

Effects of Host Size and Parasite Burden on Sex Ratio in the Mosquito Parasite *Octomyomermis muspratti*

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Abstract: The ratio of *Octomyomermis muspratti* to the host mosquito at the time of exposure had little effect on the ratio of male to female parasites that resulted. However, the ratio of males to females increased as the number of parasites/host increased. Hosts with a single nematode produced fewer than 1% males in comparison with hosts with 8 parasites which produced about 40% males; hosts with 10 or more nematodes generally produced more male than female nematodes. Males of *O. muspratti* usually emerged before females because of the earlier death of multiply-infected mosquitoes. The size of the host at the time of invasion had no significant influence on nematode sex ratios. Since mating is apparently necessary for reproduction in *O. muspratti*, the low male to female ratios that occur will be important in developing successful mass production techniques. **Key Words:** sex ratios, Mermithidae biological control, parasitism, mass production.

The sex of developing mermithid nematodes is influenced by the sex of the host (6), the host species and diet (3), the degree of infection, the host size, and the sex of the infective stage nematode that first penetrates the host (2). Therefore an understanding of these factors can aid significantly in the development of successful mass rearing procedures for some of these promising biological control agents. For example, Petersen (3) reported that, with the mosquito parasite *Romanomermis culicivorax* (= *Reesimermis nielseni* auct. partim.) Ross and Smith, the degree of infestation (worm burden), overcrowding of the hosts, or insufficient food in the rearing trays resulted in reduced numbers of postparasitic female nematodes and ultimately affected the numbers of preparasitic (infective stage) nematodes. This information has influenced the procedures employed in the mass production of *R. culicivorax* (5).

Octomyomermis muspratti (Obiamiwe and MacDonald), another mermithid parasite of mosquitoes, has greater tolerance for desiccation, salinity, and pollution than *R. culicivorax* (4) and thus may be of value in controlling mosquitoes in habitats not suitable for *R. culicivorax*. However, it has not been successfully mass produced. Little is known about the biology of *O. muspratti* (1), especially the factors that influence sex determination, but laboratory observations have indicated that the ratios of male nematodes produced differs sub-

stantially from the ratios of *R. culicivorax* males produced under similar conditions. Therefore, sex ratio data derived for *R. culicivorax* could not be applied to *O. muspratti*. Also, since egg development in females of *O. muspratti* has not been observed in the absence of males and increases with corresponding increases in the male-female ratios, it appears that mating is necessary for propagation of this parasite (author, unpublished data). A study was therefore undertaken to determine the effects of multiple parasitism, parasite-host exposure ratios, and host size on male-female ratios in *O. muspratti* as they might relate to mass production.

MATERIALS AND METHODS

The *O. muspratti* used in the study was obtained from cultures (maintained since 1974 in the Lake Charles laboratory) derived from an initial stock supplied by J. Muspratt, Johannesburg, South Africa. The mosquito *Culex pipiens quinquefasciatus* Say from the laboratory colony was used as the host for all tests because it is easily maintained and is a good host of this parasite (4).

To determine the effects of the parasite-host exposure ratio and host size on production of male nematodes, six groups of 100 first- or second-instar larvae of *C. p. quinquefasciatus* were exposed to preparasitic *O. muspratti* at ratios of 1, 2.5, 5, 7.5, 10, and 15:1; also, seven groups of 100 third-instar hosts were exposed to infective stage nematodes at ratios of 1, 2.5, 5, 7.5, 10, 15, and 20:1. To determine the effects of multiple parasitism and host size on production of male nematodes, five groups

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of 100 first-, 100 second-, and 100 third-instar hosts were each exposed to 1,000 parasitoids of *O. muspratti*. All tests were replicated 5 times.

In all tests, the mosquito larvae were exposed to 16-h-old nematodes in 100 ml of water in 150-ml beakers for 24 h and then placed into rearing trays and allowed to develop by using standard techniques. Twelve days after the exposure, the mosquitoes were removed from rearing trays and isolated in spot plate cells containing water. The plates were held in a 100% relative humidity environment (to prevent desiccation) and observed daily for nematode emergence, number and sex of the nematodes, and the time of emergence.

RESULTS AND DISCUSSION

Little difference could be detected in the male-female ratio (reported as percentage males) when parasite-host ratios at the time of exposure ranged from 1:1 to 10:1 for first- and second-instar hosts and from 1:1 to 15:1 for third-instar hosts (Table 1). However, there was a noticeable increase in production of males at the 15:1 ratio for first- and second-instar hosts and at 20:1 for third-instar hosts. Nevertheless, production of males never exceeded 32% in any of the 15 tests. Also, despite a trend toward an increase in the ratio of males to females as the ratio of parasites to hosts was increased, calculated regressions, though positive for all three instars, were not significant. Under similar conditions, when *R. culicivora* was used, parasite-host ratios of about 5:1 and 10:1 produced 47 and 66% males, respectively, in first-instar hosts (3) in comparison with 4 and 2% for *O. muspratti*.

The higher male production for second-instar hosts, especially at the higher parasite-host ratios, probably reflected the higher rate of survival of this stage host when it was multiply infected (4). These data indicate that, with *O. muspratti*, high parasite-host ratios may produce more favorable male-female ratios in the post-parasitic nematodes, but total yields of nematodes are decreased because of early host mortality. When the test data were analysed by regression analysis for the worm burden/host to determine its effect on male nematode production (Table 2), regressions

TABLE 1. Sex ratios for *Octomyomermis muspratti* which resulted when groups of 100 first-, second-, and third-instar larvae of *Culex p. quinquefasciatus* were exposed to various parasite-host ratios.^a

Parasite-host ratio	Percent host larvae infected	Mean no. of surviving nematodes per infected host	Postparasites	
			Total	Males (%)
First Instar				
1:1	21	1.2	108	1
2.5:1	44	1.5	269	1
5:1	71	2.2	545	4
7.5:1	86	2.5	650	5
10:1	88	2.8	554	2
15:1	97	4.3	366	16
Second Instar				
1:1	20	1.2	116	11
2.5:1	43	1.5	267	10
5:1	66	2.2	569	3
7.5:1	85	3.2	893	13
10:1	91	3.5	1,202	13
15:1	99	5.3	1,116	26
Third Instar				
1:1	8	1.1	31	0
2.5:1	15	1.2	73	3
5:1	28	1.4	134	5
7.5:1	41	1.8	220	4
10:1	53	1.9	296	8
15:1	77	2.7	395	9
20:1	90	3.9	355	21

^aValues are means and totals for five trials.

for the three instars were positive ($P < 0.05$) ($b = 4.41, 4.83$ and 6.93 for first-, second-, and third-instar hosts, respectively). When there was only one worm/host, production of males by all three instars was less than 1%. The ratio of males to females increased similarly in hosts exposed in first and second instars, but was more rapid in third-instar hosts. However, again, the actual numbers of males produced were much higher when hosts were exposed in the second instar because of early mortality of first-instar hosts and mortality and pupation of third-instar hosts. In contrast, second-instar *C. p. quinquefasciatus* containing two *R. culicivora* produced 26% males, and those containing five nematodes produced 85% males (3) in comparison with 4.9 and 14.8%, respectively, for *O. muspratti*. As *O. muspratti* burdens in-

TABLE 2. Effects of host (*Culex p. quinquefasciatus*) size at the time of invasion of the sex ratio of *Octomyomermis muspratti*.^a

No. nematodes per host	1st-instar hosts		2nd-instar hosts		3rd-instar hosts	
	Total	Males (%)	Total	Males (%)	Total	Males (%)
1	512	0.1	373	0.8	267	0.6
2	394	1.6	299	4.9	184	10.8
3	230	4.2	239	8.5	100	24.7
4	130	9.9	156	10.3	43	20.7
5	61	13.2	134	14.8	26	29.2
6	20	13.4	54	19.8	14	45.8
7	15	11.2	40	26.0	8	40.5
8	6	41.7	31	38.2	2	18.8
9	1	55.6	18	37.0		
10	0	—	9	47.8		
11	1	63.3	1	27.3		
12			4	59.7		
13			1	76.9		

^aValues derived from five trials.

creased, the percentage of hosts producing no male nematodes decreased (Table 3). For the most part, all the hosts with one nematode produced no males, and all the hosts with eight or more nematodes produced one or more males. Hosts with nine or more nematodes produced four or more males 52% of the time.

It is apparent from this study that production of males can be a limiting factor in the mass production of *O. muspratti*. Therefore, it is important to know the rate of emergence of male nematodes from their

TABLE 3. Effects of worm burdens on the percentage of hosts producing male *Octomyomermis muspratti*.^a

No. nematodes per host	Percentage of hosts producing indicated number of male nematodes				
	0	1	2	3	4+
1	98	2	—	—	—
2	89	8	3	—	—
3	82	12	3	3	—
4	64	31	2	0	2
5	40	50	5	1	4
6	29	33	22	13	2
7	20	30	32	12	4
8	0	23	7	38	32
9+	3	3	10	32	52

^aPercentage derived from mean for 15 trials.

hosts in relationship to the emergence of females so that the most favorable male-female ratio can be obtained. Observations showed that 35% of the nematodes emerged from their hosts (exposed as second instars) the first day of emergence, and 44% were males (Table 4). Only 24% of the nematodes emerging on the second day were males, but the actual number of males produced was nearly double that produced the first day. The third day, production of males dropped to about one-third that of the first day, and no males were produced the fourth day of emergence. Thus, early emergence of males was probably the result of early emergence of nematodes from multiply-infected hosts.

Although the mosquito parasites *O. muspratti* and *R. culicivora* have many similar behavior patterns, they differ in their potential for production of males. With *R. culicivora*, care must be taken during rearing to provide an optimum environment for the host to protect against high worm burdens that result in a preponderance of male nematodes. The opposite is apparently true for *O. muspratti*. If the same rearing procedures are used for this species, few male nematodes will be produced. Then, since mating is apparently necessary for egg production, little or no recycling of the parasite could be expected.

TABLE 4. Sex ratios of *Octomyomermis muspratti* emerging from populations of *Culex p. quinquefasciatus* exposed in the second instar.*

No. nemas per host	First day		Second day		Third day		Fourth day	
	Total	Males (%)	Total	Males (%)	Total	Males (%)	Total	Males (%)
1	0	—	3	50	102	2	215	0
2	2	100	150	4	306	6	20	0
3	6	50	372	7	180	13	0	—
4	20	0	420	11	48	17	0	—
5	30	20	490	16	20	15	0	—
6	66	30	192	18	12	17	0	—
7	105	29	119	26	7	29	0	—
8	136	39	64	36	8	38	0	—
9	117	41	27	48	0	—	0	—
10	90	42	0	—	0	—	0	—
11	11	27	0	—	0	—	0	—
12	60	63	0	—	0	—	0	—
13	13	77	0	—	0	—	0	—
14	14	57	0	—	0	—	0	—
Totals or means	670	44	1,837	24	683	17	235	0

*Values derived from five trials.

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