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Laboratory Culture and Life History of *Heleidomermis magnapapula* in Its Host, *Culicoides variipennis* (Diptera: Ceratopogonidae)

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Abstract: The mermithid parasite *Heleidomermis magnapapula* was maintained in larvae of the midge *Culicoides variipennis* for 20 months in enamel pans containing nutrient-rich water and polyester pads as a substrate. Inseminated female mermithids were introduced to the pad surface when the host was in the late second or early third-instar. Host larvae were harvested from the pans 9 days after exposure and held in tap water for nematode emergence. Preparasite yield was positively correlated with female nematode size and averaged 1,267 preparasites/female. Male and female nematodes emerged an average of 12.2 and 13.4 days after host exposure, respectively. Supplemental host food (*Panagrellus*) during the final days of parasitism did not alter time of emergence. Parasites emerging singly were 64% females, whereas superparasitized hosts yielded males (up to nine/host). Nematode carryover into the adult midge normally occurred at a level of 0.5–2.5%. Parasite load (nematodes/parasitized individual) in midge adults was lower than that of larvae from the same cohort, and adult midges were more likely to harbor female parasites. Exposure of fourth-instar host larvae resulted in higher levels of adult parasitism (up to 17%).

Key words: biological control, bluetongue, *Culicoides*, *Heleidomermis*, host–parasite relationships, Mermithidae, nematode.

Recent work on nematode parasites of insects has focused on the Steinernematidae and Heterorhabditidae, primarily due to their wide host range and potential for mass production (5). The Mermithidae, in contrast, are considerably more host-specific and more difficult to study and mass produce. Few species have been studied in depth, and this often is limited to field observations (26). The best known mermithid is the mosquito parasite *Romanomermis culicivorax*, due in large part to its successful culture in vivo (24). Field trials have been conducted with this nematode, and the biology and biological control potential have been reviewed (21,25).

The biting midge *Culicoides variipennis* is the primary vector of bluetongue virus to North American ruminants. Immature midges (four instars) develop in manure-polluted mud at the margins of ponded or slowly moving water (9). The only specific natural enemy known for this insect is the mermithid *Heleidomermis magnapapula* (13,27). The general biology of this nematode is unusual for the Mermithidae. The nematodes emerge from the ceratopogonid midge host as adults, mate, and the female retains the eggs until they hatch internally. Preparasites (second-stage juveniles) escape from the female, locate a host, and penetrate the cuticle. *Heleidomermis* is known from *C. variipennis* larvae in New York, Virginia, Alabama, and California (7,13,16,27). The most detailed distribution data are from California, where the nematode and host are widespread (16).

The present study reports culture procedures for *H. magnapapula* in *C. variipennis* and selected aspects of the host–parasite relationship in the laboratory.

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Materials and Methods

Laboratory culture: Culicoides variipennis sonorensis (AA strain) was colonized using procedures modified from Jones et al. (8). Briefly, enamel pans (42 × 28 × 5 cm) were filled with deionized water to a depth of 3–4 cm, to which nutrient broth and a "microbial starter" (fungi and bacteria) were added. Each pan held two rectangular polyester pads (18 × 13 × 3 cm), which served as substrate "islands" for the host larvae. Approximately 2,000–2,500 *C. variipennis* eggs were added per pan on day 0. Pans were held beneath two fluorescent bulbs (40 watt), and aquarium pumps were used to agitate the water and prevent formation of bacterial scum lethal to host larvae. Rearing temperature was 27 ± 2 C, with a 13 L:11 D photoperiod. Host eggs hatched within 2 days, larvae had developed to the last (fourth) instar by day 9–12, and pupation occurred after day 12 (peak day 14–22).

Parasitized *C. variipennis* larvae were collected from surface mud in a dairy wastewater pond in western Riverside County, California, in the fall of 1991. Mud was washed through a 100-mesh sieve to concentrate late-instar larvae. These were backwashed into tap water in a petri dish and held at 23 ± 2 C for *H. magnapapula* emergence. Adult males and females were removed with a wire probe into a glass dish with tap water. Adult nematode females began to show signs of oogenesis within 2 days. Females were removed individually and were placed gently on the surface of the pads in the host rearing pans (usually six females/pad) at day seven, when the hosts were in the late second or early third instar. Females either had just begun preparasite production or were within less than 24 hours of releasing preparasites. Nine days later (day 16, usually just before host pupation) the pads were removed.

Fourth-instar larvae were separated by placing the pads into a circular enamel pan (23 cm diameter and 8 cm deep). The pan was tilted, and pads were held to the bottom with a circular piece of screen (6-mm openings). The pads were submerged with a gentle, continuous stream of water, which ran into the pan, over the lip, and into a 100-mesh sieve. Larvae swam free and flowed over the lip of the pan with the water. These larvae and those from the remaining pan water were caught on the sieve. Larvae were backwashed with tap water into a glass petri dish.

Larvae were held together in glass dishes at 23 ± 2 C and natural photoperiod, and were checked daily to count and remove *H. magnapapula* into a separate glass dish for mating. Nematodes could be refrigerated (4 C) for up to 2 weeks before being used to infect hosts in the rearing pans. Nematodes in the colony were supplemented twice using wild material from the original field site.

Female size versus fecundity: Individual inseminated females were separated into dishes with a grid scored on the bottom. Preparasites emerged in tap water. When the female was essentially empty, she was removed for length and width (diameter at the vulva) measurements. In some cases up to several dozen preparasites were retained in the female. If they could be counted, the preparasites were included; otherwise the female was not used.

Time to emergence: After host harvest (9 days after host exposure), male and female nematodes were removed daily for 14–20 days (until nematode emergence ceased). This was done for 32 pans of parasitized hosts (32 trials) to generate emergence pattern data.

Fed versus unfed larval hosts: Larvae of *C. variipennis* will feed on *Panagrellus redivivus* (14). Hosts in five trials were exposed and harvested as described above. Larvae were removed randomly on the day of harvest into one of two glass petri dishes (500 larvae per dish). Larvae in one dish were held without food (standard method described above). The second dish received *P. redivivus* every 2 to 3 days. Only enough nematodes were added so that the *C. variipennis* larvae could consume them in 1 to 2 days.

This avoided anoxia from bacterial growth (dead nematodes). Fed and unfed groups were monitored daily for approximately 14 days (until nematode emergence ceased). Emerged *H. magnapapula* were removed and counted to compare yield, emergence times, and sex ratios of nematodes from fed and unfed hosts.

Parasite emergence versus time of host harvest: Time of field collection could impact parasitism estimates based on nematode emergence from hosts held in water. Young hosts, for example, might die before nematodes could emerge. In three trials, we collected portions of the pads randomly for harvest every other day beginning at day seven or nine after exposure to parasites. Larvae were extracted, and 96 larvae were held individually in ELISA plate wells per harvest day.

Parasite loads in host larvae: Larvae were pipetted individually into a 96-well ELISA plate (two plates for each of eight trials) to estimate percentage parasitism and parasite load by parasite emergence (16). This method was compared with dissection of larvae from the same cohorts (n = 150 per trial) to determine the extent to which successful emergence might underestimate these parameters. A larva was pinned to the bottom of the glass dish using a set of fine forceps, the head capsule was pinched off at the base, and the body contents were extruded and searched for nematodes.

Adult parasitism: Parasitism in both larval and adult midges was assessed in seven trials as follows. Hosts were exposed at 7 days of age, and one pad was harvested for host larvae at 9 days after exposure. Host larvae were placed individually into ELISA wells (one or two plates/trial). The second pad was left in the pan, and host pupae were collected every 2 days until pupation ceased. These pupae were held, according to time of pupation. Emerged adults then were dissected to detect nematodes in the abdomen. At least 50 male and 50 female *C. variipennis,* if available, were dissected per day.

Hosts were exposed to *H. magnapapula* at 12 days of age (fourth-instar) in two trials. Pupae were collected every 2 days until pupation ceased. Emerged adults were held and dissected.

Frequency distributions (parasitized versus unparasitized hosts, temporal patterns of parasite emergence) were examined with chi-square analysis. Nematode numbers between treatments were compared by t-test. The effect of nematode size on fecundity was examined by regression, and correlation coefficients were calculated to determine whether percentage parasitism was related among trials for adults versus larvae.

RESULTS

Laboratory culture: The exposure of late second- or early third-instar hosts on the pads worked well for maintenance. Yield of *C. variipennis* larvae varied, but usually was between 1,000 and 2,000 live fourth-instar larvae per pan. For hosts held without food in tap water (41 trials), average parasitism was 10.4% (range 2.1–50.0%), with an average sex ratio of 2.6 males/female. A pan would yield several dozen female *H. magnapapula.* Tap water was better than deionized water for holding nematodes. Nematodes in deionized water often died from unidentified fungi, especially with field-collected hosts.

Mating occurred soon after female emergence; one male was coupled with an emerging female whose gonopore had barely cleared the host body. Solitary females emerging from individually held hosts lived up to 2 weeks but showed no signs of oogenesis. When placed together in a dish of water, newly emerged male and female nematodes clustered tightly together and wrapped themselves around any object of appropriate diameter, including polyester fibers or the probes used to transfer the nematodes. After 1 to 3 days, nematodes still were active but were distributed more loosely in the dish. Most females were fertile even when the sex ratio was slightly less than 1:1.

Oogenesis required 3 to 4 days at 23 C. Preparasites hatched within the female, and most emerged during the first 12–24 hours. Active preparasites hatched in sluggish or moribund females but seldom emerged. In water with late second- or early third-instar hosts, preparasites wiggled continuously but covered little distance. As a host larva made contact, a preparasite would flex its body and try to adhere to the host cuticle. This was immediately followed by rapid, vigorous host flexion. The midge larva wrapped itself into a circle, crossing the head and tip of the abdomen in a wiping motion. The preparasite often was dislodged; if not, entry took <3 minutes.

Female size versus fecundity: For 23 laboratory-reared *H. magnapapula* females, average ± s.e. (range) length was 9.7 ± 0.4 mm (6.0–13.3 mm), and width was 0.111 ± 0.003 mm (0.09–0.15 mm). Average preparasite yield was 1,266.6 ± 122.1 preparasites/female (range 496–2,516). Preparasite yield was a function of female size, with a regression accounting for 77.6% of the variability ($y = -1{,}324.9 + 267.23\,x$) (Fig. 1).

Time to emergence: Male nematode emergence from hosts held in water without food began by day 9–10 and peaked on days 11 and 12 after host exposure (Fig. 2). Female emergence began by day 9–10 and peaked on days 12–14 (Fig. 2). Weighted mean time of emergence was 12.2 days for males and 13.4 days for females. These distributions were significantly different ($P < 0.05$).

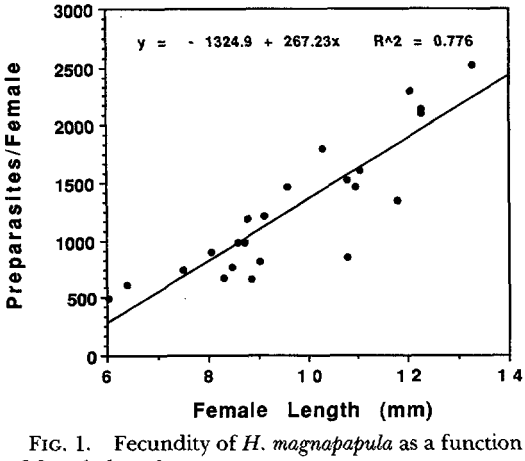

FIG. 1. Fecundity of *H. magnapapula* as a function of female length.

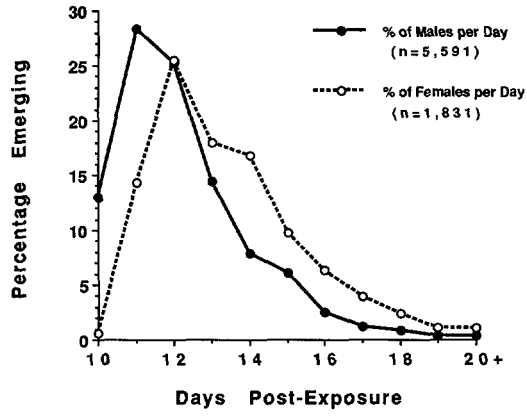

FIG. 2. Frequency distribution of *H. magnapapula* emergence from *C. variipennis* host larvae harvested 9 days after nematode exposure and held in water.

Some *H. magnapapula* emerged as late as 2 weeks after host harvest. These late-emerging individuals were smaller and less vigorous than those emerging earlier. Nearly mature *H. magnapapula*, particularly females, were observed within the host larvae. Parasitized hosts sometimes survived many days before parasite emergence, or both host and parasite would die.

Fed versus unfed larval hosts: After harvest, larvae of *C. variipennis* were either fed *Panagrellus* or not, and parasite yields were compared. The time pattern of emergence from both groups was similar to that in Figure 2. For female parasites, mean time of emergence for fed hosts (12.4 days) was identical to that from unfed hosts (12.4 days) ($P > 0.5$). Male parasites emerged slightly later from fed hosts (11.7 days) versus unfed hosts (11.5 days) ($P < 0.05$).

Average female *H. magnapapula* yield per trial was 32.6 ± 7.2 for fed hosts and 32.0 ± 7.7 for unfed hosts. Average male yield was 49.4 ± 11.3 for fed hosts and 71.8 ± 11.7 for unfed hosts. Female yield was not different from the two host groups (paired *t*-test, $P > 0.9$), though females from fed hosts tended to be larger. Male yield differed significantly ($P < 0.05$).

Parasite emergence versus time of host har-

vest: In three trials we harvested groups of hosts on alternate days between 7 and 13 days after exposure to the parasites. Percentage parasitism differed ($P < 0.05$) among days in trials one and three (higher parasitism with later host harvest), but did not differ in trial two (Table 1).

Parasite loads in host larvae: The vast majority of female *H. magnapapula* emerged as single parasites, although they rarely emerged together with a male or another female (Fig. 3). Hosts usually yielded one to three males. Loads of more than four to five emerging males were rare, although up to nine very small males emerged from a single host.

Groups of 150 host larvae/trial were dissected at time of harvest, while other hosts were placed individually in water in ELISA plates to allow parasite emergence. In one trial, percentage parasitism based on emergence (12.5%) was slightly greater than that based on dissection (11.3%). In seven trials, parasitism estimated by *H. magnapapula* emergence ranged from 18.8–76.2% less than parasitism based on dissection. Parasitism was significantly different ($P < 0.05$) in only one trial and when trials were pooled. Overall, parasitism based on emergence (10.1% of 1,440 hosts) underestimated actual parasitism (14.9% of 1,200 hosts) by 32.2%. Parasite loads were similar (paired *t*-test, $P > 0.5$) for dissected hosts (1.83 ± 0.12 nematodes/parasitized host) versus hosts held for parasite emergence (1.77 ± 0.09 nematodes/parasitized host).

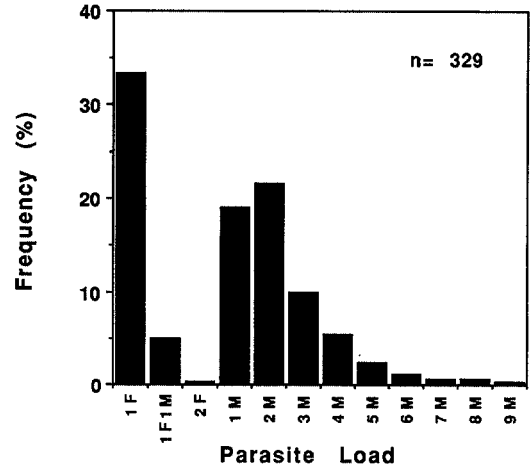

FIG. 3. Distribution of *H. magnapapula* loads (emerging parasites) in individual *C. variipennis* larvae. F = female nematode, M = male nematode.

Parasitism of adult hosts: Host larvae were exposed at 7 days of age, and pupae were collected every other day throughout the pupation period. Pupae were held for emergence and adult dissection (Fig. 4). Percentage parasitism was slightly but significantly higher ($P < 0.05$) for midges pupating earlier; 2.2 to 2.4% of midges pupating between days 7 and 11 were parasitized, versus 0.7 to 1.5% for those pupating subsequently. Adult parasitism varied between 0.6 and 4.3% among the

TABLE 1. Emergence of *H. magnapapula* from *C. variipennis* larvae harvested from rearing pans at different times following exposure.

Trial	Day of harvest	No. of hosts	No. of hosts held parasitized (%)	Parasite yield	
				Males	Females
1	7	96	10 (10.4)	13	6
	9	96	34 (35.4)	48	16
	11	96	22 (22.9)†	17	13
2	9	96	12 (12.5)	7	6
	11	96	17 (17.7)	12	10
	13	96	7 (7.3) nsd	7	3
3	9	96	16 (16.7)	12	10
	11	72	23 (31.9)†	23	10

† Relative parasitism within trial significantly different among days (chi-square test, $P < 0.05$); nsd = no significant difference ($P > 0.05$).

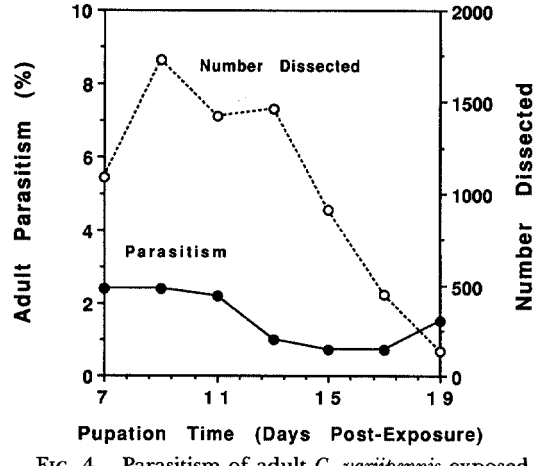

FIG. 4. Parasitism of adult *C. variipennis* exposed as 7-day old larvae to preparasites of *H. magnapapula.*

seven trials. In one case a female *H. magnapapula* dissected from a host was placed in a dish of water with males also dissected from adult hosts, and the female mated and produced preparasites. A few individual adult midges harbored both a male and a female nematode. Overall, 1.9% of adult male hosts and 1.6% of adult female hosts were parasitized at similar levels ($P > 0.05$). Parasitism of larvae in those same trials, according to 96 individually held larvae in water (no food) per trial, averaged 15.0%. There was no correlation ($P > 0.1$) between larval and adult parasitism by trial.

Parasite loads did differ ($P < 0.05$), however, for adults and larvae. In parasitized adults, the numbers harboring 1, 2, and ≥3 nematodes per host were 103, 8, and 4, respectively. Mean parasite load for adults was 1.15 nematodes/host. For larvae from the same cohorts, 45 harbored one parasite, 12 harbored two, and 15 harbored ≥ three, for a mean parasite load of 1.74 nematodes/host. While some immature nematodes dissected from adults could not be sexed, the overall sex ratio (male:female) for nematodes of known sex from adults was 0.48, versus 2.22 in larval hosts. Adult midges that were parasitized were more likely to contain a female nematode than were parasitized larvae.

In two trials we exposed hosts at 12 days of age (fourth-instar), rather than the usual 7 days. Exposure of older larvae resulted in higher carryover of *H. magnapapula* into the adult (Fig. 5). Hosts developed unusually quickly in trial two, and adult percentage parasitism was higher in those that pupated later ($P < 0.05$). Hosts developed at a more typical rate in trial one; parasitism was lower in early-pupating hosts, rose, then declined to negligible levels in very late-emerging hosts ($P < 0.05$). Overall, adult parasitism was 6.4% ($n = 751$) in trial one and 12.2% ($n = 987$) in trial two. Adult parasite load in these trials averaged 1.44 *H. magnapapula* per parasitized *C. variipennis;* we often could not determine nematode sex. Larvae harvested 7 days after exposure and held individually

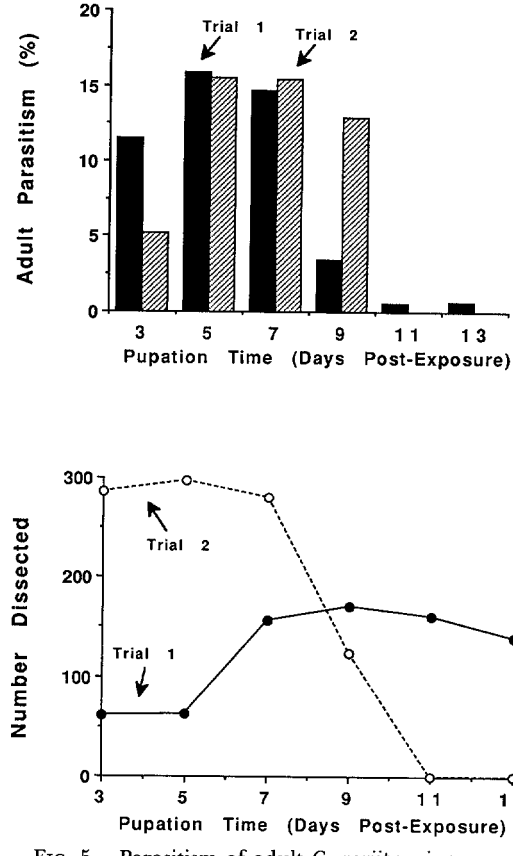

FIG. 5. Parasitism of adult *C. variipennis* exposed as 12-day old larvae to preparasites of *H. magnapapula.*

in water (no food) showed 5.2 and 8.3% parasitism, respectively, for the two trials ($n = 96$/trial). Some adult hosts in these trials harbored considerable numbers of nematodes—up to six in one parasitized male.

Parasitism in adults from late-exposed larvae differed according to host sex ($P < 0.05$). Male *C. variipennis* pupate sooner than females. Average parasitism in male hosts was 14.1% (148/1047 dissected). Parasitism in female hosts was only 2.9% (20/691 dissected).

DISCUSSION

Heleidomermis magnapapula was readily cultured in *Culicoides variipennis.* As with other mermithids, its life cycle is closely synchronized with that of the host. Main-

tenance of both hosts and parasites in polyester pads bathed in nutrient-rich water is adequate for limited mermithid production but has inherent difficulties. Hosts usually inhabit the pad surface, but substantial numbers occurred on the bottom of the pads. Adding *H. magnapapula* females shortly before they produce preparasites allowed adults to move and distribute progeny within the pads, which is important if the preparasites are infective for only a short time (18). Neither the parasites nor the young host larvae can be observed, however, and we cannot presently determine how the hosts or parasites disperse within the pads or how effectively they contact each other. The low overall parasitism rate of 10.4%, coupled with a fairly high frequency of superparasitism and an overall mermithid sex ratio of 2.6 males/female, suggests distribution of parasites relative to hosts may be clumped. The level of parasitism and sex ratio in the laboratory were similar to the field over a 4-month period in the fall at a southern California habitat (11.8% parasitism and 2.4 males/female) (16).

Assuming not all host eggs hatched and some early-stage hosts died, there were approximately 1,500 to 2,000 hosts per pan. Twelve female mermithids with an average of 1,267 preparasites each were added. Therefore the preparasite:host ratio probably was between 7 and 10, which is similar to the ratios used for mass production in *R. culicivorax* (24).

Young (late second or early third-instar) hosts are parasitized readily. We have not yet tested host age versus parasitism, but younger hosts generally are more susceptible to parasitism by mermithids (1,20,23, 28).

Sex ratio in *H. magnapapula* is dependent on the number of nematodes within the host, as also is the case with other mermithids, including those from mosquitoes or blackflies (3,11,17,19). Production of males does not appear to be a problem in *H. magnapapula.* This is more similar to *R. culicivorax* than to *Octomyomermis muspratti,* where high parasite loads are required to produce significant numbers of males (19).

Male *H. magnapapula* emerge before females, possibly reflecting greater demands on host nutrients and earlier death in hosts harboring multiple parasites (1,6,17,19). Males were produced if the host harbored more than two nematodes. Up to nine very small male nematodes emerged from a single host, but successful emergence of more than four or five was rare. Blackmore (2) documented that total nematode volume/host for *Romanomermis* was independent of parasite load, although individual parasites were smaller at high parasite loads. It is likely that hosts attacked by many preparasites die rapidly; we have observed this in glass dishes of water. Hosts harboring a single nematode yielded 64% females, whereas 36% yielded large males. The distribution of parasite loads in host larvae in the laboratory was not different ($P > 0.05$) from loads documented in host larvae in the field in southern California (16). Similarities in parasite loads, sex ratios, and levels of parasitism suggest that intensity and frequency of host–parasite contact in the laboratory rearing system were similar to the field setting.

Holding *C. variipennis* larvae in water for emergence of *H. magnapapula* had the advantage of ease of handling, host observation, and nematode harvest and sex determination. The primary disadvantage was that host larvae were starved from time of harvest to parasite emergence. When larvae were harvested close to the time of parasite emergence (9 days after exposure), the time pattern of mermithid emergence was similar for unfed versus fed host larvae. Host starvation had no effect on female parasite yield. Male nematode yield was actually higher from unfed hosts in some of our experiments, but further work is needed to confirm this and determine the mechanism involved. A higher proportion of *R. culicivorax* males result from mosquito hosts on restricted diet regimens from an early age (6,17).

Parasite emergence could be delayed by host starvation, and some hosts eventually

died with the parasite still inside. This has implications for sampling field populations. Percentage parasitism derived by holding hosts individually in water underestimated actual parasitism (determined by dissection) by about 32%. The level of conservatism probably is a function of time of host exposure to parasites and host and nematode maturity at time of collection. Our data indicate that time of sampling from a given host cohort may substantially affect parasitism as determined by parasite emergence. Higher parasitism in larvae sampled from a population later in development may reflect retarded development in parasitized hosts (4,6,15,28).

Agar, recently shown to be suitable for *C. variipennis* rearing (14), is transparent, similar to habitat mud in consistency, and offers a workable alternative for host rearing, observation and manipulation of the host–parasite system. The strong relationship between female length and preparasite number might be used to estimate preparasite numbers added to such an arena in the most natural way (i.e., adding a gravid female to the agar surface).

Heleidomermis magnapapula appeared to be primarily a parasite of the larval host. However, carryover into the adult midge occurred regularly. Adults from hosts exposed as late second or early third-instars were parasitized at a level of 0.5 to 2.5%; slightly higher among hosts pupating earlier. Parasite loads were higher in parasitized larvae when compared to parasitized adult midges from the same cohort. This may be due to less physiological stress, and a greater probability of successful pupation and adult emergence, in hosts with only one or a few parasites. The lower parasite loads resulted in a greater probability of an adult midge harboring a female nematode. This might enhance the colonizing potential of *H. magnapapula* dispersed by parasitized adult *C. variipennis*. In cases where a parasitized adult carried both sexes, only a single introduction might be necessary.

Exposure of older larvae (fourth-instar, 12 days old) resulted in greater carryover of *H. magnapapula* into the adult, as occurs with *O. muspratti* (15,20). Carryover of mermithids into adult blackflies also is common at times, and is thought to facilitate upstream dispersal (10). Parasite loads in late exposure trials were not directly compared between host larvae and adults. Clearly, however, parasitized adults had higher parasite loads than did adults from the earlier trials (exposed as second- or third-instars). In fact, up to six nematodes were dissected from one adult male midge, close to the maximum number of parasites observed successfully emerging from a host larva in earlier trials. Any parasite-induced stress might have been less severe, as these hosts were pupating within a few days of exposure of *H. magnapapula*, when the parasites were still small. Older host larvae also might be refractory to parasitism. In the first trial, hosts pupating earlier (more mature at the time of exposure) were not as heavily parasitized as those pupating a few days later. In the second trial, in which hosts developed unusually rapidly, initial parasitism in early-emerging adults was even lower. Further studies on this point are needed.

Our laboratory data agree with field trends regarding parasitism in host larvae, but not regarding adult midge parasitism at the same site and time. Adult *C. variipennis* were parasitized at an extremely low level (3 of 5,568) in the field (16). Several factors may have contributed to the inability to detect parasitized adults in the earlier field studies. First, ultraviolet light and carbon dioxide-baited traps collected mostly females, and the present laboratory data indicate the earlier-emerging midges, particularly males, were more heavily parasitized. Most of the adults from the emergence traps actually were collected over a very few weeks as one of the ponds was drying up and becoming less attractive for oviposition. Possibly, parasitized midges already had emerged prior to the main emergence sampling effort at this pond. Additionally, of course, we know nothing

at present about the behavior of parasitized adults.

Female *H. magnapapula* may live for many days if unmated, but normally mate, produce their entire complement of preparasites, and die. This strategy differs from oviparous mermithids such as *Romanomermis,* which live outside the host and produce eggs over several weeks (18). The rapid turnover might be due to the severe nature of the host habitat, minimizing the duration of contact between the manure-polluted water and the free-living nematode stages (27). Like many mosquito species, *C. variipennis* is adapted to locating and colonizing ephemeral habitats quickly. It does not, however, seem to have the ability to persist long in such areas without free water (12), requiring recolonization. The parasite also seems to lack any means of long-term persistence in the absence of live hosts. The rapid life cycle of *H. magnapapula* therefore could be an adaptation to temporally and spatially unstable habitats.

Heleidomermis magnapapula can be maintained in *C. variipennis* in the laboratory. With improvements, limited mass production of *H. magnapapula* should be possible. Compared with *R. culicivorax,* the hosts are more difficult to rear, and recovery and handling of nematodes is more difficult. *Heleidomermis magnapapula* lacks a resistant life stage (e.g., eggs) that could be stored or transported easily. *H. magnapapula* also tracks host populations closely, adjusting its sex ratio to prevent overexploitation of hosts within a given habitat over time. Nevertheless, introduction of nematodes, perhaps via parasitized hosts, could assist dispersal to new habitats. It also may be possible to release large numbers of nematodes into small habitats to achieve short-term host reduction.

The nematode *R. culicivorax* can parasitize many mosquito species in several genera (22). At this point we do not know how wide the host range of *H. magnapapula* may be, and it is possible that the nematode could parasitize other *Culicoides,* many of which are serious nuisance pests or vectors of disease agents.

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