ACANTHOCEPHALAN DEVELOPMENT: MORPHOGENESIS OF LARVAL *MONILIFORMIS DUBIUS*

by J. E. Byram and Kay W. Byram

ABSTRACT

The nine larval stages of *Moniliformis dubius* as seen in the cockroach intermediate host, *Periplaneta americana,* are illustrated, and detailed tabulations of the events of larval morphogenesis as influenced by temperature, season, photoperiod, and elevated temperature are presented. Significantly increased rates of development were seen in the summer as opposed to winter or fall and under a 12 hours light/12 hours darkness regime versus continuous dark. Morphological anomalies were present in 80% of the cockroaches harboring normal larvae and the number of anomalies was found to decrease significantly as the number of larvae per host increased. High temperature (35° C) invoked a block in morphogenesis, with few normal larvae developing beyond the II* acanthor stage, and resulted in appreciably stimulated host reactions. Attempts to circumvent this effect met with only limited success.

INTRODUCTION

The development of helminth symbionts in their arthropod intermediate hosts is influenced by a variety of environmental parameters, including those acting on the host and subsequently on the symbiont, as well as those which directly affect the symbiont. The larval development of the acanthocephalan *Moniliformis dubius* may be conveniently ordered into a series of morphologically distinct stages (Van Cleave, 1947). A comparison of the data of the early studies of Moore (1946) and of King and Robinson (1967),

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The image covers the right portion of the page with 4 photomicrographs. The left is caption text.

FIGS. 1-14. PHOTOMICROGRAPHS illustrating the various larval stages of *Moniliformis dubius* recovered from the cockroach intermediate host, *Periplaneta americana,* during the course of this study.

FIG. 1. STAGE I* ACANTHOR— isolated from the gut washings of a cockroach incubated 9 days after infection at room temperature (22-24°C). At this point, escape from the egg shell and membranes is imminent. × 565.

FIG. 2. STAGE II* ACANTHOR— seen *in situ* in the gut tissues of a cockroach incubated 11 days after infection at room temperature (22-24°C). × 560.

FIG. 3. STAGE II* ACANTHOR— as recovered from the hemocoel of a cockroach incubated 9 days after infection at 30°C. The rostellar hooks are still present on the anterior end of the larva and few details are apparent in the central nuclear mass. × 385.

FIG. 4. STAGE I ACANTHELLA (EARLY). Initial differentiation of the central nuclear mass into
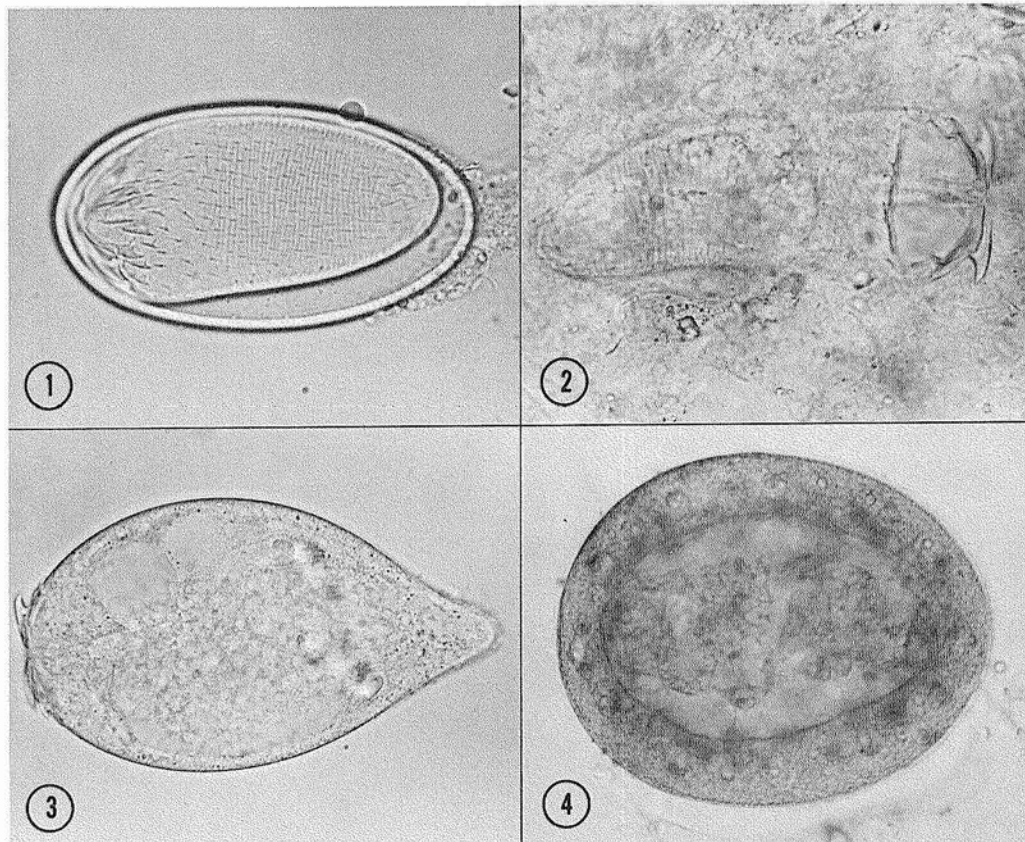
organ systems and formation of the body wall have begun. The rostellar hooks of the acanthor stage are occasionally found on the early acanthella but are typically displaced about one-eighth of the larval circumference from their previous anterior position. × 200.

FIG. 5. STAGE I ACANTHELLA (LATE). Organ systems show considerable development but the lemniscal nuclear ring characteristic of the Stage II acanthella is not yet evident. × 95.
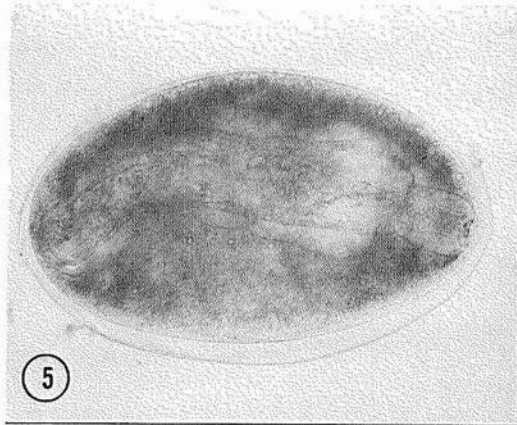
FIG. 6. STAGE II ACANTHELLA. The formation of the lemniscal nuclear ring and initial body elongation mark this stage. × 65.
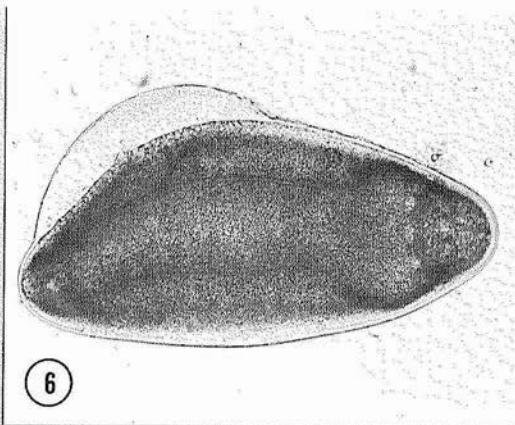
FIG. 7. STAGE III ACANTHELLA. The larval body assumes an elongated cylindrical shape and begins to fold within the enclosing envelope (not seen here). × 55.
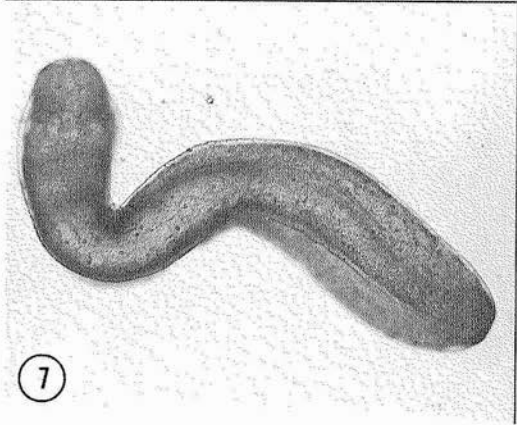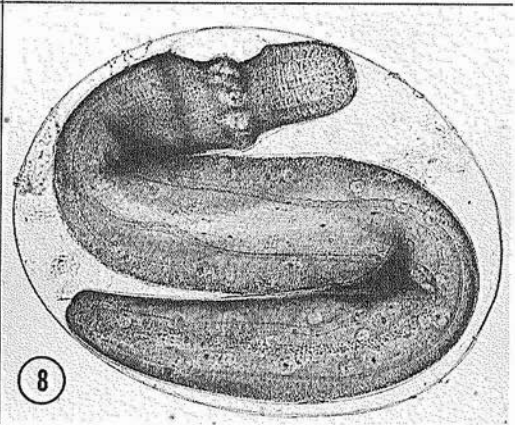
FIG. 8. STAGE III ACANTHELLA (LATE)—the characteristic z-shape of this stage. Hook nubs may be seen in the developing proboscis. × 60.
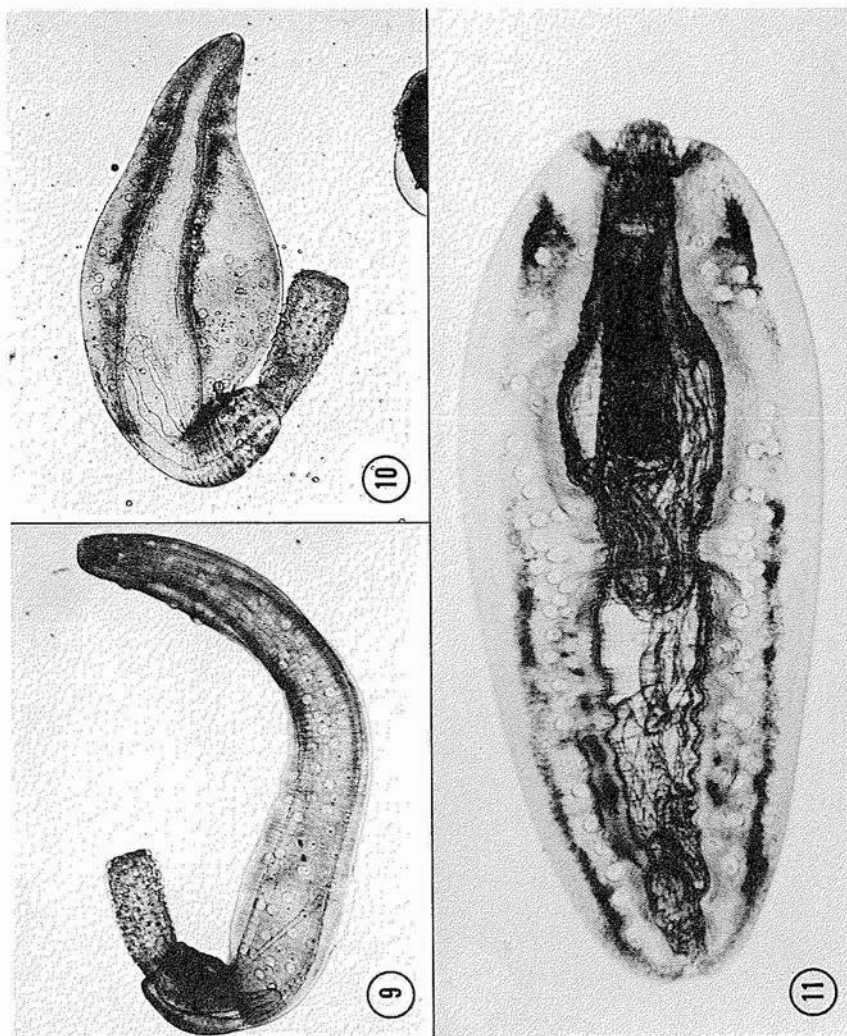
FIG. 9. STAGE IV ACANTHELLA. The appearance of the lemnisci, an elongated body shape and well developed hooks in the cytoplasm of the proboscis tegument typify this stage. × 35.

FIG. 10. STAGE V ACANTHELLA. Shortening, broadening and flattening of the larval body is seen. The proboscis musculature is complete and the tips of the hooks protrude from the proboscis surface. × 30.

FIG. 11. STAGE VI ACANTHELLA. This stage begins with the inversion of the proboscis and neck and ends with the appearance of the cystacanth. × 75.

FIG. 12. CYSTACANTH—the infective stage. The body is distinctly compacted, much shorter than the Stage VI acanthella, and compressed, with one surface being flattened or slightly concave. The proboscis and neck remain tightly withdrawn and the cortical nuclei have lost the rounded shape seen in the previous stage. × 45.

FIG. 13. CYSTACANTH. In an occasional cockroach, refractile granules of an undetermined nature were present between the cystacanth surface and the enclosing envelope. × 40.

FIG. 14. CYSTACANTH—proboscis and neck everted as is typical of a newly activated juvenile found in the intestine of the rat definitive host. Cystacanths of this appearance in the cockroach hemocoel are considered to be uninfective. × 55.
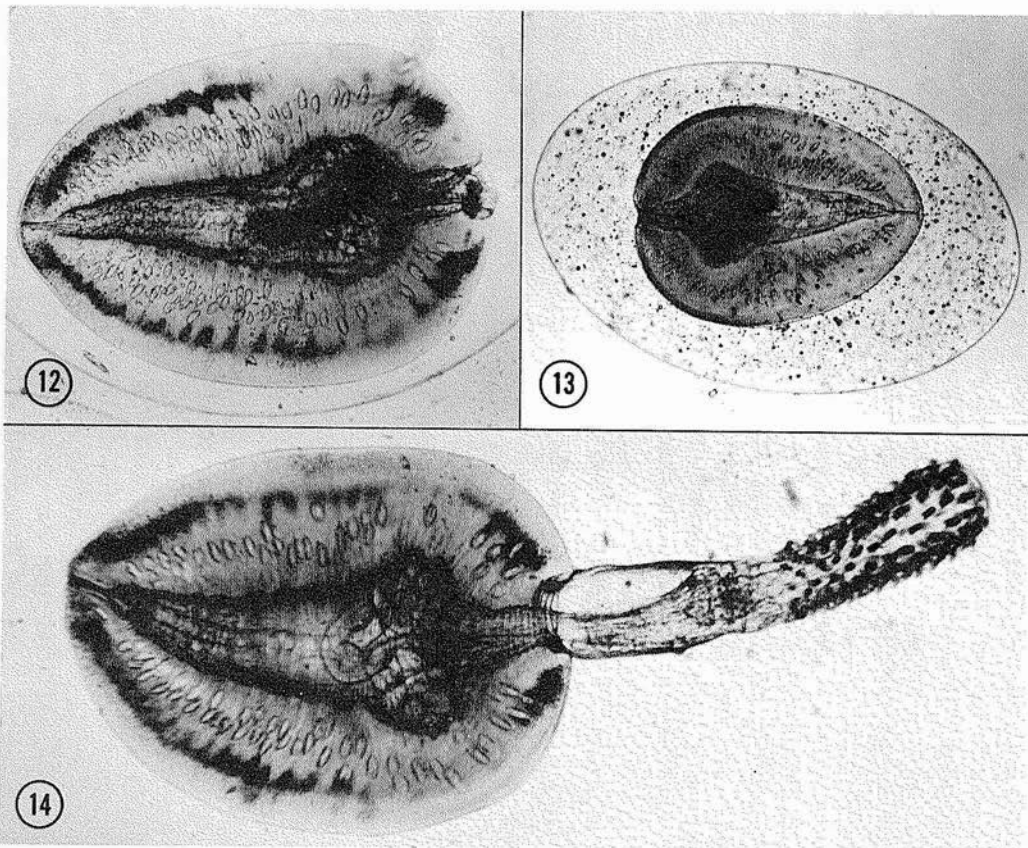
and the more recent findings of Lackie (1972a) reveal that ambient tempera-
ture governs the rate of larval morphogenesis of this symbiont. King and
Robinson (1967) presented data suggesting a similar rate influence by season.
Subsequent studies by Robinson and Jones (1971) and Lackie (1972b) re-
vealed that elevated temperatures (above 30°C) markedly altered the normal
course of larval morphogenesis. We have found that only by a detailed
tabulation of the morphogenetic events in acanthocephalan larval develop-
ment, such as is seen in King and Robinson (1967, table I) and in Lackie
(1972a, table 10), can the trends and subtleties of these events be fully
appreciated and analyzed. In this paper, selected aspects of the effects of
temperature, season, photoperiod, and temperature stress on the larval
morphogenesis of *M. dubius* are examined using such tabulations.

MATERIALS AND METHODS

The acanthocephalan, *Moniliformis dubius* Meyer, 1933, was maintained
in the laboratory as described by Byram and Fisher (1973). Adult *Periplaneta
americana* were fed eggs taken from gravid female worms 49 to 154 days
after infection of the rat definitive host.

Infected roaches were cultured under a variety of temperature and light
regimes: 1) room temperature (23°C and 26.5°C) with seasonal illumination,
2) 30°C with 24-hour darkness, 3) 30°C with 12-hour darkness/12-hour light,
4) 35°C with 12-hour darkness/12-hour light, and 5) 30°C with 24-hour
darkness to a certain developmental stage (II* acanthor and I acanthella) and
transferred to 35°C with 12-hour darkness/12-hour light to completion of
the experiment. A saturated atmosphere was insured at 30°C and 35°C.

M. dubius larvae were recovered from *P. americana* by cutting off the head
of a roach and flushing out the hemocoel contents with a Pasteur pipette filled
with tap water, or with KRTM (Read et al., 1963) when the presence of later
developmental stages was expected. The body of the roach was opened and
the tissues teased out and searched for any adhering larvae. Counts were
made of each larval stage present in an individual roach, following the
terminology of King and Robinson (1967). All larval stages and anomaly
types were photographed in fresh mounts soon after removal from the cock-
roach hemocoel, under a Zeiss microscope.

OBSERVATIONS

Even though various photomicrographs and drawings of the larval stages
of *Moniliformis dubius* have been published (Moore, 1946; King and
Robinson, 1967), a complete collection of illustrations has been lacking.
Figures 1–14 show the major features necessary to distinguish each of the nine
stages seen in the cockroach intermediate host, *Periplaneta americana,* and,
when utilized together with the concise definitions of King and Robinson

(1967), provide a convenient guide for future investigators. The increasing sizes of the various larval stages through the course of development are indicated by the decreasing magnifications of the successive figures. The Stage VI acanthella (figure 11), which previously has not been illustrated, was first recognized by King and Robinson (1967) and clearly can be differentiated from the cystacanth (figures 12–14). These photographs (figures 1–14) emphasize that the cystacanth (so fortuitously named by King and Robinson, as shown by the later studies of Rotheram and Crompton [1972], who found the envelope enclosing the larvae to be indeed a cyst) is the end result of gradual progressive larval development rather than the product of a dramatic transformation or metamorphosis (Van Cleave, 1947).

Rates of Morphogenesis

The development of larval *Moniliformis dubius* in *Periplaneta americana* required less time as the temperature increased (tables 1–6). With seasonal illumination at 23°C (table 1) and 26.5°C (table 2), development in 50-day-old infections had reached 94.6% Stage V and 54.3% Stage VI acanthella respectively. As the result of an accident, development to the cystacanth stage was not completed at 23°C, but was accomplished at 26.5°C in 55-60 days. With 12 hours darkness and 12 hours light, morphogenesis at 30°C was terminated in 35 days (table 6). The developmental rates at these three temperatures are compared graphically in figure 15. These graphic depictions of the morphogenetic rates allow an analysis of the course of development. An estimate of the duration of each stage from the 30°C samples gives the following figures: II* acanthor, 5 days; I acanthella, 3.5 days; II acanthella, 2 days; III acanthella, 2.8 days; IV acanthella, 3.7 days; V acanthella, 4.5 days; and VI acanthella, 5.5 days. Thus, it can be seen that great variations exist in the timing of the development of each stage to the next and that a regression line is of limited use in estimating when a designated stage might appear. The effects of lowering the temperature can be seen in delayed hatching of the acanthor in the cockroach gut and penetration of the gut, and

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TABLES OVERLEAF

EXPLANATION OF TABLES 1-6. The morphogenesis of larval *Moniliformis dubius* in the cockroach intermediate host, *Periplaneta americana,* under various temperature and light regimes. Each column represents the larvae recovered from one host at the indicated time after infection. The numbers in parentheses (N) at the top of each column show the number of larvae recovered from each cockroach. Anomalies are represented as the percentage of anomalous larvae in each sample. The relative composition by percentage of the morphologically normal larvae in each sample (exclusive of those showing developmental anomalies) is listed under the appropriate larval type.

TABLE 1

Temperature, 23°C; date of infection, 1/28/70; light regime, seasonal. This experiment was terminated at 50 days with 94.7% of the larvae at Stage V acanthella.

(N)	(17)	(64)	(58)	(139)	(18)	(21)	(359)
ANOMALIES	0	0	20.7	7.9	5.6	4.8	0.3
II* acanthor	100	29.7					
I acanthella		70.3	13.0	19.5			
II acanthella			87.0	72.7	47.1	5.0	2.8
III acanthella				7.8	17.6	5.0	0.6
IV acanthella					35.3	40.0	1.4
V acanthella						50.0	94.7
VI acanthella							0.6
	20	25	30	35	40	45	50

DAYS AFTER INFECTION

TABLE 2

Temperature, 26.5°C; date of infection, 6/22/70; light regime, seasonal. *a*, anomalies not recorded in day 55 sample.

(N)	(5)	(38)	(25)	(6)	(23)	(22)	(11)	(136)	(275)	(66)
ANOMALIES	0	0	8.0	33.0	4.3	4.5	0	5.2	a	1.5
II* acanthor	100	15.8								
I acanthella		84.2	43.5	25.0						
II acanthella			56.5	75.0	31.8	4.8	9.1	1.6		
III acanthella					36.4			1.6		
IV acanthella					31.8	52.4	9.1	7.8		
V acanthella						42.8	81.8	35.7	2.5	1.5
VI acanthella								53.5	19.3	9.2
cystacanth									78.2	89.3
	15	20	25	30	35	40	45	50	55	60

DAYS AFTER INFECTION

TABLE 3

Temperature, 30°C; date of infection, 2/4/70; light regime, 24 hours darkness.

(N)	(26)	(94)	(207)	(337)	(438)	(239)
ANOMALIES	11.5	6.3	1.0	0.3	2.1	1.3
II* acanthor	39.1					
I acanthella	60.9	10.2				
II acanthella		50.0	12.7	1.5	5.4	
III acanthella		5.7	3.9	0.6	2.3	
IV acanthella		34.1	10.7	7.4	11.7	
V acanthella			72.7	15.8	26.3	6.4
VI acanthella				74.7	21.0	9.3
cystacanth					33.3	84.3
	15	20	25	30	35	40

DAYS AFTER INFECTION

TABLE 4

Temperature, 30° C; date of infection, 6/30/70; light regime, 24 hours darkness.

(N)	(177)	(134)	(30)	(124)	(9)	(130)
ANOMALIES	27.7	1.5	0	3.2	0	1.5
II* acanthor	3.9					
I acanthella	76.6	0.8				
II acanthella	19.5	0.8				1.6
III acanthella						
IV acanthella		98.4	6.7	1.7		0.8
V acanthella			83.3	25.0	11.1	3.9
VI acanthella			10.0	1.7		5.4
cystacanth				71.6	88.9	88.3
	15	20	25	30	35	39
			DAYS AFTER INFECTION			

TABLE 5

Temperature, 30° C; date of infection, 10/5/70; light regime, 24 hours darkness.

(N)	(101)	(13)	(10)	(89)	(49)	(149)
ANOMALIES	10.9	15.4	10.0	2.2	12.2	5.4
II* acanthor	5.5					
I acanthella	46.7		11.1		2.3	
II acanthella	47.8	36.4	22.2	5.7	7.0	
III acanthella		9.1				
IV acanthella		54.5	55.6	2.3		0.7
V acanthella			11.1	20.7	18.6	5.7
VI acanthella				70.1	32.6	
cystacanth				1.2	39.5	93.6
	15	20	25	30	35	40
			DAYS AFTER INFECTION			

TABLE 6

Temperature, 30° C; date of infection, 10/5/70; light regime, 12 hours light/ 12 hours darkness. *a*, total number of larvae not recorded in day 8 sample.

| (N)	a	(40)	(124)	(88)	(16)	(78)
ANOMALIES	0	40.0	2.4	0	6.3	5.1
II* acanthor	100	12.5				
I acanthella		37.5				
II acanthella		50.0	12.4			
III acanthella			7.4			
IV acanthella			79.3	4.5		
V acanthella			0.8	94.3	13.3	
VI acanthella				1.2	66.7	
cystacanth					20.0	100
	8	15	20	25	30	35
			DAYS AFTER INFECTION			

TABLE 7

Probability values (p) for the significance of the difference of the mean developmental stages of the larvae examined at the indicated times when compared on the basis of season (figure 16) and light regime (figure 17). Significance probabilities were computed from the raw data used to construct tables 3-6 using the Wilcoxon two-sample test for two samples, ranked observations, not paired. *, significant.

DAY	15	20	25	30	35	40
2/4 vs. 6/30	p<0.001*	p<0.001*	p<0.02	p<0.001*	p<0.01*	- - - -
6/30 vs. 10/5	p<0.01*	p<0.1	p<0.001*	p<0.001*	p<0.05*	- - - -
2/4 vs. 10/5	p<0.001*	p<0.2	p<0.01*	p<0.9	p<0.1	p<0.2
12/12 L/D vs. 24 D	p<0.9	p<0.2	p<0.001*	p<0.05*	p<0.001*	- - - -

in the duration of each of the subsequent larval stages. In general, when the rates of morphogenesis at 30° C and 26.5° C are compared, the duration of each stage is seen to increase 1.1 to 1.5 times; however, the time spans of the Stage I acanthella and Stage IV acanthella are increased 3.1 and 2.3 times respectively. A similar selective effect of lowering the temperature on these two stages is seen at 23° C.

Tables 3–5 show an apparent seasonal effect on the infections carried out in winter, summer, and fall under constant temperature (30° C) and photoperiod (24 hours darkness) regimes. The rate of development of cystacanths in 35 days varied from a low of 33.3% in winter and 39.5% in fall to a high of 88.9% in summer. The morphogenetic rates at these seasons are compared graphically in figure 16. The mean developmental stages at the indicated points of time in development during each season were then compared statistically (table 7). Since morphogenetic progress was grouped into classes (stages), a nonparametric test of significance, the Wilcoxon two-sample test for two samples, ranked observations, not paired (Sokal and Rohlf, 1969), was utilized. The trends in table 7 are clear. The developmental rates during winter and fall do not differ significantly from one another, but development during the summer proceeds at a significantly higher rate than that during either the winter or the fall. These results support the observation of King and Robinson (1967) that at 27° C development to the cystacanth stage is more rapid in summer (5–7 weeks) than in winter (7–8 weeks).

An apparent effect of photoperiod on two parallel experiments carried out in the fall at 30° C is seen in tables 5 and 6. At 35 days, 100% of the larvae from a 12 hours light/12 hours darkness regime were at the cystacanth stage, while only 39.5% of the larvae under a 24 hour darkness regime were cystacanths. The morphogenetic rates under these two light regimes are depicted in figure
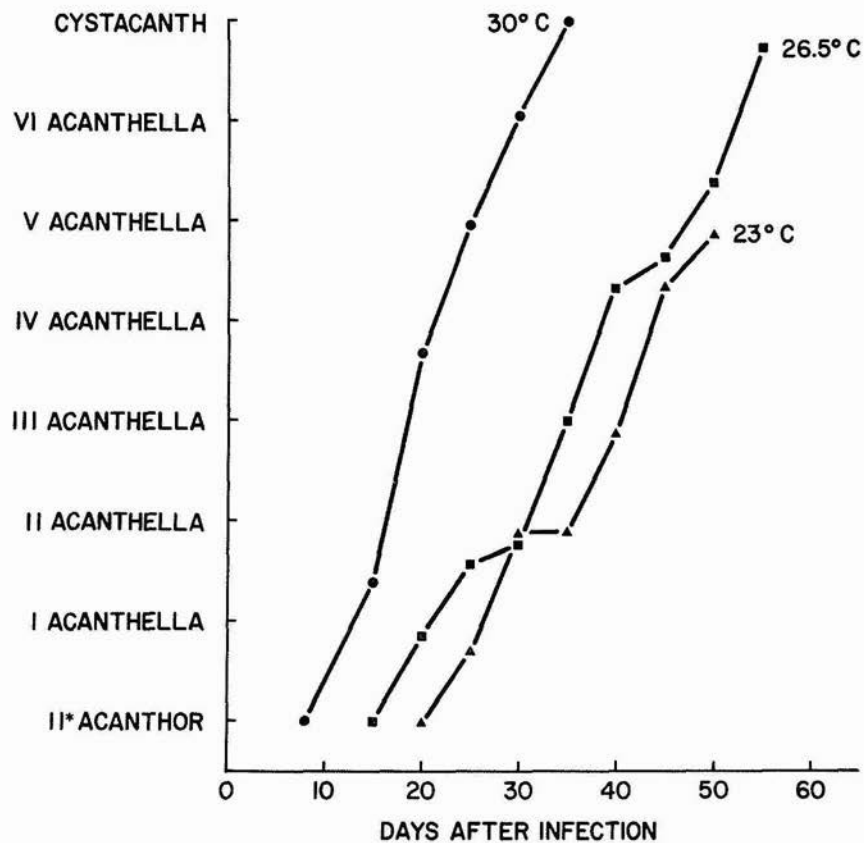
EXPLANATION OF FIGURES 15-17. The rate of morphogenesis of larval *Moniliformis dubius* in the cockroach intermediate host, *Periplaneta americana,* examined on the basis of temperature (figure 15), season (figure 16), and photoperiod (figure 17). Each point on the curves represents the mean developmental stage of the larvae recovered at the indicated time. For the purposes of comparison, each larval stage was assigned an arbitrary value (II* acanthor, 2; I acanthella, 3; II acanthella, 4; etc.) and the developmental means were computed from the raw data used to construct tables 1-6.

FIG. 15. A GRAPHIC COMPARISON of the developmental rates at three different temperatures—30°C (table 6), 26.5°C (table 2) and 23°C (table 1).
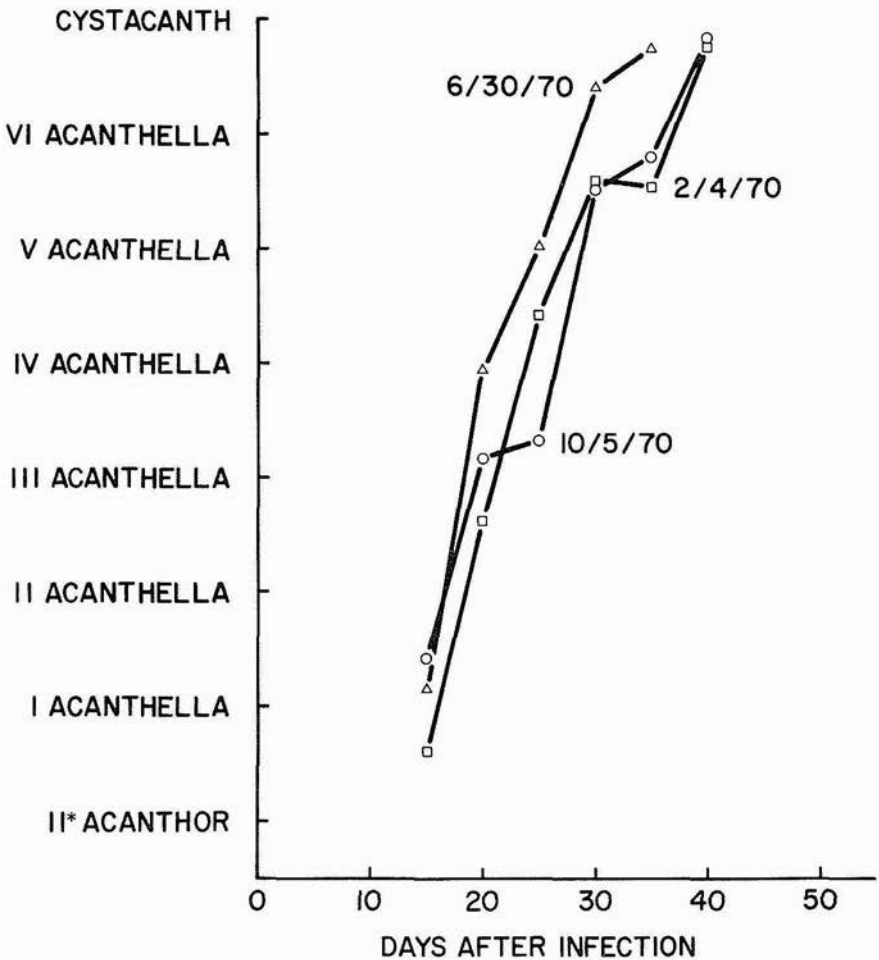
FIG. 16. A GRAPHIC COMPARISON of the developmental rates at three different seasons—winter (table 3), summer (table 4), and fall (table 5). The cockroach hosts were maintained under identical temperature (30° C) and light (24 hours darkness) conditions.
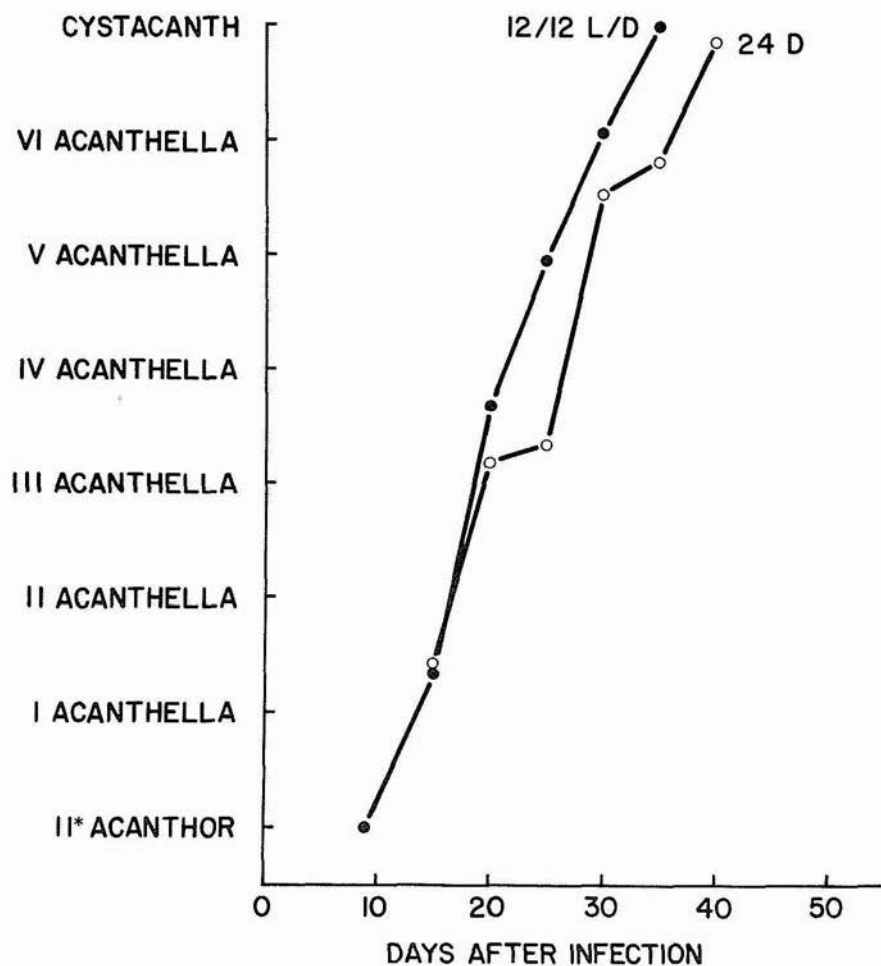
FIG. 17. A GRAPHIC COMPARISON of the developmental rates under two different photoperiod regimes—12 hours light/12 hours darkness (table 6) and 24 hours darkness (table 5). These incubations ran parallel to one another beginning from the date of infection, 10/5/70.

17. As seen in table 7, a comparison of the developmental rates at each point shows that the rates are progressively diverging and that development proceeds at a rate significantly higher under a regime of 12 hours light/12 hours darkness than under one of 24 hours darkness.

Anomalous Development

Of the 3,985 larvae studied under temperature conditions giving rise to normal development (tables 1–4), 174 or 4.37% were observed to be morphological anomalies. In general, the occurrence of anomalies paralleled normal development, with the early stage anomalies being found in the early period of development and later stage anomalies appearing later in development. This presents a puzzle, since no early stage anomalies were seen in the late phase of development. These early stage anomalies must develop to later normal or anomalous stages or be destroyed and cleaned out by a host response. Most anomalies occurred early in development, with the Stage II acanthella accounting for 42% of the total and the sum of Stage I and II acanthellas for 71%. Most later stage anomalies were distributed among the Stage IV (5.75%) and V (11.5%) acanthellas and cystacanths (6.9%). Anomalous development was seen in all stages and included features such as gross distortion of normal shape, blebs, undersized or shrunken and withered larvae, and misshapen proboscides.

The varying numbers of larvae recovered from each cockroach and the differing frequencies of anomalous development suggested the possibility of a crowding effect resulting in these anomalies, as is seen in the development of larval cestodes in the insect intermediate host (Schiller, 1959). This relationship is analyzed in the scatter diagram (figure 18). Since most of the percentages of anomalies fell below 30%, this distribution was normalized by the arcsin transformation (Sokal and Rohlf, 1969). The skew to the right in the frequency distribution of the number of larvae per cockroach was made more symmetrical by plotting these values on a logarithmic scale. The values used in figure 18 were taken from the experiments depicted in tables 1–6 and from one additional experiment run at an average temperature of 28.25° C. When all of the hosts are considered, including those from which no anomalies were recovered (0% anomalies), the regression of y on x approaches 0 ($y = 11.69 - 0.0661x$) and no correlation is seen between percentage of anomalies and larvae/host (correlation coefficient, −0.00375). If one asks whether a relationship exists between the frequency of anomalies and the number of larvae per cockroach *when anomalies are found to occur*, however, the regression of y on x is found to be negative ($y = 28.32 - 7.2703x$) and a strong correlation is evident (correlation coefficient, −0.432). Thus, a significant correlation ($p < 0.01$) exists between the percentage of anomalies and the number of larvae in a cockroach, such that the percentage of anomalies decreases as the number of larvae increases. This effect might be termed a negative crowding effect.
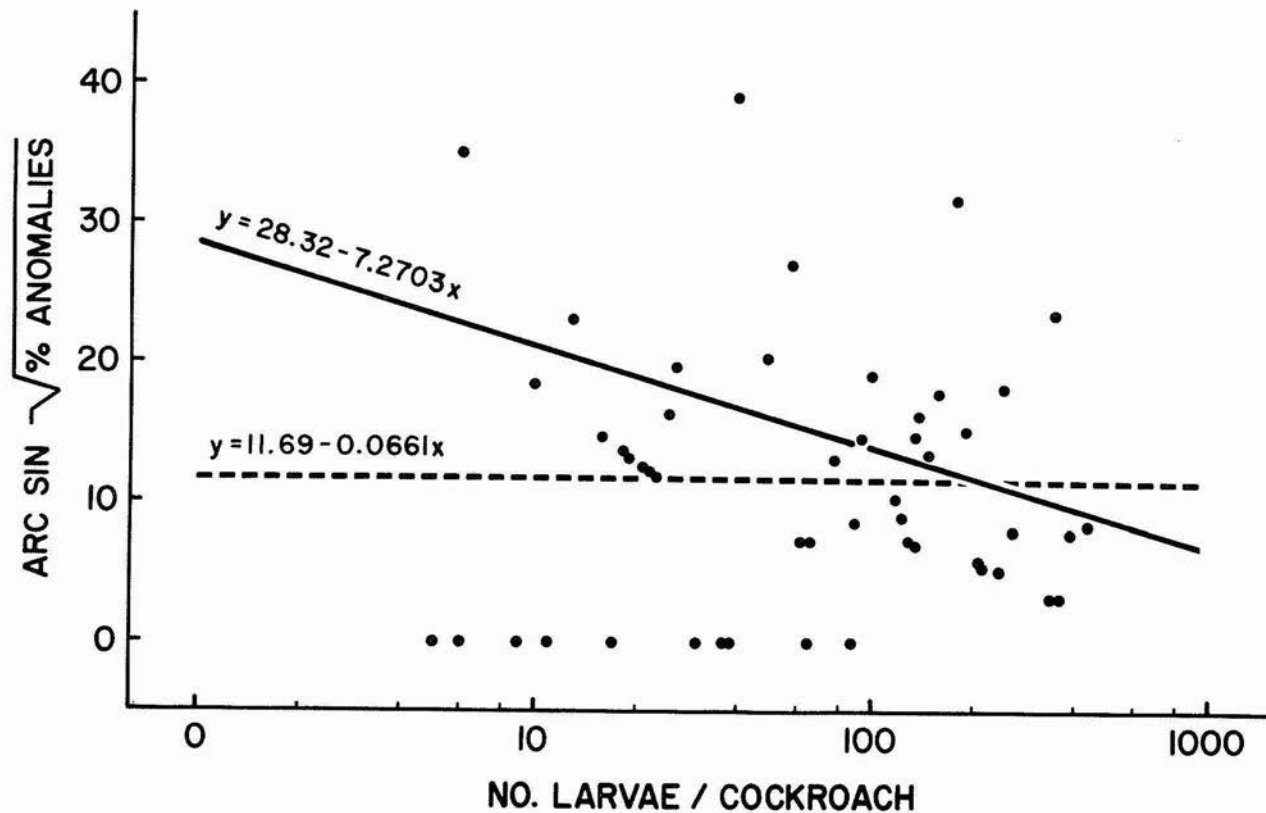
FIG. 18. SCATTER DIAGRAM of the relationship between the percentage of anomalous larvae in a given host and the total number of larvae, normal and anomalous, in that particular host. Regression lines fitted by the method of least squares. The dashed line ($y = 11.69 - 0.0661x$, N = 50) represents all cases including those hosts harboring only larvae showing normal morphology, whereas the solid line ($y = 28.32 - 7.2703x$, N = 40) is restricted to those cases in which anomalous development occurred. See text for further details.

Effects of High Temperature (35° C)

As has been shown by Robinson and Jones (1971) and Lackie (1972b), temperatures of 32-33°C and above markedly alter the normal course of larval development of *M. dubius*. The following experiments were intended to shed some light on this phenomenon.

Morphogenesis at 35°C (figure 20; see figure 19 for explanation) was followed to determine the nature and timing of abnormal development. Stage II* acanthors in the gut tissues appeared normal at day 5. A few Stage I acanthellas were found by day 13; however, development progressed no further though most of the larvae recovered through day 23 were morphologically normal. Thus the first obvious manifestation of anomalous development at high temperature is a suspension of larval morphogenesis. By day 25 most larvae were morphologically abnormal and by day 30 no normal larvae were recovered. Anomalous morphological features at high temperature included those previously listed and, in addition, a series of host reaction effects—host cellular responses, cloudiness, degeneration of the larvae, and melanization—not evidenced at 30°C and below within the time frames of these experiments.

Two further experiments were conducted to attempt to alleviate the morphogenetic block imposed by high temperature. First, the data of Robinson and Jones (1971) suggested that Stage II* acanthors might have difficulty in escaping from the gut tissues into the hemocoel at high temperatures. A possible trapping of emerging acanthors by a host reaction at the gut surface may be inferred from the study of Lackie (1972b). To circumvent this problem, cockroaches were cultured at 30°C until all Stage II* acanthors had migrated into the hemocoel (day 9) and from that point were incubated at 35°C (figure 21).

The last experiment was based on the failure of the morphogenesis of most Stage II* acanthors into Stage I acanthellas at 35°C. If, in the larval development of *M. dubius*, a true metamorphosis occurs, it is in the transformation of the acanthor into the acanthella. To determine whether the major effects of high temperature related to this transformation, cockroaches were cultivated at 30°C until a majority of the larvae had passed this point and were Stage I acanthellas (day 15) and from that point were incubated at 35°C (figure 22).

In neither of these experiments was the morphogenetic block imposed by high temperature successfully evaded. In the first experiment, a slight alteration of the 35°C results was evidenced, and in the second experiment the course of development proceeded somewhat normally through day 25; the frequency of anomalies increased steadily throughout the sampling periods, however, until all larvae at day 35 were morphologically anomalous. In addition to those anomalous features seen at 35°C, a new defect characterized by a series of pseudocoel alterations appeared in these two experiments. The
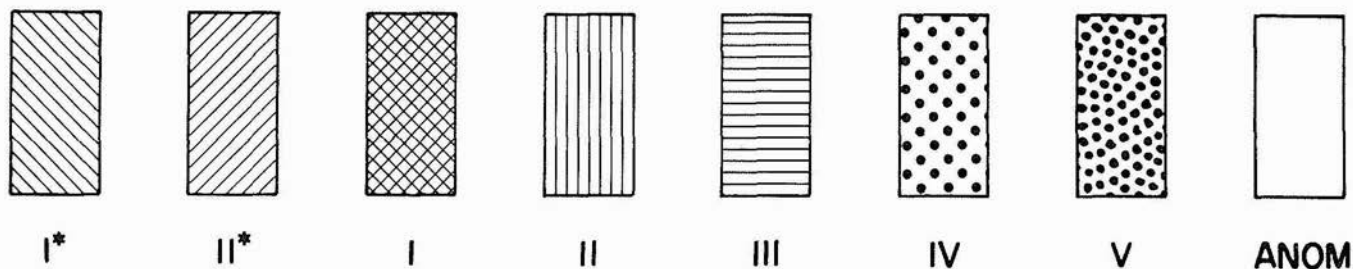
FIG. 19. A KEY TO THE LARVAL STAGES of *Moniliformis dubius* represented in Figs. 20-22. I*, Stage I* acanthor; II*, Stage II* acanthor; I, Stage I acanthella; II, Stage II acanthella; III, Stage III acanthella; IV, Stage IV acanthella; V, Stage V acanthella; ANOM, anomalies.

EXPLANATION OF FIGS. 20-22. The effects of high temperature on the morphogenesis of larval *Moniliformis dubius* in the cockroach intermediate host, *Periplaneta americana*. Each column represents the larvae recovered from one host at the indicated time after infection. The bars having no texture (located at the top of each column) represent the percentage of anomalous larvae in each sample. Absence of such a bar indicates that no anomalous larvae were recovered from that particular cockroach. The textured bar in each column represents the relative composition by larval type of the morphologically normal larvae in each sample. Successive larval types relative to their developmental appearance, if present, are always represented in an ascending order. If the percentage of a recovered larval type was too small to be shown adequately by a textured area of the bar, that particular area of the bar remains untextured and is labeled with the appropriate Roman numeral to the side of the bar. The numbers in parentheses (N) below each indicated time after infection represent the number of larvae recovered from each host.
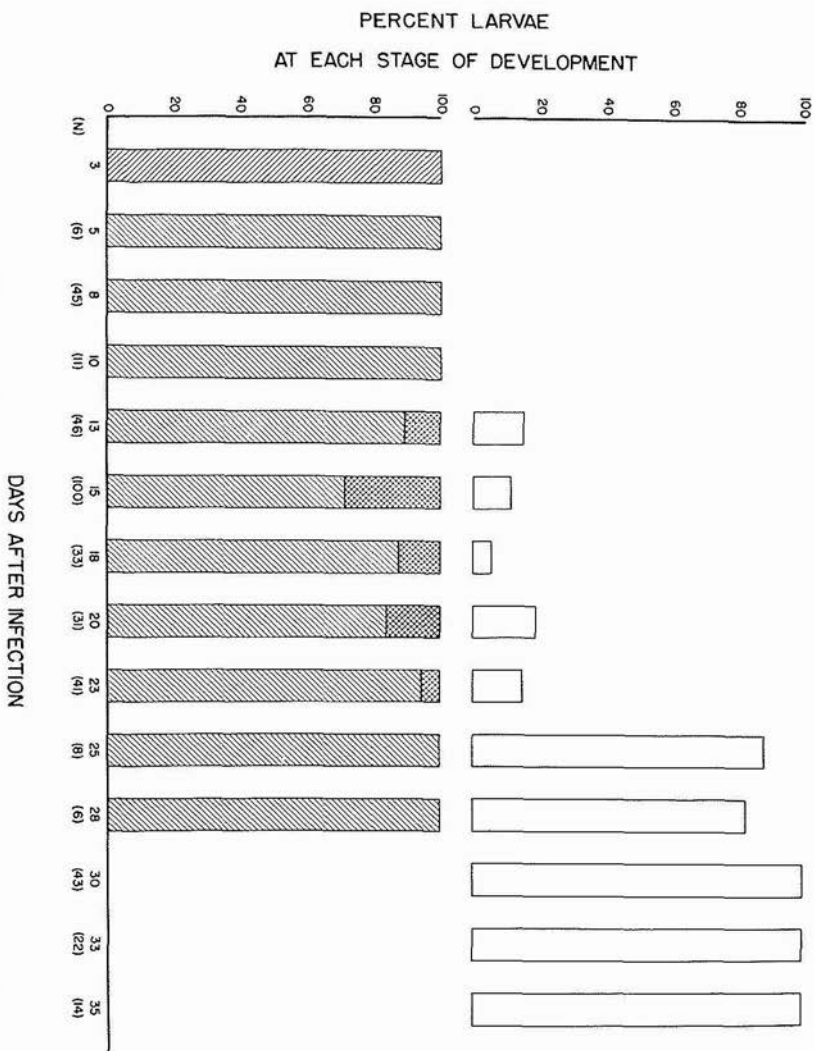
PERCENT LARVAE

AT EACH STAGE OF DEVELOPMENT

DAYS AFTER INFECTION

FIG. 20. TEMPERATURE, 35°C; date of infection, 1/9/71; light regime, 12 hours light/12 hours darkness. The larvae represented after about day 13 are clearly delayed in development but appear normal in morphology. After day 28, all larvae recovered were morphological anomalies.

DAYS AFTER INFECTION

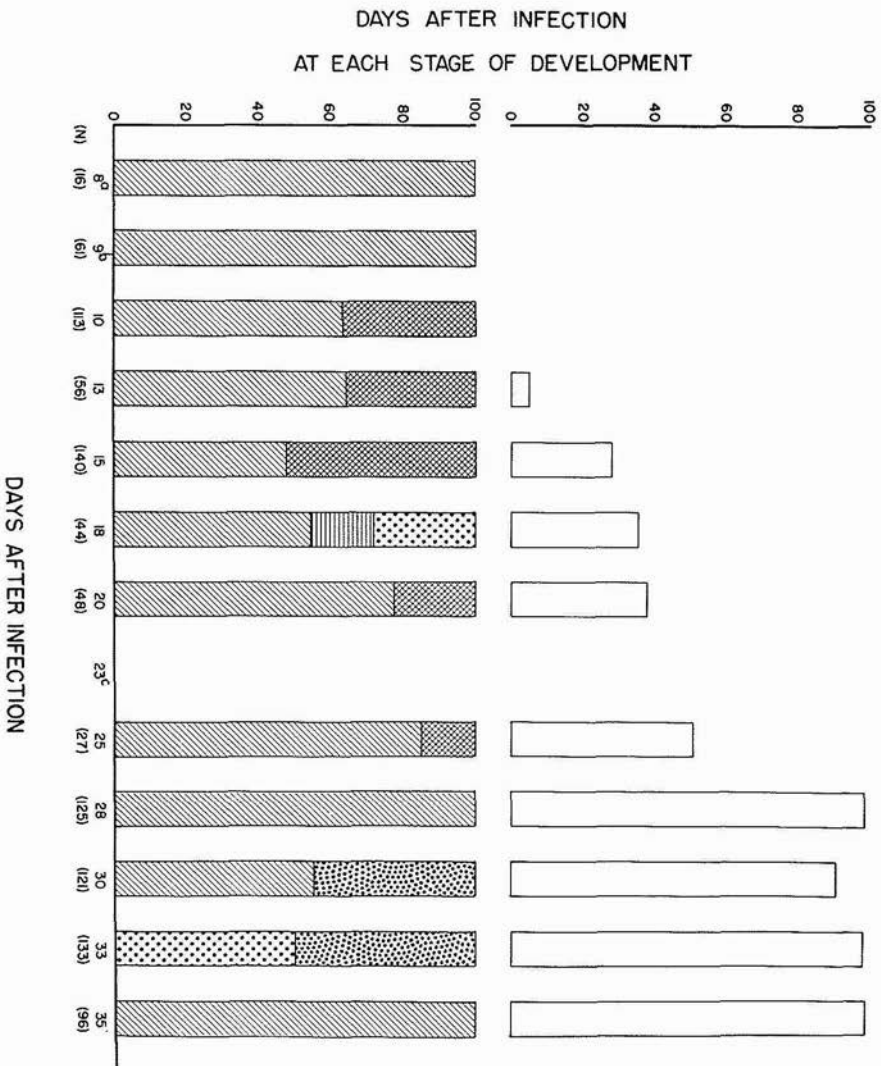AT EACH STAGE OF DEVELOPMENT

DAYS AFTER INFECTION

FIG. 21. TEMPERATURE, 30°C until all larvae had emerged from the gut wall, then 35°C for the remainder of the experiment; date of infection, 1/9/71; light regime, 24 hours darkness at 30°C, 12 hours light/12 hours darkness at 35°C. a, at day 8 all larvae were at Stage II* acanthor and located in the gut wall tissues. b, cockroaches were transferred from 30°C to 35°C at day 9, while all larvae remained at Stage II* acanthor but had emerged from the gut wall. c, no sample taken at day 23.
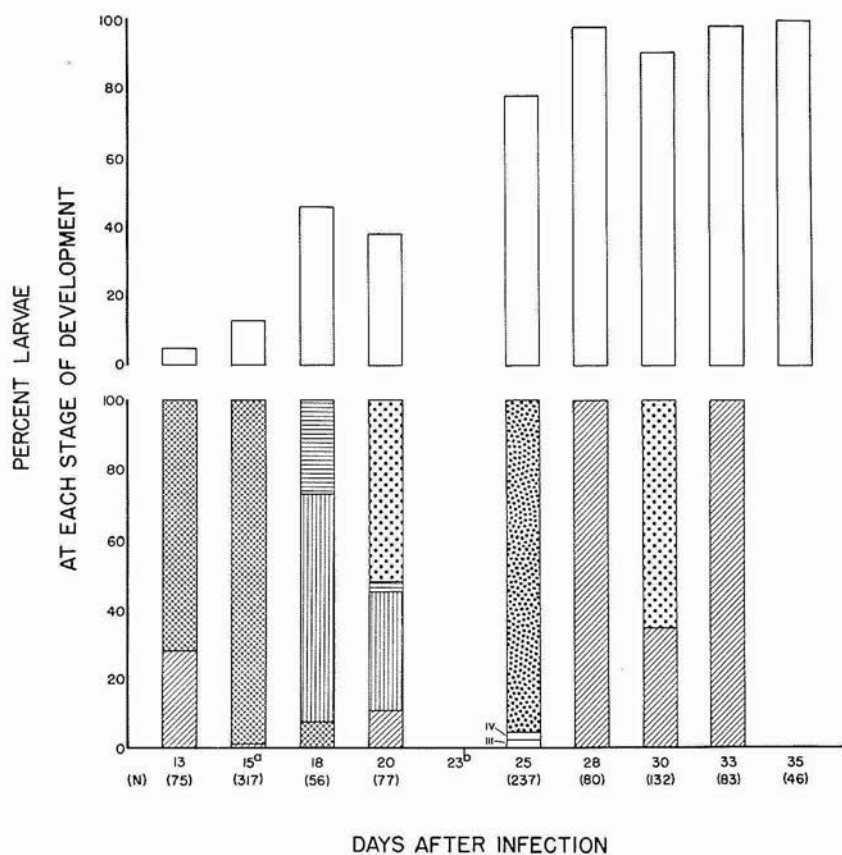
FIG. 22. TEMPERATURE, 30° C until a majority of the larvae had metamorphosed from the acanthor to acanthella stages, then 35° C for the remainder of the experiment; date of infection, 1/9/71; light regime, 24 hours darkness at 30° C, 12 hours light/ 12 hours darkness at 35° C. *a*, cockroaches were moved from 30° C to 35° C at day 15, when 99.3% of the larvae were at Stage I acanthella. *b*, no sample taken at day 23.

summary effect of 35° C temperature is apparently an increasing rate of larval death and an accelerated host response to the dead larvae.

DISCUSSION

Variability in *M. dubius* infections has been noted by several authors. Moore (1946) suggested that variation in larval size and rate of development might result from variation in hatching, gut penetration, availability of food in a particular hemocoel area, and the rate of nutrient assimilation. King and Robinson (1967) observed that the variation in larval development within a single host was as marked as that in different hosts from the same infection. Quantitative studies by Lackie (1972a) showed that, in light infections, 20-30% of the eggs administered were recovered as cystacanths, and that this percentage diminished as the infection became heavier. In a single host, 0-80% of the eggs might develop successfully. Our infections showed similar variations in the number of developing larvae and their rate of morphogenesis.

It can be seen from tables 1 and 2 and figure 15 that the rates of development we observed at 23° C and 26.5° C were relatively slower than those of Moore (1946) and Lackie (1972a) but compare favorably with that of King and Robinson (1967). Moore observed cystacanths at 23-26° C in 49-55 days while Lackie found terminal development at 27.8° C in 40 days. King and Robinson recorded completion of development at 27° C in 49-58 days. Lackie noted that the slight difference in experimental temperatures did not seem to warrant the compressed time scale he obtained in comparison to that of King and Robinson. He did not mention that his results compare more precisely with the 35-49 days required for morphogenesis in summer, however, for which King and Robinson tentatively suggested seasonal implications.

The studies mentioned previously lead to the apparent conclusion that temperature is the prime controlling factor in the development of *M. dubius* in the intermediate host, but the problem becomes more complex in view of the findings of our series of experiments at 30° C. Maintenance of roaches under continuous darkness in winter, summer, and fall resulted in rates of development that point to a host seasonal effect on the larvae. Another factor to be considered is the influence of photoperiod responses of the host on developing larvae, since there was a significant difference in rates of development at the same season and temperature but under varying light conditions.

The variables influencing morphogenesis are potentially so numerous that systems analysis of the data may be required for a more definitive study. Possible variables affecting the parasite in addition to host rhythms include sex of the host, developmental stage of the host with regard to molting, humidity at which hosts are maintained, and crowding effects in heavy parasite infections. Lackie (1972a) observed that female roaches seem to be prone to larger infections than males, but King and Robinson (1967) saw no

such trends, nor did they see any effect of age of host or of crowding. No crowding effects were apparent in the current study.

As the result of a study of the development of *Polymorphus minutus* in the amphipod *Gammarus pulex*, Butterworth (1969) defined three distinct acanthella stages—the early acanthella (corresponding to the Stage I acanthella), the middle acanthella (including the acanthella Stages II-V), and the late acanthella (equivalent to the Stage VI acanthella). Crompton (1970) charted the proportion of time spent in each of these three stages during the development of thirteen acanthocephalans including *Moniliformis dubius*. As extrapolated from Crompton's bar diagram, his estimates of the time spent in each of the acanthella stages by *M. dubius* were as follows: early acanthella, 43%; middle acanthella, 29%; and late acanthella, 28%. These figures require a revision, however, since neither of the publications (Moore, 1946; King and Robinson, 1967) on which Crompton based his calculations clearly documented the duration of the late acanthella stage. From the data in table 6 and the graphic plot of the rate of development at 30° C in figure 15, we have recalculated the relative time spent in each of the three stages by *M. dubius:* early acanthella, 47%; middle acanthella, 37%; and late acanthella, 16%. Thus, more time is spent in the middle acanthella stage and considerably less as a late acanthella than previously estimated. If we included the time spent in the acanthor stages with that spent as an early acanthella (as did Crompton), then the proportion of time spent in this early stage would exceed 50%. Crompton (1970) observed that the proportion of time spent by *P. minutus* in each of the three acanthella stages remained constant even when the total time of larval development changed at different temperatures. With *M. dubius*, the data in tables 1, 2, and 6 and figure 15 suggest that this is not the case. The selective effect of temperature on the duration of the Stage II and IV acanthellas increases the proportion of time spent in the middle acanthella stage as the temperature is lowered from 30° C to 26.5° C and 23° C. The data and analyses presented in this paper suggest that acanthocephalan development is a complex and variable process. Continued accumulation of detailed tabulations of these developmental events will allow us eventually to evolve a stochastic model of acanthocephalan larval morphogenesis using the theory of Markov processes (Bharucha-Reid, 1960).

By far the preponderance of anomalous development occurred at Stage II acanthella, a stage in which a great deal of morphogenesis normally occurs. Voge (1959), in experiments on the sensitivity of developing *Hymenolepis diminuta* to high temperature stress, concluded that the heat-sensitive period in which pathology occurs coincides with the period of maximum larval growth and development. While the anomalies seen in *M. dubius* here cannot be attributed to heat stress alone, since anomalies were present at room temperature also, it is significant that the anomalies occurring both at temperatures allowing normal morphogenesis (23° C-30° C) and at high

temperatures (35°C) definitely point to the early stages as a sensitive period particularly subject to developmental aberrations.

The morphogenetic responses of larval *Moniliformis dubius* to temperature closely correspond to the temperature relations of the cockroach intermediate host, *Periplaneta americana*. Adult male *P. americana* provided with food and water *ad libitum* exhibit a temperature preference of 24°C to 33°C (Gunn, 1935). The temperature observed to be the most preferred was 29°C and the cockroaches avoided the higher end of the spectrum more than the lower end. The upper limit of the preferred temperature range is dropped by removing the water—a compensating reaction—and re-established when the water supply is returned. Ramsay (1935) discovered that the outer wax layer of the insect cuticle undergoes a change of phase at 30°C; this modification of the cockroach surface alters water permeability. Thus, water loss from the cockroach host increases radically at about the same temperature (32-33°C) at which normal morphogenesis of the acanthocephalan larvae is disrupted. The host provides a behavioral negative feedback to temperature extremes, thereby maintaining a homeostatic plateau. Overriding the host response to temperature extremes, as we did in the 35°C experiments, results in a runaway or positive feedback in the symbiont leading to "noise" which interferes with those feedback systems normally controlling larval morphogenesis (see Read, 1970, for a discussion of cybernetic principles in symbiosis). The acceptable noise level in the symbiont is exceeded outside of the homeostatic plateau. These events result, at low temperatures, in a negligible rate of development (Lackie, 1972b); at high temperatures, in a loss of developmental regulation or a blockage of morphogenesis; and ultimately—at both ends of the temperature spectrum—in death. In conclusion, the developmental regulation of larval *Moniliformis dubius* has evolved to function best within the limits the host, *Periplaneta americana*, has established as optimal in maintaining its homeostatic plateau.

ACKNOWLEDGMENTS

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