DISTRIBUTION AND PREVALENCE OF *OCTOMYOMERMIS TROGLODYTIS* (NEMATODA: MERMITHIDAE), A PARASITE OF THE WESTERN TREE HOLE MOSQUITO, *AEDES SIERRENSIS*

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ABSTRACT. *Octomyomermis troglodytis* was found infecting *Aedes sierrensis* larvae in 14.5% of 165 tree holes sampled between 1982 and 1986. Mermithid infections were detected in tree hole waters that ranged in pH from 6.5 to 9.3 and electrical conductivities between 0.10 and 5.11 mmhos/cm. Third and fourth instar larvae were most frequently infected, and most immatures that succumbed to infections died while in the fourth instar. Most hosts contained only one nematode. Infected adults were obtained from emergence traps over tree holes, from field-collected immatures reared in the laboratory, and from mosquito collections from sentinel humans. *Octomyomermis troglodytis* escaped from adults into water vials in the laboratory, suggesting that infected adult mosquitoes serve as dispersal agents for this parasite.

INTRODUCTION

Octomyomermis troglodytis Poinar and Sanders (Nematoda: Mermithidae) is a naturally occurring parasite of the western tree hole mosquito, *Aedes sierrensis* (Ludlow). This nematode was first discovered in a survey of tree hole breeding sites of *Ae. sierrensis* (Sanders 1972). Specimens from the single tree hole in Marin County, California, where the parasite was discovered were used to describe the species (Poinar and Sanders 1974), and no subsequent occurrences of this mermithid have been reported in the literature. During an extensive survey of the natural enemy complex affecting *Ae. sierrensis* from 1982 to 1986, we sampled larval populations in 165 tree holes from different geographic areas of northcentral and southern California. Here we report results of the distribution of *O. troglodytis* in tree holes occupied by *Ae. sierrensis*, infection levels in larval and adult populations, and physical and chemical characteristics of the tree holes where mermithid infections occurred.

MATERIALS AND METHODS

During 1982–83, 19 tree holes at the University of California Hopland Field Station (HFS) in Mendicino County were sampled monthly (January–May) following methods described previously (Egerter and Anderson 1985). Similar monthly samples (October–May) were collected from 31 tree holes at HFS and from 10 tree holes at the Stanford University Jasper Ridge Biological Preserve (JRBP) in 1983–84. An additional 28 tree holes were sampled on one occasion (April) in 1984 from several sites in southern California. In 1984–85, the same tree holes at HFS were sampled four times, and tree holes at JRBP were sampled twice; we also collected a single sample (January 1985) from each of the 28 tree holes in

southern California, plus first-time samples from 27 additional holes. In February 1986, 48 tree holes at HFS and 12 tree holes at JRBP were sampled. Similarly, between January and March of 1985 and again in 1986, single larval samples were collected from the University of California Sierra Foothill Field Station, Yuba County (27 tree holes) and Blodgett Experimental Forest, El Dorado County (22 tree holes).

In the laboratory, samples of immature mosquitoes were placed in 250 ml polyethylene rearing cups filled with dilute, autoclaved tree hole water. Ground, autoclaved rat chow was provided as larval food. A sample of water from each tree hole was analyzed for pH, electrical conductivity (EC), alkalinity, and concentrations of calcium, magnesium, potassium and sodium following methods described by Egerter and Anderson (1985) and Washburn and Anderson (1986). When pupae appeared, screened tops and sucrose cubes were placed over culture cups to retain emerging adults. Larval cultures were examined weekly and dead immatures were removed and examined for cause of death. As adults emerged, they were moved to holding cages (24° C, 14:10 light: dark cycle) and provided with water and sucrose cubes. Adults in holding cages were examined daily, and dead individuals were dissected to determine if they harbored parasites. After 10 days in the holding cages, surviving adults were dissected under ether anesthesia and examined with a stereoscopic microscope (10–40X) to assess mermithid infections.

We also assessed the prevalence of *O. troglodytis* in emerging and host-seeking mosquitoes captured in the field. Adult *Ae. sierrensis* were sampled weekly using sentinel humans at HFS from May 31 to September 11, 1984. Adults of both sexes were collected along a 150 m transect in a small, wooded ravine using a backpack vacuum sampler (Meyer et al. 1983).

Mosquitoes were collected for 5 minutes at each of 10 stations along the transect three times (morning, midday, and late afternoon or early evening) on each sample date. In addition, in early April 1985, we placed emergence traps over the openings of five tree holes which contained *Octomyomermis*-infected larvae. Adult *Ae. sierrensis* were collected from the traps every 72 hr and returned to the laboratory at Berkeley. Mosquitoes were anesthetized, measured for wing length, and examined for parasites.

To determine if *O. troglodytis* parasitism affected adult eclosion or size, we compared emergence times and wing lengths of infected and normal adults collected in emergence traps in 1985. Because *Ae. sierrensis* is protandrous and sexually dimorphic in size, only groups of the same sex were compared. In addition, since the size of mosquitoes emerging from different breeding sites varied significantly (Washburn and Anderson, unpublished data), comparisons were only made with adults emerging from the same tree hole. For each tree hole, we calculated separate mean wing lengths and emergence times for uninfected males and females; each infected mosquito was compared with this standard and scored as either falling above or below the mean for uninfected adults. The null hypothesis predicts that if there is no difference between the two groups, then equal numbers of the *Octomyomermis*-infected group should fall above and below the mean for the corresponding uninfected group. Observed and expected frequencies for each comparison were evaluated using a χ^2 test (Sokal and Rohlf 1969).

RESULTS

Twenty-four of the 165 tree hole populations (14.5%) of *Ae. sierrensis* sampled between 1982 and 1986 contained individuals infected with *O. troglodytis*. The mermithid was recovered from all field sites from Mendicino to San Diego counties indicating this parasite is widely distributed between northcentral and southern California in both the coastal and Sierra foothill ranges. In tree holes that were sampled during two or more consecutive years, *Octomyomermis* was consistently recovered from the same tree holes in all but one instance (n = 15). Nematodes occurred naturally in tree holes with pH values ranging from 6.5 to 9.3 and with EC values ranging from 0.10 to 5.11 mmhos (Table 1). Alkalinity and cation concentrations in tree holes with mermithid infections also varied widely.

A total of 384 mosquitoes infected with *O. troglodytis* were collected during this study; 291 of these infections were in immatures with most infections occurring in late season, late instar larvae (Fig. 1). Larvae infected with *Octomyomermis* were absent in all samples collected from tree holes in November 1983 from HFS and JRBP, and they also were absent in all samples taken in November and December of 1984 and 1985. In 1983, only one of the

Table 1. Water characteristics of tree holes occupied by *Octomyomermis troglodytis*.

Location	Treehole	pH	EC[1] (mmhos/cm)	Ca^{++}	Mg^{++}	K$^+$	Na$^+$	Alkalinity (mg CaCO$_3$/l)
SB[2]	Bot-1	8.5	3.34	1.77	3.56	37.45	0.91	2,040[1]
	Stage-1	8.8	4.35	4.69	4.94	51.63	0.54	2,473
	Stage-2	8.4	1.79	1.52	1.81	16.24	0.28	777
	Stage-3	7.0	1.05	0.90	1.48	8.22	0.77	373
JRBP	HR-1b	9.1	1.97	1.77	7.82	13.66	0.04	1,270
	HR-3b	9.3	5.11	2.54	2.82	61.91	0.69	3,111
	HR-4	8.5	0.63	0.70	1.21	5.40	0.39	282
	HR-6	8.6	1.08	1.17	1.75	9.53	0.39	459
BEF	BL-13	8.4	1.11	1.22	1.03	10.01	0.08	715
	BL-19	6.5	0.10	0.11	0.06	0.83	0.01	97
SFFS	SF-13	9.0	4.91	9.36	4.42	55.67	0.38	3,168
HFS	BH-3	7.7	0.20	0.35	0.16	2.02	0.03	76
	II-6	7.6	0.37	0.55	0.51	3.49	0.03	138
	III-1	9.1	2.40	2.67	3.15	29.24	0.26	1,306
	III-2	8.4	0.71	1.22	1.40	6.71	0.13	321
	III-6	8.0	1.04	0.92	0.62	9.85	0.09	490
	RG-1	8.7	2.14	3.37	1.99	23.02	0.21	1,061

[1] EC = electrical conductivity.

[2] SB = Santa Barbara, JRBP = Jasper Ridge Biological Preserve, BEF = Blodgett Experimental Forest, SFFS = Sierra Foothill Field Station, HFS = Hopland Field Station.
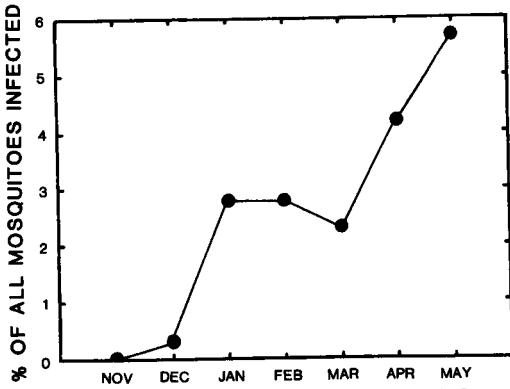
Fig. 1. The percentage of *Octomyomermis* infected *Aedes sierrensis* collected in monthly samples from 9 tree holes at the Hopland Field Station and the Jasper Ridge Biological Preserve during 1983–84. Between 781 and 900 mosquitoes were collected and reared for all monthly samples except during May when the sample consisted of 279 mosquitoes.

tree holes sampled in December contained larvae with the parasite. In all years, percentages of infected larvae increased with samples beginning in January (Fig. 1).

Levels of mermithid infection within tree holes varied widely and ranged from 0.3 to 98%. During the 1984–85 field season, an average of $15.7 \pm 21.6\%$ (SD) of the mosquito larvae from all tree holes with mermithids were infected. These values are based on January collections from HFS and JRBP and single samples from other field sites. Infection rates of 98% and 84% were observed in one tree hole population from Santa Barbara County in 1984 and 1985, respectively.

Most infected hosts collected early in the 1984–85 season (December–February) died as immatures when mermithids escaped, while
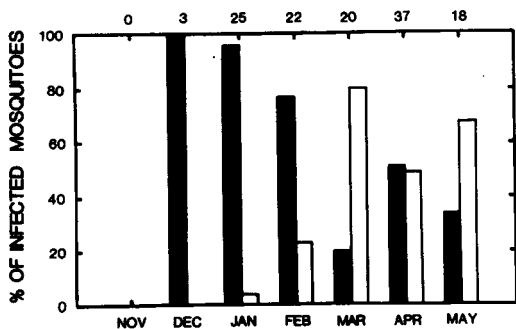

Fig. 2. The fate of mosquitoes infected with *O. troglodytis* collected during different months (1983–84). Closed bars indicate percentages of individuals in monthly samples dying as immatures; open bars indicate percentages of individuals eclosing as infected adults. Numbers of infected mosquitoes are indicated at the top.
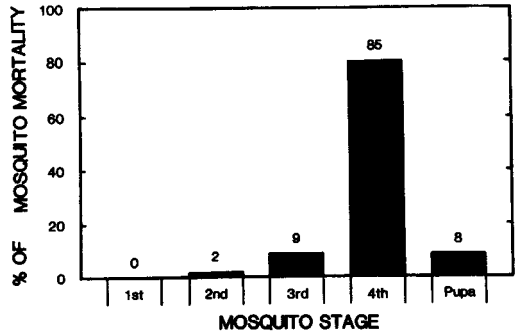

Fig. 3. Distribution of immature mosquitoes dying from *Octomyomermis* infections (1983–84). Samples sizes are shown above histograms.

greater percentages of those collected during March–May eclosed successfully into infected adults (Fig. 2). Most mosquitoes (82%) dying as immatures succumbed to mermithid infections during the fourth instar (Fig. 3). We did not find this parasite in first instar *Ae. sierrensis*, and we found it only twice in second instar larvae.

As many as 14 mermithids were present in a single mosquito, but most hosts (69.1%) contained only one parasite (Fig. 4). Similar numbers of mermithids were found in immature and adult mosquitoes, and we found no significant correlation between parasite load (i.e., number of mermithids per host) and host mortality. In general, the number of mermithids per host and the frequency of occurrence were inversely related (Fig. 4). Mermithids were found in the head, thorax and abdomen of
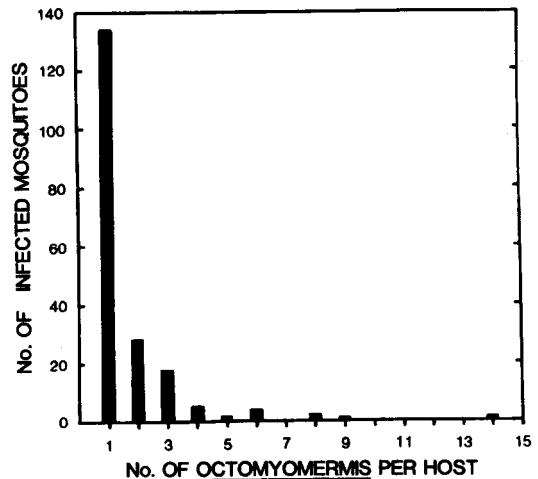

Fig. 4. Frequencies of different numbers of mermithids in mosquito hosts. Histograms are constructed from immature (n = 57) and adult (n = 137) mosquitoes from the Hopland Field Station and the Jasper Ridge Biological Preserve monthly samples (1983–85).

immature hosts. Most mermithids in adult hosts were found in the abdomen, but a few adults had nematodes in the thorax. Small, melanized nematodes were observed in the anal papillae of some third and fourth instar larvae, and in one instance a melanized nematode was found in an adult female.

Octomyomermis troglodytis was collected from adult mosquitoes eclosing from field-collected larval samples, arriving at sentinel humans, and emerging into traps placed over tree hole breeding sites. Parasite loads in adult females ranged as high as 14, whereas a maximum of 6 nematodes was found in the infected males. Of the 2,224 *Ae. sierrensis* collected in emergence traps placed over five holes known to harbor the parasite, only 36 (1.8%) were infected with *O. troglodytis*. All positive tree holes produced low numbers of infected adults; the proportion of infected adults from individual holes ranged from 0.3 to 5.2%. However, 98% (n = 50) of the larvae collected from one tree hole in Santa Barbara County eclosed into infected adults when reared in the outdoor insectary.

Wing lengths (N = 36, χ^2 = 0.44, P > 0.50) and emergence times (N = 36, χ^2 = 2.25, P > 0.10) of uninfected and *Octomyomermis*-infected adults were not significantly different between the two groups. The sex ratio of infected adults did not differ significantly from 1:1 (N = 36, χ^2 = 0.44, P > 0.50), indicating that male and female hosts eclose in equal numbers.

In 1984, we dissected 414 female and 418 male *Ae. sierrensis* that were collected from sentinel humans at HFS. None of the males and only six of the females (1.4%) were infected with *O. troglodytis*. All infected females were inseminated indicating that mermithid parasitization does not preclude mating. Further, capture of infected females at sentinels demonstrated that at least some infected mosquitoes exhibit host-seeking behavior.

Most infected adults of both sexes that emerged from field collected larvae reared in the outdoor insectary survived for 10 days under laboratory conditions before they were killed. Because adults were removed from cultures on a weekly basis, these mosquitoes were already between 0 and 7 days old when they were placed in holding cages. Thus, infected adults that survived 10 days in holding cages were actually between 10 and 17 days old at the time they were sacrificed. Such survivorship is sufficient to allow dispersal of the host from its larval source, a finding supported by capture of parasitized adults at sentinels under field conditions. No definitive correlation between parasite load and survival time of the adult host was evident. The single female that harbored 14 nematodes, for example, survived

for the entire 10 days in its holding cage before it was sacrificed.

By maintaining *O. troglodytis* infected adults in the laboratory, we documented that adult *Ae. sierrensis* are capable of dispersing this parasite. We commonly found *O. troglodytis* postparasites in water vials in cages holding infected mosquitoes. Parasites escaped by penetrating the abdominal wall, and in all cases hosts died within 24 hr, usually on the surface of the water vial.

DISCUSSION

The biological control potential of tree hole mermithids is enhanced by their ability to infect hosts in breeding sites where the water is often highly organic. In that context, tolerance of moderate salinity and rich organic waters has been cited as an advantage for *Octomyomermis muspratti* (Obiamwe and MacDonald) compared to species such as *Romanomermis culicivorax* Ross and Smith which exhibits a narrower range over which it is infective to mosquito larvae (Petersen and Willis 1970, Brown and Platzer 1978, Petersen 1981). The wide range of EC values and the varied ionic constitutions of tree holes with *O. troglodytis* (Table 1) indicate a broad tolerance for different water conditions including moderate salinity.

Our data on incidence and infection rates of *O. troglodytis* are similar to the previous reports of mermithid parasites of container breeding mosquitoes. Poinar and Sanders (1974) found *O. troglodytis* in 5.5% of 18 tree holes they examined in Marin County, California, while Muspratt (1945) reported *O. muspratti* in only 10% of the tree holes he surveyed in Africa. Petersen and Willis (1969b) mention only a single tree hole with a *Mesomermis* sp. but did not report the number of tree holes they sampled. We found *O. troglodytis* in only 14.5% of the 165 *Ae. sierrensis* tree hole populations surveyed. These few data suggest that mermithids are generally rare natural enemies in tree hole habitats.

While the average level of mermithid infections was low (15.7%), the high variance suggests that epizootics do occur under natural conditions. Low incidence rates in most tree holes (such as those shown in Fig. 1) apparently represent typical enzootic conditions in this host-parasite system. Epizootic situations were observed in other tree hole populations where levels of mermithid infections were much higher. Because populations were sampled before any adult emergence occurred, high infection levels are not an artifact of differences in developmental times between infected and uninfected mosquitoes and reflect a

significant impact of this parasite on its host populations. Muspratt (1945) found that as many as 70–80% of *Ae. marshalli* Theobald in some tree holes were infected by *O. muspratti*, and in the one tree hole positive for *O. troglodytis*, Poinar and Sanders (1974) reported a 38% infection rate. Thus, while mermithids are apparently rare among tree holes, the parasite may be abundant within tree hole mosquito populations.

The prevalence of *O. troglodytis* generally increased in monthly samples collected between January and May, and most hosts succumbed during the fourth instar when parasites exited the larvae. The majority of hosts supported only one nematode, and some larvae successfully encapsulated nematodes in their body cavities. Poinar and Sanders (1974) reported similar observations from the single tree hole population of *Ae. sierrensis* where the parasite was previously known.

Because mermithid infections were absent in mosquito samples collected early in the season when larval populations consisted primarily of first and second instars, preparasites of *O. troglodytis* apparently did not attack hosts until several months after tree holes had flooded with rainwater. The mechanism for this delay in egg hatching may be similar to that of *O. muspratti*. In this congener, egg hatch is stimulated by passage through the gut of mosquito larvae, and it is primarily the older larvae that browse in the litter at the bottom of tree holes where the eggs of the parasite are found (Platzer 1980, Platzer 1981a). Eggs of *O. muspratti* do not hatch in the gut of the mosquito, rather they are passed in the feces and subsequently release infective nematodes. The absence of early season infections of *O. troglodytis* in our study, and the similarities in its biology to that of *O. muspratti*, suggest a similar mechanism of egg hatch.

Delayed egg hatch of mermithids residing in tree holes is adaptive for several reasons. Both *O. troglodytis* and *O. muspratti* are endemic in areas with pronounced wet and dry seasons where host availability may be highly variable. In years with little precipitation, breeding habitats may dry periodically (or never fill with water), and successful mosquito development can be dramatically reduced. In response, a proportion of eggs of *Aedes sierrensis* and other container-breeding species may delay hatching for a year or more even if water is present in tree holes. The eggs of *O. muspratti* respond similarly, for preparasites can be harvested in successive floodings of laboratory cultures containing diapausing eggs (Petersen 1981). *Octomyomermis troglodytis* may increase its chances of successful development in a similar fashion

by hatching only in response to the presence of late instar hosts.

Previous studies have implicated adult mosquito hosts as the dispersal agents for parasitic nematodes (Trpis et al. 1968; Trpis 1969; Petersen and Willis 1969a, 1969b; Poinar 1977; Kurihara and Maeda 1980; Petersen 1984). Our data on survival of infected adults in the laboratory and the escape of *O. troglodytis* into water vials in holding cages indicate that infected adults are the agents of dispersal in this host-parasite system. Emergence of infected adults from tree holes and the capture of parasitized adults at sentinel humans revealed that mermithid dispersal via infected mosquito adults occurs under natural conditions. Dispersal efficacy is probably enhanced by the habit of *Ae. sierrensis* adults to rest in tree holes during periods of inactivity. Treeholes are ephemeral in time (eventually they rot through completely or fill with debris); hence, dispersal to new mosquito breeding sites is particularly critical for mermithids in these habitats. By infecting older larvae, *O. troglodytis* insures production of infected adults that may disperse to new tree holes.

Additional research is necessary to further elucidate the biology of *O. troglodytis* and assess its potential as a manipulated natural enemy of mosquitoes. *Octomyomermis muspratti* is known to develop in at least 15 species from three genera of Culicidae (Poinar 1979, Platzer 1981b), and similar host range studies are required for *O. troglodytis*. Tolerance of a broad spectrum of water conditions, efficient persistence in tree holes over the dry season, and the natural occurrence of high infection rates suggest that this mermithid has some of the desirable characteristics of a successful biological control agent.

ACKNOWLEDGMENTS

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