Effect of Aedes aegypti (Diptera:Culicidae) Age on Sex Ratios in Romanomermis culicivorax (Nematoda:Mermithidae)

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Abstract: First, second, third, early and late fourth-instar larvae, and pupae of *Aedes aegypti* were infected with *Romanomermis culicivorax* and reared at 20, 25, and 30 C. An increase in the ratio of male to female nematodes was observed with increase in host age at the time of infection at each temperature. The number of pupal and late fourth-instar infections was low, but *R. culicivorax* continued to develop in adult *A. aegypti*. Since male nematodes were recovered from both male and female hosts infected as late fourth instars or pupae, the sex of the host did not influence the sex of the nematode. *Key words: sex* determination, temperature, entomogenus nematode, biological control. Journal of Nematology 15(4):594-597. 1983.

Sex ratios of Mermithidae are affected by many factors (3). Multiple infections and host starvation generally result in a predominance of male nematodes (3). With some mermithid species, male nematodes are usually found in male hosts and females in female hosts (5). Smaller host species and possibly those with longer developmental times may result in high male: female nematode sex ratios (3).

The effect of host age on the sex ratio of a mermithid was first investigated by Parenti (2). He reported proportionately more female *Paramermis contorta* in Chironomids that were infected at late rather than early instars. However, studies with *Romanomermis culicivorax* and *Octomyomermis muspratti* in *Culex pipiens quinque-fasciatus* showed no appreciable differences in their sex ratios with increase in host age at the time of infection (3,4).

This paper documents the sex ratio of *R. culicivorax* reared in *Aedes aegypti* and shows that in this mosquito species, age at the time of infection affects sex ratios of the parasite.

MATERIALS AND METHODS

Aedes aegypti larvae and pupae were infected with *R. culicivorax* (originating from Petersen's Lake Charles, Louisiana, culture) in the host:parasite ratios of 1:5, 1:10, and 1:20. A total of 600 larvae at each stage and for each temperature were exposed to preparasites in batches of 50–100 larvae. Each batch consisted of hosts of the same instar. The larvae were exposed to preparasites for 6 hours at 27 C in 1 ml of water per host used. These hosts were then transferred to 20, 25, or 30 C. Prior to infection, mosquito larvae were reared in tap water at 28 C, but to reduce fungal contamination of the nematodes, distilled water adjusted to pH 7.4 with KOH was used during and after infection. The water was changed and hosts were fed on Tetramin twice daily. Prior to the onset of nematode emergence, each larva was placed in the well of a haemagglutination plate. The number and sex of every nematode to emerge in a well were noted.

Pupae were sexed before exposure to preparasites by placing them gently on blotting paper and noting the presence (female) or absence (male) of the projecting plate of the ninth sternite. Three replicates of 10 pupae of each sex were exposed to 200 preparasites in 50 ml distilled water (pH 7.4) for 24 hours at 27 C. These male and female pupae were then placed in separate cages and allowed to emerge at 28 C. Males were fed a 20% sucrose solution, and females obtained their bloodmeal daily from a laboratory mouse. Any pupae or adults found dead were examined for nematodes. Adults were dissected on the ninth day after pupal exposure to preparasites.

Five replicates each of 20 late fourth-instar larvae (about 60 hours after the third moult) were also exposed to 400 preparasites in 100 ml distilled water (pH 7.4) for 24 hours at 27 C and reared to the pupal stage. These pupae were then sexed and male and female pupae were placed in separate cages and allowed to emerge

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into adults. Any dead hosts were examined for nematodes and the remaining adults were dissected on the 10th day after larval exposure to preparasites.

RESULTS

The proportion of male nematodes in single infections increased as the age of the host at the time of infection increased, regardless of temperature (Table 1). To differentiate changes in sex ratios caused by differing host ages or temperatures from changes caused by multiple infections, only infections with one or two nematodes per host were considered. Observations showed that regardless of host stage and temperature, only male nematodes were recovered if there were four or more nematodes per host. Even at the comparatively low infection rate of two nematodes per host, more males than females generally emerged. Up to 22 male nematodes emerged from a single host (infected in the first instar and reared at 25 C). Male nematodes from hosts harboring 10 to 22 nematodes were smaller, and their mortality higher in the first few days after emergence, compared to males from single infections.

Temperature appeared to have little effect on sex ratios, except possibly when first instar hosts with single infections were kept at 20 C. Under these circumstances, the proportion of males produced was higher than if they were reared at 25 or 30 C. More nematodes emerged from each larval instar at 25 C (Table 1). More female nematodes and combinations of sexes were also evident from first and second instars at 25 C. The low number of nematodes emerging from early instars at 30 C resulted from the high mortality of experimental hosts that occured at that temperature. Premature emergence of nematodes from a few early instars at 30 C resulted in dead or weak nematodes. This did not occur with older hosts at 30 C. A combination of high temperature and parasitism of young hosts may have proved detrimental to both hosts and parasites. In contrast, at 20 C there were always some hosts infected as early instars which were much smaller than the majority. This lower

Table 1. Effect of temperature and the developmental stage of the host *Aedes aegypti* on the sex ratio of *Romanomermis culicivorax*.

Developmental stage (instar)	No. of Nematodes per host	20 C				25 C					30 C			
		M*	F+	MF≠	% ♂ §	M*	F+	MF≠	% ♂ §	U‖	M*	F+	MF≠	% ♂ §
1st	1	104	70	0	60	68	204	0	25	0	23	70	0	25
	2	30	9	10	71	71	15	61	69	0	33	11	9	71
2nd	1	80	51	0	61	102	88	0	54	0	10	16	0	38
	2	16	1	1	92	37	0	0	100	0	28	2	3	89
3rd	1	73	29	0	72	88	27	0	77	0	50	34	0	60
	2	41	0	1	99	45	0	4	96	0	30	0	3	95
4th	1	32	6	0	84	45	8	0	85	0	38	2	0	95
	2	15	0	0	100	21	0	0	100	0	17	0	0	100
¶Late 4th ♂	1					13	0	0	100	0				
	2					2	0	0	100	2				
¶Late 4th ♀	1					9	0	0	100	0				
	2					1	0	0	100	3				
¶Pupae ♂	1					6	0	0	100	0				
	2					0	0	0	0	0				
¶Pupae ♀	1					2	0	0	100	1				
	2					0	0	0	0	0				

*No. of hosts with male nematodes.
+No. of hosts with female nematodes.
≠No. of hosts with one male and one female nematode.
§Percentage of male nematodes.
‖No. of nematodes whose sex could not be determined.
¶Hosts reared at 28 C after infection.

temperature was not as detrimental as 30 C to emergent nematodes. As expected, the rearing time required until emergences of nematodes decreased with increase in temperature.

The number of emergent nematodes decreased as instar increased (Table 1). Also, more nematodes were recovered from late fourth instars than from pupal infections. Occasionally, two parasites were found in one host after late fourth-instar but not after pupal infections.

DISCUSSION

Female nematodes were not recovered from either late fourth-instar or pupal infections. This may be due to the low numbers of infected hosts obtained. However, in a similar study, Kurihara (1) exposed *R. culicivorax* to *C. p. molestus* and recovered low numbers of parasitized adults, yet male and female nematodes were obtained from both of the host sexes. In this study, the nine nematodes whose sex could not be determined (Table 1) were early stages and the possibility that they were females cannot be ruled out. Since male nematodes were recovered from both male and female hosts in this study, we conclude that the sex of the host does not influence the sex of the parasite. Hence the observed changes in nematode sex ratio with increase in host age can be stated as being independent of any effects related to host sex. These results are in keeping with Kurihara's study (1), where host sex does not influence the sex of the nematode. However, because of the low numbers of infected hosts obtained, it is not known if sex of the host influences the sex ratios of the nematode.

The results in Table 1 conflict with those of an earlier study by Petersen (3). He found no appreciable differences in sex ratios of *R. culicivorax* from different instars of *C. p. quinquefasciatus*. He also reported proportionately fewer males than were obtained in this study. These differences may be due to any differences that may exist between the two hosts, either physiological or behavioral. The sizes of the various instars of *C. p. quinquefasciatus* used by Petersen (3) are not known and hence cannot be compared to those of *A. aegypti*. However, it is unlikely that the difference in results are due merely to differences in host size at the time of infection, since fourth instars would be larger than first instars in both hosts. Moreover, these results apply only to *R. culicivorax*. Other mermithids may be affected differently. Thus *P. contorta,* a parasite of Chironomids, shows higher numbers of female nematodes from older hosts (2).

It should be stressed that in this study any effects of the environment are manifested merely as changes in the sex ratios of *R. culicivorax*. The exact causes for the change in nematode sex ratio with increase in host age remain unclear and cannot be stated with certainty. The observed difference in nematode sex ratio may be due to a number of causes (perhaps differential infectivity for example), including an effect on sex determination, but there is not enough evidence to state which. The presence of female nematodes from some older hosts should be borne in mind when evaluating the effect of host age as an environmental sex determinant. In this study, although proportionately higher numbers of male nematodes were found from older hosts, female nematodes and the combination of sexes in one host are not excluded and are also present. It is unlikely that older hosts are so different from each other that while from the majority of them male nematodes result, parasitism of other older hosts can also result in female nematodes (Table 1). Knowledge of sex chromosomes in *R. culicivorax*, behavior of preparasites, and factors like the mortality and reproductive ability of male nematodes resulting from different environmental conditions is lacking. Such information should provide a clearer understanding of the results seen in this study.

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