Factor Affecting Sex Ratios of a Mermithid Parasite of Mosquitoes[1]

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Abstract: The ratio of male to female *Reesimermis nielseni* Tsai and Grundmann, a nematode parasite of mosquito larvae, increased as the number of parasites per host increased. Hosts with a single nematode produced 9% males compared with essentially 100% males in hosts with more than 7 parasites; hosts with 3 nematodes produced about equal numbers of males and females. Males of *R. nielseni* generally emerged before females because of the earlier death of multiple-infected mosquitoes. The species of the host mosquito influenced the sex ratio, but the size of a specific host at the time of invasion did not. Host diet also had a noticeable influence on the sex ratio of the nematode: singly infected hosts from a starved population produced 92% males compared with 13% in the normally fed group. The importance of these factors in the mass rearing of *R. nielseni* is discussed. *Key words: Reesimermis nielseni, Romanomermis,* mosquitoes, sex ratios, Mermithidae, biological control, parasitism.

It is well established that sex determination in Mermithidae is influenced by environmental factors. Christie (1) concluded that environment is an important sex-determining factor in larvae of *Mermis subnigrescens* Cobb, a parasite of grasshoppers. Strelkov (6) reported that the sex of *Filipjevimermis singularis* Strelkov, a parasite of chironomids, correlated with that of the hosts: male parasites were most often found in male hosts and females in female hosts. Parenti (2) found that the degree of infection, the length of the host, and the sex of the parasite which first penetrated the host influenced the sex of *Paramermis contorta* Linstow, also a parasite of chironomids. Petersen *et al.* (4) and Petersen and Chapman (3) observed an influence of multiple parasitism on the sex ratio of a *Gastromermis* sp. and in *Reesimermis nielseni* Tsai and Grundmann in mosquitos: as the number of nematodes per host increased, the ratio of males to females increased; in addition, the species of host appeared to influence the sex ratio of *R. nielseni* (4).

The present study was conducted in 1970 at the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory, Lake Charles, Louisiana, to determine the importance of environmental factors on sex determination in *R. nielseni*, and to elucidate the influence these factors would have in any attempt to mass rear the parasite and use it as a biological control agent against mosquitoes. The nematode used was identified by W. R. Nickle, Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland, as *Reesimermis nielseni*. All previous publications

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concerning the Louisiana *R. nielseni* have referred to it as *Romanomermis* sp. in light of the knowledge at that time (3, 4, 5).

RESULTS AND DISCUSSION

Tests were conducted to determine the effects of multiple infections of hosts on the sex ratio of the postparasitic stage of *R. nielseni*. In one test, 10 groups of first instar *Culex pipiens quinquefasciatus* Say were exposed to eight concentrations (the lowest 2 were duplications) of preparasitic nematodes from a common laboratory culture. The exposure and rearing procedures were the same as those described earlier (5). Infected mosquito larvae were isolated just prior to the escape of the nematodes, and the numbers and sex of the emerging parasites were determined. Sexes usually were distinct in the postparasitic stage, and few, if any, intersexes occurred. If a question arose concerning the sex of a nematode, it was discarded and not included in the totals. As the concentrations of preparasitic nematodes were increased, the ratio of male to female nematodes increased (Table 1). Groups of mosquito larvae with a mean of 1.2-1.7 parasites/host produced a mean of 18% male nematodes; those with 1.7-2.5 parasites/host produced 43% males; and those with 2.7-4.7 nematodes/host produced 62% males. When the individual hosts from the ten groups were combined and separated by the number of parasites per host, only 8% of the nematodes from singly infected hosts were males compared with 45% males in larvae with 2 nematodes and 93% males in larvae with 7-9 parasites (Table 2). When the results of Trials 1 and 2 were combined, the sex ratios were found to increase from a low of 9% males in singly infected hosts to 100% males in hosts containing 9 parasites; hosts with 3 nematodes produced about equal numbers of males and females. Though the numbers of parasites per host were higher in Trial 1, a greater number of female nematodes were produced in Trial 2. In addition, the heavily infected mosquito larvae died first, and as a result, a majority of the male nematodes also emerged first.

TABLE 1. Sex ratio of *Reesimermis nielseni* resulting when groups of *Culex pipiens quinquefasciatus* were exposed to various concentrations of preparasitic nematodes.

Approx. no. preparasitic nematodes	% Surviving insect larvae infected	Mean no. of nematodes/ infected host	Postparasitic nemas	
			Total	% Males
85	63	1.2	119	16
125	76	1.2	181	13
170	89	1.3	96	29
340	96	1.7	129	38
510	100	2.5	209	47
680	100	2.7	199	64
850	100	4.4	342	58
1020	100	4.7	337	66

TABLE 2. The influence of multiple parasitism of *Culex pipiens quinquefasciatus* on the sex ratios of *Reesimermis nielseni*.

No. nemas/ host	Trial 1		Trial 2		Totals	
	No. nemas	% males	No. nemas	% males	No. nemas	% males
1	262	8	807	9	1069	9
2	204	45	360	35	564	39
3	192	51	153	54	345	52
4	192	59	84	74	276	64
5	175	63	90	80	265	69
6	42	78	0	–	42	78
7	42	88	21	86	60	87
8	24	96	0	–	24	96
9	18	100	0	–	18	100

The rate of emergence of postparasitic male and female nematodes was measured and related to the sex ratio by the exposure of ten groups of 100 first instar *C. p. quinquefasciatus* to a range of concentrations of preparasitic nematodes. In such tests, 3 days are required for a majority of the postparasitic *R. nielseni* to emerge from their hosts. On the first day of emergence, 87% of those that emerged were males; on the second day, 39%; and on the third day, 11% (Table 3). Nematodes escaped from a majority of the hosts with 6 or more parasites on the first day, and the nematodes emerged from most of the hosts with 3 or more parasites by the end of the second day. As a result, an average of 3.6 nematodes/host emerged the first day compared with 2.4 and 1.1 the second and third days, respectively. Also, nematodes that escaped from singly infected larvae the first day were mostly males; only 14% were males the second day, and no males escaped from singly infected hosts the third day.

The effects of the host species on the sex ratios of *R. nielseni* were studied by determining the percentage of male nematodes produced at various host burdens by four naturally infected species of mosquitoes, *Psorophora confinnis* (Lynch Arribalzaga), *Anopheles crucians* Wiedemann, *Aedes atlanticus* Dyar and Knab, and *Uranotaenia*

TABLE 3. The sex ratios of emerging *Reesimermis nielseni* from a population of *Culex pipiens quinquefasciatus.*

No. nemas/ host	First day		Second day		Third day		Totals	
	No. nemas	% males	No. nemas	% males	No. nemas	% males	No. nemas	% males
1	9	89	100	14	84	0	193	13
2	50	92	126	24	22	45	198	43
3	57	89	132	34	3	67	192	51
4	48	81	152	53	0	–	200	60
5	60	78	120	55	0	–	180	63
6	30	83	12	67	0	–	42	79
7	49	91	7	71	0	–	56	88
8	24	96	0	–	0	–	24	96
9	27	100	0	–	0	–	27	100
Totals or means	354	87	649	39	109	11	1112	50.4

TABLE 4. The effect of multiple parasitism and the species of host mosquito on the sex ratio of *Reesimermis nielseni.*

No. nemas/ host	No. *P.*[a] *confinnis*	% male nemas	No. *An. crucians*	% male nemas	No. *Ae. atlanticus*	% male nemas	No. *U. sapphirina*	% male nemas	No. *C.*[b] *inornata*	% male nemas
1	7	0	8	62	47	13	98	41	96	52
2	15	20	13	81	7	65	88	77	72	85
3	21	33	8	75	1	100	27	93	47	89
4	14	45	6	75	0	–	6	83	25	96
5	12	50	1	80	0	–	2	100	7	96
6	6	55	0	–	0	–	0	–	1	100
7 or more	5	69	0	–	0	–	1	100	2	100

[a] *P. = Psorophora; An. = Anopheles; Ae. = Aedes; U. = Uranotaenia; C. = Culiseta.*
[b] *Culiseta inornata* were laboratory-infected, but all others were naturally infected.

sapphirina (Osten Sacken) obtained from field collections and by one laboratory-infected species *Culiseta inornata* (Williston) (Table 4). Singly infected *P. confinnis* produced only female nematodes; those with 2-4 nematodes produced less than 50% males; and those harboring 5 or more mermithids produced 50% or more males. In contrast, *U. sapphirina* and *C. inornata* produced approximately 50% males from singly infected hosts and more than 75% males from hosts with two or more parasites. Also, in limited observations, *Anopheles crucians* produced a high percentage of male nematodes (62-80%) at all concentrations of parasites per host, and *Aedes atlanticus* produced 13% males with 1 parasite, 65% with 2, and 100% with 3. Thus, the ratio of males to females produced from *C. p. quinquefasciatus* (Table 2) was between that for *P. confinnis* and for *U. sapphirina*. These data indicate that host size has a noticeable effect on the sex ratio of *R. nielseni*: smaller pieces produce higher ratios of males. In addition, *C. inornata*, which is a relatively large species of mosquito, develops somewhat more slowly than the other species tested. As a result, the nematodes in this host often completed development before the host reached the fourth instar, and the resulting effects on the nematode appeared to be similar to that in smaller host species. Christie (1) reported that host size influenced the sex ratio in *Agamermis decaudata* Cobb, Steiner, and Christie with small hosts producing a preponderance of males. Also, Parenti (2) found that the relative frequency of male *Paramermis contorta* decreased with increasing length of the host larvae at the moment when the hosts are penetrated by the nematode.

The effects of host size, as measured by the instar, on the sex of *R. nielseni* was tested further by exposing *C. p. quinquefasciatus* in each of the four larval instars to preparasitic *R. nielseni*. No appreciable differences in the sex ratios of the nematodes were noted between instars when the hosts were infected with the same number of parasites (Table 5). Almost no

TABLE 5. The effects of host size (*Culex pipiens quinquefasciatus*) at the time of invasion on the sex ratio of *Reesimermis nielseni*.

No. nemas/ host	No. 1st instar hosts	% male nemas	No. 2nd instar hosts	% male nemas	No. 3rd instar hosts	% male nemas	No. 4th instar hosts	% male nemas
1	133	1	161	2	193	1	116	2
2	53	35	71	26	85	26	130	23
3	40	55	29	53	49	61	99	46
4	15	73	8	78	23	77	58	72
5	13	95	4	85	24	87	35	76
6	8	100	2	92	2	99	12	99
7	4	100	3	100	19	97	18	97
8	4	97	0	–	11	99	8	89
9	4	100	1	100	14	98	6	98
10	9	100	1	100	10	97	7	99
11	2	100	1	100	8	100	5	100
12	0	–	0	–	4	96	5	98
13 or more	5	100	1	100	32	100	6	100

males were produced with 1 nematode/host, and almost all males were produced with 6 or more parasites/host. However, an occasional female was produced in hosts exposed in third and fourth instar with worm burdens up to 12/host. Therefore, host size, as measured by the instar at the moment of invasion by these nematodes, appeared to have little effect on the sex of *R. nielseni*.

Other factors such as the amount of food taken in by the host might be of importance in influencing sex determination. The effect of host diet on the sex ratio of *R. nielseni* was, therefore, tested by the exposure of six groups of 100 first instar *C. p. quinquefasciatus* to about equal numbers of preparasitic nematodes from a common culture source. Three groups were fed normally (0.3 mg of finely ground rabbit chow per larva on the first day, 0.4 mg on the second, 0.6 mg on the third, and 0.9 mg each day thereafter) and three groups were fed a minimal diet (one-third the normal amount) throughout the developmental period of the parasites. Ninety-two per cent of the nematodes produced from singly infected hosts in the starved group were males compared with only 13% in the group fed normally (Table 6). Also, starved hosts containing two parasites produced a higher ratio of males (97%) than normally fed hosts that contained six parasites (83%). No starved larvae with more than 3 nematodes survived until the nematodes emerged, but 31 normally fed larvae containing 4 or more nematodes did survive. The undernourished larvae produced 95% males compared with 58% by the normally fed larvae, and escape occurred earlier from the starved hosts, with 77% on the first day compared with 7% from larvae fed an adequate diet. Uninfected fourth instar mosquitoes from the starved group were about normal in size, whereas the infected larvae were much smaller than normal.

TABLE 6. The effects of diet of the host (*Culex pipiens quinquefasciatus*) on the sex ratios of *Reesimermis nielseni*.

No. nemas/ host	No. starved[a] hosts	% male nemas	No. fed[a] hosts	% male nemas
1	97	92	26	13
2	39	97	81	46
3	3	100	60	64
4	0	–	22	67
5	0	–	5	76
6	0	–	2	83
7	0	–	1	100
8	0	–	1	100

[a]See text for explanation of starved and fed.

This study elucidates the importance of environmental influence, especially the degree of infestation, and perhaps nutrition on sex determination in *R. nielseni*. These factors would, therefore, be important in the mass rearing of the parasite for biological control. Our experience indicates that the number of preparasitic nematodes used to infect a given number of larvae should not exceed the number that will produce about 80% infection of the hosts. When the number is large enough to cause about 100% infection, too high a percentage of hosts will be multiple-infected, resulting in a high percentage of males, a low percentage of females, and ultimately a lower number of preparasitic nematodes.

Also, overcrowding of the hosts or insufficient food in the rearing pans will greatly reduce the numbers of postparasitic female nematodes. Therefore, care should be taken not to exceed the optimum number dictated by the rearing conditions when large numbers of postparasitic females are desired. Also, the host species used in the mass rearing of the parasite should be a species that is medium to large in size and that develops rapidly. Finally, other factors such as the ease of rearing and the availability of the host species must also be considered.

At present, *R. nielseni* shows promise as an effective biological control agent of mosquitoes. However, before its full potential can be determined, the procedures for mass rearing must be worked out. Information about the exact determiners of sex and the influence of various environmental conditions would aid greatly in this endeavor.

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