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Temperature Effects on Survival and Development of *Heleidomermis magnapapula* in the Laboratory

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Abstract: The mermithid *Heleidomermis magnapapula* Poinar and Mullens, a parasite of the biting midge *Culicoides variipennis* (Coquillett), was exposed to constant temperatures in the laboratory. Survival of the free-living stages and development times of eggs and the parasitic phase were inversely related to temperature. Average preparasite longevity was 70, 46, 42, and 22 hours at 15.6, 21.1, 26.7, and 32.2 C, respectively. Females survived significantly longer than males. Longevity in days (females/males) at different temperatures was 17.3/11.0 at 4.4 C, 9.0/8.2 at 15.6 C, 5.9/5.1 at 21.1 C, 5.2/4.7 at 26.7 C, and 4.4/3.6 at 32.2 C. Embryogenesis required 44 ± 2 degree days above a thermal minimum of 10.1 C, while parasitic development in host larvae required 214 ± 10 degree days above a thermal minimum of 8.9 C. Parasite responses to temperature were very closely related to temperature-dependent host development patterns.

Key words: biological control, host–parasite relationship, *Culicoides*, Mermithidae, nematode, survival, temperature, development.

Temperature is a principal factor in development and survival of poikilotherms, including nematodes and insects (1,17,19). Developmental rates plotted against temperature typically form a sigmoidal curve. The developmental rate increases with temperature until a thermal maximum is reached. Near this point the developmental rate slows, and death occurs somewhat after the thermal maximum is surpassed and critical enzyme systems are affected. At lower temperatures the rate approaches zero as the lower developmental threshold is reached. Most organisms can withstand temperatures considerably below the lower developmental threshold. While the point of freezing damage varies with the organism, most do eventually succumb to ice crystal formation and disruption of cellular membranes as temperatures drop below 0 C.

Heleidomermis magnapapula is a parasite of the biting midge *Culicoides variipennis* (Diptera: Ceratopogonidae). The life cycle has been described both in the field and laboratory and is quite unusual for the Mermithidae (13,14). Nematodes emerge from the insect host as adults and mate. Females are ovoviviparous, and the first larval molt occurs within the egg. Preparasites (second-stage larvae) emerge from the female then locate and penetrate the cuticle of a host larva. They develop to adults within the host and emerge usually within 11–16 days at temperatures of 23 C (13).

Though there are few studies on *H. magnapapula*, it appears to be widespread in *C. variipennis* in the United States and has been recovered sporadically from New York to California (7,10,14,16). Within California the nematode has been recovered from the northern Central Valley south to San Diego County, with parasitism levels as high as 69% (14). Because the host is responsible for bluetongue virus transmission to ruminants in North America (9), there is interest in *H. magnapapula* as a potential biological control agent of *C. variipennis* and perhaps other *Culicoides* spp.

Available field evidence and laboratory studies at room temperature suggest that the life cycle of *H. magnapapula* is closely synchronized with that of *C. variipennis* (13,14). Differential temperature responses, however, could influence spatial and seasonal distribution of the parasite and its potential for biological control. The present study was designed to assess temperature effects on embryogenesis, preparasite longevity, parasitic phase development, and adult longevity of *H. magnapapula*.

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Materials and Methods

Temperature effects on survival of adult *H. magnapapula* and duration of embryogenesis were studied using mermithids collected in the wild. Surface mud containing fourth (last) instar larvae of *C. variipennis* was collected from a dairy wastewater pond in western San Bernardino County, California. Host larvae were sieved (0.15-mm pore size) from the mud and held in distilled water in glass Petri dishes. Dishes were examined every 2–5 hours, and the midpoint of the interval was used as the time of adult nematode emergence from the host. One male and one female of the same age were placed together in distilled water in small watch glasses, which in turn were placed in capped plastic vials to prevent evaporation. Twenty pairs of nematodes were placed into each of the following constant temperatures: 4.4, 15.6, 21.1, 26.7, and 32.2 C. Nematodes in the 4.4 C temperature were held in darkness (refrigerator), while other temperature regimes had a 12L:12D photoperiod. Nematodes were examined every 12 hours until both adults had died. Time of death and first preparasite emergence were recorded.

Preparasite longevity and duration of the parasitic phase were determined using *H. magnapapula* from a laboratory colony; colony maintenance was described in detail earlier (13). The parasite colony was taken from a wastewater pond near the site mentioned above. Female *H. magnapapula* in the early phases of preparasite production were placed individually into dechlorinated tap water in small watch glasses. Preparasites produced over a period of 2 hours (ca. 100–200 individuals) were divided evenly (groups of 25–50) into four small watch glasses of dechlorinated tap water. These were labelled by time and female number and were placed into sealed plastic containers to minimize evaporation. They then were placed into chambers with constant temperatures of 15.6, 21.1, 26.7, or 32.2 C. Preparasites were examined at the following time intervals: 8, 12, 16, 24, 30, 36, 48, 54, 60, 72, 84, 96, 120, and 144 hours. At each interval, preparasites were scored for motility; nematodes that did not move after several minutes were considered dead.

Temperature effects on the duration of the parasitic phase were assessed by exposing groups of 10-second instar, laboratory-reared *C. variipennis* larvae in 35-mm diameter Petri dishes with a 3 mm thick 1% water agar substrate. Each dish received approximately 50 young preparasites (<8 hours after emergence) in a small amount of dechlorinated tap water. Host larvae could swim freely in the thin water film above the agar and were attacked by the nematode preparasites. Five dishes, including two unexposed controls, were used at each temperature in the first experimental replication. Seven dishes, plus two unexposed controls, were used in the second experimental replication.

After a 24-hour exposure period at 22 C, the dishes were moved into constant temperatures of 15.6, 21.1, 26.7, or 32.2 C. Host larvae were fed as needed (1–3 day intervals, depending on the host size and temperature) with the bacterial feeding nematode *Panagrellus redivivus* (L.)(12). Dishes were examined daily. Emerged *H. magnapapula* adults were removed and recorded by emergence time and sex. Pupating *C. variipennis* were allowed to emerge and were dissected to detect nematodes persisting into the adult stage. For the purposes of this study, the "parasitic phase" of *H. magnapapula* included second-stage juveniles after host penetration, third- and fourth-stage juveniles, and adults before they emerge. Emerged mermithid adults were seen easily on or in the transparent agar and were readily differentiated from the smaller *P. redivivus* used as host food.

The duration of embryogenesis, preparasite longevity, and adult longevity were examined using analysis of variance. Wild-collected adult mermithids that died from fungal infections were excluded from the analysis. Because variances were related to means, data were log-transformed before analysis. Means were separated using Tukey's studentized range test (HSD) at an

alpha level of 0.05. Data on duration of parasitic development were analyzed similarly but did not require transformation. Linear regression was applied to the dependent developmental rate data (1/days) versus temperature for both embryogenesis and parasitic phase duration. Intercepts with the x (temperature) axis were used as approximations of the lower developmental thresholds (t) and for calculation of degree days necessary for development (1,19).

Results

Lifespan of mated pairs of adult *H. magnapapula* was inversely related to temperature (Table 1). Analysis of variance, using temperature and nematode sex as main effects plus the interaction, indicated that male longevity overall was significantly less than that of females ($P < 0.01$), regardless of temperature (interaction $P > 0.4$). Male longevity ranged from 3.6 days at 32.2 C to 11 days at 4.4 C, while the range for females was 4.4 to 17.3 days.

Preparasite longevity also varied inversely with temperature (Table 2). At 32.2 C, average longevity was 21.7 hours, approximately 90% were dead by 36 hours, and none lived beyond 48 hours. At 26.7 C, survival was 83% at 36 hours, but 90% were dead by 54 hours, and none survived beyond 72 hours. At 21.1 C, most preparasites (86%) still were vigorous at 36 hours, and average longevity of 46 hours was not significantly different from that for 26.7 C (42 hours). Mortality also increased rapidly after 36 hours at 21.1 C; approximately 97% were dead by 84 hours, and none survived beyond 96 hours. At 15.6 C, 75% were still alive and vigorous at 60 hours, but 95% were dead by 120 hours, and only 3 sluggish individuals (0.6%) were alive at 144 hours, when the study was terminated.

Table 1. Temperature effects on lifespan (mean ± SE) of adult *Heleidomermis magnapapula* held in water.

Temperature (C)	Sex	n	Lifespan (hours)
4.4	F	20	414.4 ± 0.3 a
	M	20	262.8 ± 0.3 b
15.6	F	19	214.6 ± 0.2 bc
	M	19	195.5 ± 0.2 bc
21.1	F	19	142.3 ± 0.2 cd
	M	17	122.0 ± 0.2 de
26.7	F	19	124.1 ± 0.1 de
	M	18	112.5 ± 0.2 de
32.2	F	20	106.3 ± 0.2 de
	M	17	85.7 ± 0.2 e

Column means followed by the same letter are not significantly different ($P > 0.05$, Tukey's studentized range test). Analysis on log-transformed data. Means backtransformed; standard errors in log units (Zar 1984).

Embryogenesis was significantly longer at lower temperatures (Table 2). No egg development was noted at temperatures of 4.4 C. Preparasites were produced after 7.4 days at 15.6 C, but this developmental period decreased to only 2 days at 32.2 C.

Hosts were parasitized and reared on agar to determine the duration of parasitic development. A two-way analysis of variance of total mermithid emergence per replication (main effects were temperature and trial, plus the interaction) indicated that there was no significant difference among the temperatures ($P > 0.3$) and no significant temperature × trial interaction ($P > 0.6$). Trials differed in total nematode yield, with an average of 6.6 ± 0.9 nematodes per dish in trial one and 4.4 ± 0.8 nematodes in trial two.

Time of nematode emergence (at a fixed temperature) did not differ between the trials. Thus, the trials were pooled for further analysis. Duration of parasitic development differed significantly among temperatures (Table 2). The ranges of adult mermithid emergence from *C. variipennis* larvae were as follows: 23 to 47 days at 15.6 C, 13–23 days at 21.1 C, 9–23 days at 26.7 C, and 8–11 days at 32.2 C. At each temperature nematode emergence began just as unparasitized hosts in the control dishes (or in the treated dishes) began pupating. All *H. magnapapula* emerged from host larvae, except for a single male *C. variipennis* adult, which harbored two male parasites upon dissection. These two nematodes were not included in the time of emergence calculations. Most emerging mermithids were males. No female *H. mag-*

TABLE 2. Temperature effects on life history parameters (mean ± SE) of immature *Heleidomermis magnapapula*. Number (*n*) noted in parentheses beneath each mean value.

Temperature (C)	Duration of embryogenesis (hours)	Preparasite lifespan (hours)	Parasitic development (days)
15.6	178.0 ± 0.05 a	70.1 ± 0.5 a	34.7 ± 6.2 a
	(14 females)	(486)	(64)
21.1	104.6 ± 0.05 b	45.5 ± 0.6 b	15.8 ± 2.2 b
	(14 females)	(562)	(64)
26.7	60.3 ± 0.06 c	42.1 ± 0.4 b	12.6 ± 3.3 c
	(17 females)	(510)	(72)
32.2	47.7 ± 0.04 d	21.7 ± 0.5 c	9.1 ± 1.0 d
	(18 females)	(532)	(38)

Column means followed by the same letter are not significantly different ($P > 0.05$, Tukey's studentized range test). Analysis of log-transformed data for embryogenesis and lifespan. Means backtransformed: standard errors in log units (Zar 1984). Analysis on raw data for parasitic development.

napapula were recovered at 32.2 C, and only three females/temperature emerged at 21.1 and 26.7 C.

Developmental rates (1/days) for embryogenesis and parasitic phase, regressed against temperature, resulted in excellent linear fits (Fig. 1). There was no indication of declining rates at the highest or lowest temperatures used. For embryogenesis, the relationship was described by the equation $y = -0.233 + 0.023 \times$(temperature), with an r^2 value of 0.99. For parasitic development, the regression was $y = -0.042 + 0.005 \times (r^2 = 0.99)$. Extrapolating to the *x*-axis intercept, approximate developmental threshold values (*t*) were 10.1 C for embryogenesis and 8.9 C for parasitic phase development.

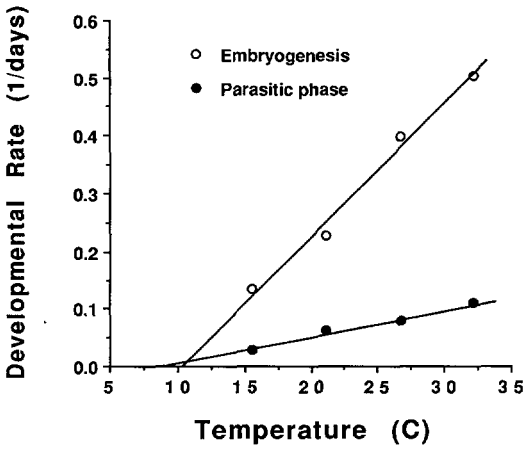

FIG. 1. Developmental rates (1/developmental duration in days) of embryos and parasitic phase of *Heleidomermis magnapapula* at different constant laboratory temperatures.

DISCUSSION

As expected, temperature significantly affected all the life stages of *H. magnapapula*. Females, which were larger, survived longer than males. Adults survived as long as 11 days (males) to 17 days (females) at refrigerator temperatures (4 C). Mating in *H. magnapapula* can occur immediately after exit from the host. It is likely that many of the females used in the present study had mated even before being placed together with a male for the adult longevity studies. Mated females stored at 4 C for 1–2 weeks are viable and often are used for parasite culture maintenance (13). Mated females, in particular, typically die soon after giving birth to a complement of preparasites. Unmated females of *H. magnapapula* live up to 2 weeks in the laboratory at temperatures of 23 C (13), which is substantially longer than survival of mated females. It is conceivable that free-living, unmated adults might survive several weeks in host habitat mud during cooler periods.

Longevity of preparasites, the most ephemeral life stage, declined rapidly at high temperatures. Nematode infectivity was not measured in these studies, but would be expected to be proportionally less than motility. Studies on effects of

temperature on preparasites of the mosquito parasite *Romanomermis culicivorax* Ross and Smith demonstrated that nematodes survived a maximum of about 2 days at 32 C and 6 days at 1–12 C (3). Infectivity and motility of *R. culicivorax* were related and prolonged at lower temperatures, but infectivity declined much faster than motility. Motility of less than approximately 40% corresponded to very low infectivity for mosquito larvae. In the present studies, preparasites of *H. magnapapula* survived well up to a certain point, after which mortality increased quickly. Motile preparasites toward the end of the period were quite sluggish, and their infectivity is suspect. Based on motility and apparent vigor of the preparasites, we hypothesize that infectivity of *H. magnapapula* should be good for about 12–18 hours at 32 C, 24–36 hours at 26.7 C, 24–48 hours at 21.1 C, and 60 hours at 15.6 C. This remains to be tested experimentally.

Two developmental rate functions were calculated, one for embryogenesis and one for duration of the parasitic phase. Embryogenesis of *H. magnapapula* responded to temperature and was quite extended (7.4 days) at 15.6 C. Lack of embryogenesis at 4.4 C could have been caused by lack of mating. It is likely, however, that at least some of the females had mated in the general collecting dish before being placed with individual males at the lowest temperature (4.4 C) or perhaps mated with the individual male before temperatures cooled in the dish. As no detectable egg development took place at 4.4 C, the true developmental threshold temperature (t) must lie between 4.4 and 15.6 C. The t value for embryogenesis, as estimated by linear regression, was 10.1 C. Parasitic phase development was considerably longer, requiring an average of up to 35 days at 15.6 C. The estimated t for the parasitic phase was 8.9 C, requiring about 214 degree days for development.

Thermal requirements of *H. magnapapula* are very similar to those for the host *C. variipennis* larvae (t = 10.7 C and 213 degree days), and the developmental rate slopes are nearly identical (0.00467 for the mermithid and 0.00453 for the host) (11). This degree of synchrony almost certainly is adaptive for the parasite, allowing a given cohort of nematodes to begin to emerge at about the time hosts are pupating. The period of *H. magnapapula* embryogenesis is slightly longer than the required pupal period for the host (11), probably providing reasonable synchrony between preparasites in the environment and susceptible young host larvae (13). Similarly matched thermal requirements for insect hosts and their mermithid parasitic phase also have been noted for *R. culicivorax* in *Culex pipiens* (8) and for *Hexamermis* sp. parasitizing the grasshopper *Chortoicetes terminifera* (Walker) (6). Parasite–host developmental synchrony should help prevent overuse of host resources by a more rapidly developing parasite, which could result in premature death of both host and parasite. Conversely, the synchrony also should act to prevent possibly maladaptive carryover of a parasite into a later host stage.

Nearly all emerging adult *H. magnapapula* in the present studies were males, which are known to emerge slightly but significantly earlier than females (13). The paucity of females was somewhat surprising and precluded our calculating possible temperature effects on nematode sex ratio. Low temperatures and low parasite: host ratios favor production of females in *R. culicivorax,* (15,18). The mechanism of temperature or parasite load effects on sex ratio probably is nutrient availability for the parasites shortly after host penetration (5). Total nematode volume per parasitized host in *R. culicivorax* increases slightly (4) or is fairly constant with increasing parasite loads (2), suggesting intraspecific competition for nutrients. Additionally, the documented male-biased sex ratio of *R. culicivorax* from starved mosquito hosts (15) tends to support the conclusion that nutrient availability to the host influences parasite sex ratio. Superparasitized *C. vari-*

ipennis larvae also produce predominantly male *H. magnapapula* (13,14).

The level of parasitism in our temperature experiments intentionally was held at 30–50% to avoid excessive superparasitism. Larvae of *C. variipennis* reared on agar exhibit a lagged period of maturation compared with larvae reared on microorganisms in opaque fibrous pads (12). This apparently is due to the fact that the tiny early instar *C. variipennis* larvae are inefficient predators on *P. redivivus*. It is possible that nutritional stress among host larvae during the few days following parasitism caused the male bias among emerging mermithids. The developmental rates reported here for the parasitic phase may vary somewhat under different parasite loads or host nutritional standards.

We saw no evidence of declining developmental rates at the highest temperature tested (32.2 C), and the upper rate maximum for *H. magnapapula* could be considerably higher. The upper range of temperature tolerance for *C. variipennis* larvae is also unknown, but local ponds containing both the host and parasite commonly reach temperatures of 34–36 C in summer. The host overwinters mainly in the larval stage. The *t* values for host and parasite are similar, and we have demonstrated the potential for extending the developmental period of the parasite to at least 47 days in host larvae at low temperatures. The free-living stages (adults and especially preparasites) are relatively short-lived. We therefore consider it likely that *H. magnapapula* passes the winter primarily within parasitized hosts, especially in more northerly regions. In subtropical regions such as southern California, both *C. variipennis* and *H. magnapapula* may be active during moderately cool winter months.

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