

Influence of Host Nutrients on the Parasitic Development of *Mermis nigrescens* (Mermithidae)

STUART M. CRAIG¹ AND JOHN M. WEBSTER²

Abstract: *Schistocerca gregaria* nymphs and adults of both sexes were infected with eggs of *Mermis nigrescens*. Mermithid larvae grew more slowly in nymphal hosts, and emerging larvae were smaller than those from adult hosts. The longer the larvae remained in the host, the greater their size. Those developing in adult female hosts were longest. Single mermithid larvae that were transferred to a second host continued to grow and were significantly longer at emergence than larvae that developed solely in one host. In adult hosts that were infected with 40–300 *M. nigrescens* eggs, the percentage of mermithids that became males was strongly dependent on host weight at infective doses of 90 eggs or more. Results are discussed in relation to nutrient stress on the larvae and its importance in developing in vitro culture techniques. **Key words:** sex ratios; parasite burden, *Mermis nigrescens*, *Schistocerca gregaria*, nutrient stress, larval development.

Journal of Nematology 14(3):398-405. 1982.

The development of larval mermithids in their insect hosts is influenced by environmental factors. Hence, the number of male *Mermis nigrescens* (syn: *Mermis subnigrescens*) that develop is greater with increasing size of the infective burden of mermithids per grasshopper host (3,5); a larger proportion of male *Filipjevimermis singularis* develop in male than in female chironomids (15); the number of male *Paramermis contorta* developing in *Chironomus contorta* is proportional to the size of the host at the time of infection (9); and the host species influences the sex ratio of *Romanomermis culicivorax* (syn: *Resimermis nielseni*) (9). However, Petersen (11) found that host diet rather than size or the age of the mosquito host, *Culex pipiens quinquefasciatus*, affected the sex of the parasitizing *R. culicivorax* larvae, and Gordon et al. (7) demonstrated the importance of the quantity of host food and its protein content for parasitic development of *R. culicivorax*.

It seems likely that the common factor affecting the development and sex ratio of particular mermithid species is the total host nutrients available to the parasite. In a series of experiments to assess the effect of nutrient availability on parasite development, host weight was used as a measure of

total available nutrients. The experiments were performed on the Desert Locust, *Schistocerca gregaria* Forskal, infected by *M. nigrescens* Dujardin.

MATERIALS AND METHODS

Desert Locusts (*S. gregaria*) were reared and fed with infective *M. nigrescens* eggs as previously described (4), and a series of experiments was done.

Effect of host stage on nematode development: 1-day-old third-instar nymphs, 4-day-old fourth-instar nymphs, 2-day-old fifth-instar nymphs, and 1-day-old and 28-day-old adult locusts of each sex were infected with 50 nematode eggs. Twenty locusts of each age group and sex were reared in separate cages at 35 C. Growth of the larvae during their parasitic development was determined by dissecting a locust of each age group and sex every 4 days and measuring larval length. At the time of emergence of the first nematode larva from a host of a particular age, between four and seven locusts of that age were killed and dissected, and the larval length, number of larvae developing, and their duration of parasitic development were recorded.

Effect of successive hosts on nematode development: 1-day-old adult male and female locusts were infected with 50 *M. nigrescens* eggs. After 25 days the larval nematodes were removed from the carbon dioxide anesthetized hosts and transferred in groups of 1, 3, 5, or 10 into the hemocoel of a similarly anesthetized uninfected 1-day-old adult locusts through a 5-mm abdominal incision. The incision was coated with chloramphenicol crystals to minimize

Received for publication 1 October 1981.

¹Pesticide Control Branch, British Columbia Ministry of Environment, 1873 Spall Road, Kelowna, B.C., Canada V1Y 4R2.

²Pestology Centre, Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, B.C., Canada V5A 1S6.

The authors appreciate the assistance of the Departmental Insectary staff and financial support from the Natural Sciences and Engineering Research Council grant (A4679).

bacterial infection and sealed with melted paraffin wax. The controls consisted of 1-day-old adult locusts that had been infected by oral ingestion of eggs so that the parasite burden was either 1, 3, 5, or 10 nematodes. The overall duration of parasitic development and larval length at the termination of the experiment were recorded.

Effect of parasite burden and host stage on nematode development: 10 one-day-old adult locusts of each sex were each infected with 40–300 *M. nigrescens* eggs, and 10 one-day-old fifth-instar nymphs were each infected with 50–250 eggs. Immediately after ingesting the eggs the locusts were weighed and then placed in rearing cages at 35 C. After 18 days, the locusts were killed and the larvae from each were counted and sexed on the basis of the form of the genital primordia (1,2). The percentage of *M. nigrescens* mortality at each infective burden was calculated for hosts of each age and sex.

RESULTS

Effect of host stage on nematode development: Significantly fewer ($P < 0.001$) larvae developed in the third-instar hosts than in other age groups and the duration of parasitic development of these larvae in the third instar was significantly shorter than for most other host age groups (Table 1). The duration of larval development was longest in the 28-day-old adult female (Table 1).

After day 10 of parasitism, the nematode's length at the time of emergence differed greatly depending on host age (Fig. 1). Mermithid larvae grew more slowly in

nymphal hosts and their length at the time of emergence was less than for larvae parasitizing adult hosts. Parasites in adult hosts showed an 8–9-fold increase in length between days 11 and 17. There was a parallel increase in maximum width of the larvae in adult locusts, but the greatest increase occurred between days 8 and 14. By day 21, nematodes were significantly ($P < 0.05$) longer in female than in male hosts. In general, the longer the *M. nigrescens* larvae remained in a host, the greater their size (Fig. 1).

Effect of successive hosts on nematode development: Single nematodes that were transferred to a second host continued to grow, and, at emergence, were significantly longer than nematodes that had developed solely in one host (Table 2). The total duration of parasitic development of those larvae from successive hosts also was significantly longer. Transfer of groups of three nematodes into a second host increased their overall duration of parasitic development but not their size. It was also noted that groups of either 5 or 10 nematodes transferred to a second host caused no significant ($P < 0.05$) difference in either development time or larval size.

Effect of parasite burden and host stage on nematode development: At each host age the differentiation of nematode larvae into males was proportional to the inoculum dose. Only male nematodes developed when 1-day-old fifth-instar *S. gregaria* were infected with 120 eggs, or 1-day-old male locusts with 180 eggs, or 1-day-old female locusts with 240 eggs (Figs. 2 and 3).

Mean weights of the hosts at the time of egg ingestion were as follows: 1-day-old fifth

Table 1. Effect of sex and age of *Schistocerca gregaria* on duration of the parasitic phase of *Mermis nigrescens* larvae and on the number of larvae developing.

Age at infection	Sex	Number of replicates	Mean no. developing \pm SE. (% developing)	Mean duration of parasitism (days) \pm SE*
1-day-old third instar	...	(7)	30.10 \pm 2.37 (60.2%)	22.33 \pm 2.04 a
4-day-old fourth instar	...	(6)	40.90 \pm 2.91 (81.8%)	30.50 \pm 1.57 b
2-day-old fifth instar	...	(4)	40.62 \pm 1.36 (81.2%)	33.00 \pm 3.11 b, c
1-day-old adult	male	(6)	46.50 \pm 2.36 (93.0%)	23.33 \pm 1.37 a
1-day-old adult	female	(6)	41.60 \pm 2.55 (83.3%)	30.00 \pm 2.15 b
28-day-old adult	male	(6)	44.70 \pm 2.41 (89.4%)	27.83 \pm 2.28 b
28-day-old adult	female	(5)	40.00 \pm 2.73 (80.0%)	38.20 \pm 3.36 c

*Means followed by the same letter are not significantly ($P = 0.05$) different from each other.

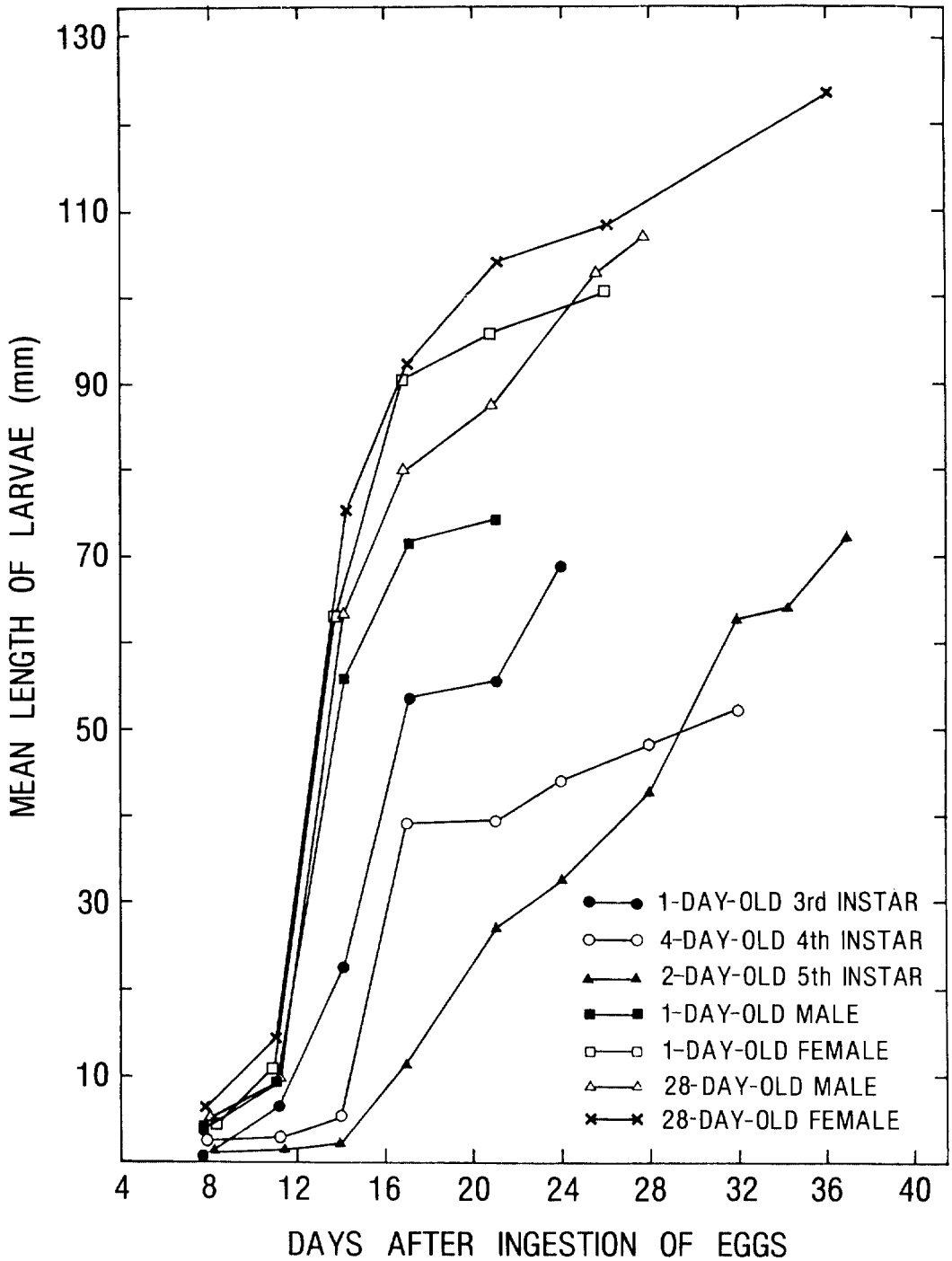


Fig. 1. Rate of growth of *Mermis nigrescens* in *Schistocerca gregaria* of different ages with an inoculum of 50 eggs per host.

instar, 0.83 ± 0.04 g; 1-day-old adult male, 1.09 ± 0.07 g; 1-day-old adult female, 1.47 ± 0.16 g. Fifth-instar nymphs weight significantly ($P < 0.01$) less than adults of

either sex, and adult male locusts weighed significantly ($P < 0.01$) less than female locusts. Male nematode larvae were smaller than females of the same age. The percent-

Table 2. The effect of transferring *Mermis nigrescens* larvae to a second host (*Schistocerca gregaria*) on the duration of parasitism and mean body length of the parasite.

Number† and sex of host(s)	Number of replicates	Infective burden per host	Mean duration of parasitism (days) ± SE	Mean larval length ± SE* (mm)
1, male	6	1	31.0 ± 1.15	87.35 ± 4.00
2, male	9	1	38.0 ± 1.89	104.53 ± 6.80
1, male	5	3	30.0 ± 1.67	85.36 ± 2.90*
2, male	8	3	34.4 ± 1.80	86.89 ± 1.12
1, female	7	1	37.0 ± 2.39	116.96 ± 3.12
2, female	9	1	44.2 ± 1.81	128.71 ± 3.45
1, female	7	3	34.3 ± 1.91	106.68 ± 5.30*
2, female	5	3	41.1 ± 2.71	107.28 ± 4.11

†Parasite developed within either one host (1) or two consecutive hosts (2).

*Means of these couplets are not significantly ($P = 0.05$) different.

age of *M. nigrescens* larvae that became males was strongly dependent on host weight at infective doses of 90 eggs and more and was weakly dependent on host weight at infective doses of 80 eggs or less per host (Fig. 3).

The percentage of larval mortality increased with increases in infective dose of eggs and at higher doses was significantly greater in fifth-instar hosts than in adults (Fig. 4).

DISCUSSION

Parasitic *M. nigrescens* larvae absorb glucose and amino acids from the hemocoel of their host, *S. gregaria*. The host does not compensate for this loss by increased food consumption (6). It seems likely, therefore, that the period of time that a host is able to support parasites probably depends on the availability of specific nutrients or on the total available nutrients in the host. To address this latter possibility we considered the host weight as a gross measure of total available nutrients. Our findings (Table 1) show that *M. nigrescens* larvae remained within mature adult female hosts for a significantly longer period than within adult male or young larval instar hosts, which weighed less. It seems likely, therefore, that the duration of parasitism of *M. nigrescens* is influenced by host size and by the total available host nutrients. The strength of this hypothesis was tested by increasing the normal amount of available nutrients by

surgically transferring developing larvae from the original host from which some of the available nutrients had been absorbed to a new host with a complete complement of available nutrients (Table 2). The overall duration of the parasitic phase was greater in two consecutive hosts than within a single host. Further, the duration of parasitism was greater in the mature female hosts (Table 1) than in any other age group or in males. It may be that an inadequate nutrient supply from the host may trigger nematode emergence, providing that the nematode is sufficiently well developed. This is supported by the fact that multiple infections in transfer hosts did not increase the larval length despite an increased duration of parasitism. The parasitic larvae developed significantly faster in younger host instars (Table 1) and in hosts with a larger parasite burden. This suggests a response to nutrient stress, and the influence of stress could be alleviated by the nematodes not growing so long and by a shift to maleness in the sex ratio. Male *Octomyomermis muspratti* usually emerge before females because of the earlier death of multiple infected mosquitoes (12). Larval *M. nigrescens* emerge faster from heavily infected than from lightly infected hosts, and more males are produced under these circumstances. Hence, it is likely that males develop faster than females. Furthermore, the duration of parasitic development of the larvae in the three instar hosts is shorter than in adult hosts (Table 1).

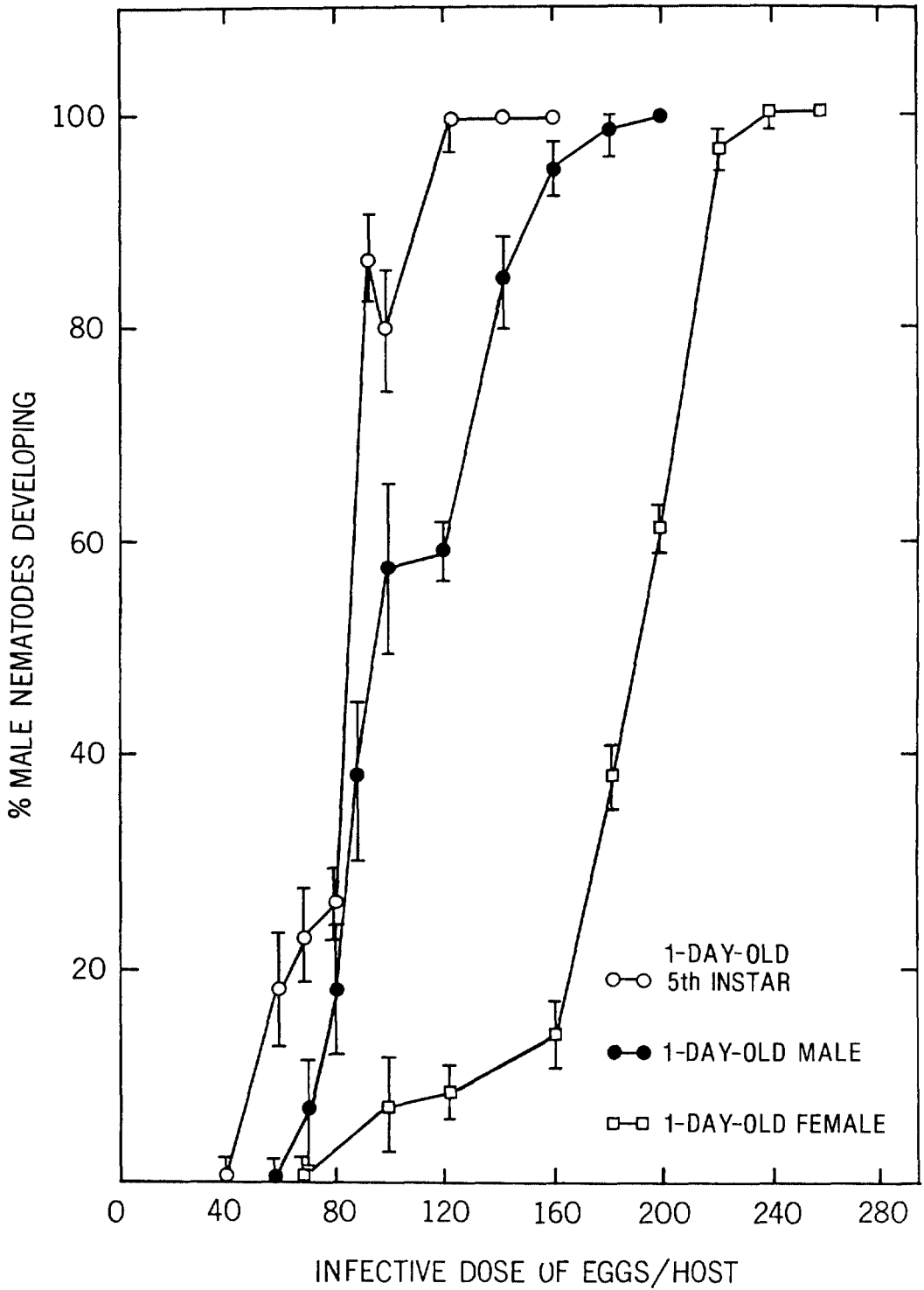


Fig. 2. Effect of inoculum size on the sex ratio of *Mermis nigrescens* in *Schistocerca gregaria*.

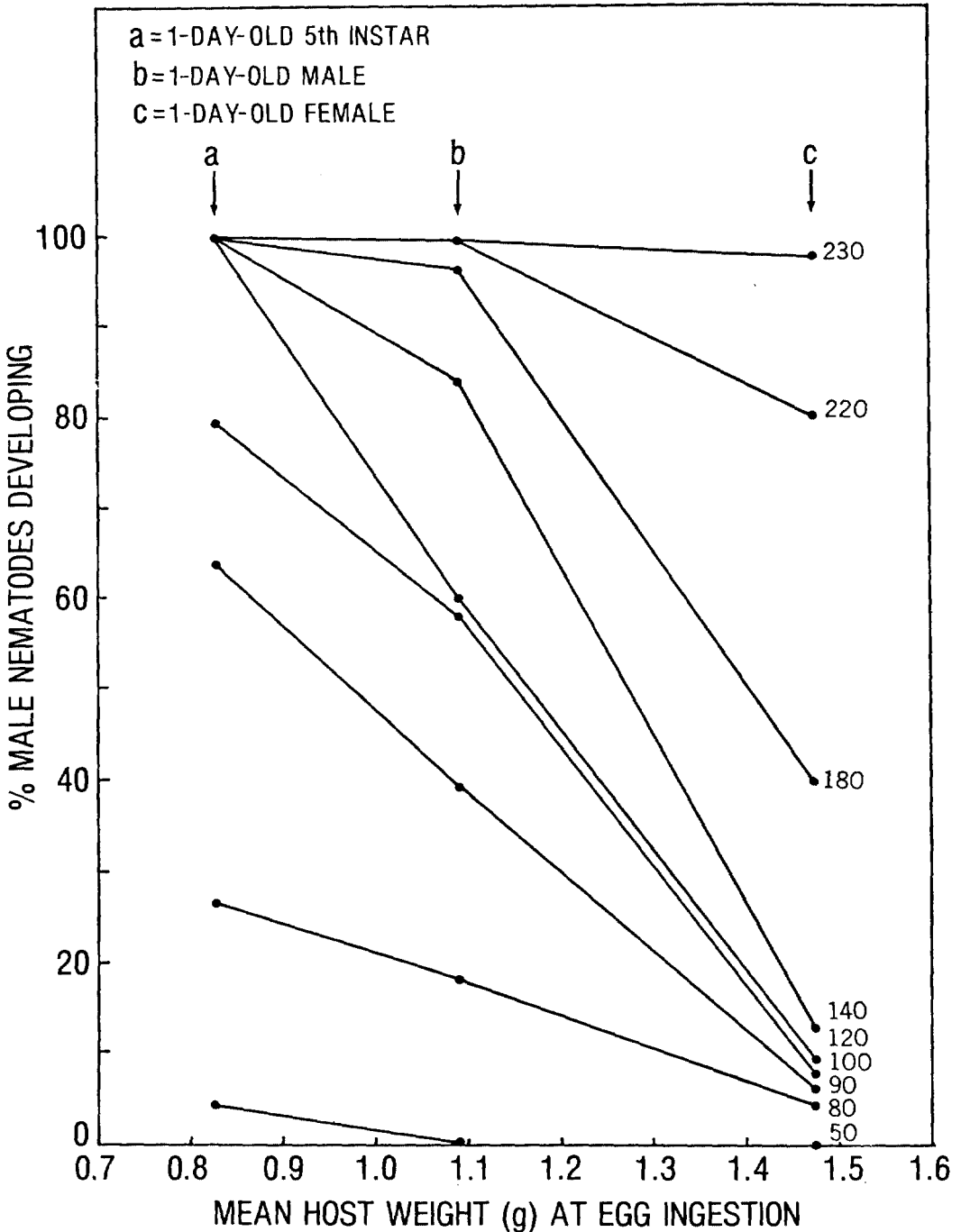


Fig. 3. Effect of host weight and inoculum size on the sex ratio of *Mermis nigrescens* in fifth instar, male, and female *Schistocerca gregaria*. Infective dose of parasites is given for each line.

M. nigrescens larvae increase most in length in adult hosts between days 11 and 12 after egg ingestion (Fig. 1), and they take up nutrients for their own growth and development most rapidly during this period (6). Adult female locusts have higher

haemolymph protein and carbohydrate concentrations than do males and are about 50% heavier than males. It is probable, therefore, that *M. nigrescens* larvae attain greater body length in female than in male hosts because female hosts contain more

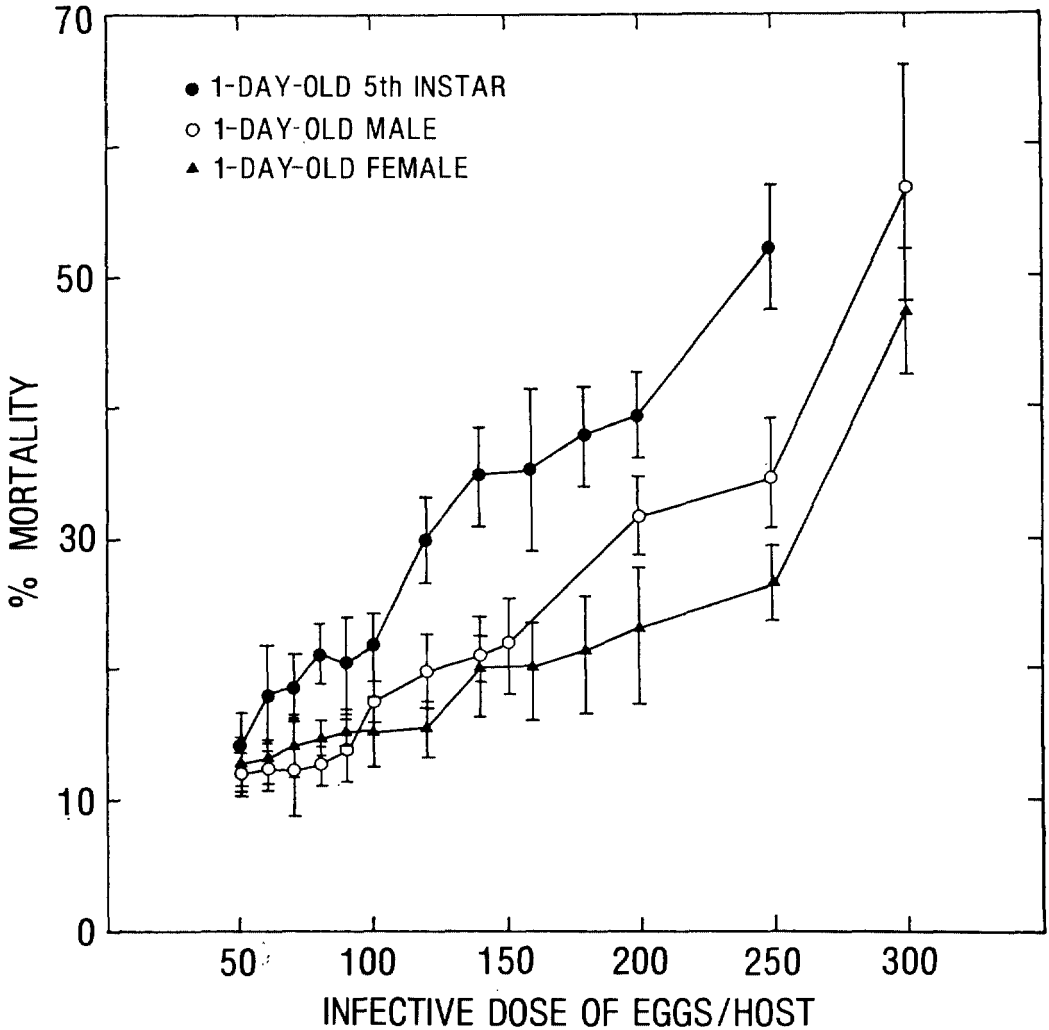


Fig. 4. Effect of infective dose on the percentage mortality of developing *Mermis nigrescens* larvae in fifth instar, male, and female *Schistoserca gregaria*.

total available nutrients than do males. Similarly, more mermithid larvae grew larger in older, heavier hosts, probably because these hosts contained more nutrients available for the developing larval mermithids.

A positive correlation between infective dose and the percentage of developing male *M. nigrescens* is in agreement with the findings in other host-parasite systems (1,3,10, 11,13,14). Our evidence suggests, however, that the size of the infective dose alone does not govern this phenomenon, since hosts of different ages (and, therefore, of different sizes and weights) harboring the same number of *M. nigrescens* larvae produced significantly different percentages of male

nematodes (Fig. 3). It seems likely that a large, heavy host provides more nutrients for a parasite than does a small host. Such a hypothesis would be in agreement with reports that host sex (10,15), host species (3, 5,11), and host length (9) influence the sex ratio of the mermithid parasites. This is supported by Petersen (11), who showed that when *R. culicivoxax* infected undernourished *C. pipiens quinquefasciatus*, more male nematodes develop even at the lower infective burdens. The present study suggests that male nematodes appear in greater numbers when the supply of host nutrients, as measured by host age, sex, and weight, is limited. Hence, the minimum infective dose required to cause more male

nematodes to develop than females is independent of host weight below a certain dose level and strongly dependent on host weight above that dose level within each host age class. However, Petersen's (11) finding that neither the size nor the age of the mosquito, *C. pipiens quinquefasciatus*, affected the size of the *R. nielsenii* larvae may suggest that the level of host nutrients was not a limiting factor in his experiments.

A change in the sex ratio of some nematode parasites under environmental stress may be due to a mortality differential between the sexes as in the case of the beet cyst nematode, *Heterodera schachtii* (8). This is not so with *M. nigrescens*, where larval mortality was not sufficiently high to account for the complete absence of female mermithids in 1-day-old fifth instars and 1-day-old adult males receiving doses of 100 and 180 mermithid eggs, respectively (Figs. 2-4). It is not known, however, whether the mortality of *M. nigrescens* at higher infective doses was due to egg or larval mortality.

In an abundance of food, the duration of parasitic development is longer and the resulting parasites, which are nearly all females, are larger in size and presumably lay more eggs. When available nutrients are a limiting factor, progressive increases in food stress could be expected to cause the larval *M. nigrescens* to develop along one of the following patterns according to the severity of the stress: smaller females, shorter duration of parasitism, more males, and 100% males. The shorter duration of parasitism would be a reflection of the greater percentage of males, which likely develop faster than females. The sex ratio, and the percentage of females in a given population determines the potential for increase in number. It is unlikely that adult hosts in the field would ingest 100 or more eggs. However, comparable stress responses could occur in the field if young instars ingest large numbers of eggs.

The implication of nutrient stress affecting sex ratios and duration of parasitic de-

velopment is of particular importance in developing *in vitro* culture techniques for *M. nigrescens*, even allowing for the fact that this nematode is parthenogenetic.

LITERATURE CITED

1. Baylis, H. A. 1944. Observations on the nematode *Mermis nigrescens* and related species. *Parasitology* 36:122-132.
2. Baylis, H. A. 1947. The larval stages of the nematode *Mermis nigrescens*. *Parasitology* 38:10-16.
3. Christie, J. R. 1929. Some observations on sex in the Mermithidae. *J. Exp. Zool.* 53:59-76.
4. Craig, S. M., and J. M. Webster. 1974. Inhibition of molting of the desert locust, *Schistocerca gregaria*, by the nematode parasite *Mermis nigrescens*. *Can. J. Zool.* 52:1535-1539.
5. Denner, M. W. 1968. Biology of the nematode *Mermis subnigrescens* Cobb. Ph.D. Thesis, Iowa State University, Ames.
6. Gordon, R., and J. M. Webster. 1971. *Mermis nigrescens*: Physiological relationship with its host, the desert locust, *Schistocerca gregaria*. *Expl. Parasit.* 29:66-79.
7. Gordon, R., J. M. Squires, S. J. Babie, and I. R. Burford. 1981. Effects of host diet on *Romanomermis culicivorax*, a mermithid parasite of mosquitoes. *J. Nematol.* 13:285-290.
8. Johnson, R. N., and D. R. Viglierchio. 1969. Sugar beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants. II. Selected environmental and nutritional factors affecting development and sex ratio. *Nematologica* 15:144-152.
9. Parenti, U. 1962. Ricerche sulla sessualita dei Mermithidi. II. Infestazione sperimentale di *Paramermis contorta* in *Chironomus tentans*. *Boll. Zool.* 29:453-462.
10. Parenti, U. 1965. Male and female influence of adult individuals on undifferentiated larvae of the parasitic nematode *Paramermis contorta*. *Nature* 207:1105-1106.
11. Petersen, J. J. 1972. Factors affecting the sex ratios of mermithid parasites of mosquitoes. *J. Nematol.* 4:83-87.
12. Petersen, J. J. 1977. Effects of host size and parasite burden on sex ratio in the mosquito parasite *Octomyomermis muspratti*. *J. Nematol.* 9:343-346.
13. Petersen, J. J., and H. C. Chapman. 1970. Parasitism of *Anopheles* mosquitoes by a *Gastromermis* sp. (Nematoda: Mermithidae) in southwestern Louisiana. *Mosquito News* 30:421-424.
14. Petersen, J. J., H. C. Chapman, D. B. Woodward. 1968. The bionomics of a mermithid nematode of larval mosquitoes in southwestern Louisiana. *Mosquito News* 28:346-352.
15. Strelkov, A. 1964. Biology of *Filipjevimermis singularis* sp. nov. found in the Rybinski Reservoir, Vestn. Leningrad. Univ. Serv. Biol. No. 3. 19:55-69.