THE HOST-PARASITE RELATIONSHIP BETWEEN MERMIS NIGRESCENS DUJARDIN AND THE DESERT LOCUST, SCHISTOCERCA GREGARIA FORSKÅL.

by

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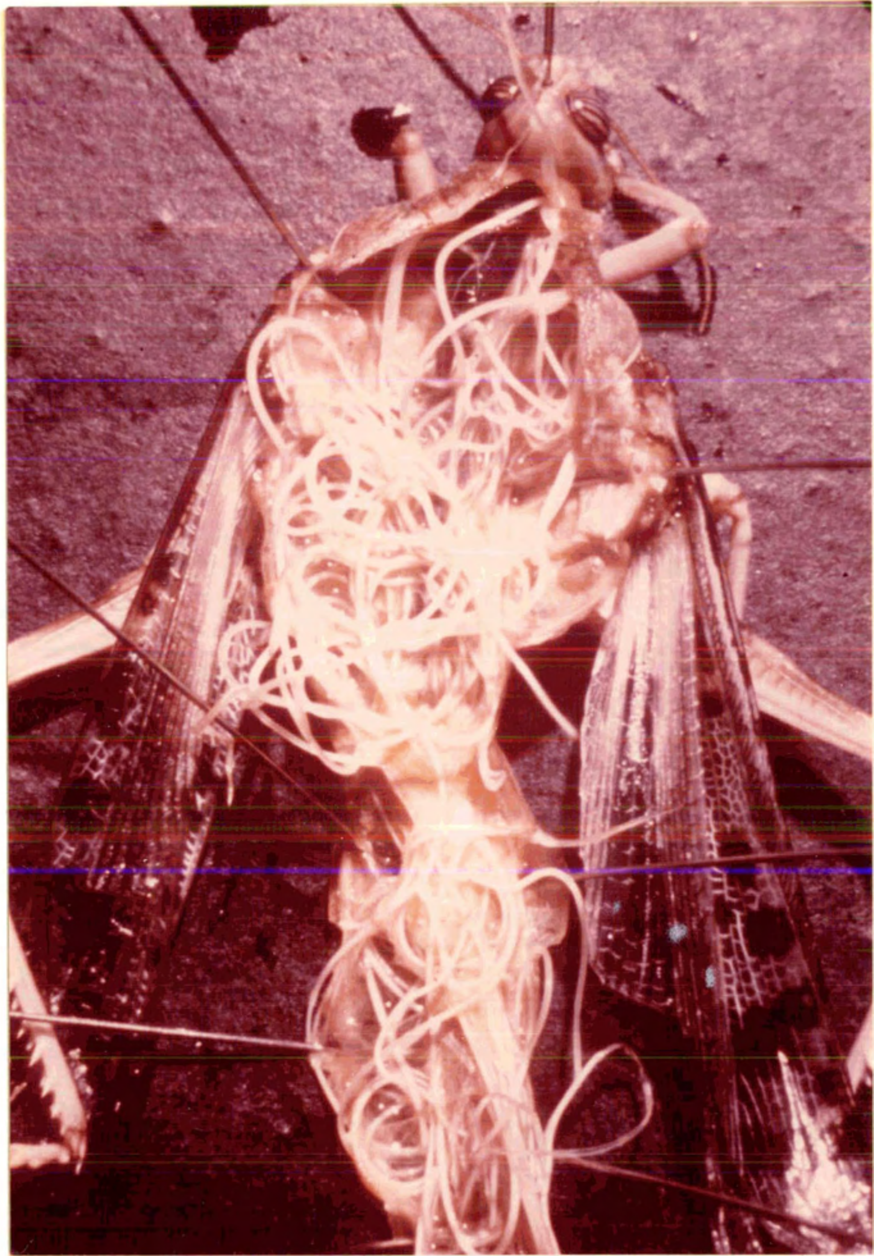
Frontispiece

Adult desert locust infected with *Mermis nigrescens*



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ABSTRACT

There are several important aspects of the host-parasite association between *Mermis nigrescens* and the desert locust, *Schistocerca gregaria*, which must be investigated before this nematode can be cultivated *in vivo* for use in the biological control of the desert locust. The purpose of this investigation was to study the development of *M. nigrescens* in *S. gregaria* in the laboratory, to determine the interaction between *M. nigrescens* and *S. gregaria*, and to determine the mechanisms of *M. nigrescens* pathogenicity on *S. gregaria*.

M. nigrescens eggs hatched within 3 1/2 to 6 hours after they were fed to *S. gregaria* adults, in the region of the host gut between the posterior end of the crop and the point of origin of the Malpighian tubules. The nematode penetrated into the haemocoel in the same region as egg hatching occurred, but 1 to 20 hours later. Parasite eggs remained viable for up to 27 months in dry storage at 5°C and 55-60% R.H.

Several factors influenced the duration of the parasitic phase and the ultimate size of the nematode in the locust. As the number of parasites increased the total development time shortened. Infected locusts reared at 35°C shortened the total parasite development time and significantly

lengthened the nematodes as compared with those reared at 22°C. Parasites remained for a significantly longer period of time and grew significantly more in length in female hosts than in male hosts. They remained for a significantly longer time in older adult hosts than in ones that had just completed the imaginal moult. Larvae developing from eggs fed to adult locusts had a faster growth rate and grew significantly more in length than those developing from eggs fed to nymphal hosts. The duration of the parasitic phase was dependent on the nutritional state of the host. Nematodes increased significantly in length and remained a significantly longer period of time in two consecutive hosts than did nematodes that had developed solely in one host.

The sex ratio of the developing nematode larvae was influenced by the number of nematode parasites in the host, and by the weight or age of the host. Increase in the infective burden (i.e., the number of nematodes) resulted in more males, but the minimum infective dose required to cause male nematodes to develop was independent of host weight below a certain dose level, and strongly dependent on host weight above that level within each host age class.

Depending on host age at infection, there was a

certain minimal infective dose above which the next-but-one host moult was inhibited. The older the host at infection, the higher the burden of parasites required to inhibit moulting. *M. nigrescens* was demonstrated to inhibit host moulting by significantly decreasing the rate of fat body protein synthesis, by decreasing the rate of amino acid precursor incorporation from the haemolymph and concurrently, by possibly stimulating catabolism of fat body proteins to provide a dietary source of amino nitrogen for its own development.

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GENERAL INTRODUCTION

Schistocerca gregaria Forskål., the desert locust, has been an important crop pest for at least 3000 years throughout the hotter part of the Old World including most of Africa. Populations of the desert locust fluctuate in numbers as a result of the effects of many factors including those of predators, parasites and microorganisms. However, these factors have been insufficient to reduce the damage potential caused by *S. gregaria* to an ecocomically acceptable level over a long period. Those few reports that have claimed high mortality to desert locust numbers have been the result of laboratory studies and/or small field tests. Although several organisms show potential in controlling high density locust populations in the field, quantitative estimates of their effectiveness is lacking.

Entomophilic nematodes have great potential for use as biological control agents against agricultural insect pests (Welch, 1962a, 1965; Poinar, 1971; Nickle, 1972). One family, the Mermithidae, parasitize crustaceans, spiders and snails (Chatterjee and Singh, 1965) but, are primarily insect parasites. Mermithids always kill their hosts upon emergence from them. They occur as internal larval parasites, but as adults are free-living in soil or fresh water. In some species, the larvae grow up to 15 cm in length. They derive their

nutrients from the host's haemocoel and subsequently cause major changes in host metabolism, physiology and behavior (Welch, 1965; Poinar, 1971) and thus, retard host development and egg production (Strickland, 1911; Christie, 1936; Welch, 1960; Denner, 1968). Strickland (1911) noted abnormal organ development and partial degeneration of the thoracic flight muscles in mermithid infected blackflies (Diptera: Simuliidae). Welch (1960) reported total fat body depletion in mosquitoes infected with _Hydromermis churchilliensis_. Earlier work frequently described suppression or resorption of infected host oocytes (Christie, 1936, 1937; Crowcroft, 1948). Phelps and DeFoliart (1964) found total fat body depletion and oocyte resorption in female hosts and suppression of testes in the males in mermithid-parasitized blackflies. Poinar and Gyrisco (1962) described similar changes in the alfalfa weevil attacked by _Hexamermis arvalis_. A single mermithid worm invariably kills the host when emerging from it, probably as a result of the loss of host body fluids. Apart from the observed effects of mermithids on host tissues, the most commonly manifested result of mermithid parasitism is physiological castration of the female hosts. Thus, mermithids have the potential of regulating pest species by both rendering the host sterile and eventually killing the survivors.

Since mermithids have a world-wide distribution, their presence is coincident with that of many susceptible host insects. Mermithids are obligate parasites, are relatively host specific and up to 100% parasite incidence has been reported (Sugiyama, 1956; Wülker, 1961; Welch, 1962b; Rubtsov, 1964; Welch and Rubtsov, 1965). Although little is known about the effects of the physical environment on survival of mermithid eggs, some laboratory studies indicate that prolonged storage is feasible (Petersen, Chapman and Woodard, 1968; Petersen, 1972).

Although there are no records of naturally occurring nematode parasites of the desert locust, there are several reports of the natural occurrence of mermithids among grasshoppers (Christie, 1936, 1937; Baylis, 1944, 1947; Smith, 1944, 1947; Denner, 1968). Mermis subnigrescens is a common parasite of grasshoppers in the United States (Christie, 1937). Gravid females migrate to the soil surface, climb the vegetation and deposit their eggs, which are ingested by feeding grasshoppers. The eggs hatch in the alimentary tract of the host, and the young larvae penetrate the gut wall and develop in the body cavity. The parasite remains in the host from 4 to 10 weeks. Parasites kill the host on emergence and complete the remainder of their development in the soil. Denner (1968) observed a

decrease in egg production in mermithid-infected grasshoppers which he attributed to the presence of nematode exudates and secretions. The length of the *M. subnigrescens* parasite was dependent on host size and on the number of parasites per host. Pathological effects of *M. subnigrescens* infection are inhibition of gonad development, sterility and longer nymphal instars. *M. subnigrescens* is an important factor in grasshopper control throughout the parasite's host range (Christie, 1937).

The life cycle of *M. nigrescens* is very similar to that of *M. subnigrescens*. Both parasites have been reported as playing an important natural role in suppressing grasshopper populations (Christie, 1937; Baylis, 1944, 1947; Denner, 1968), and *M. nigrescens* has also been reported to parasitize the earwig, *Forficula auricularia* (Crowcroft, 1948). *M. nigrescens* and *M. subnigrescens* may be the same species (Baylis, 1947; Nickle, 1972) or may be separate species (Cobb, 1926; Denner, 1968).

Gordon and Webster (1971) studied the physiological relationship between *M. nigrescens* and *S. gregaria*, an unnatural host. *M. nigrescens* parasitism caused a depletion of total haemolymph carbohydrates in male and female hosts during the time when the most active nematode growth was occurring. Suppression of oocyte development and oocyte

resorption were noted in female hosts. Fat body proteins and amino acids were decreased in infected hosts during the latter stages of infection, and parasitism caused a significant reduction in the level of glycogen and non-glycogen carbohydrates in the host fat body (Gordon and Webster, 1971; Gordon, Webster and Mead, 1972). They hypothesized that the nematode, by feeding on the blood carbohydrates of the host, caused decreased glycogenesis in the host fat body, thus depriving that organ of carbohydrates.

Despite this recent evidence, there remains several important aspects of the host-parasite association to be investigated before *M. nigrescens* can be cultivated *in vivo* for use in the biological control of *S. gregaria*. Therefore, my objectives were: I. To study the development of *M. nigrescens* in *S. gregaria* in the laboratory. II. To determine the interaction between *M. nigrescens* and *S. gregaria*, and III. To determine the mechanisms of *M. nigrescens* pathogenicity on *S. gregaria*.

GENERAL MATERIALS AND METHODS

1. Rearing of *Schistocerca gregaria*

The locust colony, obtained initially from the University of British Columbia, was reared in the insectary under constant light at a temperature of 34-36°C, a relative humidity of 60-70%, and on a mixed diet of whole wheat bran and laboratory-grown grass. The diet was chosen so as to preclude the possibility of exposing the locusts to a natural mermithid infection. The diet throughout the study remained the same. The bran mixture was composed of 6 parts whole wheat bran, 2 parts grass meal, 1 part white brewer's yeast and 1 part powdered milk (Hunter-Jones, 1956).

In all experiments, male and female locusts were maintained in separate cages.

2. Collection of *Mermis nigrescens* eggs

Adult *M. nigrescens* females were collected from a newly ploughed field in Chilliwack[1], British Columbia, in early June of 1970, 1971, and 1972. The nematodes were found on the

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[1] Collections made by courtesy of Mr. E. Bollerup, 50105 Patterson Road, Chilliwack, British Columbia.

soil surface either during or immediately after a period of early morning rain. The females which emerged from the soil to commence egg laying on the nearby foliage were collected and placed on a moistened, filter-paper-lined, Petri dish in which they laid eggs. The infective eggs were washed three times with sterile water and centrifuged at low speeds after each washing to remove debris and surface contaminants, and then stored dry on filter-paper-lined Petri dishes at 5°C until required.

3. Infection of *Schistocerca gregaria* with mermithid eggs

Mermithid eggs were transferred, using a fine-tipped disposable Pasteur pipette under a stereoscopic microscope, onto small pieces of laboratory-grown grass coated with a thin layer of a Gelgard M - water suspension (Webster and Bronskill, 1968) to facilitate adhesion of the eggs. This enabled a precise dosage of *M. nigrescens* eggs to be given to each locust. Locusts, prestarved for 4 hours, were fed the mermithid-infected grass. Non-infected control groups were set up in separate cages for each experiment.

4. Statistical analysis of results

The data presented in this study were analyzed statistically using Student's t-test, with significance at the 0.05 (5%), 0.01 (1%) and 0.001 (0.1%) levels being accepted and indicated in the tables and figures by the symbols a, b and c as follows:

Significance at the 0.05 (5%) level .... a

Significance at the 0.01 (1%) level .... b

Significance at the 0.001 (0.1%) level.. c

SECTION I

The effects of selected external factors and the age, sex, and nutritional state of the desert locust on the development of *Mermis nigrescens*.

INTRODUCTION

The eggs of *M. nigrescens*, unlike those of most other mermithids (Phelps and DeFoliart, 1964; Trpis, Haufe and Shemanchuk, 1968), are swallowed by the host during feeding and they then hatch in the gut and the larvae penetrate into the haemocoel. Previously documented studies on the development of *M. nigrescens*, however, are unclear as to the development of the parasite during the first 4 days after infection. Of particular importance is the fact that there is inconclusive evidence as to the site of egg hatching and subsequent gut wall penetration. Christie (1937) states that the eggs hatched 1-1 1/2 hours after ingestion by the host and that the larvae penetrated the intestinal wall of the grasshopper, *Melanoplus femur-rubrum* (DeGeer) 20 minutes later. Baylis (1947) found that when the eggs of *M. nigrescens* infect *F. auricularia*, they hatch in the gut, and the larvae actively migrate through the gut wall into the body cavity. Inside the haemocoel, the larvae changed little for 2 days, except that the cellular contents of the body showed signs of being arranged in a longitudinal series of irregular, solid masses. Neither the region(s) of the gut where penetration of the *M. nigrescens* larvae into the haemocoel occurs nor the factor(s) that govern egg hatching have been elucidated.

There is little information on the viability of mermithid eggs under laboratory conditions. Petersen *et al*., (1968) found that the eggs of *Romanomermis* sp., which parasitize the aquatic larvae of Simuliids, could be stored in sand moistened with distilled water for at least 6 months at ambient temperatures with no significant loss in egg viability. Muspratt (1965) noted that the eggs of an unidentified mermithid parasite of the mosquito, *Culex pipiens*, survived storage in the laboratory for several months if the eggs were placed in a box of sand of high water content and slowly desiccated. There is no published record of long-term storage of *M. nigrescens* eggs in the laboratory.

The size of the *M. nigrescens* larvae upon emergence from the host has been postulated as being governed by the initial infective burden (Christie, 1929; Baylis, 1944; Denner, 1968). Gupta and Burr (unpublished observation) have shown that the infective dose of eggs and the host category (by age and weight) of *S. gregaria* govern the emergence size of the *M. nigrescens* larvae. Christie (1937) claims that *M. subnigrescens* larvae grew larger and remained longer in a large grasshopper than in a small one, and also that the fewer nematodes that a grasshopper harboured, the larger these worms became and the longer they remained within the host. Other researchers, using

a variety of other hosts of mermithids, have reached similar conclusions (Stabler, 1952; Welch, 1960, 1965; Denner, 1968; Trpis *et al*., 1968; Petersen and Willis, 1970).

Several reports have indicated that mermithid parasites are more often found in one developmental stage of the host than in another (Christie, 1936, 1937; Welch, 1960; Poinar and Gyrisco, 1962; Petersen *et al*., 1968; Petersen and Willis, 1970). Whether one developmental stage of *S. gregaria* is more susceptible to *M. nigrescens* parasitism than another stage is not known.

There are conflicting reports in the literature on the effect of the sex of the host on the development of the mermithid parasite. Parasitism of the grasshoppers *Melanoplus atlanis* and *M. bivittatus* by the nematode *Mermis ferruginia* resulted in 45% of the female hosts being infected but only 9% of the males (Glaser and Wilcox, 1918). Christie (1936), however, found no significant difference in percent parasitism of male and female hosts in several species of short-horned grasshoppers (Orthoptera: Acrididae) when infected with *A. decaudata*.

In order to clarify some of the confusion in the literature, a series of experiments were undertaken using *M. nigrescens* in the desert locust under controlled conditions. An experiment was set up to determine the site of parasite egg hatching

and subsequent larval penetration into the host haemocoel and the factors that control these events. The effects of long-term storage of M. nigrescens eggs on their viability and the temperature at which parasite development occurs most rapidly are two areas about which nothing is known, yet which must be elucidated before M. nigrescens can be mass produced in the laboratory. Effects of parasite burden, age, sex and weight of the desert locust on the duration of M. nigrescens development and on the size of the nematode larvae upon emergence from the host are also areas which must be clarified. The results of these investigations are presented in this section.

MATERIALS AND METHODS

1. M. nigrescens egg viability

Thirty-five, 7-day-old adult female locusts were each infected with fifty nematode eggs, dissected 25 days later and the total number of developing nematode larvae in the haemocoel counted. M. nigrescens eggs which had been stored for 3, 15, 27 and 39 months at 5°C and 55-60% R. H. were used to infect the locusts.

2. Development of the parasitic phase of M. nigrescens

All developmental studies were done using 1-day-old adult male locusts as hosts, each infected with fifty eggs. All measurements were done using a dissecting microscope and an ocular micrometer. In growth determinations, each locust was dissected in insect saline solution (Appendix I) at different times after infection. The larvae from each host were sexed and then heat-killed prior to measuring. Nematodes that had developed for less than 7 days were processed as described below.

To determine egg hatching time, M. nigrescens eggs were dipped in a 1% solution of Sudan black or methylene blue and the excess stain removed by three water washings. Locusts were killed at different times after infection, ranging from 20 minutes to 24 hours. At each time interval, the gut was removed and the total number of egg casings recorded. To identify the gut region in which most of the eggs hatched, locusts infected with fifty eggs were dissected immediately from the ventral surface in insect saline solution and the gut ligatured in one of the following places:- Anterior end of the esophagus, posterior end of the esophagus, posterior end of the crop, posterior end of the proventriculus, posterior to the point of origin of the Malpighian tubules, midway along the hindgut and at the junction of the hindgut and rectum (See Figure I).

Each section of the gut was dissected open either 7, 12, or 24 hours after ligaturing, and the number of egg casings counted. Seventy locusts were used in the study.

Nematode development up to Day 4 of infection was studied by two methods. Two hours after infection, locusts were dissected and the gut ligatured anterior to the pharynx and posterior to the rectum. The entire gut was then placed in insect saline solution in a glass Petri dish. Passage of nematodes through the gut wall was observed under the compound microscope using transmitted light. In this experiment, thirty-six locusts were used. In another experiment, infected locusts were decapitated at intervals from 6 hours to 5 days after infection, the anterior of the gut ligatured, and the entire locust body centrifuged at low speed to extract the insect haemolymph and nematode larvae. Measurements of the mean length and mean maximum width of the larvae were made as previously. In some instances, the addition of 0.5% Cotton blue facilitated location of larvae in the extracted haemolymph. Twenty-nine locusts were used in this experiment.

The effect of parasite burden on nematode development was studied in 1-day-old adult male and female locusts infected with ten, thirty or fifty nematode eggs. The mean

number of emergent nematode larvae and the duration of their parasitic development were observed for each locust. Between three and six locusts were used in each study.

All hosts used in temperature effect studies were 1-day-old adults of both sexes. Each locust was infected with fifty nematodes and reared at 22°C or 35°C. The mean number of emergent larvae, their mean parasitic development time and their mean length and mean maximum width were recorded.

The effects of host sex on *M. nigrescens* development was studied using twenty, 1-day-old adult locusts of each sex and twenty, 28-day-old adult locusts of each sex. Each host was infected with ten, thirty or fifty nematode eggs each, respectively, and reared at 22°C or 35°C.

The effect of host age on *M. nigrescens* was studied in locusts of five ages: One-day-old third instar nymphs, 4-day-old fourth instar nymphs, 5-day-old fifth instar nymphs, 1-day-old adult male and female locusts and 28-day-old adult male and female locusts. Twenty locusts from each group and sex were reared in separate cages. Each host was fed fifty parasite eggs and the mean number of emergent larvae, their development time and their mean length and mean maximum width were recorded.

3. <u>Effect of transferring *M. nigrescens* larvae to a second host on the total development time and the size of the parasite</u>.

Twenty-five days after infection of twelve, 1-day-old adult male and female locusts with fifty nematode eggs, the hosts were killed, the nematodes removed, and their mean lengths and mean maximum widths recorded. Uninfected 1-day-old adult locusts were anaesthetized in CO_2, and one, three, five or ten nematodes inserted into the haemocoel through a 5 mm abdominal incision. The incision was daubed with chloramphenicol crystals to stop infection and then sealed with melted paraffin wax. One set of controls consisted of 1-day-old adult locusts infected with either one, three, five or ten nematodes. The total development time and the mean length and mean maximum width of emergent worms were recorded. A second set of controls were sham operated but not infected.

RESULTS

1. *M. nigrescens* egg viability

Eggs stored dry at 5°C and 55-60% R.H. for up to 15 months showed no significant loss in viability, as indicated by the number of nematode larvae found in the haemocoel (Table I). Eggs stored for 27 or 39 months under similar (pre-infection) conditions showed a significant ($P<0.001$) decrease in viability. The longer the time of storage, the greater was the range of viability of the eggs from host to host. Eggs used after 39 months storage ranged in viability from 20-60%, as opposed to 85-95% after 3 months.

2. Development of the parasitic phase of *M. nigrescens*

The nematode eggs hatched in the region of the host gut between the posterior end of the crop and anterior to the point of origin of the Malpighian tubules 3 1/2 to 6 hours after ingestion (Fig. 1). Ligaturing experiments indicated that the hatching of the *M. nigrescens* eggs in the desert locust gut was site specific. Eggs did not hatch in other parts of the gut, even 24 hours after infection. When a ligature was made at the posterior end of the crop immediately after feeding the eggs to the host, intact eggs

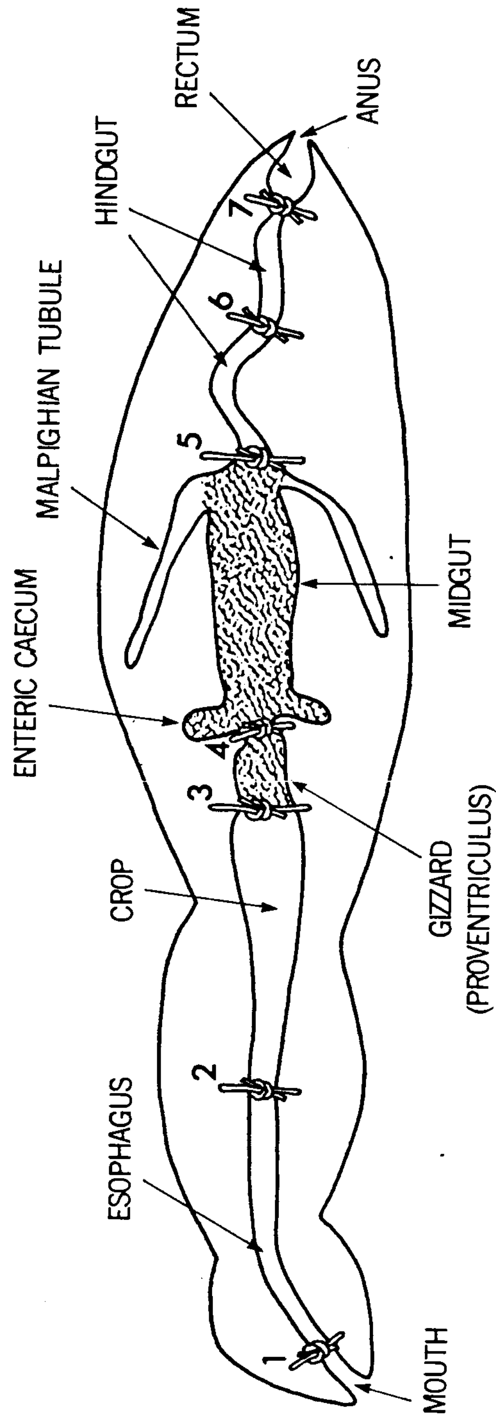
TABLE I

The effect of duration of storage at 5°C on the viability of fifty *Mermis nigrescens* eggs in 7-day-old adult *Schistocerca gregaria* hosts. Locusts examined 25 days after infection.

Period of Storage	No. Locusts Examined	Mean Number of Parasites Developing in the Host ± S.E.[1]
3 months	10	45.00 ± 2.57 c',c (90.0%)
15 months	8	41.38 ± 3.94 a,b (82.8%)
27 months	7	32.00 ± 3.12 b,c (64.0%)
39 months	8	22.00 ± 8.89 a,c' (44.0%)

[1] Means followed by the same letter are significantly different.

Figure 1. Diagrammatic representation of the digestive system of *Schistocerca gregaria*, showing ligation sites (numbered one to seven). Region marked by shading indicates where *Mermis nigrescens* eggs hatch and larvae penetrate through the gut wall into the haemocoel.


RECTUM
ANUS
HINDGUT
7
6
MALPIGHIAN TUBULE
5
MIDGUT
ENTERIC CAECUM
4
3
GIZZARD
(PROVENTRICULUS)
CROP
2
ESOPHAGUS
1
MOUTH

but no empty egg casings were recovered anterior to the ligature either 7 or 24 hours after feeding. Similarly, when a ligature was made midway along the hindgut immediately after infection and the gut was dissected 12 hours later, the majority of empty egg casings and all of the nematode larvae detected were observed in the midgut. When ligatures were made at positions three and five (Fig. 1) and 12 hours later the ligature at position three was severed, all the empty egg casings were found in the midgut of the host upon dissection 4 hours later. Since the locust faeces were not examined for egg casings, it was not possible to determine what percentage of the *M. nigrescens* eggs failed to hatch.

Penetration of *M. nigrescens* larvae from the gut into the haemocoel occurred in the same region as egg hatching, and from 1 to 20 hours later. Partial exit of the nematodes from the isolated, infected guts into insect saline solution was observed on several occasions, but complete exit from the gut was never observed. Newly hatched larvae in the gut possessed a stylet, but it was not possible to discern whether gut wall penetration was intercellular or intracellular.

Centrifugation of decapitated locusts to extract the

haemolymph plus nematode larvae enabled almost complete recovery of the young larvae from the haemocoel. Larvae appeared in the haemolymph 48 to 72 hours after ingestion of the eggs by the desert locust. Very little growth or differentiation of the larvae occurred during the first 4 days after infection.

As the infective dose of *M. nigrescens* eggs was increased, the duration of the parasitic phase was significantly shortened in both male and female hosts (Table II). There was no significant difference in the percentage of nematodes that emerged from each host infected with different numbers of nematode eggs (Table II).

In 1-day-old adult male, but not female hosts, the duration of the parasitic phase was significantly shorter at 35°C than at 22°C (Table III). There was no significant effect of rearing temperature on the total number of emergent parasites (Table III). Although the nematode's development time was shorter at 35°C than at 22°C, there was no significant difference in their daily growth patterns in hosts of either sex (Fig. 2) (as based on mean length and mean maximum width). The greatest increase in length of the developing larvae occurred between Days 11 and 17 after egg ingestion when a nine to ten-fold increase occurred. Similarly, the

TABLE II

The effect of the infective dose of *Mormis nigrescens* on the duration of the parasitic phase. One-day-old *Schistocerca gregaria* adults infected, and reared at 35°C.

Sex of Host	No. Locusts Infected	Infective Dose	Mean No. Larvae Emerging ± S.E.	Mean Duration of Parasitic Phase (Days) ± S.E.[1]
Male	4	10	7.75 ± 0.83 (77.5%)	32.75 ± 1.79 c'
Male	3	30	26.67 ± 2.49 (80.0%)	31.33 ± 0.92 c
Male	6	50	46.50 ± 2.36 (93.0%)	23.33 ± 1.37 c,c'
Female	3	10	8.67 ± 0.47 (86.7%)	37.33 ± 2.05 b
Female	3	30	26.33 ± 2.36 (79.0%)	34.00 ± 1.63 a
Female	6	50	41.67 ± 2.56 (83.3%)	30.00 ± 2.16 b,a

[1] Means followed by the same letter are significantly different.

TABLE III

The effect of rearing temperature on the duration of development of *Mermis nigrescens* larvae in 1-day-old *Schistocerca gregaria* adults infected with fifty eggs.

Sex of Host	No. Locusts Infected	Rearing Temperature	Mean No. Larvae Emerging ± S.E.	Mean Duration of Development (Days) ± S.E.[1]
Male	7	22°C	45.3 ± 1.67 (90.6%)	29.86 ± 1.12 c
Male	6	35°C	46.5 ± 2.36 (93.0%)	23.33 ± 1.37 c
Female	6	22°C	41.0 ± 2.89 (82.0%)	32.83 ± 2.54
Female	6	35°C	41.7 ± 2.56 (83.4%)	30.00 ± 2.16

[1]Means followed by the same letter are significantly different.

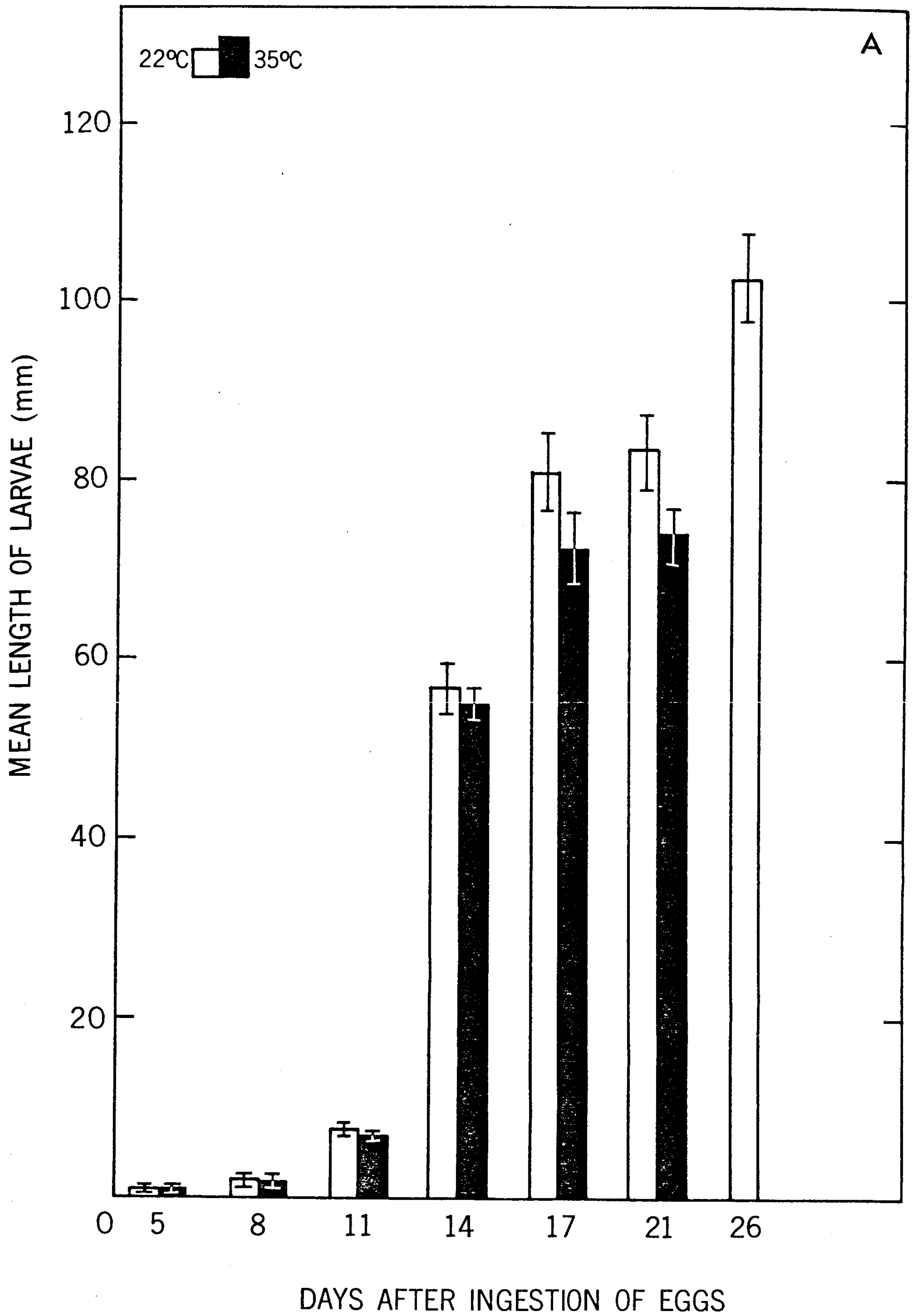
Figure 2. Effect of rearing temperature on the mean length and mean maximum width of *Mermis nigrescens* larvae in *Schistocerca gregaria* infected with fifty parasite eggs one day after the imaginal moult. Vertical bars represent ± 2 standard errors.

A: Mean length of *M. nigrescens* larvae in a male host; rearing at 22°C and 35°C.

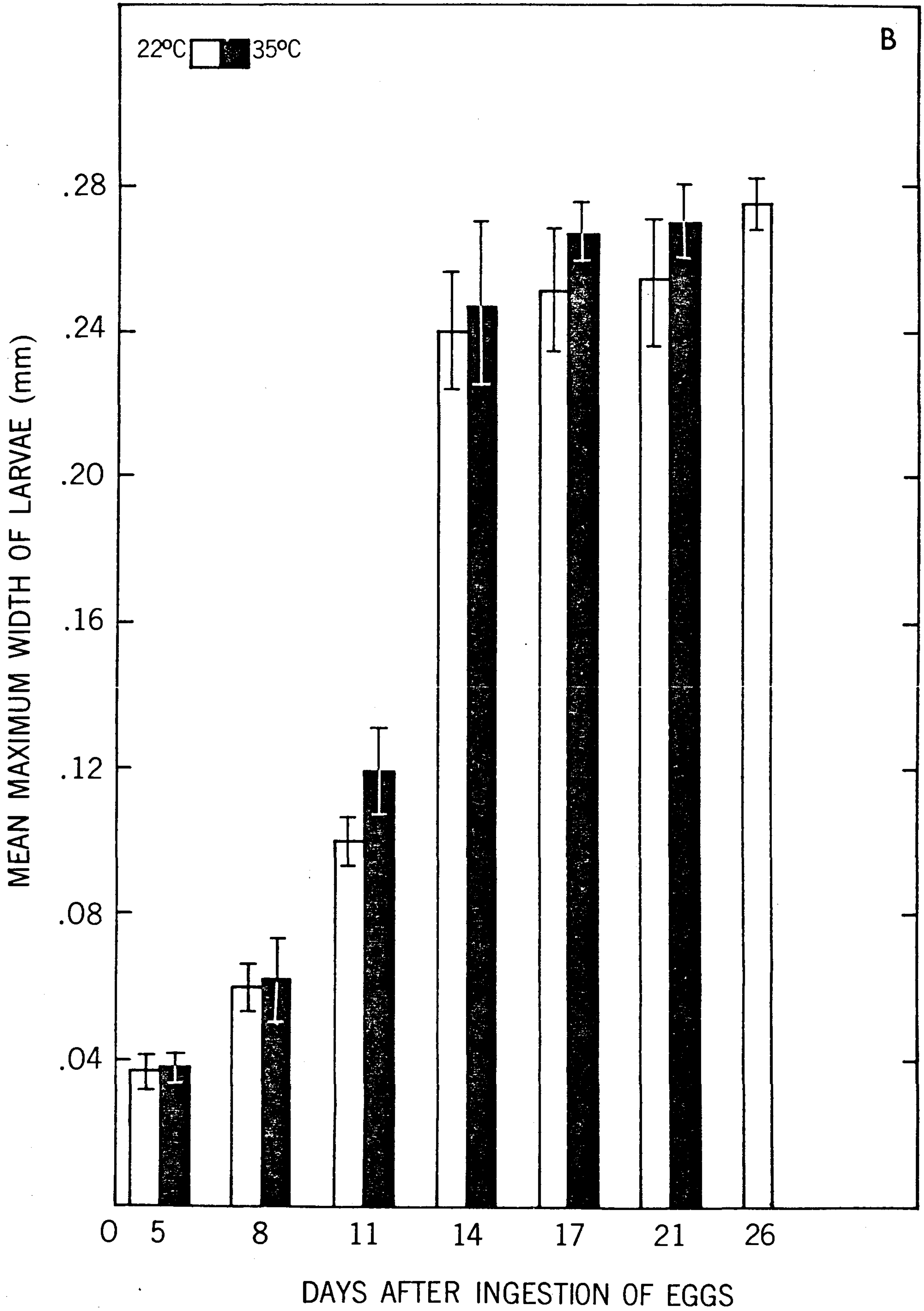
B: Mean maximum width of *M. nigrescens* larvae in a male host; rearing at 22°C and 35°C.

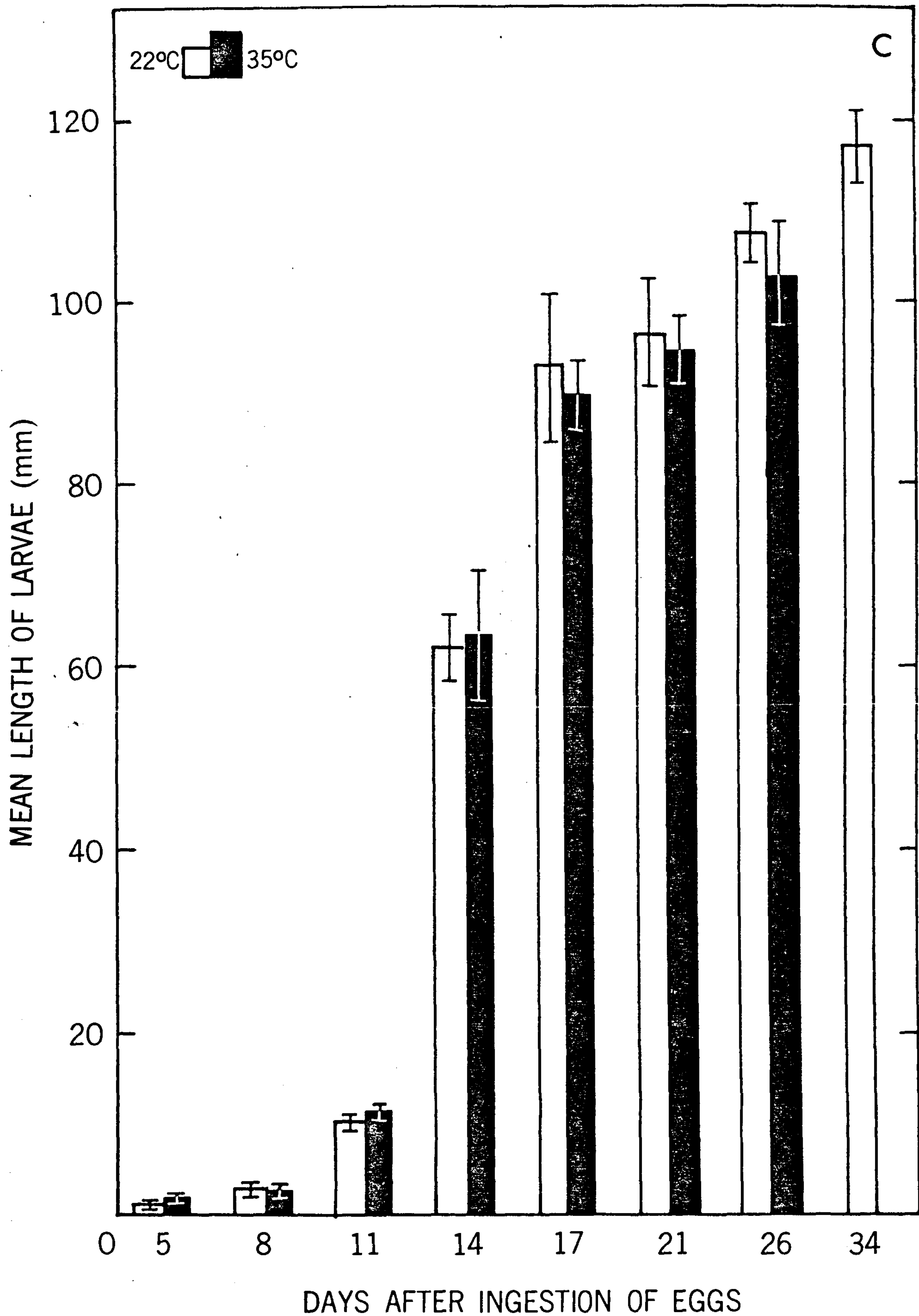
C: Mean length of *M. nigrescens* larvae in a female host; rearing at 22°C and 35°C.

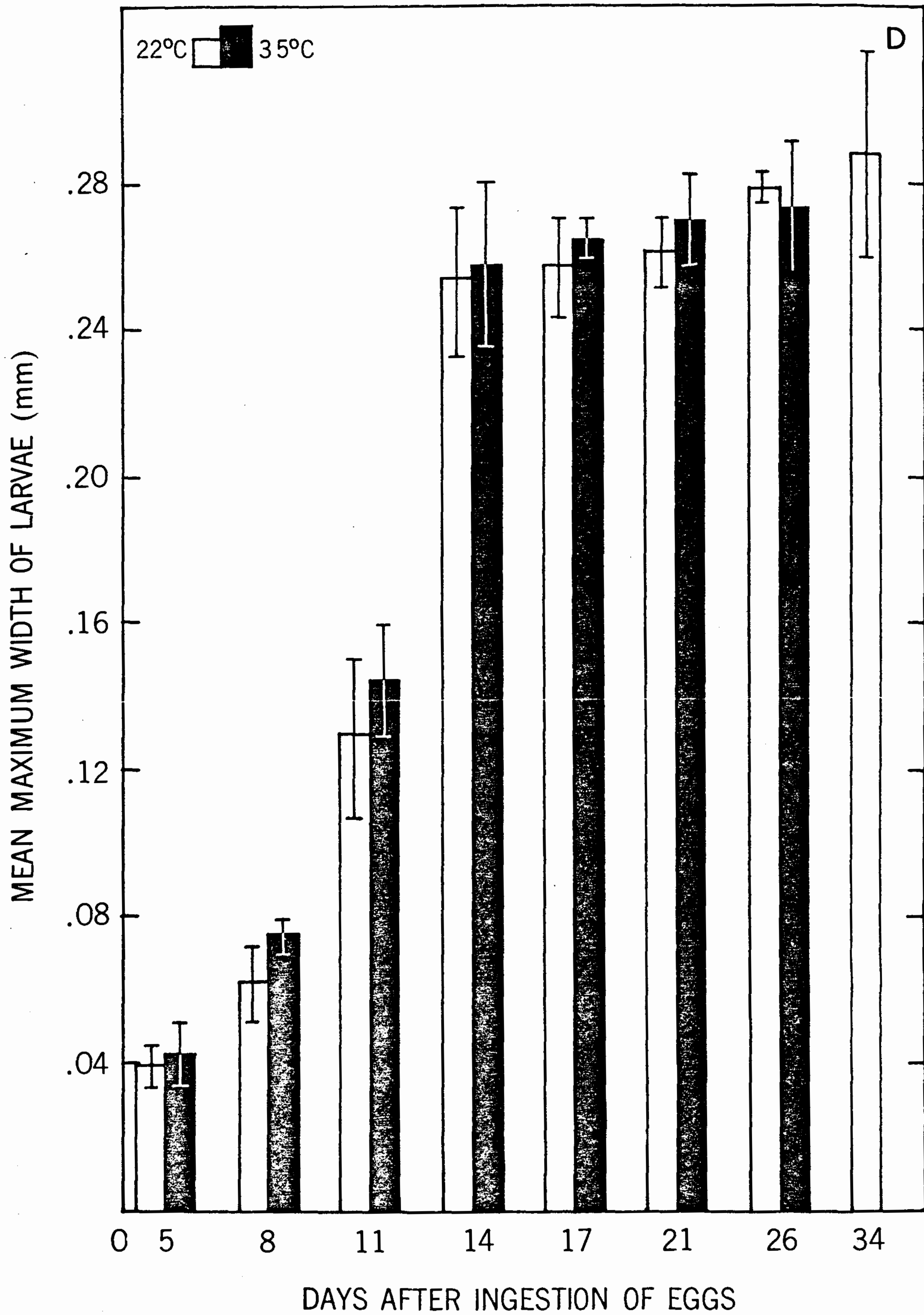
D: Mean maximum width of *M. nigrescens* larvae in a female host; rearing at 22°C and 35°C.



22ºC
35ºC
A
MEAN LENGTH OF LARVAE (mm)
120
100
80
60
40
20
0
5
8
11
14
17
21
26
DAYS AFTER INGESTION OF EGGS


22°C
35°C
B
MEAN MAXIMUM WIDTH OF LARVAE (mm)
.28
.24
.20
.16
.12
.08
.04
0
5
8
11
14
17
21
26
DAYS AFTER INGESTION OF EGGS


22°C
35°C
C
MEAN LENGTH OF LARVAE (mm)
120
100
80
60
40
20
0
5
8
11
14
17
21
26
34
DAYS AFTER INGESTION OF EGGS


22°C
35°C
D
MEAN MAXIMUM WIDTH OF LARVAE (mm)
.28
.24
.20
.16
.12
.08
.04
0
5
8
11
14
17
21
26
34
DAYS AFTER INGESTION OF EGGS

greatest increase in maximum width occurred between Days 11 and 14 after egg ingestion, a 2 1/2-fold increase at both rearing temperatures.

Development time of M. nigrescens larvae was significantly longer in female than in male hosts infected either 1 or 28 days after the imaginal moult with fifty (but not ten or thirty) parasite eggs and reared at 35°C (Table IV). The mean body lengths of the nematodes in female hosts of either infection age were often significantly longer than those in male hosts of the same age (Table V). Host sex had no significant effect on parasite width at any time during larval development.

The age of the host significantly affected the rate of development of the M. nigrescens larvae within locusts infected with fifty nematode eggs. The total development time of the larvae within a third instar host was significantly shorter than within either a fourth or fifth instar host under identical experimental conditions (Table VI). Parasites remained within 28-day-old adult male and female locusts significantly longer than in 1-day-old adults.

The comparative growth rate of M. nigrescens changed with age of the host, with the rapid growth periods being host-age-dependent (Figs. 3, 4). Significant differences

TABLE IV

The effect of the sex of *Schistocerca gregaria* adults on the duration of the parasitic phase of *Mermis nigrescens* larvae reared at 35°C.

Age	Sex	No. of Hosts Infected	Infective Dose	Mean # Emerging from Host ± S.E.	Mean Time of Emergence (Days) ± S.E.[1]
1-day-old Adult	male	6	50	46.5 ± 2.36	23.33 ± 1.37 c
1-day-old Adult	female	6	50	41.7 ± 2.56	30.00 ± 2.16 c
28-day-old Adult	male	6	50	44.7 ± 2.41	27.83 ± 2.28 b
28-day-old Adult	female	5	50	40.0 ± 2.73	38.20 ± 3.36 b
1-day-old Adult	male	4	10	7.8 ± 0.83	32.75 ± 1.79
1-day-old Adult	female	3	10	8.7 ± 0.47	37.33 ± 2.05
1-day-old Adult	male	3	30	26.7 ± 2.49	31.33 ± 0.92
1-day-old Adult	female	3	30	26.3 ± 2.36	34.00 ± 1.63

[1] Means followed by the same letter are significantly different.

TABLE V

Effect of sex of _Schistocerca gregaria_ on the size of _Mermis nigrescens_ larvae. Rearing temperature , 35°C; infective dose, fifty eggs.

Day After Infection	Sex of Host	Host 1-Day-Old Adult At Infection: *Mermis nigrescens* Mean Length ± S.E.[1] (mm.)	Host 1-Day-Old Adult At Infection: *Mermis nigrescens* Mean Max. Width ± S.E. (mm.)	Host 28-Days-Old Adult At Infection: *Mermis nigrescens* Mean Length ± S.E.[1] (mm.)	Host 28-Days-Old Adult At Infection: *Mermis nigrescens* Mean Max. Width ± S.E. (mm.)
Day 5	male	0.71 ± 0.04	0.032 ± 0.003	1.01 ± 0.09	0.044 ± 0.007
Day 5	female	0.82 ± 0.10	0.042 ± 0.006	1.09 ± 0.07	0.053 ± 0.003
Day 8	male	2.39 ± 0.13 c	0.062 ± 0.007	3.57 ± 0.13a	0.071 ± 0.006
Day 8	female	2.84 ± 0.17 c	0.073 ± 0.003	4.02 ± 0.09a	0.083 ± 0.010
Day 11	male	7.59 ± 0.49c	0.119 ± 0.011	9.22 ± 0.27b	0.127 ± 0.011
Day 11	female	10.02 ± 0.52 c	0.144 ± 0.016	11.18 ± 0.41b	0.155 ± 0.017
Day 14	male	55.30 ± 2.34	0.244 ± 0.028	63.03 ± 3.28a	0.250 ± 0.031
Day 14	female	62.15 ± 7.31	0.263 ± 0.031	75.09 ± 2.03a	0.271 ± 0.024
Day 17	male	72.30 ± 4.10c	0.266 ± 0.009	81.85 ± 5.21a	0.257 ± 0.034
Day 17	female	90.40 ± 3.45c	0.271 ± 0.007	92.52 ± 2.79a	0.274 ± 0.034
Day 21	male	74.31 ± 3.93c	0.268 ± 0.012	87.52 ± 4.17a	0.266 ± 0.026
Day 21	female	95.85 ± 4.23c	0.272 ± 0.013	104.31 ± 4.09a	0.280 ± 0.051

continued....

Table V (continued)

Day After Infection	Sex of Host	Host 1-Day-Old Adult At Infection: *Mermis nigrescens* Mean Length ± S.E. (mm.)	Host 1-Day-Old Adult At Infection: *Mermis nigrescens* Mean Max. Width ± S.E. (mm.)	Host 28-Days-Old At Infection: *Mermis nigrescens* Mean Length ± S.E.[1] (mm.)	Host 28-Days-Old At Infection: *Mermis nigrescens* Mean Max. Width ± S.E. (mm.)
Day 26	male	--- ---	--- ---	102.00 ± 5.21	0.272 ± 0.062
Day 26	female	101.40 ± 7.21	0.276 ± 0.019	106.39 ± 6.03	0.291 ± 0.030
Day 28	male	--- ---	--- ---	107.10 ± 4.00 b	0.290 ± 0.024
Day 36	female	--- ---	--- ---	123.65 ± 9.22 b	0.302 ± 0.009

[1] Means within each couplet followed by the same letter are significantly different.

TABLE VI

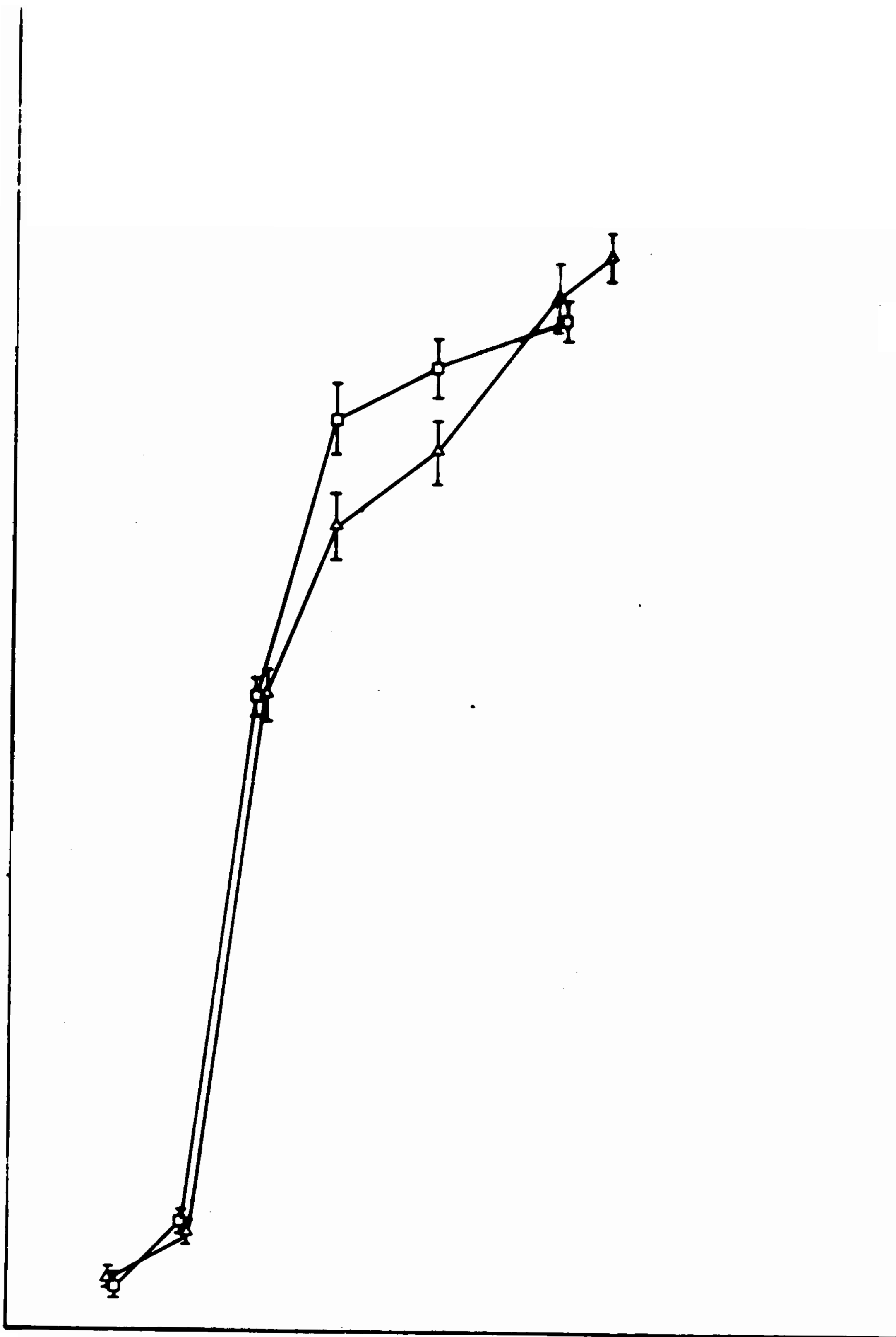
The effect of the age of _Schistocerca gregaria_ on the duration of the parasitic phase of _Mermis nigrescens_ larvae, reared at 35°C., with an infective dose of fifty eggs.

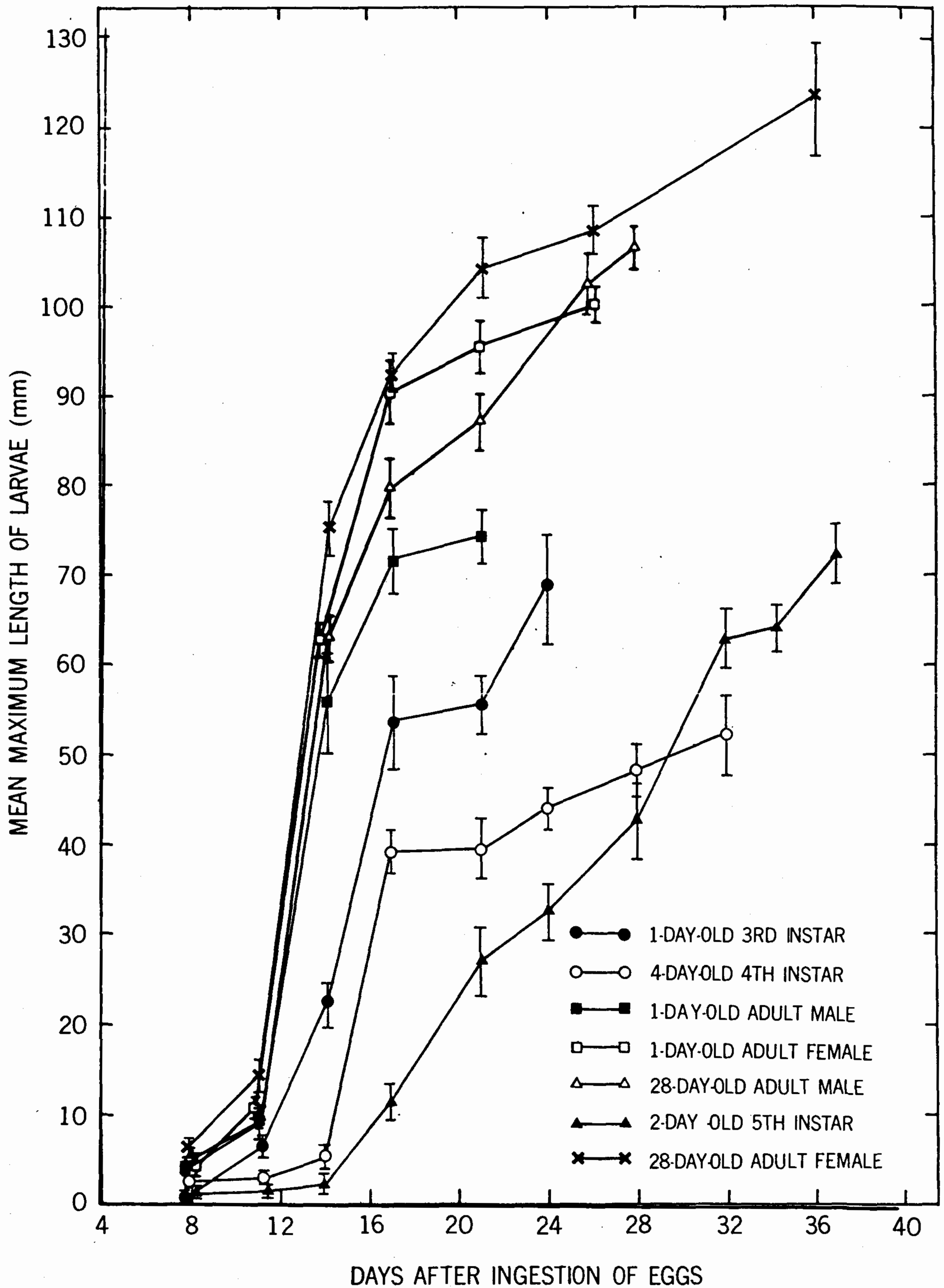
Schistocerca gregaria			*Mermis nigrescens*	
Age at Infection	Sex	No. of Hosts Infected	Mean # Emerging from Host ± S.E.[1]	Mean Time of Emergence (Days) ± S.E.[1]
1-day-old Adult	male	6	46.50 ± 2.36b,c,a'	23.33 ± 1.37 a,c',b'
28-day-old Adult	male	6	44.70 ± 2.41a	27.83 ± 2.28 a,a"
1-day-old Adult	female	6	41.60 ± 2.55	30.00 ± 2.15 c,b"
28-day-old Adult	female	5	40.00 ± 2.73b	38.20 ± 3.36 c,c",c"'
2-day-old 5th Instar Nymph	---	4	40.62 ± 1.36a,c	33.00 ± 3.11 b,c'
4-day-old 4th Instar Nymph	---	6	40.90 ± 2.91	30.50 ± 1.57 a',c",b'
1-day-old 3rd Instar Nymph	---	7	30.10 ± 2.37[2]	22.33 ± 2.04 a', b, a", b",c"'

1 Means followed by the same letter are significantly different.

2 Significantly different ($p<0.001$) from the number of _M. nigrescens_ larvae emerging from hosts of all other ages.

Figure 3. Effect of the age of Schistocerca gregaria on the mean length of Mermis nigrescens larvae. Rearing temperature, 35°C; infective dose, fifty eggs. Vertical bars represent ± 2 standard errors.




MEAN MAXIMUM LENGTH OF LARVAE (mm)
DAYS AFTER INGESTION OF EGGS
1-DAY-OLD 3RD INSTAR
4-DAY-OLD 4TH INSTAR
1-DAY-OLD ADULT MALE
1-DAY-OLD ADULT FEMALE
28-DAY-OLD ADULT MALE
2-DAY OLD 5TH INSTAR
28-DAY-OLD ADULT FEMALE
0
10
20
30
40
50
60
70
80
90
100
110
120
130
4
8
12
16
20
24
28
32
36
40

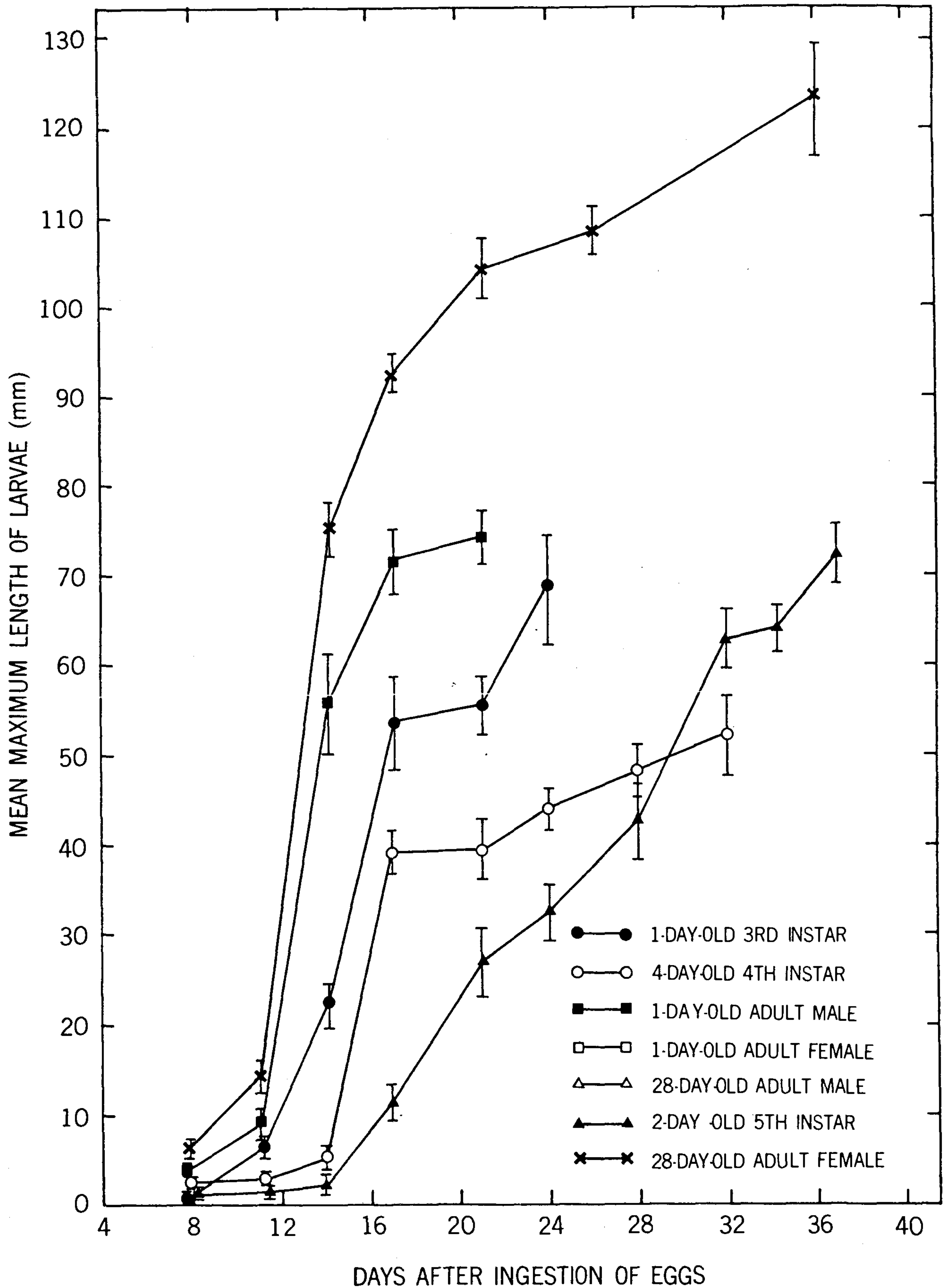
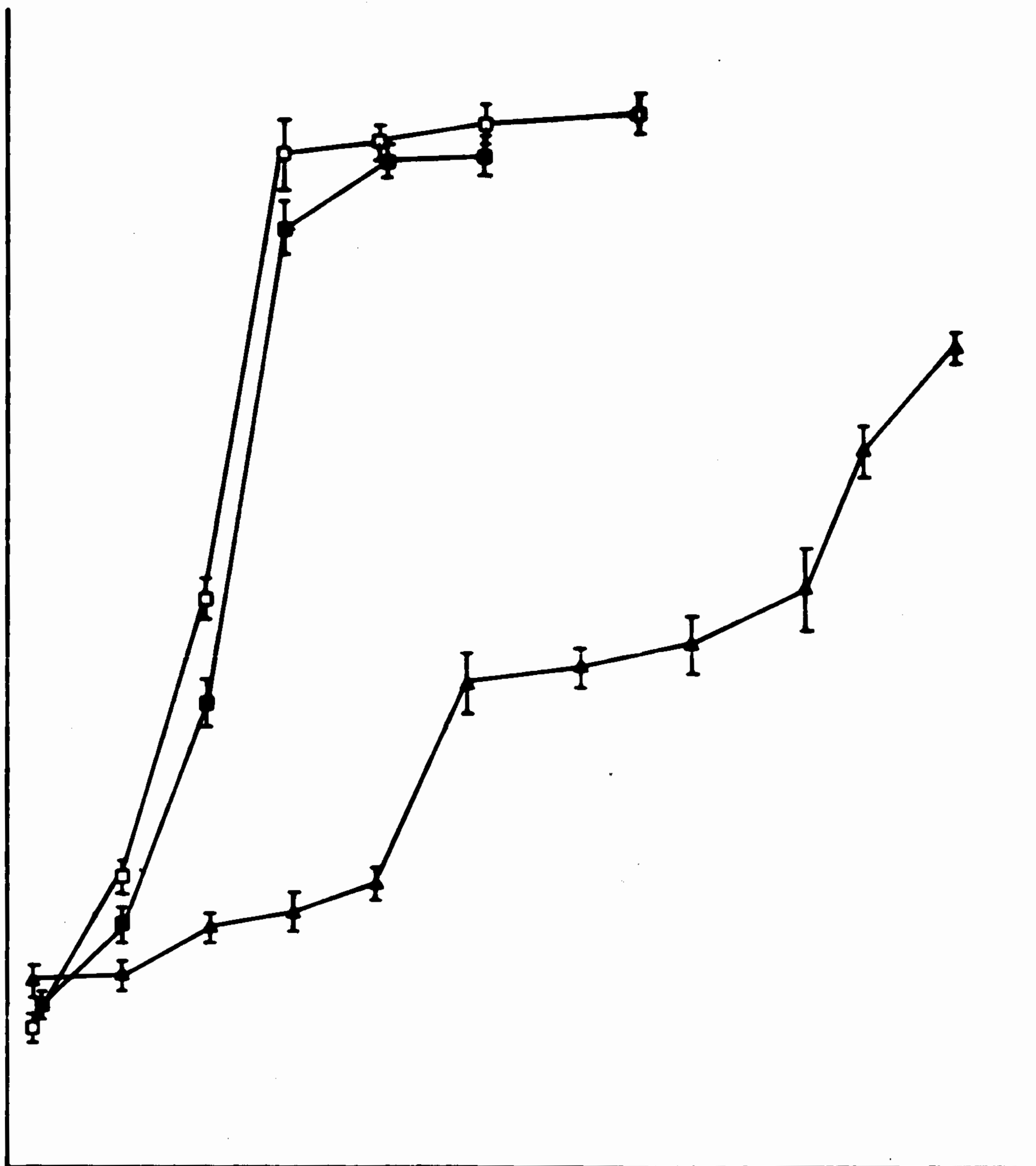
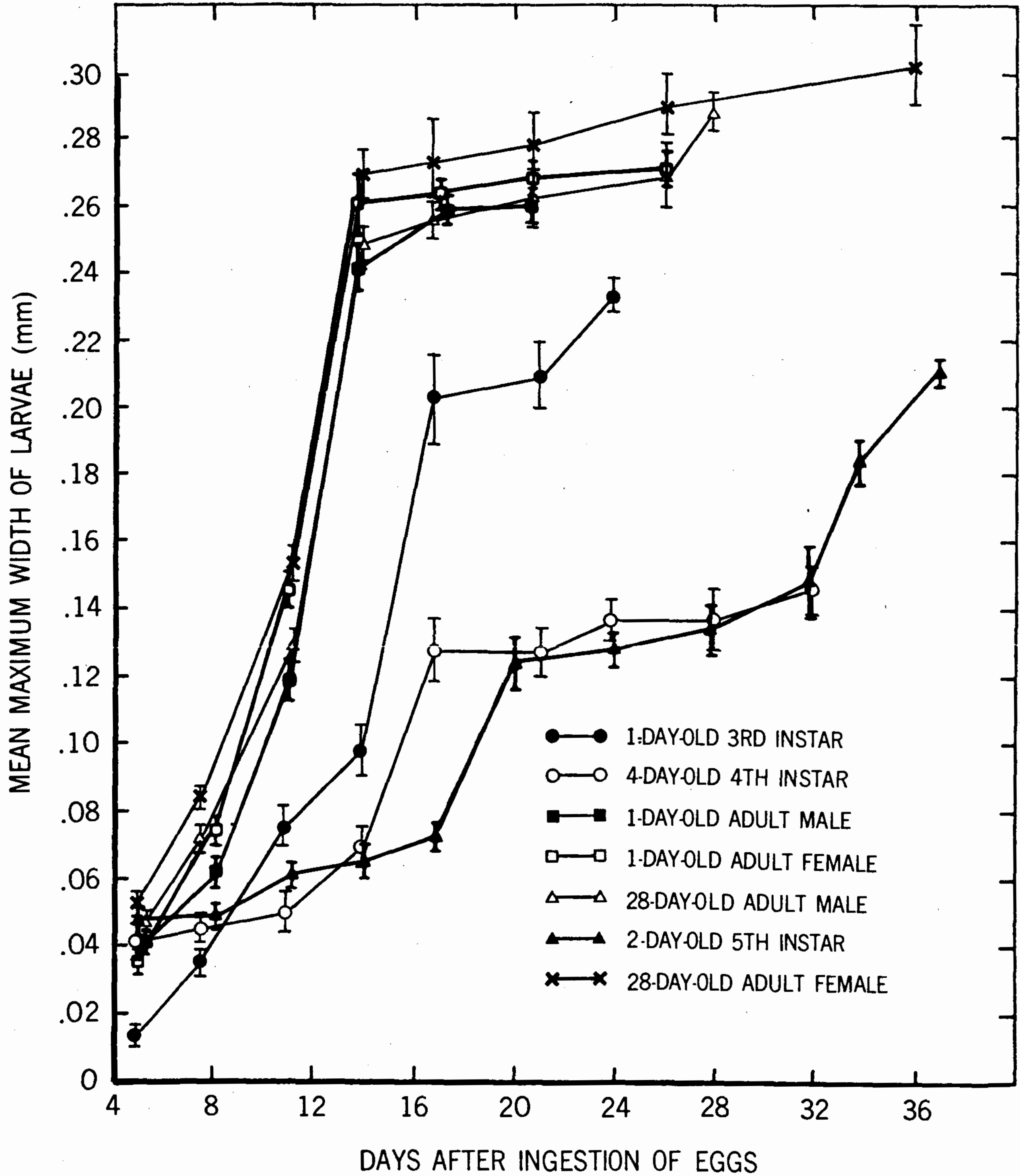
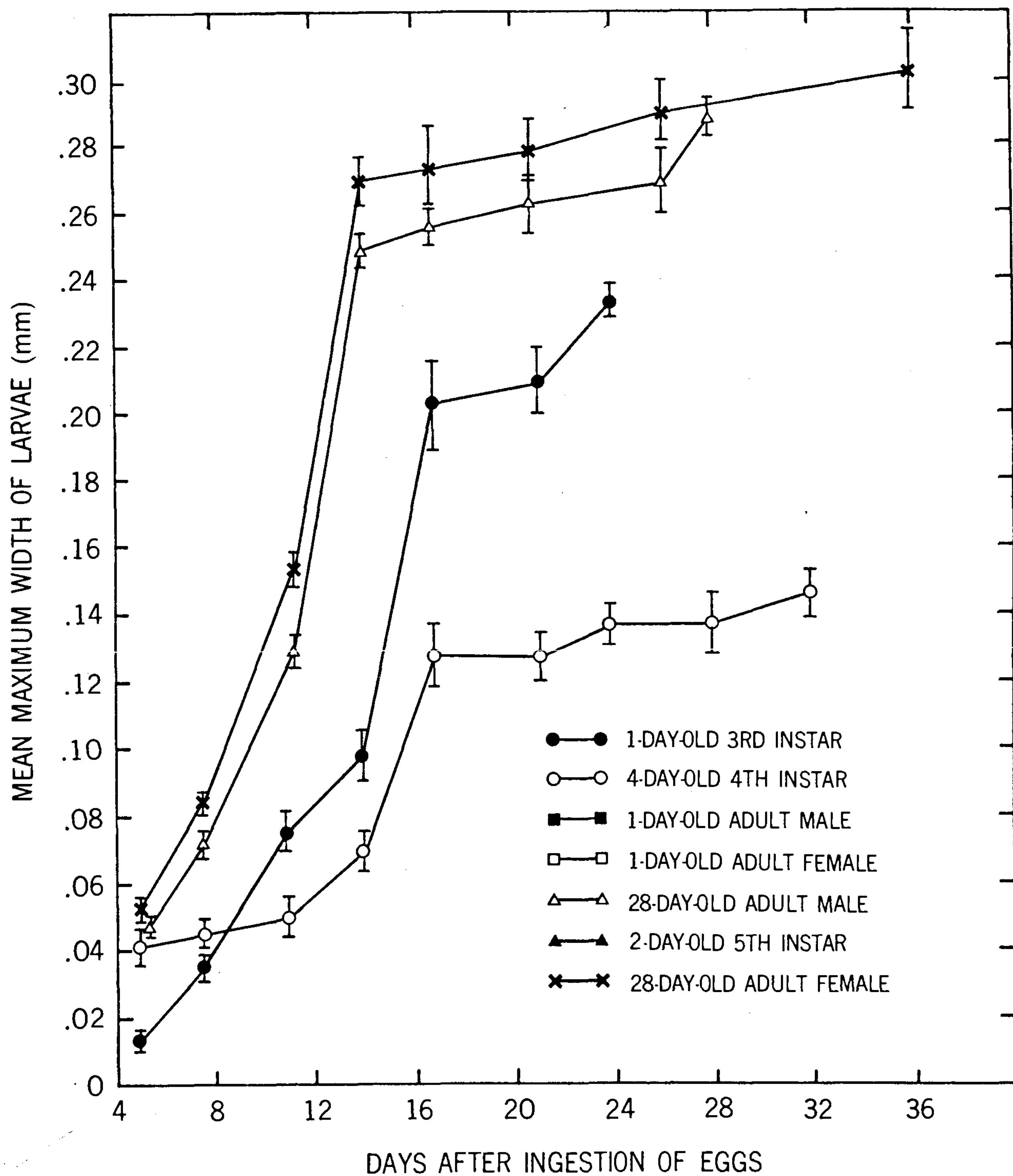

MEAN MAXIMUM LENGTH OF LARVAE (mm)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER INGESTION OF EGGS
1-DAY-OLD 3RD INSTAR
4-DAY-OLD 4TH INSTAR
1-DAY-OLD ADULT MALE
1-DAY-OLD ADULT FEMALE
28-DAY-OLD ADULT MALE
2-DAY -OLD 5TH INSTAR
28-DAY-OLD ADULT FEMALE

Figure 4. Effect of the age of *Schistocerca gregaria* on the mean maximum width of *Mermis nigrescens* larvae. Rearing temperature, 35°C; infective dose, fifty eggs.




MEAN MAXIMUM WIDTH OF LARVAE (mm)
DAYS AFTER INGESTION OF EGGS
1-DAY-OLD 3RD INSTAR
4-DAY-OLD 4TH INSTAR
1-DAY-OLD ADULT MALE
1-DAY-OLD ADULT FEMALE
28-DAY-OLD ADULT MALE
2-DAY-OLD 5TH INSTAR
28-DAY-OLD ADULT FEMALE
0
.02
.04
.06
.08
.10
.12
.14
.16
.18
.20
.22
.24
.26
.28
.30
4
8
12
16
20
24
28
32
36



MEAN MAXIMUM WIDTH OF LARVAE (mm)
0
.02
.04
.06
.08
.10
.12
.14
.16
.18
.20
.22
.24
.26
.28
.30
4
8
12
16
20
24
28
32
36
DAYS AFTER INGESTION OF EGGS
1-DAY-OLD 3RD INSTAR
4-DAY-OLD 4TH INSTAR
1-DAY-OLD ADULT MALE
1-DAY-OLD ADULT FEMALE
28-DAY-OLD ADULT MALE
2-DAY-OLD 5TH INSTAR
28-DAY-OLD ADULT FEMALE

with respect to the age of the host were found at any day subsequent to Day 5 after egg ingestion. At 14 days after egg ingestion, for example, nematodes ranged in length from 55.30 mm in adult males 1-day-old at time of infection to 75.09 mm in adult females 28-days-old at time of infection. In nymphal hosts, nematode lengths ranged from 2.83 mm to 21.90 mm; significant reductions in length compared to adult-infesting nematodes. The width of the larvae at the same times after host infection also differed significantly (Figs. 3, 4).

Growth patterns of nematodes in nymphal hosts were different from those in adult hosts, both in rate of increase in length and width (Figs. 3, 4). The size of the nematodes in nymphal hosts increased very slowly and usually only reached 80% of their full parasitic length and width shortly before they emerged from the host. In contrast, parasites in adult hosts showed an eight to nine-fold increase in length between Days 11 and 17. Increase in maximum width of the larvae in adult locusts occurred in a similar manner, but between Days 8 and 14. In the growth of the parasite between nymphal and adult hosts, with the exception of nematodes developing in 1-day-old third instar nymphs, the longer the *M. nigrescens* larvae spent within a host, the greater was their mean length and width (Figs. 3, 4).

3. Effect of transferring *M. nigrescens* larvae to a second host on the total development time and size of the parasite.

Single nematodes transferred to a second host after having developed for 25 days in the first host increased significantly in mean length and remained significantly longer in two hosts combined than did nematodes that had developed solely in one host (Table VII). Transfer of three nematodes into a second host resulted in a significant increase in development time but no increase in size of the larvae. Transfer of either five or ten nematodes to a second host caused no significant difference in either development time or size of the larvae.

TABLE VII

The effect of transferring *Mermis nigrescens* larvae to a second healthy host (*Schistocerca gregaria*) on the total development time, mean length and mean maximum width of the parasite.

Number of Hosts, Sex of Host	# of Replicate Hosts	Infective Burden Per Host	*Mermis nigrescens*: Development Time of Larvae (Days) ± S.E.[1]	*Mermis nigrescens*: Mean Length ± S.E.[1] (mm.)	*Mermis nigrescens*: Mean Max. Width ± S.E.
1[†], male	6	1	31.0 ± 1.15 b	87.35 ± 4.00 b	0.259 ± 0.006
2 , male	9	1	38.0 ± 1.89 b	104.53 ± 6.80 b	0.271 ± 0.003
1 , male	5	3	30.0 ± 1.67 b	85.36 ± 2.90	0.257 ± 0.007
2 , male	8	3	34.4 ± 1.80 b	86.89 ± 1.12	0.262 ± 0.002
1 , female	7	1	37.0 ± 2.39 b	116.96 ± 3.12 b	0.294 ± 0.008
2 , female	9	1	44.2 ± 1.81 b	128.71 ± 3.45 b	0.297 ± 0.006
1 , female	7	3	34.3 ± 1.91 b	106.68 ± 5.30	0.286 ± 0.018
2 , female	5	3	41.1 ± 2.71 b	107.28 ± 4.11	0.286 ± 0.007

[1] Means within each couplet in the same column followed by the same letter are significantly different.

[†] This column denotes that the parasite developed either within one host (1) or developed within two consecutive hosts (2).

DISCUSSION

Although prolonged storage lowered M. nigrescens egg viability (Table I), there was no indication why such eggs failed to produce a high percentage of maturing larvae. Prolonged storage may effect egg development or the ability of the larvae to emerge at hatching. Christie (1929) stated that nearly 50% of M. subnigrescens eggs which had been stored for 12 months prior to infection passed through the alimentary tract of M. femur-rubrum unhatched. It is not known whether this occurred in my experiments since locust faeces were not examined. Alternatively, the effects of storage may manifest itself behaviorally in the larva's inability to successfully find and penetrate the gut wall of the host.

The experiments on hatching of M. nigrescens eggs indicate that hatching is site-specific, but do not indicate whether or not hatching occurs in response to a host stimulus. Although Crofton (1966) states that among free-living nematodes hatching is controlled by a nematode secretory factor, Rogers (1960) points out that many animal-parasitic nematodes have lost the ability to control the hatching process. The fact that eggs did not hatch in guts ligated anterior to the normal hatching site suggests that the host provides the initial

hatching stimulus for M. nigrescens. This may be in the form of a substance which acts to break down the inner, membranous covering (Denner, 1968) of the egg, the outer membrane having been removed during egg washing prior to their storage. If host secretion only partially broke down the inner membrane, larval stylet action or action of a nematode-released substance could complete egg membrane breakdown. Rogers and Somerville (1957) demonstrated that rumen fluid from the host triggered parasitic trichostrongyle larvae of sheep to produce exsheathing fluid, while Rogers (1960, 1966) showed that high pH and high CO_2 concentration induced infective eggs of Ascaris lumbricoides to produce a hatching fluid that broke down the egg shell. However, neither the pH nor the CO_2 concentration of the locust gut were measured in this study. It must be remembered that M. nigrescens eggs in the free-living stage still have an outer membrane. Therefore, unless this outer membrane is removed during the egg's passage from the locust mouth to the midgut, the hypothesis must be modified to account for degradation of this membrane. However, since this outer membrane was easily removed during egg washing in the laboratory, the passage of the eggs through the moist host gut, plus the mechanical actions of host digestion, particularly in the proventriculus, would likely remove this membrane in naturally

infected locusts.

It sometimes took up to 48 hours after egg ingestion to find the parasitic larvae in the host haemolymph. However, Christie (1937) claimed that newly-hatched larvae could be found within the host haemocoel within 20 to 30 minutes. Although eggs hatched between 1 1/2 and 3 hours after ingestion, Denner (1968) was able to find only 3-day-old larvae in the host haemocoel. I did not observe initiation of gut wall penetration, but larvae were seen emerging from the gut wall tissue in insect saline solution up to 20 hours after egg hatch. It is not known how long gut wall penetration took, since larvae were not observed in the haemolymph until 48 hours after egg hatch, and intermediate observations between 24, 48 and 72 hours after feeding were not made. The only observational technique employed during these first 3 days was to centrifuge decapitated locusts and search the haemolymph for nematode larvae. Although one to 3-day-old larvae are less than 0.3 mm long and their cuticle is extremely fragile, it is inconceivable that centrifugation at such a low speed could rupture the larval cuticle.

My results show that the parasitic phase of *M. nigrescens* is shorter in duration at higher infective doses. Christie (1929) found a similar phenomenon for *M. subnigrescens* larvae in 'heavily' infected *Romalea microptera* and *M. femur-rubrum*, particularly in female grasshoppers. In *M. nigrescens* infections of *F. auricularia*, nematode larvae emerged from ear-wigs infected with 120 eggs in 11 to 13 days, from those with thirteen to fifteen eggs in 35 to 37 days, and in 55 days when only a single parasite egg was fed to the host (Baylis, 1944). Unlike Christie (1929), Baylis found no tend-ency for shorter parasite development time in female hosts.

My findings support the hypothesis that the total available nutrients in the host govern the length of time spent by the parasite within the host; particularly since Gordon and Webster (1971) have shown that *M. nigrescens* caused a significant depletion of haemolymph carbohydrates and fat body proteins and amino acids in the desert locust, especially during the later stages of infection. Since they also found that the host does not compensate for the drain on its nutrients by increased food intake, it follows that the higher the burden of parasites, the greater will be the quantity of

nutrients lost per unit time by the host to the parasite. Emergence from the desert locust, therefore, probably occurs when the supply of nutrients in the host becomes insufficient to satisfy the nutritional requirements of the larvae. This hypothesis gains further support from the results of the transfer experiments. These results strongly support the hypothesis that the host influences the total parasite development time. Once the host is unable to supply an adequate quantity of nutrients to permit further nematode growth, M. nigrescens emerges from the host. Therefore, a change in the concentration of the nutrients in the host haemolymph, or a change in the haemolymph osmolarity as a result of nutrient depletion may trigger this process.

M. nigrescens completed its parasitic phase in the desert locust in a significantly shorter time when reared at 35°C than at 22°C. On several occasions it was also noted that S. gregaria nymphs developed at a slower rate at lower temperatures, with a longer stadia in each instar. Many studies (Chitwood and Jacobs, 1938; Baylis, 1947; Wigglesworth, 1961; Mahler and Cordes, 1966; Gordon and Webster, 1971, 1972) indicate that there is a more rapid turnover and utilization of nutrients by the host at higher rearing temperatures, hence

resulting in a greater supply of available nutrients for the parasite.

Female hosts apparently favored M. nigrescens larval development. The larvae grew and remained within female hosts significantly longer than in males. Since the nematode must assimilate and store sufficient nutrients during its parasitic life to guarantee successful egg production during its free-living state in the soil (Gordon and Webster, 1971), a host that can supply such nutrients in the greatest quantity should be the most suitable.

Healthy adult female locusts have higher haemolymph protein and carbohydrate concentrations than males (Gordon and Webster, 1971). Kulkarni and Mehrotra (1970) demonstrated that adult S. gregaria females have higher proline, total amino acid nitrogen and haemolymph protein levels during certain periods of their adult life than males. Lee (1965) claims that parasitic nematodes may utilize host carbohydrates as substrates for their own energy metabolism. Host nutrients also provide the energy source required for rapid M. nigrescens development and protein storage in the trophosome (Baylis, 1944; 1947). Either host haemolymph carbohydrates or specific proteins in the locust haemolymph may be this crucial energy source, since these are significantly decreased in adult desert

locusts infected with _M. nigrescens_ (Gordon and Webster, 1971; Gordon, Webster and Hislop, personal communication). Since the biosynthesis of aminao acids from carbohydrates and inorganic nitrogen has been demonstrated for _A. lumbricoides_ and _Caenorhabditis briggsae_ (Pollak, 1957; Rothstein and Tomlinson, 1961), a proportion of the proteins required by _M. nigrescens_ may originate from host carbohydrates. The fact that _M. nigrescens_ larvae can incorporate large quantities of C^{14} leucine into protein, but not large molecules such as proteins, also indicates that low molecular weight amino acids in the host haemolymph may form the raw materials for both energy production and rapid growth. _M. nigrescens_ larvae increase most in length between Days 11 and 21 after egg ingestion (Fig. 4), corresponding closely with the time interval during which the locust haemolymph carbohydrate level was significantly lower than that in uninfected controls (Gordon and Webster, 1971). Adult female _S. gregaria_ weighed significantly more than males of the same age (Gupta and Burr, unpublished observation). Thus, it is probable that M. nigrescens larvae attained greater lengths in female hosts since they possess a greater available nutrient source than male locusts, as indicated by their significantly greater body weight and the presence of proportionally more available haemolymph nutrients as an energy source.

Although this study has shown that host sex (Tables IV, V) exerts a marked influence on nematode development, it can not explain other reports that host sex influenced locust susceptibility to mermithid attack. It is hard to envisage that one sex of S. gregaria can avoid ingesting mermithid eggs as implied by Denner (1968). The same percentage of M. nigrescens larvae develop in either sex at any infective dose (Table IV). Since the endocrine systems of male and female insects are similar in hormonal composition (Novak, 1967; Highnam, 1967; Highnam and Hill, 1969; Wigglesworth, 1970), it is unlikely that hormonal differences can account for the sex-specific differences in locust susceptibility to mermithid infection.

Adult and nymphal S. gregaria seem to constitute two distinct host classes for M. nigrescens larvae since the mean length and mean maximum width growth curves are significantly different (Figs. 3, 4). The comparative growth rates of parasitic juveniles of M. subnigrescens in various unspecified grasshopper hosts (infective dose, one to six parasites per host) (Denner, 1968) closely resemble the growth curve of M. nigrescens in S. gregaria nymphs. However, my results are all based on a larger infective dose of fifty parasite eggs per host. Possibly, the nutrient supply in the

grasshoppers which Denner (1968) used was low (due to the smaller size of his hosts), and nematode growth characteristic of that of *M. nigrescens* in adult *S. gregaria* was never attained.

SECTION II

Effect of parasite number and host weight on the sex ratio of *Mermis nigrescens* in *Schistocerca gregaria*.

INTRODUCTION

There have been many suggestions as to the control of sex ratio in mermithid nematodes. As the number of *M. subnigrescens* per grasshopper host increased, the proportion of male nematodes increased (Christie, 1929; Denner, 1968). Christie (1929) concluded that the haemolymph environment was important in sex determination. Petersen *et al*. (1968) and Petersen and Chapman (1970) concluded similarly for *Gastromermis* sp. and *Reesimermis* (=*Romanomermis*) *nielseni* Tsai and Grundmann parasites of mosquitoes. However, Petersen and Chapman (1970) also noted that the species of host influenced *R. nielseni* sex ratio. Gupta and Burr (unpublished observations) demonstrated that an increase in the infective burden of *M. nigrescens* in *S. gregaria* caused an increase in the percentage of male nematodes and that the percentage of nematodes that developed into males was independent of host weight below a certain infective burden and strongly dependent on host weight above that level. Strelkov (1964) noted that the sex of *Filipjevimermis singularis* Strelkov, a parasite of chironomids, was correlated with host sex in that male parasites were more often found in male hosts and females in female hosts. Frequency of

male Paramermis contorta Linslow, a parasite of the chironomid, Chironomus contorta, increased with an increase in infestation level (Parenti, 1962a). At a given infestation level, the frequency of male nematodes decreased with increasing lengths of the Chironomus larvae at the time when they were penetrated by the nematode (Parenti, 1962b). Parenti (1965) also found that the sex of the developing nematode was influenced by the sex of the parasite that first entered the host. Once a host had been infected by a nematode of one sex, subsequent nematodes that penetrated tended to differentiate into the opposite sex. Petersen (1972), however, found that neither the size nor the age of the mosquito, Culex pipiens quinquefasciatus Say, affected the sex ratio of the R. nielseni larvae. Host diet had a noticeable influence on the sex ratio of this nematode, since starved and unstarved hosts infected with a single nematode developed 92% and 13% males, respectively.

The conclusions reached by previous researchers all have a common basis in that they can all be correlated with the total available host nutrients. Host age, sex, weight and species may all be indices of host nutritional state. Depending on the total available host nutrients and the burden of parasites per host, the quantity of nutrients available to each parasite will differ. Therefore, a series of experiments

were done using *M. nigrescens* in the desert locust to investigate the effect of parasite burden, host age and host weight on the sex ratio of this nematode.

MATERIALS AND METHODS

Ten, 1-day-old adult locusts of both sexes were each infected with one to 300 *M. nigrescens* eggs and ten, 1-day-old fifth instar nymphs were each infected with from fifty to 250 eggs. Immediately after ingesting the eggs, the locusts were weighed and then placed in insectary rearing cages. The hosts were killed 18 days later and the larvae from each host were sexed by identification of the form of the genital primordia. Male nematodes were identified as early as Days 11 to 14 after egg ingestion, the genital primordium appearing as a loose, cellular configuration at the posterior end of the trophosome, which gave the latter structure a characteristic curvature visible under the dissecting microscope. These observations were consistent with findings of earlier work (Baylis, 1944, 1947; Gupta and Burr, unpublished observations). Male nematodes were visibly smaller than female nematodes of the same age.

The percentage survival of M. nigrescens at each infective burden was calculated for hosts of each age and sex.

RESULTS

Infection of 1-day-old fifth instar S. gregaria with 120 eggs resulted in 100% male nematodes developing but as many as 180 and 240 eggs had to be fed to 1-day-old adult male and female hosts, respectively, to achieve 100% male nematodes developing (Fig. 5). At each host age, an increase in the infective dose of eggs resulted in a higher percentage of male nematodes developing.

Mean weights (fourteen replicates) of the hosts at the time of egg ingestion were as follows:
1-day-old fifth instar, 0.825 ± 0.040 gms; 1-day-old adult male, 1.092 ± 0.071 gms; 1-day-old adult female, 1.473 ± 0.162 gms. Fifth instar nymphs weighed significantly less ($P<0.01$) than adults of either sex and adult males weighed significantly less ($P<0.001$) than adult females.

The percent of M. nigrescens larvae that became males was strongly dependent on host weight at infective doses of

Figure 5. Effect of infective dose on the sex ratio of *Mermis nigrescens* in *Schistocerca gregaria*. Vertical bars represent ± 2 standard errors.

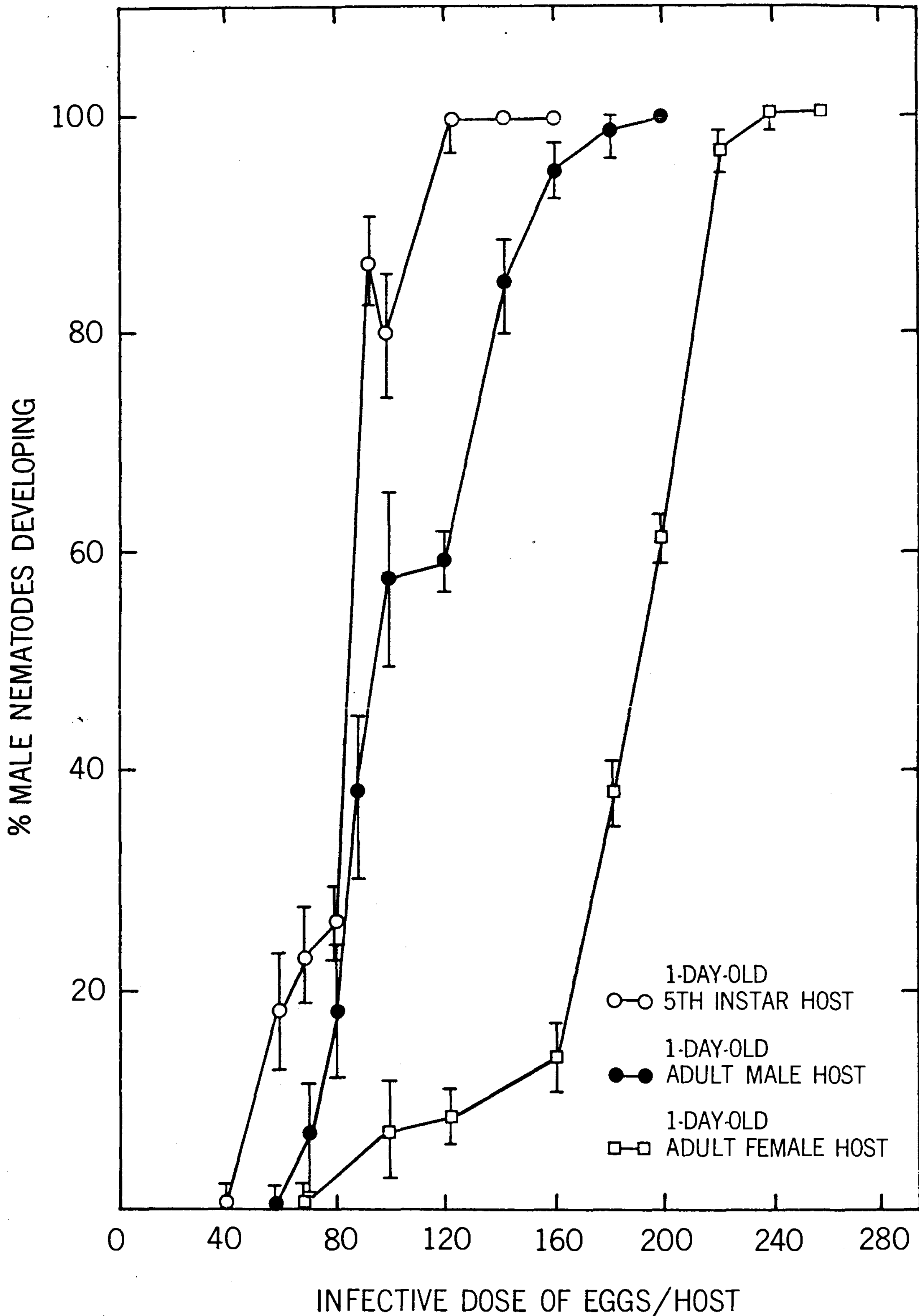
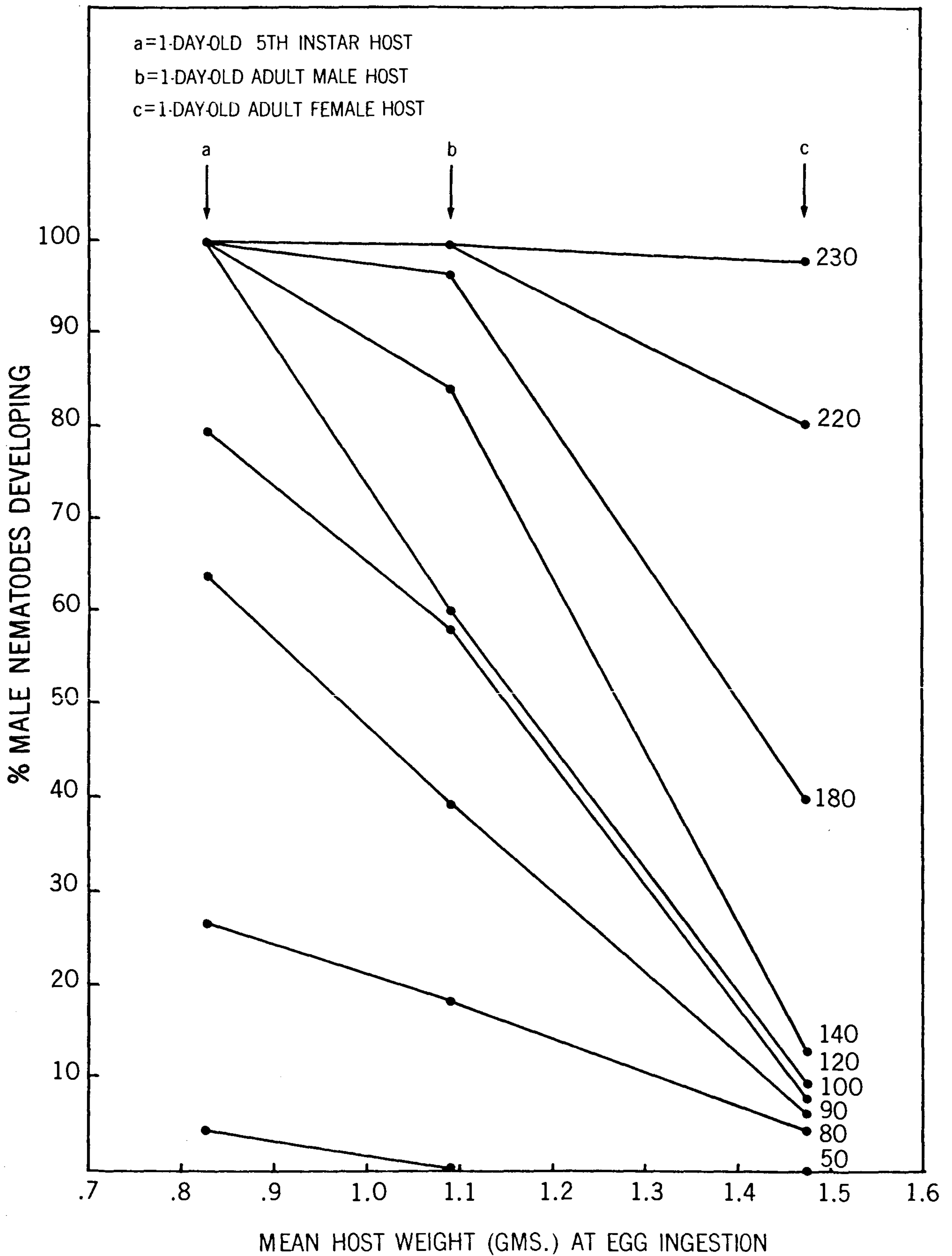

% MALE NEMATODES DEVELOPING
INFECTIVE DOSE OF EGGS/HOST
0
20
40
60
80
100
0
40
80
120
160
200
240
280
1-DAY-OLD
5TH INSTAR HOST
1-DAY-OLD
ADULT MALE HOST
1-DAY-OLD
ADULT FEMALE HOST

Figure 6 Effect of host weight and infective dose on the sex ratio of *Mermis nigrescens* in *Schistocerca gregaria*. Infective dose of parasites given for each line. Vertical bars represent ± 2 standard errors.


a=1-DAY-OLD 5TH INSTAR HOST
b=1-DAY-OLD ADULT MALE HOST
c=1-DAY-OLD ADULT FEMALE HOST
a
b
c
% MALE NEMATODES DEVELOPING
100
90
80
70
60
50
40
30
20
10
230
220
180
140
120
100
90
80
50
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
MEAN HOST WEIGHT (GMS.) AT EGG INGESTION

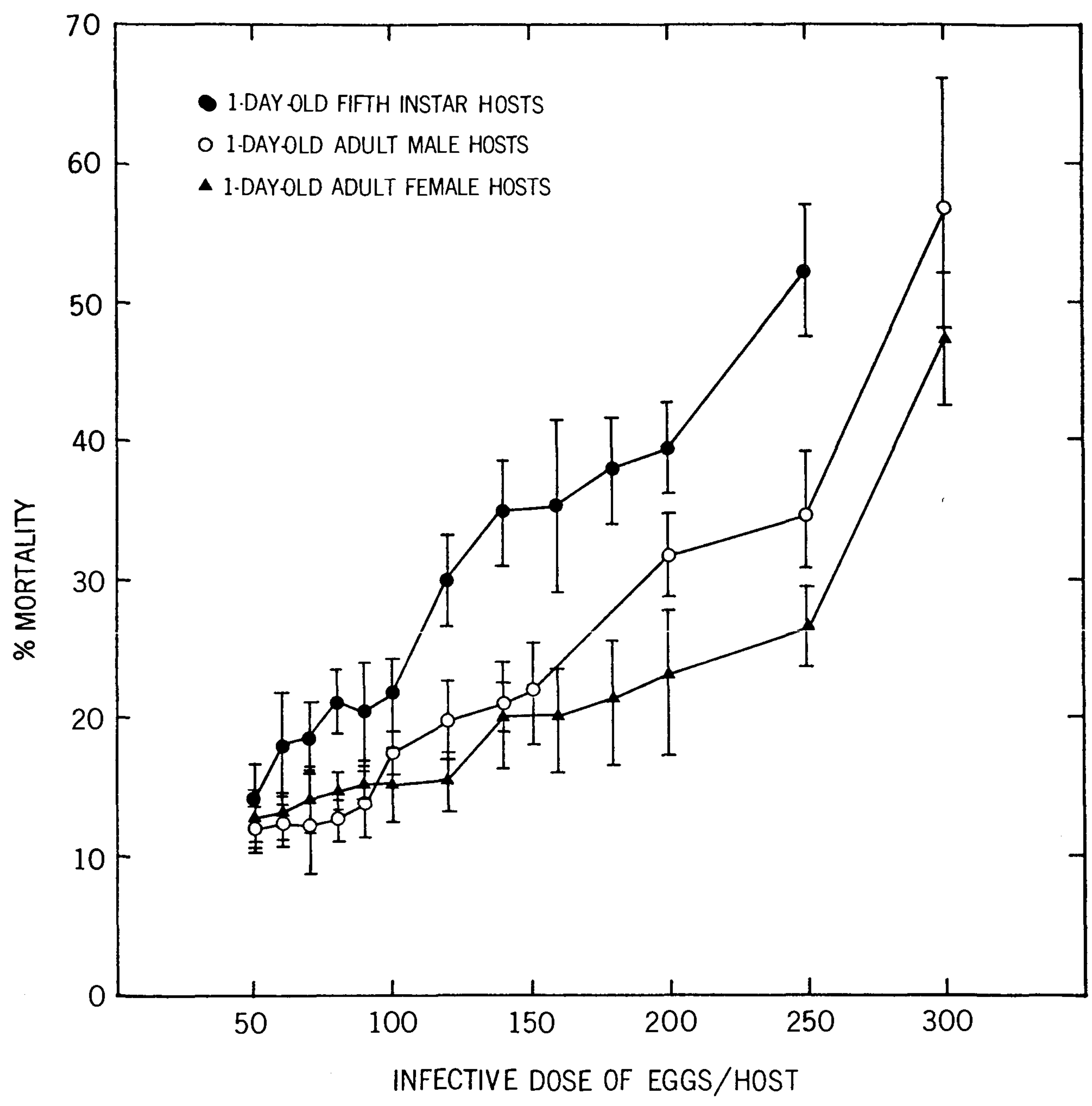
M. nigrescens larvae of 90 and above, and was very weakly dependent on host weight at infective doses of M. nigrescens larvae of 80 or less per host (Fig. 6).

For each age and sex of host used, the percentage mortality of M. nigrescens increased with increase in infective dose (Fig. 7). At an infective dose of 250 parasite eggs per host, for example, there was a mortality of 26%, 34% and 53% for 1-day-old fifth instar hosts, 1-day-old adult male hosts and 1-day-old adult female hosts, respectively.

DISCUSSION

Infective dose and host weight at egg ingestion clearly influenced the sex ratio of the developing M. nigrescens larvae in S. gregaria. A positive correlation between infective dose and percentage of male nematodes developing is in agreement with previous findings (Christie, 1929; Baylis, 1944; Parenti, 1965; Petersen et al., 1968; Petersen and Chapman, 1970; Petersen, 1972). However, the size of the infective dose alone does not govern this phenomenon. Infection of the earwig, F. auricularia, with five or more M. nigrescens eggs resulted in 100% male nematodes (Baylis, 1944). Petersen (1972)

Figure 7 Effect of infective dose on *Mermis nigrescens* mortality in *Schistocerca gregaria*. Vertical bars represent $\pm$ 2 standard errors.


1-DAY-OLD FIFTH INSTAR HOSTS
1-DAY-OLD ADULT MALE HOSTS
1-DAY-OLD ADULT FEMALE HOSTS
% MORTALITY
INFECTIVE DOSE OF EGGS/HOST
0
10
20
30
40
50
60
70
50
100
150
200
250
300

found similar results for _R_. _nielseni_ in _C_. _pipiens quinquefasciatus_. However, in _M_. _subnigrescens_ parasitic in _R_. _microptera_, if the number of parasites was three or less they were female, when there were from four to twenty-three there was a mixture of both sexes, and in all instances of twenty-four or more parasites per host all the developing larvae were males (Christie, 1929). I found that higher infective doses were required to cause 100% maleness of _M_. _nigrescens_ in _S_. _gregaria_.

It was postulated (Section I) that the total available nutrients in the host governed the duration of the parasitic phase of _M_. _nigrescens_. A similar hypothesis is now advanced to explain the development of 100% male nematodes at high infective doses. A large host, as identified by weight, would provide more available nutrients for the parasite than does a small host. Petersen (1972) has shown that when the host is undernourished, male nematodes develop at lower infective burdens. _C_. _pipiens quinquefascuatus_ fed a normal diet required seven _R_. _nielseni_ parasites per host to produce all male nematodes, while hosts maintained on one-third the normal diet throughout the parasite's developmental period required only one parasite per host to induce 92% maleness and three parasites per host to induce 100% maleness. Hence,

in the case of *M. nigrescens* in *S. gregaria*, as the infective dose is increased, the quantity of host nutrients available to each parasite will be proportionally decreased, and the result will be the production of a greater percentage of male nematodes. The higher mortality of the *M. nigrescens* larvae observed at larger infective doses may be a result of competition for available host nutrients. It is not known, however, at what time after egg ingestion that parasite egg mortality occurred. It is possible that larval establishment in the host haemocoel and/or successful larval penetration of the host gut wall was delayed at high infective doses as a result of larval competition for space. The exact mechanism is not known.

SECTION III

Inhibition of moulting in *Schistocerca gregaria* infected by *Mermis nigrescens*.

INTRODUCTION

In mermithid-parasitized insects, many changes occur in processes which are normally controlled in part by the insect's hormonal balance and/or nutritional state. The parasitoid *Stylops* sp. can suppress both ovarian growth and corpora allata activity in the solitary bee host, *Andrena vaga*, probably by decreasing host protein synthesis and possibly indirectly affecting endocrine activity (Brandenburg, 1956). *Sphaerularia bombi*, a nematode parasite of bumble bees, suppresses host growth and corpora allata activity, possibly by secreting a toxic material (Palm, 1948).

Welch (1960) postulated that mermithid nematodes in a larval host inhibit histoblast development, either directly by secreting a hormone into the host or indirectly by disturbing the host's own hormonal balance. Until recently, however, little information was available on the nutritional relationship between *M. nigrescens* and the desert locust, and of the resulting effects upon host physiology. Gordon and Webster (1971) found a reduction in total fat body protein concentration in desert locusts parasitized by *M. nigrescens*. Significantly, both haemolymph and fat body protein synthesis in *S. gregaria* are closely implicated in the moulting process

and both are under neuroendocrine control (Hill, 1962, 1965).

Kearns and Nair (1972) showed that the imaginal moult of S. gregaria was inhibited by an injection of the chemosterilant, tepa, which caused a decrease in leucine-C^{14} incorporation into the fat body and subsequent protein release. Proteins are required in the epidermal cells just prior to moulting (Wigglesworth, 1970). The intense mitotic activity in the insect epidermal cells just prior to moulting requires the action of the growth hormone, ecdysone, both for the initiation of moulting and to stimulate sufficient quantity of fat body proteins to be released into the haemolymph for continuation of this mitosis.

My objectives were to elucidate the mechanism of inhibition of moulting in S. gregaria as a result of M. nigrescens parasitism by: (1) measuring ecdysone levels in healthy and infected locusts at the time of their moulting, (2) determining haemolymph and fat body protein levels in healthy and infected locusts, and (3) measuring the incorporation of leucine-C^{14} into fat body proteins of healthy and infected locusts and their subsequent release into the haemolymph.

MATERIALS AND METHODS

In order to determine whether or not inhibition of the imaginal moult in infected locusts was due to a decrease in haemolymph ecdysone level, the following procedures were undertaken[2]. Using a disposable micropipette, 25 μl of haemolymph per locust were removed from each healthy locust through a small puncture in the arthrodial membrane at the base of the second pair of legs. This was done 7 days after they had moulted into the fifth instar and so corresponded to the 12 to 24 hour period prior to the imaginal moult. The ecdysone was extracted four times from the haemolymph with 3 ml of 65% methanol in water. The extract was evaporated at 64-66°C, extracted a further three times with 10 ml of pure methanol, and then evaporated to a final volume of 1 ml.

Volumes of from 1 to 20 μl of extract from healthy locusts were injected into the posterior end of 5-day-old *Musca domestica* larvae which had previously been ligatured midway along the longitudinal body axis (Fig. 8). The volume of extract that resulted in at least 50% of the *M. domestica* larvae

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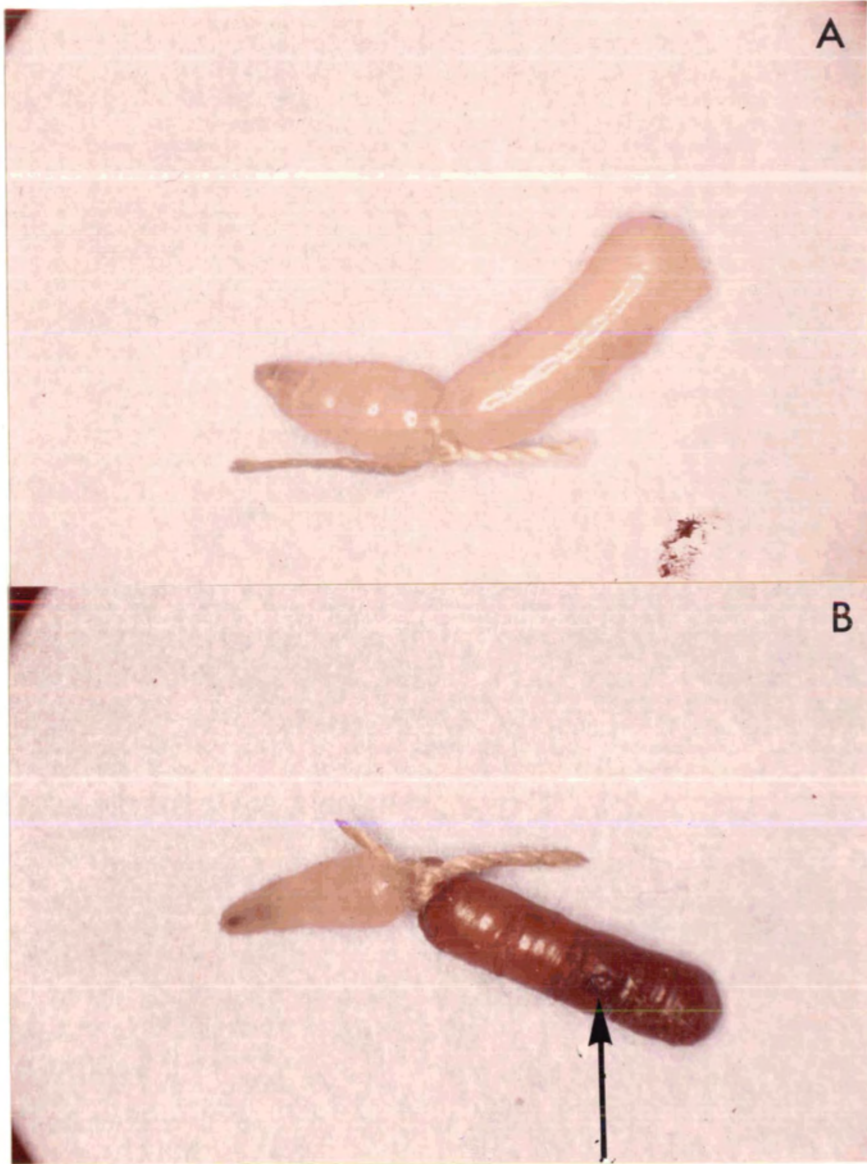
2 Ecdysone extraction technique by courtesy of Dr. D.S. King, Zoecon Corporation, 975 California Avenue, Palo Alto, California.

Figure 8. *Musca domestica* larvae used in the bioassay of ecdysone levels during the imaginal moult in healthy and infected *Schistocerca gregaria*.

A: Five-day-old, last instar *M. domestica* larvae, indicating the ligature midway along the longitudinal axis.

B: *M. domestica*, 48 hours after injection of ecdysone extract from healthy locusts into posterior end of larva. Injection site indicated by arrow.


A
B

developing pupal characteristics in the region posterior to the ligature within 48 hours after injection was used as the criterion for ecdysone level in the extracts.

Haemolymph protein measurements were made daily, beginning 24 hours after the locust nymph had moulted into the fifth instar, and terminating when all the control insects had completed the imaginal moult. Using a disposable micropipette, 5µl samples of haemolymph were obtained from each locust as described in the haemolymph ecdysone level experiment. Each locust was used once, and six locusts were used at each time.

Each haemolymph sample was added to 2 ml of 5% trichloroacetic acid (TCA) in order to precipitate the protein. The precipitate was washed twice in an ethanol : ether (1:1) mixture, and centrifuged after each washing. The samples were then assayed for total haemolymph protein according to the method of Lowry, Rosebrough, Farr and Randall (1951). A calibration curve was done using known concentrations of bovine serum albumin (BSA) as standards. The optical density of each sample was read at 500 mµ using a Unicam SP500 spectrophotometer.

For fat body protein analysis, approximately 1 mg of lyophilized visceral fat body was weighed on a Cahn gram electrobalance and homogenized in 5 ml of 5% TCA. After centrifugation, the total fat body protein was determined as described for the

haemolymph proteins.

There was significantly less protein in the fat body of infected 7-day-old fifth instar nymphs than in healthy ones of the same age. Thus, fat body from locusts of this age was used to determine the effects of parasitism on protein synthesis. The fat body tissue was incubated in a medium containing all essential amino acids (Medium A, Stephenson and Wyatt, 1962; See Appendix II) with leucine-C^{14} (specific activity 311 mCi/mM) substituted for leucine. Osborne, Carlisle and Ellis (1968) noted that this medium was suitable for incubation of _S. gregaria_ fat body. Measurement of fat body protein synthesis was done according to Kearns and Nair (1972) with the following exceptions. In estimating the amount of protein in each sample after incubation (Lowry _et al_., 1951), 0.25 ml of extract was used. For each sample, the C^{14}-activity was measured over a 10 minute period at a counting efficiency of 84%, using a Packard Tri-Carb-3003 Liquid Scintillation Spectrometer. The specific activity of the total protein in each locust fat body, after correcting for background activity, was computed in counts per minute (CPM) per mg of protein.

At the end of each incubation period, the proteins released by each fat body into the incubation medium were twice

precipitated by addition of 2 ml of 5% TCA. The amount of protein and its C^{14}-activity were determined as previously described.

RESULTS

My results confirm that above a certain minimal dose, and depending on the age of the host at the time of infection, M. nigrescens infection results in an inhibition of S. gregaria moulting. Table VIII indicates that for each host age group, there is a minimum dose above which the next-but-one moult is inhibited. This minimum infective dose is twenty, thirty and forty-five nematodes per host, for 1-day-old third instar, 1-day-old fourth instar and 4-day-old fourth instar nymphs, respectively. When a 4-day-old fourth instar nymph is infected with forty nematodes, it moults into an adult which has deformed wings (Fig. 9) and fails to reproduce successfully, even if it escapes death by cannibalism.

The results of the bioassay for ecdysone showed that there was no significant difference in ecdysone level in infected locusts during the time period when they should have moulted into adults. A volume of 7.0 μl of ecdysone extract

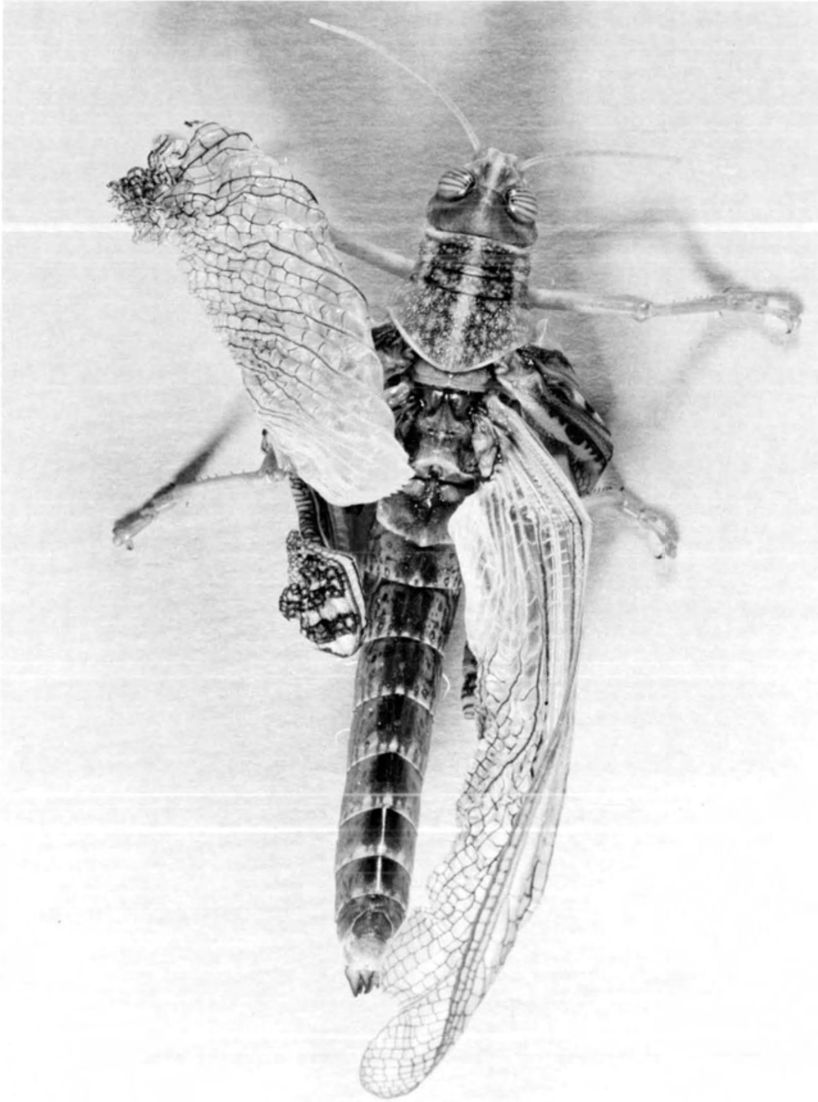
TABLE VIII

Effect of *Mermis nigrescens* parasitism on the moulting of *Schistocerca gregaria*. At least sixteen replicates for each host age and infective dose.

Age of Host at time of Infection	Infective Dose	Presence of Moult		
		3rd to 4th Instar	4th to 5th Instar	5th to Adult
1-day-old 3rd Instar	15 or Less	YES	YES	YES
1-day-old 3rd Instar	20 or More	YES	NO	NO
1-day-old 4th Instar	20 or Less	---	YES	YES
1-day-old 4th Instar	30 or More	---	YES	NO
4-day-old 4th Instar	35 or More	---	YES	YES
4-day-old 4th Instar	40	---	YES	SOME[1]
4-day-old 4th Instar	45 or More	---	YES	NO

[1] Began the Imaginal Moult but Failed to Complete it.

Figure 9. Mermithid-infected adult *Schistocerca gregaria*, showing deformed wings resulting from parasitism.



from healthy, 7-day-old fifth instar S. gregaria was required to cause 50.8 ± 1.8% pupal characteristics to appear in 48 hours in M. domestica larvae. An identical volume of ecdysone extract from infected locusts resulted in 49.0 ± 2.1% of the M. domestica larvae displaying pupal characteristics.

There was a significantly lower ($P<0.001$) haemolymph protein concentration in infected than in healthy locusts at Days 6 and 7 of the fifth instar (Fig. 10). Since uninfected locusts completed their imaginal moult between Days 6 and 7 1/2 of the instar, this represented a significantly lower level of proteins available to the locust during the moulting process.

Although the total fat body protein concentration in infected locusts paralleled that of the healthy locusts, it did so at a significantly lower ($P<0.001$) level between Days 6 and 7 of the fifth instar (Fig. 11). This corresponded to the same time period when the haemolymph protein concentration was similarly decreased (Fig. 10).

In both healthy and infected 7-day-old fifth instar locusts, incorporation of leucine-C^{14} into fat body proteins increased with increase in incubation time. The rate of incorporation of leucine-C^{14} into fat body proteins was, however, significantly lower ($P<0.001$) in the infected locusts after

Figure 10. Effect of _Mermis nigrescens_ parasitism on the total haemolymph protein levels in fifth instar _Schistocerca gregaria_, infected with fifty parasite eggs 1 day after they had moulted into the fourth instar and reared at 35°C. Vertical bars represent ± 2 standard errors.

Figure 11. Effect of _Mermis nigrescens_ parasitism on the total fat body protein levels in fifth instar _Schistocerca gregaria_, infected with fifty parasite eggs 1 day after they had moulted into the fourth instar and reared at 35°C. Vertical bars represent ± 2 standard errors.

GM. EQUIV. B.S.A./100 ML. HAEMOLYMPH
5.0
4.0
3.0
2.0
1.0
● HEALTHY
○ INFECTED
DAY 7 OF INFECTION
IMAGINAL MOULT

GM. EQUIV. B.S.A./100 GM. DRY WT. FAT BODY
10
8
6
4
2
● HEALTHY
○ INFECTED
DAY 7 OF INFECTION
IMAGINAL MOULT

0 1 2 3 4 5 6 7 8 9
DAYS AFTER ECDYSIS INTO FIFTH INSTAR

incubation for both 30 and 60 minutes (Table IX).

The quantity of proteins released into the incubation medium from the fat body of both healthy and infected locusts increased with increase in incubation time. However, the quantity of proteins released from the fat body of infected locusts was significantly lower than that released from the fat body of healthy locusts after 15, 30 and 60 minutes incubation (Table X).

Although the specific activity of the proteins released from the fat body of both healthy and infected locusts increased significantly ($P<0.001$) with respect to incubation time, there was no significant difference in the specific activity of the released proteins between the two groups after either 15, 30 or 60 minutes incubation in the medium (Table XI).

DISCUSSION

The results of this study have shown that there is no decrease in ecdysone level at the time of moulting in infected locusts. Consequently, M. nigrescens probably inhibits moulting in the desert locust by inhibiting protein synthesis and the subsequent release of fat body proteins into the haemolymph.

TABLE IX

Effect of *Mermis nigrescens* parasitism on the incorporation of leucine C^{14} into the fat body proteins of *Schistocerca gregaria*. Six replicates per host and six hosts for each incubation time.

Incubation Time (Min)	Specific Activity of Proteins x 10^3	
	Healthy ($\bar{x} \pm$ S.E.)[1]	Infected ($\bar{x} \pm$ S.E.)[1]
15	23.88 ± 1.05	23.52 ± 2.06
30	46.79 ± 0.92 c	38.28 ± 1.47 c
60	82.38 ± 2.62 c'	61.30 ± 3.22 c'

[1] Means followed by the same letter are significantly different.

TABLE X

Amount of protein released into the incubation medium by fat body from control and infected 5th instar *Schistocerca gregaria*. Six replicates per host and six hosts for each incubation time.

Incubation Time (Min)	Released Protein as % of Fat Body Protein	
	Control ($\bar{x} \pm$ S.E.)[1]	Infected ($\bar{x} \pm$ S.E.)[1]
15	9.31 ± 0.55 c	5.29 ± 0.53 c
30	10.66 ± 0.94 c'	7.66 ± 0.73 c'
60	10.81 ± 1.26 c"	7.41 ± 0.95 c"

[1]Means followed by the same letter are significantly different.

TABLE XI

Effect of *Mermis nigrescens* parasitism on the incorporation of leucine C^{14} into proteins released from the fat body of *Schistocerca gregaria*. Six replicates per host and six hosts for each incubation time.

Incubation Time (Min)	Specific Activity of Proteins x 10^3 Healthy ($\bar{x} \pm$ S.E.)	Specific Activity of Proteins x 10^3 Infected ($\bar{x} \pm$ S.E.)	Comparison of Means
15	23.95 $\pm$ 4.07	25.23 $\pm$ 1.85	Not Sig. ($P>0.10$)
30	38.21 $\pm$ 4.53	36.78 $\pm$ 4.74	Not Sig. ($P>0.10$)
60	76.08 $\pm$ 6.52	74.00 $\pm$ 3.98	Not Sig. ($P>0.10$)

The finding that there is no effect of mermithid parasitism on the incorporation of leucine-C^{14} into proteins released by the fat body further indicates that it is the fat body, the site of protein synthesis in the insect, that is affected by the parasite.

My findings have corroborated the work of Gordon and Webster (1971), who showed that the total fat body protein level of adult female S. gregaria was significantly depleted by M. nigrescens parasitism between Days 16 and 21 after infection. In the same study these authors also noted that the total haemolymph protein levels of infected hosts did not differ significantly from the levels in uninfected hosts throughout the infective period, a result seemingly contradictory to that found in this study. In subsequent experiments using polyacrylamide disc electrophoresis and hosts of a different age, however, Gordon, Webster and Hislop (personal communication) indicated a significant depletion in haemolymph protein components at Day 21 after infection. From their findings they concluded that the developmental stage of the host at the time of infection was extremely important in determining the extent to which M. nigrescens modified host protein metabolism. I also indicated that the age of the host at the time of infection was important in that it significantly influenced the duration

of parasitic development (Table VI) and the comparative growth rate of the M. nigrescens larvae (Figs. 3, 4).

Both the total haemolymph and total fat body protein levels demonstrated for uninfected locusts are similar to those levels indicated by previous workers (Kulkarni and Mehrotra, 1970; Gordon and Webster, 1971; Kearns and Nair, 1972). No comparison can be made, however, since my results are based on the fifth instar nymph while previous work utilized only adult S. gregaria.

There are two means whereby protein synthesis in the fat body can be inhibited. It is known that there is a neuro-secretory control of protein synthesis in the fat body of S. gregaria (Highnam and Hill, 1969; Engelmann, 1970). However, since this study has confirmed that there is no effect on haemolymph ecdysone levels of infected locusts, it is unlikely that fat body protein synthesis is inhibited as a result of an effect on the host ecdysone production. Fat body protein synthesis may also be inhibited due to a decrease in the quantity of protein precursors in the haemolymph. As Hill (1962) has demonstrated, the free amino acids in the insect haemolymph provide the amino acid pool for fat body protein synthesis. Gordon and Webster (1971), however, found that M. nigrescens did not significantly affect the total amino acid level in the

haemolymph of infected male and female S. gregaria. Although such a finding would seem to imply that the inhibition of protein synthesis was not due to a decrease in the quantity of amino acid precursors in the infected host haemolymph, the evidence may be interpreted in several ways when discussed in relation to M. nigrescens development and associated nutritional requirements.

Gordon and Webster (1972) indicated that Days 14 to 21 after infection appeared to be the maximal period of growth/ protein synthesis by M. nigrescens larvae. Therefore, in attempting to fulfill their high requirements for dietary amino nitrogen at this stage in their development, the nematode would exert its greatest effect on protein turnover in S. gregaria. Since the M. nigrescens larvae used in my experiments were between Days 13 and 15 of their parasitic development when they inhibited locust moulting, their nutrient requirements would be maximal (Gordon and Webster, 1972). However, since total haemolymph amino acid levels are unaffected by M. nigrescens parasitism (Gordon and Webster, 1971), I must conclude that the nematode indirectly utilizes host fat body proteins by inducing changes in their metabolism. M. nigrescens may stimulate protein release from the fat body. Since the nematode is unable to assimilate proteins and there is no evidence of the presence

of a proteolytic enzyme in homogenates of *M. nigrescens* (Gordon and Webster, unpublished observations), these proteins would eventually accumulate within the host haemocoel. Since I found, however, that M. *nigrescens* caused a significant depletion of total haemolymph protein concentration in the locust (Fig. 10),it is unlikely that this occurs. Decreased protein synthesis by the infected host fat body may also be due to a parasitically-induced impairment of the fat body cells to incorporate amino acid precursors. This could occur if the *M. nigrescens* larvae secreted an inhibitory substance. Although Denner (1968) gave inconclusive evidence of *M. subnigrescens* exudates and secretions causing decreased egg fecundity in several grasshopper species, I found no evidence of an inhibitory effect on *S. gregaria* as a result of a substance originating from the *M. nigrescens* larvae (unpublished observation).

I consider it more probable that *M. nigrescens* feeds upon host haemolymph nutrients and thus deprives *S. gregaria* of some of the necessary amino acid precursors for protein synthesis. For this system to be operative, however, the parasite would have to stimulate the locust fat body to catabolize proteins and so release amino acids into the haemolymph; both for the nematode's nutrition and to compensate for the depletion

of haemolymph amino acids resulting from larval feeding in the parasitized *S. gregaria*. This hypothesis has experimental support in that it was shown that catabolism of *S. gregaria* fat body proteins at 2 weeks after infection resulted in a subsequent depletion of haemolymph proteins 1 week later (Gordon and Webster, 1971).

The mechanism(s) whereby *M. nigrescens* may stimulate protein catabolism (or suppress protein anabolism) within the fat body of *S. gregaria* is not known. It can occur as a result of an imbalance in metabolite level between the haemolymph and fat body or as a result of an alteration in the hormonal milieu of the locust. While I have shown that there is no depletion in ecdysone levels in infected locusts during the moulting process, the possibility cannot be discounted that *M. nigrescens* secretes its own hormones into the locust haemolymph. Investigations by Fisher and Sanborn (1964) and Rajulu, Kulasekarapandian and Krishnan (1972) have demonstrated that nematodes can produce substances with juvenile hormone activity and a steroid hormone, respectively.

My hypothesis on the mechanism whereby *M. nigrescens* inhibits moulting in the desert locust is as follows: *M. nigrescens* inhibits fat body protein synthesis in infected locusts by significantly impairing the incorporation of amino

acid precursors from the haemolymph and by concurrently stimulating catabolism of fat body proteins; thus providing a dietary source of amino nitrogen for *M. nigrescens* larval growth and development.

Research Topics Arising out of this Project.

Although *M. nigrescens* has considerable potential as a biological control agent against the desert locust, some aspects of this relationship require further clarification.

1) There is currently some confusion regarding the taxonomy of *M. nigrescens* and *M. subnigrescens*. Although Cobb (1926) and Denner (1968) maintain that *M. subnigrescens* is a valid species and should be separated from *M. nigrescens*, Nickle (1972) has synonymized the two species. It is important that the discrepancy in the taxonomy of these species be resolved, so that the results of my investigations and those already in the literature can be used to best advantage.

2) My investigations have indicated that the stimulus for the *M. nigrescens* eggs to hatch in the gut of the desert locust probably originates in the host. It would be useful to demonstrate the nature of this stimulus, since it will be necessary to induce *M. nigrescens* eggs to hatch as a first step in establishing an *in vitro* culture of the nematode larvae.

3) Since the present study shows that the burden of parasites which a locust harbours dictates the number of male nematodes that develop, it will be necessary to confirm earlier indications (Cobb, 1926) that *M. nigrescens* is parthenogenetic for successful *in vitro* culture. If it is parthenogenetic, then

only the nutrient requirements for the production of female nematodes would have to be determined when investigating the composition of an *in vitro* culture medium.

4) Although previous workers have indicated that the post-parasitic stage of *M. nigrescens* lasts for 2 or 3 years, it would be useful in a biological control program to be able to shorten this soil-living period. However, since virtually nothing is known of the factor(s) that govern the duration of the post-parasitic phase in the soil, an avenue of investigations in this area would be beneficial.

5) My results show that moulting is inhibited in nymphal desert locusts due to the inhibition of protein synthesis in the fat body. It would be useful to test my hypothesis on the mechanism whereby fat body protein synthesis is inhibited by *M. nigrescens*.

6) McKinley and Randall (1971) have demonstrated that there are large differences in the moisture content between each of the commonly used locust and grasshopper diets. This results in significant differences in locust survival and body weight and consequently, large differences in the quantity of nutrients per unit volume of food consumed by the *M. nigrescens* larvae. Such factors affect nematode development and warrant further investigation.

7) Although it was noted that *M. nigrescens* larvae attained a greater size under longer rearing conditions, it is not known what effect the duration of parasitic development had on either the number of eggs laid by the mature nematode females or on the viability of these eggs. It may be possible to remove a parasite before it completes its parasitic development and still have viable eggs laid at the termination of post-parasitic development.

8) The results of my experiments elaborate on the quantitative rather than the qualitative nutrient requirements of *M. nigrescens*. Future research should stress the importance of elucidating the qualitative nutrient requirements as a necessary first step to the establishment of an *in vitro* culture.

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APPENDIX 1

Composition of insect saline solution for *Mermis nigrescens* developmental studies.

Constituent	mg/100 ml
$NaCl$	650
KCl	140
$CaCl_2$	12
$NaHCO_3$	10
Na_2HPO_4	1

APPENDIX 2

Composition of medium A used for incubating *Schistocerca gregaria* fat body.

Constituent	mg/100 ml	Constituent	mg/100 ml
KCl	298	L-glutamine	60
$CaCl_2$	81	Glycine	65
$MgCl_2$	304	L-Histidine HCl	250
$MgSO_4$	370	L-isoleucine	10
NaH_2PO_4	110	L-lysine HCl	125
Malic acid	67	L-methionine	10
L-alanine	45	L-phenylalanine	15
L-arginine HCl	70	L-proline	35
L-asparagine	35	L-serine	110
L-aspartic acid	35	L-threonine	35
L-cysteine	8	L-tryptophan	10
L-cystine	2.5	L-tyrosine	5
L-glutamic acid	60	L-valine	20

The solution also contained streptomycin sulfate (25 μg/ml) and penicillin G (250 units/ml), and the pH was adjusted to 6.5 with NaOH. The $CaCl_2$ was added after mixing the amino acids with the other salts to avoid precipitation.

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