Temperature Effects on the Parasitic Phase of Romanomermis culicivorax in Culex pipiens

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Abstract: The developmental period for the parasitic stage of *Romanomermis culicivorax* in *Culex pipiens* was determined at constant and fluctuating temperatures. The median developmental times at 15, 18, 20, 27, and 32 C were 27.6, 17.2, 11.5, 7.1, and 5.8 days, respectively. The optimum temperature range for development of the parasitic stage in *C. pipiens* was 20 to 32 C. The threshold for development was calculated as 10.4 C, and the heat units required for development were 122.2 ± 3.6 day-degrees. Development at fluctuating temperatures conformed to that predicted by the constant temperature data. *Key Words:* development, temperature, biological control.

Petersen (6) has shown that *Romanomermis culicivorax* Ross and Smith is a promising biocontrol agent against larval stages of some mosquito species. Further development of the potentials of this mermithid and other mermithid species in biological control requires a thorough understanding of the ecology of the parasite and host. Petersen (6) has established some of the effects of density dependent and independent factors on the infectivity of *R. culicivorax*. Temperature effects on the infectivity of *R. culicivorax* have been well defined (1, 4). In the present investigation, we have studied the effects of constant and fluctuating temperatures on the duration of the parasitic stage of *R. culicivorax* in *Culex pipiens* Linnaeus. These data should aid in predicting the generation time for *R. culicivorax* under field conditions.

MATERIALS AND METHODS

Romanomermis culicivorax was obtained from Dr. J. J. Petersen (USDA, Lake Charles, Louisiana) and an autogenous and hybrid strain of *Culex pipiens* was obtained from Dr. A. R. Barr (University of California, Los Angeles). Nematodes were maintained according to the procedures of Petersen and Willis (9).

Infective larvae of *R. culicivorax* were obtained by transferring approximately 70 gm of moist sand with eggs from a sand culture of *R. culicivorax* (9) to a plastic container and adding 100 ml dechlorinated tap water. After 3-4 h, the water was decanted into a 100-ml graduate cylinder and left for 1 h. Unhatched eggs settled to the bottom, and infective larvae were carefully drawn off in the top 60 ml. The larvae in three 0.5-ml samples were counted, and the average number per ml was used to calculate the volume required for infection of the mosquito larvae.

Thirty, first-instar larvae of *C. pipiens* were placed in each of three 500-ml polystyrene food containers with 250 ml dechlorinated tap water in each container. Ninety infective larvae of *R. culicivorax* were added to each container and were left for 12 h. The infection process was terminated by pouring the contents of each container into a seive with 180-μm openings. The mosquito larvae were rinsed with water, returned to clean 500-ml containers with 250 ml water in each, and fed a mixture of 3 parts finely ground rabbit chow (Purina) and 1 part brewer's yeast (ICN Pharmaceuticals). The containers were placed in an incubator at one of the six experimental temperatures. The variable temperature incubator was set for 20 C, 12 h and 27 C, 12 h. Temperature control was ±0.5 C at all temperatures.

When fourth-instar mosquito larvae were observed in the containers, infected mosquito larvae were transferred to individual 10-ml polystyrene vials containing 8 ml water, fed, and checked for the emergence of postparasitic nematodes every 12 h. The emergence of postparasites signified the completion of the parasitic phase. The sex of the postparasites was not determined.

RESULTS AND DISCUSSION

The results summarized in Fig 1. represent the pooled data for three separate experiments at each temperature: 20, 27, 32,

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and 20/27 C. The data for 15 and 18 C were derived from single experiments and were in agreement with preliminary experiments. The data from multiple infections of five or more *R. culicivorax* were eliminated from consideration since postparasitic larvae emerged prematurely from such highly infected hosts (5).

The time for emergence of postparasites from the mosquito hosts was shortest, 2.5 to 3.0 days, at the higher temperatures after the parasitic phase was complete. These times are comparable to those reported by Petersen (5). In contrast, emergence was prolonged at the lower temperatures, 15 to 20 C, and required 6.5 to 7.5 days. This response also occurred in the population kept at the fluctuating temperature, 20/27 C.

The variability in the ranges of time taken for emergence necessitated the calculation of the median development time (DT_{50}) as a measure of the duration of the parasitic phase of *R. culicivorax* in *C. pipiens*. This statistic and the 95% confidence limits were computed by probit analysis (3).

The median developmental times were significantly different at each temperature studied (Table 1). A graphical plot of median developmental times versus temperature yielded a hyperbolic curve and the data from the fluctuating temperature experiments fit the curve closely (Fig. 2). The optimum temperature range for the development of the parasitic stage of *R. culicivorax* in *C. pipiens* was 20 to 32 C. This temperature range is the same as that found for the infective stage of *R. culicivorax* (1).

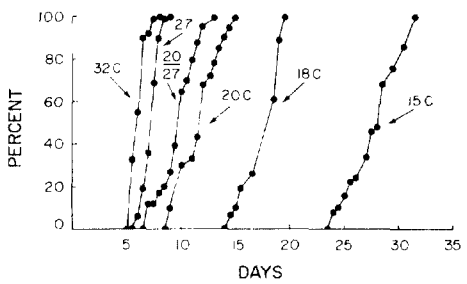

FIG. 1. The accumulated percentage of *Culex pipiens* larvae dying from emerging postparasitic *Romanomermis culicivorax* at constant and fluctuating temperature.

TABLE 1. Median developmental time in days for postparasites of *Romanomermis culicivorax* at different temperatures.

Temperature (C)	N[a]	DT_{50}[b]	Confidence Limits (95%)	Heat Units[c] (Day-Degrees)
15	50	27.6	27.3-27.9	126.9
18	72	17.2	16.4-18.1	130.7
20	173	11.5	11.2-11.7	110.4
27	105	7.1	7.0-7.2	117.8
32	209	5.8	5.2-6.1	125.2
20/27	124	9.6	9.3-9.8	125.7

[a]Number of mosquitoes from which nematodes emerged.
[b]Determined by probit analysis of data from Fig. 1.
[c]Heat Units = DT_{50} x (experimental temperature-threshold temperature). Average of heat units from constant temperatures is 122.2±3.6 (SE).

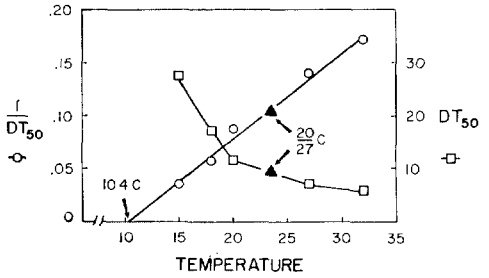

FIG. 2. Development of the parasitic stage of *Romanomermis culicivorax* in *Culex pipiens*. The data for fluctuating temperature (△) were not used to fit the curves. Time-temperature curve, -□-; rate-temperature curve, -○-.

Although the upper and lower temperature limits for the development of *R. culicivorax* in *C. pipiens* were not determined empirically in these experiments, the theoretical threshold for development could be estimated (12). This technique assumes that the relationship of development with temperature follows an equilateral hyperbola, and therefore, the relationship of the developmental rate (1/time) with temperature is linear. The intercept with the temperature axis gives an estimate of the developmental threshold. The reciprocals of the median developmental times were calculated and plotted versus temperature. The line was fitted by the least squares method, and a developmental threshold or "developmental zero" (12) of 10.4 C was obtained for the parasitic stage of *R.*

culicivorax. This value was a reasonable estimate since *C. pipiens* does not develop below 11 C (2). From this relationship, the fraction of development of the parasitic stage at a fixed or variable temperature can be estimated for any time interval (12).

The results for the fluctuating temperature experiment fit both the graphical plot (Fig. 2) and calculation of heat units. *R. culicivorax* in *C. pipiens* requires 122.2±3.6 day-degrees for development of the parasitic stage. A comparable statistic for the mosquito host could not be calculated since the only data available in the literature for a *C. pipiens* subspecies (10) did not yield a constant value for heat units when a similar analysis was attempted.

These experiments indicate that the development time of *R. culicivorax* in a host mosquito at both constant and fluctuating temperatures is predictable. Thus, parasite development in field studies should be predictable when diurnal changes in temperatures are known. Similar studies have been carried out on *Meloidogyne hapla* Chitwood in field studies (11). Further temperature studies, as initiated by Petersen (7, 8), are required on the development of postparasitic stages and the eggs of *R. culicivorax,* so prediction of the complete life cycle will be possible for field conditions. Such information would facilitate the management of mosquito populations through appropriately timed releases of the infectious larvae of *R. culicivorax,* and the biology of this parasite might be incorporated into models of mosquito population dynamics.

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