J. Parasitol., 92(4), 2006, pp. 756–763

SHAPE VARIATION OF CYSTICERCOIDS OF *HYMENOLEPIS DIMINUTA* (CYCLOPHYLLIDEA) FROM FED, PARTIALLY FED, AND FASTED *TRIBOLIUM CONFUSUM* (COLEOPTERA)

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ABSTRACT: Quantitative studies of a crowding effect on cysticercoids of *Hymenolepis diminuta* in the intermediate host are few and limited in scope. In this study, we developed a technique to rapidly collect morphological information on large numbers of parasites, and verified the utility of geometric models for simple and accurate estimation of cysticercoid size for quantitative studies. These models were tested using measurements from 4,899 *H. diminuta* obtained from 666 *Tribolium confusum* exposed 1–4 wk previously. Length, width, and depth of the body and cercomer (when present) can be used in conjunction with these models to provide the most accurate estimation of parasite size. However, parasite body length alone can be used, with adjustment for effects of host diet and infection intensity, to predict the remaining measurements in incomplete specimens. Parasites that developed in higher intensity infections, or in hosts with reduced food intake, were narrower and had a proportionately shorter cercomer. Host age, sex, and mating status, and parasite age also had statistically significant, but small-magnitude, effects on parasite shape.

Hymenolepis diminuta infection of beetles is a popular model system to examine the relationships between cestodes and their insect intermediate hosts. This parasite has been implicated in various effects on host behavior (Robb and Reid, 1996), reproduction (Hurd, 2001; Hurd and Ardin, 2003), and survival (Webster et al., 2000; Hurd et al., 2001). The mechanisms underlying these effects are areas of active investigation, but host–parasite energetic relations probably play a role. Direct biochemical studies to assess energetic relationships (Novak et al., 1993) are difficult to perform on individual hosts. Alternatively, surrogate measures such as host egg production or parasite size may be used to assess energetic relationships within individual hosts.

Early studies on *H. diminuta* in the intermediate host *Tribolium confusum* included qualitative reports (Voge and Heyneman, 1957; Dunkley and Mettrick, 1971) of a crowding effect (Read, 1951) on cysticercoid size. More recent quantitative studies provide mixed support for a crowding effect. Studies that measured length and width of the cysticercoid body (Soltice et al., 1971; Hurd and Arme, 1987) reported no correlation with intensity, but those that measured total cysticercoid length, including the cercomer (Keymer, 1980), reported an inverse relationship to intensity. This relation corroborates the qualitative observation (Voge and Heyneman, 1957) that crowding affects not only the thickness of the tissue layers surrounding the scolex but also the size of the cercomer. Surprisingly, differential measurements of the cysticercoid body and cercomer with respect to intensity have not been reported, even though they could help clarify the nature of the crowding effect on *H. diminuta* in its intermediate host. Depth measurements have not been reported, although the cysticercoid body looks elliptical in cross section (Goodchild and Harrison, 1961). Questions on parasite growth, such as space versus food as limiting resources for the parasite (Keymer, 1980), also might be resolved if body measurements enable estimation of parasite volume. Performing extensive morphological measurements on fresh cysticercoids increases the time required to perform a host necropsy, and therefore reduces the number of hosts that can be incorporated into an experimental design. One can preserve hosts for later necropsy (Hurd and Arme, 1987), but this approach introduces the potential for fixation artifacts.

Received 14 October 2005; revised 9 January 2006; accepted 10 January 2006.

Our first objective was to develop a technique to rapidly and accurately record morphological information on parasites at the time of necropsy. Our second objective was to develop geometric models of developmental stages of *H. diminuta* that would be typically encountered during necropsy of intermediate hosts. These models would ideally balance an accurate representation of parasite shape, with a minimal number of required morphological measurements, to render them practical for use in large-scale studies. Our third objective was to assess the effect of several commonly used experimental variables on the shape of larval stages of *H. diminuta* occurring in *T. confusum*. We will report elsewhere on the use of these techniques to evaluate the crowding effect in this system.

MATERIALS AND METHODS

Animals

Hymenolepis diminuta (source: Rice University, Houston, Texas, supplemented >15 yr ago with eggs from a biological supply house) were kept in male Sprague-Dawley rats infected with 10 cysticercoids and maintained for up to 1 yr. Fecal pellets from the cage bottom were soaked in tap water for 1–2 hr and teased apart to recover intact proglottids, or briefly pulsed in a blender, washed through sieves (retaining the 45–80-μm fraction), layered over 1 M sucrose, and centrifuged 10 min at 1,000 rpm to obtain purified eggs. Eggs or proglottids were stored in distilled water at 4 C for ≤3 days before use.

Tribolium confusum of unknown origin has been maintained in our laboratory for >15 yr. Beetles were stored in the dark at 26 C in medium made up of unbleached, additive-free white flour supplemented with 5% brewer's yeast. Stock cultures contained mixed sex beetles of unknown age; medium is changed 1–2 times/yr. Mated beetles of known age were produced by isolating mixed sex pupae and storing them in groups of ≤300 on 15 ml of medium, changing medium, and removing all nonadult stages at intervals <4 wk to maintain the original population of adults. Unmated beetles of known age and sex were produced by sexing pupae and storing them in same sex groups (≤10 per vial) on 1 ml of medium, which was changed 2–3 times/yr.

Infection protocols

Twenty-five different experiments were performed between November 2002 and September 2005. In each experiment, 29–152 beetles, fasted for 4 days, were exposed to parasite eggs. Most beetles were exposed individually for 16–20 hr in 16-ml glass vials containing 2–4 proglottids at the base of the vial, or a 20-μl aliquot of egg suspension in water containing 1,000 or 2,000 eggs placed on a 5- × 5-mm piece of Whatman no. 1 filter paper, and then maintained individually until necropsy. The remaining beetles were part of controlled mating experiments and were exposed to parasite eggs and subsequently maintained

until necropsy, as pairs. Beetles were kept in the dark at 26 C throughout experiments, except when handling was necessary.

The experiments evaluated effects of various factors (host diet, age, sex, and mating status, and parasite age and infection intensity) on the host–parasite relationship. Host diet was varied by providing hosts medium either ad libitum (except for the 4-day preexposure fast) or partially (a repeating sequence of 2 days on medium, 5 days fasting), or by fasting beetles throughout the experiment. Beetles of known age were 2, 4, 5, 6, 8, 16, 28, 40, or 52 wk old at time of exposure. Beetles were of unknown mating status from mixed sex cultures, or else known virgin females and males. Most beetles were exposed to parasite eggs on only one occasion, and at necropsy they harbored parasites that were either 1, 2, 3, or 4 wk old. Some beetles were exposed twice and harbored mixed age infections of 1- and 2-wk-old parasites, 1- and 3-wk-old parasites, 2- and 3-wk-old parasites, or 2- and 4-wk-old parasites. Variation in infection intensity (1–44 parasites per host) was achieved as a by-product of normal variation in the infection process. Each of the 25 experiments performed a different combination of these treatments, and collectively, 57 of 864 possible combinations of the abovementioned treatments (excluding variation in intensity) were done.

Necropsy procedure

Beetles were killed by crushing the head. Gentle pressure was applied to the abdomen to evert the genitalia for sex determination. The body was teased apart in a watch glass containing insect Ringer's saline (Kennedy and Behnke, 2001) and scanned at ×40 magnification to enumerate parasites and categorize them according to developmental stage (Voge and Heyneman, 1957). Briefly, stage 2 larvae are oval- to pear-shaped and undifferentiated (Fig. 1A); stage 3 larvae possess a tripartite body with scolex in various stages of development, but not yet withdrawn (Fig. 1C); stage 4 larvae possess a withdrawn scolex, but with incomplete deposition of cell layers around the scolex (Fig. 1E); and stage 5 larvae are fully developed cysticercoids (Fig. 1F, G). All parasites were moved to the center of the watch glass. In 1 experiment to assess the 3-dimensional shape of the parasites, measurements were made immediately using an ocular micrometer. In all other experiments, the watch glass was placed over a 1-mm grid (photocopied onto an acetate sheet), and the group of parasites was photographed at high resolution (2,048 by 1,536 pixels) by using a digital camera mounted on a dissecting microscope. Although this procedure provided only 2-dimensional views of the parasites, it permitted measurements to be recorded on a much larger number of specimens.

Morphological measurements

Stage 2 (Fig. 1A) and stage 3 (Fig. 1C) larvae were measured for maximum body length and body width, which we termed BL and BW, respectively, and body depth, BD, was defined as the measurement perpendicular to BW. The body of some stage 3 larvae was curved; thus, we measured BL along the curve (Fig. 1C). A typical stage 5 larva (Fig. 1F, G) comprises an anterior multilayered region, containing the scolex, which we term the body (''capsule'' or ''cyst''), and a posterior cercomer (''tail''). The body has a thick outer layer that tapers as it joins the cercomer, and an isthmus at that junction or a few micrometers anterior to it (Fig. 1F). The isthmus was a common point of separation of the cercomer in broken specimens. We used the narrowest part of the isthmus as the demarcation between body and cercomer and also as the point at which cercomer width (CW) was measured (Fig. 1F). Most stage 5 (>86%) larvae came to rest presenting their greatest width, which we defined as BW, and in this view clusters of granules anterior to the scolex showed up as 2 distinct narrow arcs (Fig. 1F). Others presented an oblique or side view, which we defined as BD and cercomer depth (CD) when perpendicular to BW, and in this view the granules showed up as a single broad arc (Fig. 1G). The cercomer was seldom straight, and cercomer length (CL) was measured along the midline of the curve, but BL was always measured as a straight line (Fig. 1F). Stage 4 larvae (Fig. 1E) were measured as for stage 5 larvae; although stage 4 larvae seldom had an isthmus, there was an inflection point between body and cercomer that we used to demarcate the boundary. We measured BL on all parasites, CL only if the cercomer was unbroken, BW and CW only if the parasite was lying flat, and BD and CD only on fresh specimens that could be rotated to ensure that we were viewing the specimen directly from the side.

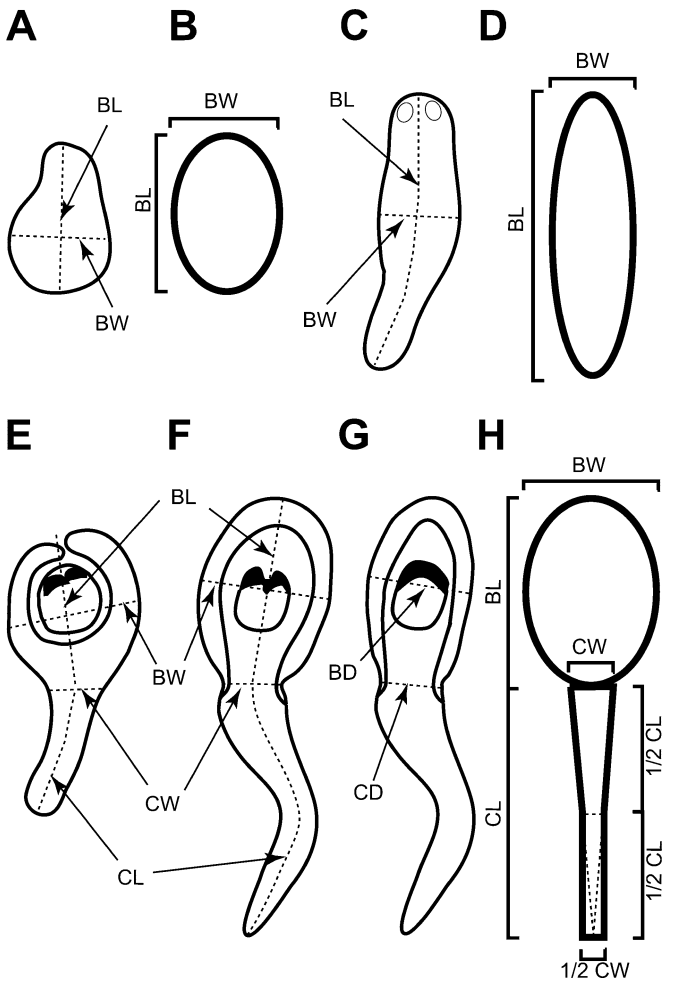

FIGURE 1. Morphological measurements made on stages 2–5 *H. diminuta* from *T. confusum*. (**A**) Frontal view of stage 2 parasite. Dotted lines indicate path of measurement. (**B**) Geometric approximation of shape of stage 2 parasite in frontal view. (**C**) Frontal view of stage 3 parasite. Dotted lines indicate path of measurement. (**D**) Geometric approximation of shape of stage 3 parasite in frontal view. (**E**) Frontal view of stage 4 parasite. Dotted lines indicate path of measurement. (**F**) Frontal view of stage 5 parasite. Dotted lines indicate path of measurement. (**G**) Side view of stage 5. Dotted lines indicate path of measurement. (**H**) Geometric approximation of shape of stages 4–5 in frontal view. Dotted lines indicate overlap between hypothetical triangle and rectangle used to represent the cercomer. BD, body depth; BL, body length; BW, body width; CD, cercomer depth; CL, cercomer length; CW, cercomer width.

Measurements of fresh specimens were done from beetles that were infected and then maintained either fasted or on medium ad libitum. Beetles were necropsied in saline at 7, 8, or 14 days postinfection (PI), and parasites of stages 2–5 were recovered. Up to 15 parasites of each stage from each beetle were measured in the order in which they were encountered. Using a dissecting microscope at ×40 magnification, parasites were manipulated with a fine pin to reveal their widest aspects, from which BL, BW, CL, and CW were measured using an ocular micrometer, and then rotated to reveal their narrowest aspect, from which BD and CD were measured. Measurements were done to the nearest 0.5 units on the ocular micrometer, which at ×40 equaled 12.5 μm. Parasite stages and total intensity were recorded for each beetle.

Measurements on photographed parasites were done using Photoshop software (Adobe Systems, San Jose, California) by measuring dimensions in pixels and converting them to distance by using the 1-mm background grid in the photograph for scale. To test the accuracy of

TABLE I. Agreement of repeated morphological measurements on the same photographed specimen of *Hymenolepis diminuta* from *Tribolium confusum.*

Parasite stage	Dimension*	Ratio†
2	BL	1.01 ± 0.023 (15)‡
	BW	1.00 ± 0.027 (15)
3	BL	1.00 ± 0.037 (30)
	BW	1.00 ± 0.027 (30)
4	BL	0.99 ± 0.038 (30)
	BW	1.00 ± 0.035 (30)
	CL	1.04 ± 0.110 (28)
	CW	1.03 ± 0.072 (28)
5	BL	0.99 ± 0.033 (30)
	BW	1.00 ± 0.035 (30)
	CL	0.99 ± 0.108 (23)
	CW	1.02 ± 0.102 (23)

* BL, body length; BW, body width; CL, cercomer length, CW, cercomer width.
† Second measurement of a specimen/first measurement of that specimen.
‡ Prediction error, $\bar{x}$ ± SD (n).

TABLE II. Agreement of areas of *Hymenolepis diminuta* from *Tribolium confusum* as estimated by geometric models for their shape, with their actual areas as measured by integration.

Parasite stage	Area	Model	Ratio*
2	Body	Ellipse† × 0.91	1.00 ± 0.069 (13)‡
3	Body	Ellipse† × 0.82	1.00 ± 0.081 (30)
4	Body	Ellipse†	0.99 ± 0.062 (15)
	Cercomer	Compound§	0.97 ± 0.116 (15)
5	Body	Ellipse†	1.00 ± 0.036 (40)
	Cercomer	Compound§	1.01 ± 0.087 (30)

* Area predicted from model/area measured by integration.
† ¼ × π × body length × body width.
‡ Prediction error, $\bar{x}$ ± SD (n).
§ As illustrated in Figure 1H; effectively, 5/8 × cercomer length × cercomer width.

this method, a metal pin about the size of a large stage 5 larva was placed in the same apparatus used to photograph parasites, and photographs were taken at a range of magnifications and after moving the pin to various locations within the field of view. Given the possibility of subjectivity in decisions on anatomical boundaries on the parasites, a randomly chosen sample of photographs of all stages was remeasured (blind) to test repeatability of measurements.

The areas in profile of a random sample of parasites of different developmental stages were measured by an integration procedure to test the ability of various geometric approximations to represent actual parasite size. Body widths of all parasites were taken at the midpoints of 10 evenly spaced segments, i.e., at 5, 15, 25 . . . 95% of total length, multiplied by 0.1× BL, and summed to determine body area. A separate determination of cercomer areas on stages 4 and 5 parasites used the same method.

Data analysis

We use intensity to refer to the total number of parasites per infected beetle, whether parasites were of the same stage or not, and whether they resulted from single or multiple exposures to parasite eggs. Statistical testing used SYSTAT software (SPSS Inc., Chicago, Illinois). Tests involving multiple factors used a factorial analysis of variance (ANOVA) model, including interactions. Intensity was incorporated as a covariate. Residual plots were examined visually for departures from normality or equality of variances. None of the variables tested required transformation. Post hoc pairwise comparisons used Tukey tests. Individual parasites were treated as independent replicates. Differences among proportions were evaluated by chi-square test. Significance was determined using $\alpha = 0.05$. Values are presented as $\bar{x}$ ± SD unless indicated otherwise. Because data were obtained from multiple experiments of differing design, tests for effects of various factors were done on all available subsets of data in which other factors could be held comparable.

We define prediction error (PE) of a measurement simply as E/O, where E is the expected measurement as predicted by some method under test, and O is the actual observed value of the measurement. An ideal predictor would have PE = 1 ± 0. Bias (consistent under- or overestimation) would be indicated by mean PE significantly <1 or >1. Increasing random variation in predicted values would be indicated by increasing SD of PE.

RESULTS

In total, 627 infected beetles produced 21 stage 2, 593 stage 3, 90 stage 4, and 3,903 stage 5 parasites that were photographed for later measurement. An additional 39 infected beetles produced 1 stage 2, 67 stage 3, 80 stage 4, and 144 stage 5 parasites that were measured immediately using an ocular micrometer.

Although the objective of this study was to evaluate parasite shape, with detailed analysis of absolute size to be presented elsewhere, we report here for completeness the ranges for measurements (all in micrometers) recorded on parasites in our study. Stage 2 larval measurements were BL, 170–619; BW, 140–241; and BD, 168. Stage 3 larval measurements were BL, 230–1,131; BW, 96–240; and BD, 108–216. Stage 4 larval measurements were BL, 192–416; BD, 144–240; CL, 161–978; CW, 56–155; and CD, 84–144. Stage 5 larval measurements were BL, 171–619; BW, 118–352; BD, 120–276; CL, 156–1,552; CW, 47–194; and CD, 72–156.

All measurements of the test pin on photographs were within 1.5% of the length as measured directly on the pin by using an ocular micrometer. Repeated measurements on photographed specimens of all stages were consistent. There was no significant mean difference between first and second measurements for BL and BW measured on 15 stage 2 and 30 stage 3 larvae, or for BL, BW, CL, and CW measured on 30 stage 4 and 30 stage 5 larvae (paired *t*-tests, $P > 0.05$ in all cases). Variation among individuals (Table I) was low; SD < 0.04 for body measurements and SD < 0.11 for cercomer measurements.

The shapes of early developmental stages varied, with stage 2 parasites ranging from elliptical to pear-shaped, and stage 3 parasites with anterior (future scolex) and posterior (future cercomer) projections that ranged from short and wide to long and slender. No single, simple, geometric model encompassed all the variation observed. The assumption of an elliptical shape (Fig. 1B, D) produced overestimates of parasite area compared with the area determined by integration, but multiplication of that estimate by a correction factor (Table II) resulted in estimates with a relatively low SD among individuals. The geometric representations we chose for stages 4 and 5 (Fig. 1H) parasites assume an ellipse for the shape of the body, and a cercomer comprising proximally the basal half of a triangle of base CW and height CL, and distally a rectangle of length 1/2 CL and width 1/2 CW. Without need for correction factors, these produced estimates that on average were within 0–3% of the area as determined by integration (Table II) and had rela-

tively low SD among individuals, although again the SD were higher for cercomer measurements than for the body.

Complete morphological measurements could be done on only a small proportion of parasites. As noted, 14% of photographed stage 5 larvae seemed to be lying to some extent on their side, and accurate BW could not be measured. Moreover, many stage 5 larvae had either a broken cercomer or it was absent. The proportion of 3,902 stage 5 larvae with intact cercomers declined with increased host feeding: 40% in fasted beetles, 26% in partially fed beetles, and 24% in beetles fed ad libitum (chi-square test, $P < 0.001$). The proportion of stage 5 larvae with intact cercomers also declined with age. Of 802 stage 5 larvae from partially fed beetles, 30% were intact at 2 wk, 25% at 3 wk, and 17% at 4 wk (chi-square test, $P < 0.05$). Of 1,964 stage 5 larvae from beetles fed ad libitum, 25% were intact at 2 wk, 25% at 3 wk, and 3% at 4 wk (chi-square test, $P < 0.05$). Although other measurements could often not be made, clear BL measurements could be made on 99.9% of 4,610 parasites photographed in this study. Therefore, we explored the extent to which other measurements could be predicted from BL.

Data on complete stage 5 larvae from photographs in various treatment combinations were selected to test effects of those treatments on morphological ratios BW/BL, CL/BL, and CW/BL. Random variation among hosts was tested using 1-way ANOVAs on 68 unique combinations of host diet, host sex, and intensity, in which multiple hosts were necropsied from that combination. Few significant differences among hosts were detected for BW/BL (9/68 combinations differed), CL/BL (4/68), or CW/BL (11/68). The number of significant differences was slightly more than expected from type I error (3–4 occurrences expected from 68 tests when using $\alpha = 0.05$), but cases of significant difference were spread among the 68 treatment combinations in no apparent pattern with respect to host sex or diet or intensity. Random variation among experimental replicates was significant in some comparisons (Table III). The ratio CW/BL, but not the other ratios, differed between 2 replicate experiments with fasted beetles. The first of 4 replicate experiments using beetles fed ad libitum produced significantly smaller CL/BL and CW/BL than the subsequent 3 replicates (Tukey test, $P < 0.05$), but the magnitude of the effect was small (Table III).

Effects of fixed factors were tested in a series of ANOVAs with intensity as a covariate. All factors tested had significant effects on at least 1 of BW/BL, CL/BL, and CW/BL under at least some conditions (Table III). Intensity usually had a significant effect on all measurements in hosts fed ad libitum, occasionally in partially fed hosts, and was never significant in fasted hosts. A differential response according to host diet was observed with most other factors as well. Some but not all analyses indicated significant effects of host sex, mating status and age, and of parasite age, but the differences among treatment groups were usually of small magnitude. The fewest significant tests occurred for BW/BL, more for CL/BL, and the most for CW/BL, but CL/BL expressed the greatest variation among treatment groups.

Although all factors we tested had some effects on the shape of stage 5 parasites in photographs, 2 factors, host diet and infection intensity, seemed to predominate. These factors were used to develop predictive formulae for BW/BL, CL/BL, and CW/BL (Table IV). We analyzed separately by host diet. If the effect of intensity was not significant, we assumed the ratio to be a constant. If intensity had a significant effect, we used the regression formula as a predictive equation. We also selected only host diet and infection intensity to determine formulae for stages 2–4 larvae (because of the limited variation in experimental designs used to produce stages 2–4 larvae, we could not perform the range of background analyses presented in Table III for stage 5 larvae). BW/BL was independent of intensity and similar among host diets in stages 2–4 larvae, but, in stage 5 larvae, an increasing host diet resulted in larger ratios (increasing intercept) and an effect of intensity (significant negative slope). Similarly, in stages 4 and 5 larvae, CL/BL increased with increasing host diet and was affected by intensity. CW/BL in stage 4 larvae was unaffected by intensity or host diet, whereas CW/BL in stage 5 larvae increased with host diet and was affected by intensity in hosts fed ad libitum. The ratios of BW/BL, CL/BL, and CW/BL determined on photographed specimens (Table IV) were corroborated by ocular micrometer measurements on a different set of fresh specimens. Mean values determined by the 2 methods were significantly different (ANOVA, $P < 0.05$) in only 3 of 15 comparisons: BW/BL was 18% smaller in photographed specimens of stage 3 larvae from hosts fed ad libitum, CL/BL was 20% larger in photographed specimens of stage 4 larvae from hosts fed ad libitum, and 14% larger in photographed specimens of stage 5 larvae from fasted hosts.

Ocular micrometer measurements on fresh specimens revealed that stages 2–5 larvae were round to oval in cross section (Table IV); earlier stages were more rounded (BD/BW or CD/CW near 1), and the later stages progressively more flattened (BD/BW or CD/CW < 1). Parasites from beetles fed ad libitum were either the same shape or flatter than parasites from fasted beetles. Cross-sectional shape was constant across all infection intensities (linear regression, $P > 0.05$ in all cases). Predictions of BD and CD from either BL, or BW and CW (Table V), were all unbiased (mean PE did not differ significantly from 1, *t*-test).

We tested the ability of the relationships in Table IV (which were derived from specimens with complete measurements), to predict BW, CL, and CW from BL on an independent data set of stages 3–5 larvae (the remaining specimens that had incomplete measurements except for BL). The predicted measurements in the test data set were then compared with the actual measurements on those specimens for which they could be obtained and were not compromised by a lateral view or broken cercomer. (Relationships for stage 2 larvae were not tested because all their data were already used to generate the values in Table IV.) Predictions were unbiased except for 1 case, from stage 5 larvae in partially fed hosts, where the predicted CL was significantly smaller than measured CL (*t*-test, $P < 0.05$). SD of the predictions were generally highest for stage 3, lower for stage 4, and lowest for stage 5 larvae, and lowest for BW, higher for CW, and highest for CL (Table VI). Because sex was 1 factor that frequently had a significant effect on body shape (Table III), we attempted to reduce the SD of the predictions by deriving separate relationships for parasites in female and male beetles. This attempted reduction was unsuccessful; both $\bar{x}$ and SD of the PEs (data not shown) remained similar to those in Table VI where host sex was ignored.

DISCUSSION

The simple technique of photographing *H. diminuta* as a group immediately after dissecting them from the beetle inter-

TABLE III. Summary of ANOVA tests of effects of various factors on morphological ratios of stage 5 *Hymenolepis diminuta* from *Tribolium confusum* on different diets. Intensity was incorporated as a covariate.

			BW/BL*		CL/BL*		CW/BL*	
Diet	Source of variation	df	MS	*P*	MS	*P*	MS	*P*
Fasted	Experimental replicate	1	0.0025	0.304	0.205268	0.108	0.00983 (0.31–0.34)†	0.013
	Host sex	1	0.0019	0.372	0.180719	0.131	0.00014	0.764
	Interaction	1	0.0027	0.279	0.000029	0.984	0.00066	0.516
	Intensity	1	0.0064	0.099	0.008464	0.743	0.00029	0.667
	Error	198	0.0023		0.078642		0.00156	
Ad libitum	Experiment replicate	3	0.0034	0.228	0.446 (1.3–1.5)	0.047	0.0199 (0.30–0.34)	<0.001
	Host sex	1	0.0163 (0.64–0.66)	0.009	2.500 (1.4–1.5)	<0.001	0.0092 (0.31–0.32)	0.006
	Interaction	3	0.0043	0.142	0.026	0.924	0.0029	0.068
	Intensity	1	0.0296	<0.001	11.761	<0.001	0.0261	<0.001
	Error	376	0.0023		0.167		0.0012	
All	Host diet	2	0.0104 (0.64–0.66)	0.012	13.23 (1.1–1.5)	<0.001	0.0017 (0.31–0.32)	<0.001
	Host sex	1	0.0023	0.319	0.30	0.134	0.0016	0.284
	Interaction	2	0.0036	0.220	1.06 (1.0–1.6)	<0.001	0.0059 (0.31–0.32)	0.018
	Intensity	1	0.0250	0.001	12.34	<0.001	0.0121	0.004
	Error	833	0.0023		0.13		0.0015	
Ad libitum	Host sex	1	0.00861	0.057	1.15 (1.3–1.4)	0.007	0.0014	0.336
	Mating status	1	0.00071	0.583	9.91 (1.2–1.5)	<0.001	0.0012	0.369
	Interaction	1	0.00437	0.175	0.38	0.118	0.0025	0.198
	Intensity	1	0.02570	0.001	11.60	<0.001	0.0092	0.013
	Error	835	0.00237		0.16		0.0015	
Fasted	Host age	2	0.0059	0.084	0.068	0.465	0.00612 (0.29–0.32)	0.025
	Intensity	1	0.0042	0.184	0.038	0.511	0.00009	0.817
	Error	178	0.0023		0.088		0.00162	
Ad libitum	Host age	7	0.0016	0.526	0.28	0.168	0.00215 (0.31–0.33)	0.033
	Intensity	1	0.0278	<0.001	6.69	<0.001	0.01508	<0.001
	Error	194	0.0019		0.19		0.00097	
Partial	Parasite age	6	0.006 (0.62–0.66)	0.013	0.18	0.175	0.0062 (0.30–0.33)	<0.001
	Intensity	1	0.0064	0.092	1.38	<0.001	0.0017	0.257
	Error	244	0.0022		0.118		0.0013	
Ad libitum	Parasite age	5	0.0052	0.054	0.34	0.083	0.0097 (0.32–0.36)	<0.001
	Intensity	1	0.0183	0.006	10.50	<0.001	0.0259	<0.001
	Error	378	0.0024		0.17		0.0013	

* BL, body length; BW, body width; CL, cercomer length; CW, cercomer width.
† Values in parentheses are the range of values among treatment groups as an indicator of the magnitude of the significant treatment effect.

mediate host enabled accurate, repeatable morphological measurements to be made at a later time. Measurements made on photographed specimens were substantially the same as those obtained by the traditional technique of direct measurement by ocular micrometer. The use of photographs, therefore, allows incorporation of larger sample sizes or more complex designs in experiments that require necropsy of hosts on a fixed schedule. Moreover, the permanent record provided by the photograph provides an opportunity to reevaluate measurements that subsequent analysis may indicate as outliers. The ability to reexamine photographs was instrumental in allowing us to recognize the different appearance of the granules overlying the scolex as a marker of a poorly oriented cysticercoid; although this difference may have been noticed by other researchers, we have found no published reports of it. The presence of paired granular areas may be a feature of *H. diminuta*, but not other species in the genus (Rothman, 1957).

The technique does have limitations. Parasites that are out of

TABLE IV. Morphological ratios of *Hymenolepis diminuta* from *Tribolium confusum* on different diets.

Parasite stage	Diet	BW/BL*†	BD/BL*‡	BD/BW*‡	CL/BL*†	CW/BL*†	CD/BL*‡	CD/CW*‡
2	Fasted	0.57§	0.44	0.93				
	Partial	0.57						
	Ad libitum	0.57						
3	Fasted	0.31§	0.31	95				
	Partial	0.31						
	Ad libitum	0.28	0.31	0.88				
4	Fasted	0.81	0.69	0.87	0.82	0.40	0.41	0.95
	Partial	0.81			1.27	0.40		
	Ad libitum	0.81	0.69	0.87	2.34–0.0038 *x*	0.40	0.41	0.95
5	Fasted	0.64	0.56	0.85	1.03	0.31	0.32	1.00
	Partial	0.65–0.0018 *x*§			1.33–0.016 *x*	0.30		
	Ad libitum	0.66–0.00079 *x*	0.53	0.81	1.71–0.016 *x*	0.39–0.00076 *x*	0.32	1.00

* BL, body length; BW, body width; BD, body depth; CL, cercomer length; CW, cercomer width; CD, cercomer depth.
† Measured digitally from photographed specimens.
‡ Measured by ocular micrometer on fresh specimens.
§ Where no significant difference occurred among host diets, the pooled mean is used to represent all diets for that stage. Where there were significant differences among diets (Tukey test), separate means for each diet are shown. Regression formulae as a function of infection intensity (x) are shown only when the effect of intensity was significant.

focus, not lying flat, or outside the recorded area of the photo cannot be measured, and this problem may not be evident until well after the necropsy when the specimens have been discarded. It was practical to record up to 44 parasites on a single photograph. This system allowed us to position parasites so that they did not overlap the grid lines on the background scale and still photograph them at sufficient magnification to record morphological detail. Infections much larger than that, which occur routinely in *Tenebrio molitor* (Hurd and Arme, 1987) or as a result of multiple infections (Keymer, 1980), would need to be photographed in batches.

Early developmental stages of *H. diminuta* change rapidly (Voge and Heyneman, 1957), and it is not surprising that their variable shape could not be described by a simple geometric representation. The assumption of an ellipse or ellipsoid shape overestimated parasite size, and a correction factor was necessary to obtain a reasonably accurate approximation to actual area or volume of stages 2 and 3 parasites as determined by integration. For the greatest accuracy, these stages should be measured by integration. By contrast, the bodies of stages 4 and 5 larvae were well represented by an ellipse or ellipsoid, even though their shape has been described as cardioid (Rothman, 1957). We have found no reports of attempts to characterize the cercomer other than by length (Keymer, 1980). Our simple geometric approximation requires measurement only of length and basal width of the cercomer, yet produced unbiased and low variability estimates of its area. We have found no previous reports of cross-sectional shape or measurements of the cysticercoid of *H. diminuta*, except for photographs (Goodchild and Harrison, 1961) of histological sections of an infective cysticercoid with an ellipsoid shape and BD/BW that we measure to be 0.7, slightly narrower than our determinations. Somewhat surprisingly, although we found that the body of *H. diminuta* became progressively flatter throughout development, the cercomer remained virtually round in cross section. Although we could not do an integrative measure of volume, the low variability we obtained in depth measurements suggests that extending our 2-dimensional geometric models to 3 dimensions (by assuming an ellipsoid or circular cross-sectional shape for the parasite) should give similarly accurate results.

Our analysis revealed a new layer of complexity to parasite shape, particularly in the later stages of development, because of variation in length and width measurements of body and cercomer relative to 1 another. For a parasite of given body length, an increase in intensity or decrease in host food intake resulted in parasites with a narrower body and also a shorter,

TABLE V. Agreement of body and cercomer depth estimates of larval stages of *Hymenolepis diminuta* from *Tribolium confusum* on different diets as predicted by different morphological ratios.

Parasite stage	Diet	Prediction of BD*		Prediction of CD*	
		from BD/BL*	from BD/BW*	from CD/BL*	from CD/CW*
3	Fasted	1.03 ± 0.20 (43)†	1.01 ± 0.06 (43)		
	Ad libitum	1.07 ± 0.20 (24)	1.01 ± 0.06 (24)		
4	Fasted	1.04 ± 0.13 (36)	1.00 ± 0.07 (36)	1.02 ± 0.15 (8)	0.98 ± 0.06 (8)
	Ad libitum	1.00 ± 0.13 (44)	1.01 ± 0.06 (44)	1.05 ± 0.19 (11)	1.02 ± 0.09 (11)
5	Fasted	1.01 ± 0.07 (52)	1.00 ± 0.06 (52)	1.04 ± 0.11 (25)	1.02 ± 0.09 (25)
	Ad libitum	1.01 ± 0.13 (92)	1.01 ± 0.11 (92)	0.99 ± 0.10 (39)	0.99 ± 0.05 (39)

* BL, body length; BW, body width; BD, body depth; CW, cercomer width; CD, cercomer depth.
† Prediction error, $\bar{x} \pm$ SD (n).

TABLE VI. Ability of morphological ratios from Table IV, to predict body width, cercomer length, and cercomer width of *Hymenolepis diminuta* from *Tribolium confusum* on different diets. Predictions were made on specimens that were not used to generate the ratios in Table IV.

Stage	Diet	Prediction of BW* from BW/BL	Prediction of CL* from CL/BL	Prediction of CW* from CW/BL
3	Fasted	1.05 ± 0.23 (118)†		
	Partial	0.99 ± 0.23 (83)		
	Ad libitum	1.06 ± 0.23 (49)		
4	Fasted	1.26 ± 0.31 (8)		1.08 ± 0.35 (8)
	Partial	0.98 ± 0.16 (11)		0.97 ± 0.09 (11)
	Ad libitum	1.02 ± 0.12 (50)	1.12 ± 0.32 (17)	1.06 ± 0.13 (50)
5	Fasted	1.00 ± 0.09 (460)	0.96 ± 0.25 (166)	1.02 ± 0.14 (460)
	Partial	1.00 ± 0.09 (926)	0.94 ± 0.28 (183)	1.00 ± 0.12 (926)
	Ad libitum	1.00 ± 0.09 (1,458)	1.03 ± 0.28 (184)	1.01 ± 0.14 (1,160)

* BL, body length; BW, body width; BD, body depth; CL, cercomer length; CW, cercomer width.
† Prediction error, $\bar{x}$ ± SD (n).

narrower cercomer. This relation confirms qualitative observations (Voge and Heyneman, 1957) that crowding disproportionately affects cercomer length of *H. diminuta* and may explain why quantitative studies report a crowding effect when total length, including cercomer, is measured (Keymer, 1980), but not when body length alone is measured (Dunkley and Mettrick, 1971; Hurd and Arme, 1987). The effects we observed of crowding and host diet on BW/BL and CW/BL have not been reported previously.

The proximate mechanisms controlling cysticercoid shape were not investigated. The major effects we observed for the fixed treatment effects of host diet and intensity are indicative of parasite competition for nutrient resources, which would presumably be more limited in fasted hosts and in higher intensity infections. Moreover, the disproportionate shortening and narrowing of the cercomer relative to body length that occurred when host food was reduced may indicate that the parasite is selectively allocating limited nutrient resources to the body region. Other fixed effects (host sex, host age and mating status, and infection age) also were associated with body shape variation, although to a lesser extent than host diet and infection intensity. It is possible that these factors indirectly influence host–parasite nutritional relationships. There are reports of sex-related differences in host food intake (Shea, 2005) and sex- and age-related differences in hemolymph amino acid composition (Hurd and Arme, 1987) for a related beetle, *T. molitor*. It is likely that any mechanism affecting cysticercoid shape involves more than just the quantity of nutrients available to the parasite. Mean body lengths and widths for *H. diminuta* in the much larger *T. molitor* indicate a narrow BW/BL of 0.57–0.59, even in the apparent absence of a crowding effect (Hurd and Arme, 1987), although our study found more robust parasites (BW/BL ≈ 0.64), even in fasted *T. confusum*. Two possibilities are that nutritional pathways may differ between host species, or there may be a difference in normal growth forms between the sources of *H. diminuta* used by Hurd and Arme (1987) and in the present study.

In addition to strong fixed treatment effects, we identified two sources of random variation in parasite shape. There was a low frequency of occurrence of significant variation in parasite shape among different beetles of the same sex, diet, and infection intensity. These differences were small, and in a majority of comparisons no difference was detected. We feel that, in general, this finding supports our use of individual parasites as independent units of replication in our other statistical analyses, even though they occur with other parasites in the same host. There also were slight variations in average shape of parasites recorded among replicated experiments. Clearly, there are additional, as yet unidentified, factors that may influence the shape of *H. diminuta*. It would be prudent, before making use in a new system of the morphological ratios we present here, to verify them on a sample of completely measured specimens.

Regardless of its causes, the shape variation we demonstrated for *H. diminuta* indicates that length measurements alone are not sufficient to reveal all effects on parasite size, because we have shown that parasites of the same length can have significantly different widths, depths, or both, and hence volumes. Not only have we demonstrated the variability in parasite shape and some of the factors that contribute to it, but also we have developed and verified algorithms to estimate widths and depths when they cannot be measured directly. Furthermore, we have shown how these measurements can be used in conjunction with simple geometric models to estimate overall parasite size. With this new set of tools, it should now be possible to study growth of *H. diminuta* in the intermediate host with much greater resolution than has been done previously.

ACKNOWLEDGMENTS

We thank two anonymous reviewers whose comments greatly increased the clarity of the manuscript.

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