

OBSERVATIONS ON THE BIOLOGY OF *ROMANOMERMIS* SP. (NEMATODA: MERMITHIDAE) PARASITES OF *Aedes* IN WESTERN WYOMING

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ABSTRACT. Univoltine *Aedes* mosquito larvae infected by *Romanomermis* sp. were found at 2 localities in Yellowstone National Park, Wyoming. *Aedes nevadensis*, *Ae. impiger*, *Ae. pullatus* and *Ae. punctator* were the predominant host species. Only *Ae. nevadensis* was common to all sites. Rates of parasitism ranged from 55 to 93% and differed significantly between localities. Significant annual variation in the rate of parasitism occurred within populations, but neither infection rate nor mean parasite load differed among host species. Parasite populations were distributed among host species in proportion to abundance, but distributions of parasites within host species were over-dispersed. Inter-specific variation in survival of infected larvae was associated with the degree to which immune responses were manifested in different host species.

INTRODUCTION

The genus *Romanomermis* Coman (Nematoda: Mermithidae) contains 13 described species, 12 of which parasitize mosquitoes. One species, *R. culicivora* Ross and Smith, has been studied intensively, but most of these investigations were directed toward the use of this organism for mosquito control (Petersen 1985). Many aspects of the biology of mermithids were elucidated as a result of these studies, but relatively little effort has been made to examine the dynamics of interactions between the nematodes and their hosts in natural populations.

Romanomermis nielsenii (Tsai and Grundmann) is the only mermithid mosquito parasite reported from Wyoming and is not known to occur outside the immediate vicinity of the type locality near Lone Tree, Uinta County. Recently, mosquito larvae collected from vernal pools at 2 localities in Yellowstone National Park have been found to harbor mermithid parasites morphologically similar to *R. nielsenii*. Pilot studies conducted in 1984 and 1985 established that the life cycles of these newly discovered populations corresponded closely to that reported for *R. nielsenii* by Tsai and Grundmann (1969). Differences between these populations and the Lone Tree population of *R. nielsenii* included variations in habitat, host species and adult size.

In addition to describing the occurrence and extent of mermithid infections among the mosquito populations at the Yellowstone National Park localities, this paper deals with the patterns of infection among different mosquito species present at these sites. These observations are compared with similar data for the *R. nielsenii* population at Lone Tree, Wyoming.

MATERIALS AND METHODS

Three mosquito populations in western Wyoming were studied. The southernmost, located

at Lone Tree, is described in Tsai and Grundmann (1969). Three pools at 2 localities in Yellowstone National Park, Park Co., Wyoming, also were sampled. Two of these sites are located 500 m east of Grebe Lake (44° 45' N; 110° 35' W) at an elevation of 2,447 m, and the third site is situated on the northern side of DeLacy Creek about 350 m northwest of Shoshone Point (44° 27' N; 110° 42' W) at an elevation of 2,438 m. For convenience these localities will be referred to as the Lone Tree, Grebe Lake and DeLacy Creek sites, respectively.

The Lone Tree and DeLacy Creek populations occupy pools associated with permanent streams. During the spring runoff these pools may be incorporated into the streams. They contain much silt and are rich in organic matter. The Grebe Lake population occurs in a contiguous series of vernal pools that are formed from melting snowdrifts at the edge of a meadow. An additional pool (site 2) discovered at the Grebe Lake locality in 1986 was also sampled in 1987. Although this second pool lies less than 30 m from the primary Grebe Lake site, they are disjunct even during the peak snowmelt. These sites remain dry most of the summer and are covered with grasses. The soil underlying both Grebe Lake sites is a gravelly clay.

Mosquito larvae were collected from 3 pools at Lone Tree, Wyoming, every 4 days during March, April and May 1986, 1987. Two of these pools were contiguous when water levels were high, and data from these were combined in the analyses presented here. Samples from other pools in the area were taken less frequently to assess the distribution of mermithids, but these data were not included in the principal analyses. Mosquito larvae obtained by dipping were transported to the laboratory and reared individually in 2-dram (15 × 60 mm) vials containing 7-ml deionized water. Cultures were maintained at 15°C and a photoperiod of 12L:12D in a growth chamber. Diet consisted of fish food supplied *ad libitum*.

Mosquitoes were collected on alternate days during May, June and July 1986 from one pool at each of the Yellowstone National Park sites. Collections at these sites in 1987 were made between April and June. The difference in sampling periods was a result of between-year differences in timing of the spring snowmelt and generally warmer, drier conditions in 1987. Laboratory rearing of the Yellowstone mosquitoes was handled as previously described except that the cultures were housed in an unheated building and kept near windows to provide natural photoperiod and illumination.

After eclosion or death each mosquito was identified to species and dissected to determine parasite load. All nematodes associated with each mosquito, including emerged postparasites and unemerged or encapsulated parasites, were counted. Head capsule widths of dead larvae and larval exuviae were measured with an ocular micrometer.

Data were analyzed as follows: *G*-tests were used to compare the proportion of each species infected at each of the sites and the survivorship of those species. Differences in the mean number of parasites per infected host were evaluated using Mann-Whitney *U* and Kruskal-Wallis tests. Parasite distributions for each host species were compared to the Poisson distribution using single classification *G*-tests for goodness of fit (Sokal and Rohlf 1981).

RESULTS

At the initial site at Grebe Lake, 1,379 mosquitoes were collected, of which 96% were either *Aedes nevadensis* Chapman or Barr or *Ae. impiger* Walker (Fig. 1A). Less common species present included *Ae. hexodontus* Dyar, *Ae. diantaeus* Howard, Dyar and Knab, *Ae. cinereus* Meigen, *Ae. cataphylla* (Dyar), *Ae. pionips* Dyar and *Ae. ventrovittis* Dyar. Insufficient numbers of the latter 6 species were collected to fully evaluate their susceptibility to *Romanomermis* but parasites were discovered in all species except *Ae. cinereus* and *Ae. diantaeus*. Encapsulated nematodes were found in *Ae. nevadensis*, *Ae. hexodontus* and *Ae. ventrovittis*.

At site 2 at Grebe Lake, 474 mosquitoes were collected, of which 84.5% were *Ae. nevadensis* with small numbers of *Ae. hexodontus* and *Ae. impiger* present (Fig. 1B). Other species collected at this site (*Ae. cataphylla*, *Ae. pionips*, *Ae. pullatus* (Coq.)) comprised less than 2% of the mosquitoes present. All species present were infected with mermithids. *Aedes cataphylla*, *Ae. nevadensis* and *Ae. hexodontus* contained encapsulated nematodes.

Aedes nevadensis, *Ae. punctator* (Kirby), *Ae. hexodontus* and *Ae. pullatus* were abundant (Fig.

1C) at the DeLacy Creek site during most of the study. *Culiseta impatiens* (Walker) and *Cs. incidens* (Thomson) occurred at this site late in the season, but few were present until well after the *Aedes* spp. had pupated. Although all *Aedes* species were parasitized, no infected individuals of either *Culiseta* species were found among 118 examined. Of the infected species, only *Ae. punctator* did not encapsulate nematodes.

At Lone Tree, Wyoming, *Romanomermis nielsenii* infects *Ae. nevadensis*, *Ae. pullatus*, *Ae. increpitus* Dyar, *Ae. implicatus* Vockeroth, *Ae. fitchii* (Felt and Young), *Ae. schizopinax* Dyar, *Ae. cinereus* and *Ae. cataphylla*. *Aedes nevadensis*, *Ae. increpitus* and *Ae. pullatus* were predominant throughout the area (Fig. 1D), but the abundance of each varied in different parts of the site. Only *Ae. nevadensis*, *Ae. fitchii*, *Ae. cinereus* and *Ae. cataphylla* had encapsulated parasites.

Rates of parasitism ranged from 55 to 93% among the populations sampled during 1986 and 1987 (Table 1). The proportion of infected mosquitoes differed significantly between localities in both years, and significant annual variation in parasitism was observed within each population. Neither the infection rate nor the mean number of parasites per infected mosquito (parasite load) differed significantly among the species present at a particular site during the same year (Table 1).

Parasite populations at each site were distributed among the different host species in proportion to the relative abundance of each species (Fig. 2), but the variance to mean ratios (coefficient of dispersion) of all populations exceeded unity (Table 1), indicating that the distribution of parasites within different host species was generally over-dispersed. The only host species in which the observed parasite distributions were not all significantly different from Poisson was *Ae. pullatus*.

Melanotic encapsulation of *Romanomermis* was found in individuals of only 2 of the 6 common species: *Ae. nevadensis* and *Ae. hexodontus*. Individuals of these species varied considerably in the degree to which this immune response was manifested. Although 65% of infected *Ae. nevadensis* and 57% of *Ae. hexodontus* had encapsulated nematodes, many of these individuals also harbored unencapsulated, normally developing parasites. Interspecific differences in the stage of development attained by parasites before encapsulation were observed. Most of the worms encapsulated by *Ae. nevadensis* retained either the filiform shape of the preparasite or comma shape characteristic of the earliest stages of parasitic development (Gordon et al. 1974), suggesting that they were encapsulated relatively quickly. Parasites in *Ae. hexo-*

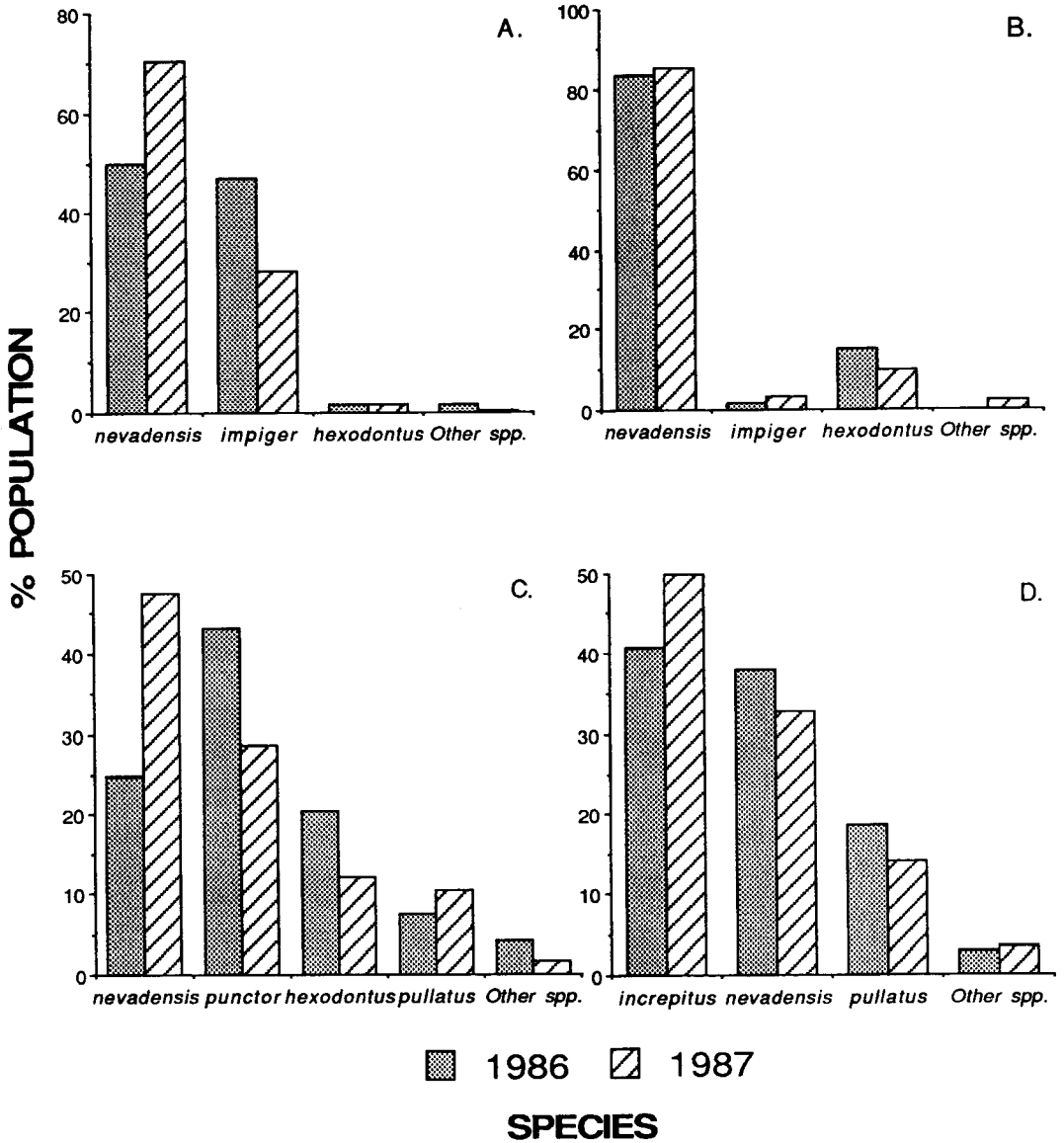


Fig. 1. Relative abundance of *Aedes* mosquito species collected in 1986 and 1987 at 4 sites in western Wyoming: A. Grebe Lake site 1; B. Grebe Lake site 2; C. DeLacy Creek; D. Lone Tree, Wyoming.

dentus generally progressed much further in development and attained much larger sizes before being encapsulated. Encapsulated nematodes were found in all 4 larval instars, pupae and adults of both *Ae. nevadensis* and *Ae. hexodontus*. Unencapsulated nematodes were not observed in adult mosquitoes of any species.

Postparasite emergence was restricted to the fourth stadium in most species except *Ae. increpitus*. *Romanomermis nielsenii* often emerged from third instar *Ae. increpitus* although emergence during the fourth stadium was more com-

mon. Instances of emergences from third instar *Ae. punctor* and *Ae. pullatus* were observed, but these occurrences were rare. Postparasite emergence always resulted in host death, but infections did not always culminate this way. To examine the impact of parasitism on different host species, survivorship of infected and uninfected fourth instar larvae to the pupal stage was compared for each population. All infected *Ae. increpitus*, *Ae. pullatus* and *Ae. punctor* died regardless of whether parasites emerged (Fig. 3). Four infected *Ae. impiger* pupated, but these

Table 1. Characteristics of mermithid infections in 4 populations of univoltine *Aedes* spp. mosquitoes in western Wyoming.

Locality Species	% infected		Mean no./host (\pm SE)		CD*	
	1986	1987	1986	1987	1986	1987
Grebe Lake site 1						
<i>Ae. impiger</i>	79.6	92.9	4.9 (0.3)	8.9 (0.4)	3.7	4.4
<i>Ae. nevadensis</i>	72.2	91.9	5.8 (0.3)	8.9 (0.4)	6.0	7.0
Grebe Lake site 2						
<i>Ae. nevadensis</i>	—	88.2	—	4.3 (0.2)	—	2.2
All other spp.	—	80.5	—	3.1 (0.4)	—	2.4
Delacy Creek						
<i>Ae. hexodontus</i>	60.0	86.1	2.6 (0.3)	2.9 (0.4)	2.1	2.5
<i>Ae. nevadensis</i>	60.6	72.7	2.3 (0.2)	3.0 (0.2)	1.4	2.1
<i>Ae. pullatus</i>	59.0	67.7	1.8 (0.3)	2.4 (0.3)	1.3	1.7
<i>Ae. punctator</i>	55.0	73.3	2.9 (0.3)	3.0 (0.3)	2.8	2.3
Lone Tree						
<i>Ae. increpitus</i>	71.5	—	2.9 (0.6)	2.3 (0.2)	2.7	3.5
<i>Ae. nevadensis</i>	79.8	—	5.4 (0.1)	2.2 (0.2)	4.4	2.6
<i>Ae. pullatus</i>	63.8	—	7.5 (0.2)	5.0 (0.2)	10.8	2.0

* Coefficient of dispersion = variance/mean.

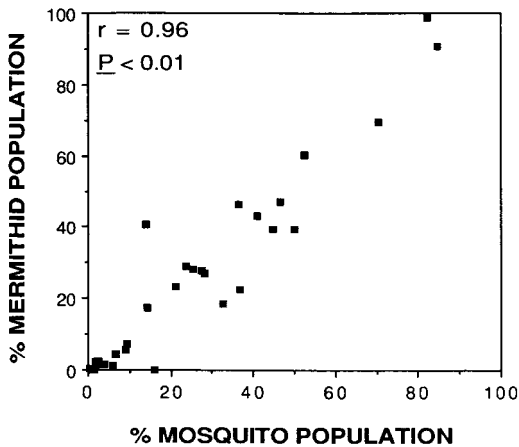


Fig. 2. Parasite distribution among host species in relation to host availability. Data shown are for each host species present at each site, 1986 and 1987.

represented less than 2% of the infected larvae that reached the fourth instar. A significantly greater ($P < 0.01$) proportion of infected *Ae. nevadensis* and *Ae. hexodontus* larvae pupated. In all 4 populations, uninfected larvae of different species did not differ significantly ($P > 0.05$) in interstadial survivorship.

DISCUSSION

Each of the mosquito populations examined in this study was composed of a unique combination of species. Despite significant site-specific differences in species composition, infection rates and parasite load, no interspecific differences in the pattern of parasitism were observed. At all 4 localities all species were

parasitized, and within sites the proportion of infected mosquitoes did not vary between species. Parasites were apportioned among host species according to the relative abundances of those species. These observations support the hypothesis that preparasites indiscriminately attack all species present. This pattern is somewhat surprising given the differences in host immune responses that were observed. Because a high proportion of *Ae. nevadensis* and *Ae. hexodontus* encapsulate parasites, nematodes infecting larvae of these species are less likely to complete their development and reproduce compared with those which parasitize larvae of other mosquito species in which this immune response is not elicited (Blackmore¹). The apparently indiscriminant pattern of host selection suggests that other factors affect the selection pressure exerted by differential survival in different mosquito species.

The behavior of infected mosquitoes may contribute to the over-dispersed distribution of parasites within host populations. Welch (1960) found that infected *Ae. communis* feed less, become more sluggish in their movements and are about half as active as uninfected larvae. Shamseldean and Platzer (1989) recorded paralyses in larvae lasting several minutes coincident with penetration by *R. culicivoxax* preparasites. Such behavioral changes increase the likelihood that additional preparasites will locate, attach to and

¹ Blackmore, M. S. 1989. Ecology and host-parasite interactions of nematodes (Mermithidae) infecting larvae of univoltine *Aedes* mosquitoes in western Wyoming. Ph.D. dissertation, University of Utah, Salt Lake City, UT.

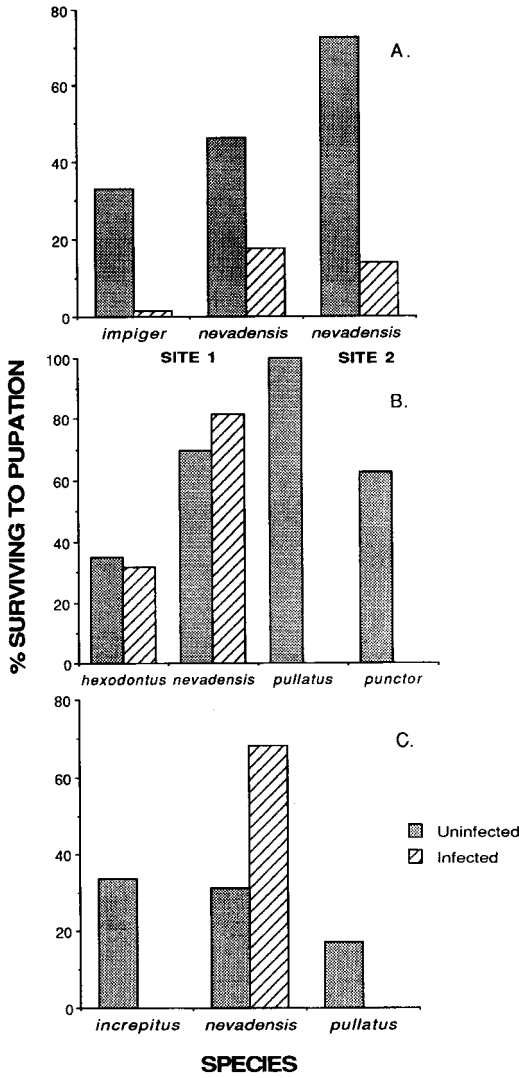


Fig. 3. Survivorship of *Aedes* mosquito larvae infected by *Romanomeris* sp. parasites (striped bars) compared to uninfected conspecifics (solid bars). Missing bars indicate 100% mortality prior to pupation. A. Grebe Lake; B. DeLacy Creek; C. Lone Tree, Wyoming.

infect larvae that are already infected and increase over-dispersion of parasites. Galloway and Brust (1985) found that infection by even a single nematode slowed the development of *Ae. vexans* (Meigen) larvae. They suggested that the longer period of exposure made it more likely that infected larvae would be multiply infected and that this might account for nonrandom parasite distributions.

Over-dispersion means that the majority of parasites develop in hosts that are superparasitized. Due to competitive interactions between

parasites within superparasitized mosquitoes, those hosts are potentially poorer quality developmental environments than hosts with single parasites. Postparasite size is negatively correlated with parasite load (Blackmore¹), and multiple parasitism may contribute to premature host mortality (Chen 1976, Petersen and Chapman 1970 and Petersen et al. 1967). Both factors should negatively affect nematode fitness and select for parasites that avoid infected mosquitoes. The infection patterns observed in this study suggest that such selection is mitigated by other selection pressures in these populations.

In contrast to the situation for parasites, multiple parasitism could have a slight beneficial effect for the fitness of some infected mosquitoes. Populations of mosquito larvae contain many related individuals due to adult oviposition behavior. An infected individual that becomes superparasitized reduces the number of preparasites remaining in the pool. If this reduces the likelihood of siblings becoming infected, it increases the inclusive fitness of the superparasitized host. Such fitness benefits would only be realized in those species in which parasite load is not correlated with host survival (i.e., those species that do not encapsulate *Romanomeris*).

For *Ae. nevadensis* and *Ae. hexodontus*, the consequences of infection and superinfection are very different. Because these species can encapsulate parasites, a significant proportion of infected larvae survive to adulthood (Blackmore 1989), but their chances for survival are related to the number of parasites they bear (Blackmore¹). Selection would favor the avoidance of infection by individuals of these species even after an initial infection because this would maximize the chances of a host encapsulating its entire parasite load and thereby surviving.

If avoidance of parasites entails some cost (e.g., expending more energy to evade preparasites, or foraging in suboptimal areas of the pool), individuals of species that can not encapsulate may be more likely to accede to those costs than species that encapsulate. Species with effective immune responses might be able to risk being infected. There also may be metabolic costs associated with encapsulation ability. These factors could potentially be important in competitive interactions between mosquitoes of different species occupying the same site.

The means by which mermithids are dispersed between habitats is not known. The extreme patchiness of mermithid populations observed at all three Wyoming localities suggests that these nematodes are seldom successfully disseminated into different sites. Some of the mosquitoes observed in this study were able to complete development to the adult stage after becoming

infected, but all of those individuals had encapsulated all of their nematodes. Because living parasites were not found in adult hosts, it appears that adult mosquitoes rarely, if ever, transfer these mermithids between disjunct larval habitats.

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