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Effect of Temperature on the Development of *Thelastoma bulhoesi* (Oxyurata, Thelastomatida) and Other Nematodes

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Abstract: Embryonation of *Thelastoma bulhoesi* was monitored at eight temperatures between 0 and 35 C. Cell division did not occur below 15 C or at 35 C. Development was most rapid at 25 and 30 C. The effect of temperature on the rate of embryological development of *T. bulhoesi* at different stages was measured using the temperature coefficient, Q_{10}. The developmental temperature response curve obtained for *T. bulhoesi* was similar to enzyme temperature response curves. Our evidence supports the thesis that nematode embryonation, as affected by temperature, varies between species and between stages of development.

Key words: temperature coefficient, Q_{10}.

When Wallace (15) reviewed the literature on the effect of temperature on nematodes in 1961, he stated "a mathematical interpretation of the influence of temperature on development seems a useful approach . . ." and "the minimum and optimum temperatures for development are also related to enzyme activity." A mathematical model still does not appear to have been developed. Many of these studies treat development from egg to juvenile as a single event. In fact, several separate pro-

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TABLE 1. Percentage of egg development of *Thelastoma bulhoesi* in 0.75% NaCl, pH 7.0, at 15 C.

	Stage of development									
	No. cells present					Later stages*				Center
Hours	(i): 1	2	4	8	16	G	PV	J_1	R	of percentages
	1	2	3	4	5	6	7	8	9	
0	100	0	0	0	0	0	0	0	0	1.00
1	91	3	0	0	0	0	0	0	0	1.03
2	82	12	0	0	0	0	0	0	0	1.13
4	56	36	1	0	0	0	0	0	0	1.40
8	32	43	17	0	0	0	0	0	0	1.84
16	14	53	22	0	0	0	0	0	0	2.04
32	2	9	43	29	0	0	0	0	0	3.14
64	No data available									
128	2	0	0	9	42	26	0	0	0	5.12
256	2	0	0	0	0	27	43	0	0	6.48
512	2	0	0	0	0	2	2	35	15	7.64

* G = gastrula. PV = prevermiform. J_1 = first-stage juvenile. R = resistant.

cesses can be identified; ultimately each cell division can be viewed independently. Examining the effect of temperature on subprocesses such as cleavage, gastrulation, and maturation may be beneficial. Factors limiting development during discrete embryological stages should be compared for a variety of nematode species.

The temperature coefficient, Q_{10}, can indicate what limits a given reaction: low values (1.2 to 1.4) indicate physical reactions that depend on molecular diffusion or photochemical effects, whereas enzyme-catalyzed reactions typically have values from 1.3 to 5, generally close to 2 (1). Temperature coefficients may have value as ecological tools for comparing temperature effects on different species. In this paper we calculate Q_{10} values during embryonation of *Thelastoma bulhoesi* to define conditions limiting the various stages of embryological development.

MATERIALS AND METHODS

Female *T. bulhoesi* were removed from the hindgut of cockroaches (*Periplaneta americana*), placed in sterile saline (0.75% NaCl) on a depression slide, and macerated with a needle to liberate unembryonated ova from the uteri. A cover glass was placed over the eggs to form a wet mount, and the slide was placed in a petri dish with moist filter paper in the bottom. Slides, each containing about 175 eggs, were incubated at 0, 5, 10, 15, 20, 25, 30, and 35 C, and eggs were examined microscopically (100 ×) after 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 hours.

TABLE 2. Percentage of egg development of *Thelastoma bulhoesi* in 0.75% NaCl, pH 7.0, at 25 C.

	Stage of development									
	No. cells present					Later stages*				Center
Hours	(i): 1	2	4	8	16	G	PV	J_1	R	of percentages
	1	2	3	4	5	6	7	8	0	
0	100	0	0	0	0	0	0	0	0	0
1	94	1	0	0	0	0	0	0	0	1.01
2	43	49	3	0	0	0	0	0	0	1.59
4	1	27	52	15	0	0	0	0	0	2.85
8	0	8	24	24	37	2	0	0	0	3.88
16	0	0	0	0	15	78	3	0	0	5.87
32	0	0	0	0	11	55	29	0	0	6.19
64	0	0	0	0	0	0	4	91	0	7.69
128	0	0	0	0	0	0	1	1	93	8.97

* G = gastrula. PV = prevermiform. J_1 = first-stage juvenile. R = resistant.

TABLE 3. Effect of temperature (as Q_{10}) on successive stages of development of *Thelastoma bulhoesi* eggs.

Stage of development	Q_{10} for three temperature ranges		
	15–20 C	20–25 C	25–30 C
1–2 cells	16.0	1.0	1.0
2–4 cells	2.9	49.9	1.0
4–8 cells	4.0	64.0	0.3
8–16 cells	7.2	256.8	9.1
16 cells to gastrula	1.0	64.2	0.3
Gastrula to prevermiform	0.3	16.0	4.0
Prevermiform to J_1	1.0	64.0	4.0

The number of eggs in the 1-, 2-, 4-, 8-, and 16-cell; gastrula (G); prevermiform (PV); first-juvenile (J_1); and resistant (R) stages were counted at each of the above times. The number of eggs in each stage was divided by the total number of eggs to yield the percentage in a given stage for each temperature and time interval.

The Q_{10} values for the temperature ranges 15–20 C, 20–25 C, and 25–30 C were calculated according to the formula (10):

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{\frac{10}{t_1 - t_2}}$$

where K_1 and K_2 are rates of nematode development expressed as reciprocals of hours elapsed between consecutive developmental stages, at temperatures t_1 and t_2. The $Q_{15-25\,C}$ for the interval one-cell stage to J_1 was calculated from rate values extracted from the literature for *Haemonchus contortus, Meloidogyne javanica, Bunostomum trigonocephalum, Strongyloides fulleborni, Dochmoides stenocephala, Trichostrongylus retortaeformis, Meloidogyne incognita,* and *Cooperia punctata.* These values were compared with that for *T. bulhoesi.*

Because data must be extremely good statistically to be meaningful as a Q_{10} value, the data have also been represented graphically. Each developmental stage was assigned a numerical value, and the calculated center of percentages for stages was plotted for each temperature and time combination using the formula:

$$\frac{\Sigma\, i - P_i}{\Sigma\, P_i}$$

TABLE 4. Effects of a 5 C change in temperature on the embryonic development of several nematodes.

Species	Reference	Rate as reciprocal (days)	$Q_{15-20\,C}$
Haemonchus contortus	(12)	0.0	7.0
Meloidogyne javanica	(3)	0.0	4.4
Thelastoma bulhoesi		0.0	4.2
Bunostomum trigonocephalum	(2)	0.2	4.0
Strongyloides fulleborni	(6)	1.0	3.0
Dochmoides stenocephala	(8)	0.3	3.0
Trichostrongylus retortaeformis	(11)	0.0	1.9
Meloidogyne incognita	(13)	0.0	1.9
Cooperia punctata	(5)	0.1	1.6

where P_i is the percentage of nematodes at the i^{th} stage.

RESULTS

Cell division did not occur at 0 C, and only two-cell divisions took place over 256 hours at 5 C. Four-cell divisions occurred at 10 C, although it required 256 hours for 65% of the eggs to reach this stage. Complete development occurred at 15 C, although only 15% of the nematodes reached the resistant (quiescent) stage after 512 hours (more than 21 days) (Table 1). Development was most rapid at 25 (Table 2) and 30 C, but the largest number of nematodes reached the quiescent stage at 20 and 30 C. At 35 C embryonation proceeded only to the gastrula, with only 3% of the eggs developing to that stage.

Table 3 lists the calculated Q_{10} values for the three temperature ranges and the various stages of development. The Q_{10} is largest at lower temperatures (15–20 C) and early cell division, the middle temperatures (20–25 C) for intermediate stages, and high temperatures (25–30 C) for the final stages of larval development.

When Q_{10} values were calculated for the entire developmental interval from the one-cell stage to the first juvenile stage for a variety of nematode species, no phyloge-

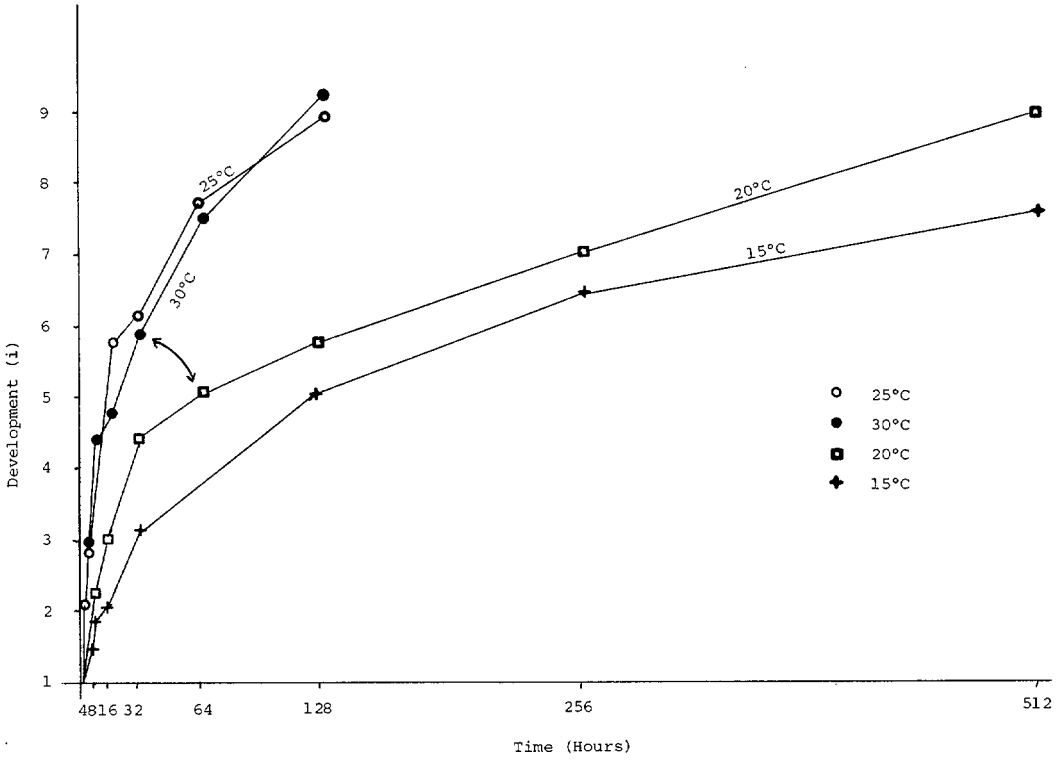

FIG. 1. The rate of development of *Thelastoma bulhoesi* at four temperatures. Developmental stages (i) of 1, 2, 4, 8, and 16 cells, gastrula, prevermiform, first-stage juvenile and resistant larvae were assigned the numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, respectively.

netic relationships were observed (Table 4).

Figure 1 shows the rate of development at four temperatures. There is an abrupt change in rate of development from 8 cells to 16 cells over 20–25 C. This developmental interval also shows an abrupt change in Q_{10} values in Table 3.

DISCUSSION

Wallace (15) noted that generalizations on temperature requirements of nematodes are difficult to make because of the large body of literature and frequently conflicting results of different workers using the same species. Recent literature supports his suggested ranges for optimum (20–30 C), minimum (10–15 C), and maximum (extremely variable with species) temperatures. These temperatures are similar to the shape of an enzyme temperature response curve with a rapid rate rise at lower temperature, a steady increase above 15 C, an optimum between 20 and 30 C, and a decline above 35 C. This pattern is also documented for seed germination (10).

The various intervals of development of *T. bulhoesi* have different optimum temperatures, as is evidenced by the completion of early cell division at lower temperatures than are required for later stages of development. The relationship between temperature and development does not appear to be a simple straight line. Bishop (4), Wallace (14), and Gordon (9) have also shown that alternating temperatures can increase the rate of development in some nematodes.

Temperatures also affect the rate of cell division. Completion of embryonation in *T. bulhoesi* appears to require at least 128 hours (5.3 days) and may require 512 hours (21.3 days) at below optimum temperatures. High temperatures also slow the rate of early cell division and inhibit later cell division.

Since nematodes have determinate cleavage, each cell division represents, to some extent, differentiation as well as

growth. In vertebrate embryology the first several cell divisions are indeterminate with little differentiation occurring. However, even in nematodes, later cell divisions (differentiation) may involve a larger variety of enzymes. The limiting factor of a "multiple-reaction event" is the reaction, or enzyme, most sensitive to that particular temperature range. The enzymes of differentiation are probably not the same enzymes as in cell division; this could explain why early cell divisions are possible at both lower and higher temperatures than are tolerated by later differentiating cell divisions. Presumably a greater number of enzymes are required in differentiation. One enzyme is temperature dependent and thus becomes a limiting factor in the entire cell division reaction. The temperature coefficient formula is a calculation of the temperature effect on the most susceptible enzyme system functioning at a given time. Large Q_{10} values indicate a temperature effect on an enzyme system for that particular temperature range and stage of development. If the Q_{10} is less than 1, it indicates that the limiting factors involved are physical, not biological.

The temperature coefficient has certain drawbacks as a statistic since it is a ratio. If one considers the ratio A/B and is 95% certain that A lies within the range 0.9A to 1.1A and similarly for B, then one is (0.95)(0.95) (= 90%) certain that A/B lies in the range 0.82A/B to 1.22A/B. One can only be 81% certain that the value of A/B for the next stage lies in the range 0.67A/B to 1.49A/B. Data must be extremely good statistically for Q_{10} values to be reliable. Thus a visual format (Fig. 1) was used to confirm the trend indicated by Q_{10} values.

It seems that increasing the temperature from 15 C to 20 C is beneficial to early cell division in *T. bulhoesi* (Q_{10} 16.0) but less necessary to later stages of development (Q_{10} 1.0) which presumably include more differentiation reactions. The abrupt change in rate of development at 8 cells and 16 cells over 20–25 C, as seen in Figure 1, substantiates the evidence, provided by Q_{10} values, that later cell divisions are more critically dependent on optimum temperatures. This suggests different temperature sensitive enzymes active during early and late development. If this is so, then an increasing temperature from 15 C to 30 C during development might yield a more rapid development than will a constant temperature within that range.

Comparison of the Q_{10} values for nine nematode species (Table 4) revealed no phylogenetic similarities in the influence of temperature on embryological development in those species. Ecological relationships may exist, but there has not been enough cross-discipline work between plant and animal nematologists to determine any such relationship.

Crofton (7) identified three ecological groups of sheep nematodes. A cold-weather group included members of the genera *Ostertagia, Haemonchus, Chabertia,* and *Nematodirus. Trichostrongylus* spp. were intermediate while *Cooperia* spp. and *Bunostomum* spp. were high-temperature nematodes. Table 4 shows that *Haemonchus* spp. are greatly benefitted by a 5 C rise in temperature over the range 15–20 C, which is unusual for an animal which presumably is adapted to cold climates. *Cooperia* sp., a high temperature nematode, seems to be nearly independent of this temperature difference and is considerably different from *Bunostomum* sp. Much of this lack of agreement may be attributed to the variety of ways in which the data were gathered. However, it may also be a result of measuring total nematode development.

Further research on temperature effects on embryological development should be limited to discrete stages of development smaller than unembryonated egg through first-stage juvenile, since it appears that development is not uniform in all stages at all temperatures. Perhaps temperature coefficients may prove useful in clarifying ecological niches of particular nematode species or predicting location of species according to geography or time of the year.

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