodes to survive in the terraria culture demands some attention to the possible underlying causes. Pupae introduced into the terraria were taken from a pool showing the highest rate of infestation (78.1%) and both terraria and the four sand cultures were kept in the same laboratory. The highest recorded temperature of the terraria was 27°C, 4°C higher than that of the sand cultures. The difference in temperature was due to the fact that the terraria were kept by a window where there might have been the possibility of the sun's incident rays falling on them - hence the higher temperatures. Failure of survival then might have been caused by the decomposition of any worms present in the dead mosquitoes as well as any that succeeded in emerging into the surrounding media. Jenkins and West (1954) state that death by decomposition occurred after a month in a similar attempt to culture Hydromermis sp. from A. communis. They found that when containers were held in a refrigerator at approximately 6°C the same behavior was observed except that few individuals remained active on the surface of the sand for three to four months. Muspratt (1964) expresses the desire to know whether the worms can mature when continuously submerged or if partial desiccation is necessary. As a departure from his own technique the sand cultures here were virtually submerged continually, and after six or more weeks the nematodes had developed characters which indicate they are nearing the adult stage.

Considerations On The Mode of Infestation And Growth Pattern Of The Parasite. Data so far, shows the mermithid parasite under consideration, to be first seen in the head of the second instar larva, and is the probable site of entry if infestation is active. Many of the reports in the literature on the mode of infestation are purely speculative, only in a few cases has experimentation by exposure of various larval instars to the primary infective stage of a worm yielded any conclusive evidence of the mode of infestation. In nematodes other than mermithids the mode of infestation is usually passive, by consumption of the nematode eggs, or whole juveniles, followed by the worms piercing the gut wall on their way to the haemocoele. Welch and Bronskill (1962) observed such passive infestation in Aedes aegypti (L) by a neoaplectanid nematode, DD136.

Although Muspratt (1947, 1964) has conducted infestation tests with a mermithid nematode, he does not make mention of any observation by direct penetration of the hosts body cuticle. Many authors, however, believe there is penetration of the host cuticle by the second stage juvenile mermithid by means of its spear-shaped odontostyle.

The migratory tendency of this mermithid to change its locale of development within the mosquito host has not been previously reported for mermithids that parasitize mosquitoes. Where infestation has been definitely established to be passive, the mermithid penetrates the gut wall to grow in the haemocoele only at that section of the host's body. Such migratory behaviour is also seen in some other groups like the simuliids, where the author has personally made observations to confirm existing reports. In the blackfly, Simulium damnosum Theobald, this parallel pattern commences after a blood meal by the female fly. The microfilaria of Onchocerca volvulus penetrate the gut wall to enter the haemocoele and subsequently wander in an upward ascent until they reach the thorax where they remain to undergo development into other juvenile stages, such as the "sausage" stage, and the infective stage which then finds its way to the proboscis for reinfection when the fly next bites for a meal.

The life cycle of this nematode is insufficiently known. The parasitic phase has been followed fairly closely in the mosquito host. The emphasis on culturing was to obtain an adult worm for identification purposes hence cultures could not be sacrificed to follow up the post-parasitic phase to determine the specific timing of mating, fertilization and oviposition. This could be accomplished by more elaborate culturing and examination of the cultures at shorter intervals of time, and to attempt to obtain pre-parasitic juveniles, which according to the literature have been seen on very

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rare instances in other mermithids. Persistent efforts to recover adult worms from their natural habitats is also necessary to obtain a fuller picture of the life cycle.

The infestation rates shown by the Michigan pools falls within the range reported in the literature, which is 0 - 100%. A notable feature revealed in this study was that all the eight pools examined showed a measure of infestation. References in the literature, however, show haphazardness in pool infestations whereby one pool may have infested larvae whereas a neighboring pool may not. There are also references to situations where an infested pool may show a considerably reduced infestation, or no infestation at all, the following year.

The parasite's means of dispersal is often unknown in nematodes which kill their hosts by emergence at the larval stage, thus preventing pupation. In such situations the method of dispersal of the nematode is yet to be determined. This mermithid parasite of A. stimulans in Michigan is carried through to the adult mosquito and may be the parasite's means of dispersal in nature.

The relationship between the mermithid parasite and Aedes stimulans should be pursued as well as any such relationship between the nematode and some other species, to build up a complete picture of relationships. Only then can the true relationships be revealed and understood to enable those engaged in applied work to determine the feasibility of establishing the nematode for pest control.

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SUMMARY

Parasitic mermithid nematodes were discovered infesting all the stages of the snow mosquito, Aedes stimulans (Walker).

Dissections and histological sections showed that infestation began with the second instar larva and continued into the adult mosquito.

Post-parasitic life of the nematode began with emergence from the adult mosquito. Emergence was never observed in the larva or pupa of the mosquito.

The growth pattern of the parasite revealed a migratory tendency in its development as evidenced in the change in locale of development within the mosquito host. In the second through the fourth instar larva of A. stimulans, the mermithid was confined to the larval head, and was seen in various locations within the head. Infestation in the pupa occurred mostly in the abdomen and at times in the posterior end of the cephalothorax. Nematode infestation in the adult mosquito was invariably in the abdominal haemocoele.

There was only a measure of success with the culturing of freely emerged nematodes. Nematodes could be held for 12 weeks in moist soil. By the fourteenth week they had decomposed.

Incidence of parasitism was fairly high. The range of infestation was in the order of 4 - 78.1% for the total area sampled.

Multiple parasitism was common in many observed cases of

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infestation.

Dissection of 162 adult mosquitoes from one area revealed 76.5% of infestation to occur in the female mosquito. The maximum number of parasites was also recorded in the female mosquito.

The taxonomy of the nematode could not be determined but this mermithid parasite of *A. stimulans* was found to be close to two acquatic genera namely *Mesomermis* and *Gastromermis*.

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