Effects of Host Diet on Romanomermis culicivorax, a Mermithid Parasite of Mosquitoes

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Abstract: When larval mosquitoes (*Aedes aegypti*) infected with the mermithid nematode *Romanomermis culicivorax* were fed on a diet low in quantity or protein content or both, the number of postparasites which emerged from the hosts decreased and host mortality increased marginally. Parasitic development was prolonged and became asynchronous in nutritionally deprived hosts. Nematodes emerged from insects infected by more than one nematode before the remaining juveniles comprising such infections had completed parasitic development; this resulted in substantial reductions in postparasite numbers. Host development was retarded by low protein and/or reduced diets. Postparasites emerging from second and third instars were reduced in size and in the amount of stored nutriment compared to those recovered from hosts fed on a high protein diet ad libitum. A greater proportion of the mermithids developed into males in hosts fed on reduced diets but not in hosts fed on low protein diets. *Key words: Aedes aegypti,* asynchronous development, protein diet. Mermithidae, postparasites, restricted diet, sex ratios.

The mermithid nematode *Romanomermis culicivorax,* a parasite of larval mosquitoes, is normally mass cultivated by an in vivo procedure (13). The establishment of in vitro techniques for mass cultivating this nematode has been advocated (4,12,16) as being potentially cheaper, more efficient, and easier to maintain than the current in vivo method. Attempts to culture the parasite in vitro have been handicapped by lack of knowledge of the nematode's nutrition and have met with only limited success (3, 14,15). The quantity of food available to the host is known to affect the determination of sex in *R. culicivorax* (10,11), but no information is available on the consequences of altering the quality of the host diet or on aspects of development other than sex determination.

The purpose of this study was to determine the effects of nutrient deficiency on the parasitic development of *R. culicivorax* through in vivo studies involving manipulation of the amount or protein content or both of the host diet.

MATERIALS AND METHODS

Cultures of the egg and adult stages of *R. culicivorax,* stored in sand, were supplied by Dr. J. J. Petersen, USDA. The mosquito *Aedes aegypti* (mixed strain) from our laboratory colony was used as the host.

Laboratory infections of first instar *A. aegypti* larvae were accomplished by modification of a previously described procedure (2). Mosquito larvae were exposed to inoculum for 16–19 h in Pyrex casserole dishes (37 × 23 × 4 cm) at a host:preparasite population density of 1:10–16 per ml distilled water (day 0 infection). The mosquitoes, not fed during the inoculation period, were transferred in groups of 100 into finger bowls containing 100 ml distilled water and reared at 27 C. Feeding began at this time (day 1 infection). Hosts were fed on a synthetic diet (7) of either high protein (55% casein)-low carbohydrate (30% corn starch) or low protein (3.5% casein)-high carbohydrate (81.5% corn starch) content. The quantity of food provided constituted a second variable. Hosts were fed either ad libitum or in restricted amounts according to the starvation schedule outlined by Petersen (10) for *Culex quinquefasciatus* fed on rabbit chow. Thus, mosquito larvae were placed on one of four types of diets (treatments): high protein, ad libitum (HP); high protein, restricted supply (HPR); low protein, ad libitum (LP); low protein, restricted supply (LPR). Each experiment involved setting up at least five bowls (replicates) of 100 infected mosquitoes per bowl for each of the four feeding regimes; i.e., 20 bowls in all.

All of the postparasitic nematodes were counted, measured, and sexed, and the duration of their parasitic development relative to day 0 infection was recorded. From day 1 to day 6 of infection (the time that postparasites began to emerge from HP hosts), daily dissections were done (20 insects per treatment, taken from individual replications) to compare incidences and levels of

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parasitism. The insects were dissected in half strength Dulbecco's phosphate buffered saline (Grand Island Biological Company, Grand Island, New York) and microscopically examined for differences in developmental morphology between the four groups of nematodes. The lengths of the developing nematode juveniles were measured (6–11 nematodes representative of each group) daily throughout the course of the infection.

Host mortality was recorded 1 d before postparasites began to emerge. To compare the effects of the diets on the development of the infected hosts, measurements of the head capsule widths of five insects per dietary group were made daily from day 1 until postparasites commenced emergence. Head capsule width is an index of larval instar (1). The time periods, relative to day 0, over which pupation occurred in insects which escaped infection were recorded.

Data were analyzed by two-way analysis of variance with replication. Dietary groups were compared in pairs using the Student's 't' test.

RESULTS

The total number of postparasites which emerged from the variously fed insects decreased in the order HP > HPR > LP > LPR (Table 1). One sixth as many postparasites were recovered from LPR as compared to HP hosts. A significant decline in numbers of postparasites resulted when hosts were fed on a diet deficient in (a) protein content or (b) quantity ($P < 0.001$ in each case). There was no protein content × food quantity interaction ($P > 0.75$). Thus, the effect on postparasite numbers of a host diet deficient in both protein and amount (i.e., LPR group) was additive and explicable in terms of the separate effects of the two factors involved. Regardless of the host diet, a high percentage ($\geqq 88.5\%$) of the postparasites were males (Table 1). The production of male nematodes was increased significantly ($P < 0.001$) when hosts were fed a restricted diet. However, the protein content of the host's diet did not significantly affect the sex ratio of the mermithid. There was no significant interaction between protein content and quantity of food on the sex ratio of the postparasites.

The average size of the postparasites also decreased among the groups in the order HP > HPR > LP > LPR (Table 1). When hosts were either starved or fed on a protein deficient diet, significantly ($P < 0.001$) smaller postparasites developed. Combined starvation and dietary protein deficiency had a cumulative effect on postparasite size; there was no protein content × food quantity interaction ($P > 0.25$).

The time required for the nematode to complete parasitic development varied among hosts of the same dietary group, so the postparasites emerged from such similarly fed insects over an extended time interval. The mermithid took longer to complete parasitic development in nutritionally

Table 1. Effects of host *(Aedes aegypti)* diet on host mortality and postparasitic juveniles of *Romanomermis culicivorax*.

Host diet*	Number of postparasites recovered†	Length of postparasites (mm)†	Males (%)†	Period of postparasitic emergence (days after start of infection)‡	Host mortality§
HP	126.2 ± 5.1	11.52 ± 0.70	88.5 ± 1.5	6-11	4.6 ± 0.5
HPR	86.0 ± 6.0	7.87 ± 0.41	96.4 ± 0.6	7-15	8.2 ± 1.2
LP	60.0 ± 4.2	6.51 ± 0.37	92.4 ± 1.3	8-20	10.0 ± 1.4
LPR	21.7 ± 3.6	4.53 ± 0.81	95.3 ± 1.6	10-30‖	15.4 ± 0.6

*HP = high protein; HPR = high protein reduced; LP = low protein; LPR = low protein reduced.

†Values are means ± standard errors from six replicates of 100 mosquito larvae each.

‡Values are ranges, indicating the times during which postparasites emerged from each group (six replicates).

§Values are mean percentages ± standard errors from five replicates of 100 mosquito larvae each.

‖Two of the nematodes had still not emerged by 30 d after the start of infection.

disadvantaged hosts, however. Postparasites started to emerge first from HP-fed insects then from hosts fed HPR, LP, and LPR diets, in that order. The time interval over which emergence of the postparasites occurred was increased according to the same sequence of host groups. Thus, while postparasites emerged from HP hosts over a 5-d period (6–11 d after day 0 infection), the comparable time span for LRP derived nematodes was in excess of 20 d (10–30+ d after day 0 infection).

The mortality rate of the hosts followed the same succession, with least mortality in HP and greatest mortality in LPR-fed insects (Table 1). Though these data indicated that a decline in dietary protein content or food intake or both significantly increased host mortality ($P < 0.001$ for each parameter), the magnitude of these effects was insufficient to account for the drastic reductions in postparasite numbers that were observed.

There was no decline in the numbers of developing parasitic stages among nutritionally deprived groups to account for the decreased numbers of postparasites. The incidence of parasitism, the incidence of parasitism by more than one nematode, and the mean level of parasitism were approximately the same in all four host groups, averaging ca. 96%, 69%, and 2.2 nematodes/host, respectively. No encapsulation or other defence mechanism was observed.

The parasitic stages developed more slowly in hosts that were fed on a protein deficient and/or restricted diet; parasitic development was slowed down progressively according to the series HP-HPR-LP-LPR (Fig. 1). The sudden burst of growth, which is thought to characterize a molt in this nematode (5), took place at day 3 of infection in HP, at day 4 of infection in HPR and LP, but not until day 9 of infection in LPR insects. At the time that postparasites began to emerge from the various groups, the parasitic stages differed in size in the same fashion (HP-HPR-LP-LPR) as did the postparasites themselves. Thus, the delayed emergence and smaller size of postparasites which emerged from nutritionally disadvantaged hosts were due to retardation of parasitic development. The hosts themselves were similarly retarded in their development through inadequate nutrition. While pupation of noninfected HP hosts took place at times corresponding to days 8–14 of infection, it occurred at days 10–21, days 14–22, and days 20–30+ in groups HPR, LP, and LPR, respectively. Due to this differential retardation of host development, postparasites invariably emerged from fourth-instar mosquito larvae in group HP, but began emerging from third-instar hosts in group HPR and from second instars in groups LP and LPR (Fig. 1).

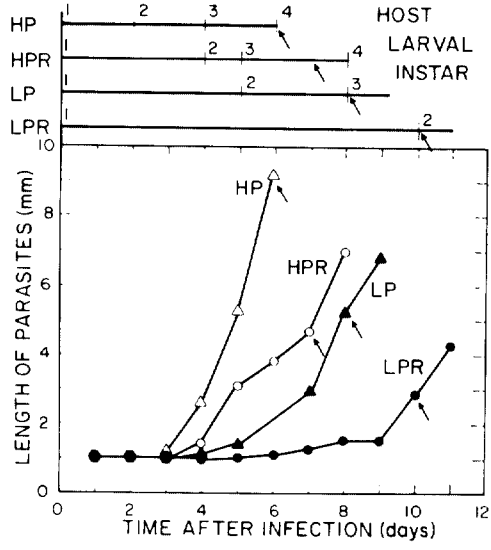
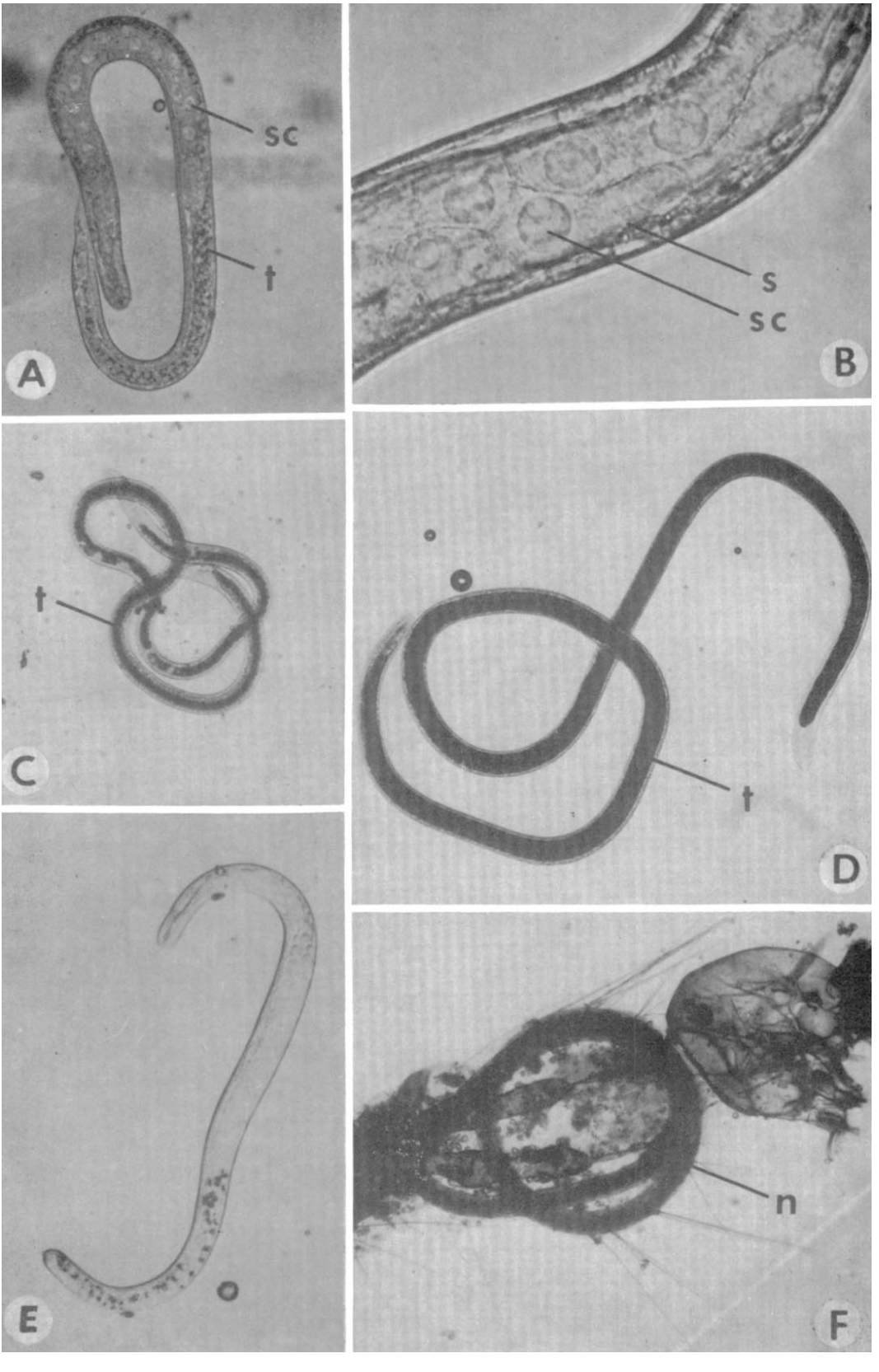


Fig. 1. Growth of *Romanomermis culicivorax* in *Aedes aegypti* larvae fed on a high protein diet ad libitum (HP △-△); high protein diet, reduced amount (HPR o-o); low protein diet, ad libitum (LP ▲-▲); low protein diet, reduced amount (LPR ●-●). Values on graph represent mean length of nematodes at intervals following infection. Shown above the graph are the stages of larval development, attained by the variously fed hosts, that correspond to these times. The numbers indicate that a portion of the host population had molted to the instar in question at that time after infection. Arrows on graph and instar diagram indicate times at which postparasites began to emerge from the hosts.

The sequence of events associated with the development of the parasitic stages in HP hosts was identical to that previously described (6) for this mermithid in hosts fed on guinea pig chow. These same changes in tissue organization and structure occurred, but over an extended period in nematodes that developed in hosts fed on a less nutritious diet. The majority of LP and LPR parasites at days 5–6 of infection (Figs. 2A, B) resembled 2–3-day HP parasites in size, paired arrangement of stichocytes, and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lack of storage material in the trophosome. The trophosomes of nematodes which developed in LP and LPR hosts (Fig. 2C) did not become so densely packed with storage nutriment as did those in parasites of HP and HPR hosts (Fig. 2D). There was a major difference between the groups in the degree to which parasitic development was synchronized. At all stages of the infection, nematodes developing in HP hosts displayed approximately the same degree of development as one another. Development in other hosts became asynchronous after the nematodes had commenced their rapid phase of growth. Thus, within a particular dietary group, nematodes from singly infected hosts as well as those from hosts infected by more than one nematode showed staggered development. This asynchrony became more pronounced as the infection progressed and was increasingly evident through the series HPR-LP-LPR. In singly infected hosts, the parasites eventually completed their development and emerged as postparasites, the staggered parasitic development manifesting itself in a more protracted period of postparasite emergence. Infections involving more than one nematode/host frequently were characterized by parasites at varying stages of development (Figs. 2C, E). In such infections, one nematode completed parasitic development and emerged from the host; the remaining underdeveloped nematodes were thus killed (Fig. 2F).

DISCUSSION

This study has shown that the development of larval *A. aegypti* and of its mermithid parasite, *R. culicivorax,* are markedly affected by the protein content of the host diet and by the quantity of food that the host consumes.

Uninfected hosts fed a protein deficient or reduced diet or both took longer to pupate but survived almost as well as did those that received a high protein diet ad libitum. In contrast to *C. quinquefasciatus* (10,11), heavily infected and undernourished *A. aegypti* larvae survived until the time that postparasites began to emerge. Prolongation of larval development is characteristic of *A. aegypti* that are starved (9) or maintained on nonsynthetic diets of low protein content (8). Undernourished mosquito larvae are also smaller and contain less lipid than well-fed insects (9). We found that host diet affected the size of the host; they were progressively smaller on HP > HPR > LP > LPR diets. Thus, it seems that nutritionally deprived hosts restrict nutrient availability to parasites and result in smaller postparasites. Nutritionally deprived hosts only developed to the second (hosts fed the LP and LPR diets) or third (in HPR hosts) instar by the time postparasites began to emerge. It is understandable, therefore, that such postparasites were reduced in size and depleted in trophosomal storage material (in LP and LPR hosts).

The data obtained in this study support the concept that sex determination in *R. culicivorax* is influenced by host food intake (10,11). The male:female nematode ratio was significantly greater when the amount of food available to the hosts was restricted. The sex ratio of the nematode, however, did not appear to be significantly affected by the protein content of the host's diet. In this study, synthetic diets were used; casein constituted the sole dietary source of protein nitrogen and other nutrients were probably not as plentiful as would be the case in an undefined diet such as rabbit chow. In the group that received the most nutritious diet (HP), almost 90 percent of the postparasites were males. This approxi-

Fig. 2. A) *Romanomermis culicivorax* from low protein (LP) fed host, day 5 of infection. Stichocytes (sc) arranged in pairs and trophosome (t) poorly developed (× 438). B) *R. culicivorax* from LP host, day 6 of infection, showed paired arrangement of stichocytes (sc) within membrane-bound stichosome (s). Phase contrast (× 1750). C) *R. culicivorax* from host infected by more than one nematode and maintained on low protein, reduced amount (LPR) diet. Day 10 of infection; trophosome (t) (× 188). D) *R. culicivorax* from host fed on a diet high in protein but reduced in amount (HPR), day 7 of infection. Note increased amount of storage material in trophosome (t) compared to nematode shown in Fig. 2C (× 175). E) *R. culicivorax* from the same host (LPR) as that shown in Fig. 2C, day 10 of infection. Note the much earlier stage of tissue organization that this nematode represents compared to that shown by the nematode in Fig. 2C (× 469). F) Dead *Aedes aegypti* larva (LPR), day 10 of infection, from which nematode(s) have emerged. Inside the thorax is a dead nematode (n), incomplete in parasitic development (× 188).

mates the sex ratio of postparasites that emerged from *C. quinquefasciatus* fed on a restricted diet of rabbit chow (11) and indicates our diets were of much lower nutritional value; even nematodes developing in HP hosts were undernourished. Recently, Harlos et al. (6) found that a mermithid parasite of adult *Aedes vexans* developed a greater proportion of female nematodes when hosts were fed blood instead of sucrose alone. This suggests that the sex of the mermithid is governed to a large degree by the quality (and quantity) of the host diet. Additional studies are needed with *R. culicivorax* using a synthetic host diet of higher nutritional value than the one used in this study, one which would promote a lowered male:female postparasite ratio. Changes (if any) resulting from omissions and/or reductions of dietary components would undoubtedly be in the direction of increasing maleness and thus be detectable.

When hosts were maintained on a restricted and/or low protein diet, parasitic development was prolonged and became asynchronous. Such a staggering of parasitic development ultimately led to pronounced decreases in the numbers of postparasites recovered from nutritionally deprived hosts. Postparasites began to emerge from hosts harbouring more than one nematode before the remaining juveniles had fully developed. The finding that inadequate nutrition caused the nematodes to develop in an asynchronous fashion has considerable bearing upon the efficiency of any eventual system that may be devised for mass cultivating *R. culicivorax* in vitro. Efforts to culture this nematode in vitro should insure that the nutritional composition of the medium is sufficient to avoid asynchronous nematode development as well as overproduction of males.

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