

Female ixodid ticks grow endocuticle during the rapid phase of engorgement

Peter C. Flynn · W. Reuben Kaufman

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Abstract Lees (Proc Zool Soc Lond 121:759–772, 1952) concluded that the ixodid tick *Ixodes ricinus* grows endocuticle during the slow but not during the rapid, phase of engorgement, a conclusion supported by Andersen and Roepstorff (Insect Biochem Mol Biol 35:1181–1188, 2005) for the same species. In this study analysis of dimensional data and cuticle weight measurements from female ixodid ticks (*Amblyomma hebraeum*) were used to test this hypothesis. Both approaches showed that endocuticle growth continues during the rapid phase, tapering to zero at a fed/unfed weight ratio of ~ 60 . Of the total mass of cuticle in the engorged tick 32–43% was formed during the rapid phase. We demonstrate that if cuticle growth stopped at the end of the slow phase, there would not be sufficient cuticle to account for the thickness of cuticle observed at the end of engorgement. This finding is consistent with prior studies of *Rhipicephalus (Boophilus) microplus*, and with a dimensional analysis of the cuticle thickness data of Lees for *I. ricinus*, in contradiction to his conclusion from an analysis of tick cuticle weight measurements. An examination of cuticle weight measurements for *I. ricinus* by Andersen and Roepstorff similarly supports the finding of cuticle growth during the rapid phase. All ixodid ticks undergo major body expansion, typically tenfold or more, during a rapid phase of engorgement and require sufficient cuticle at the end of that process to contain their body. The fact that cuticle grows during the rapid phase of engorgement in three species suggests that this is a general characteristic of the family Ixodidae.

Keywords Cuticle synthesis · Ixodid ticks · *Amblyomma hebraeum* · *Ixodes ricinus* · *Rhipicephalus (Boophilus) microplus* · Rapid phase of engorgement

P. C. Flynn
Department of Mechanical Engineering, University of Alberta, Edmonton, AB T6G 2G8, Canada

W. R. Kaufman (✉)
Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada
e-mail: Reuben.Kaufman@ualberta.ca

Introduction

Ixodid ticks (Acari: Ixodidae) are obligatory haematophagous arthropods during each stage of a larval, nymph and adult life cycle. Once copulated, adult females typically engorge to ~100 times the unfed body weight. The feeding period is divided into three stages: (1) a brief ‘preparatory stage’ during which the feeding lesion is produced and the cement cone secreted (2) a ‘slow phase’ during which a fed-to-unfed weight ratio (henceforth simplified to ‘weight ratio’) of 10–12 is achieved over about 8 days in *Amblyomma hebraeum*, and (3) a ‘rapid phase’ of 12–24 h during which the weight ratio approaches 100.

Lees (1952) concluded that adult female *Ixodes ricinus* synthesized endocuticle during the slow but not rapid period of engorgement, in contrast to what he observed for two species of argasid ticks: adult female *Ornithodoros moubata* and larvae of *O. delanoei acinus*. In argasid ticks engorgement occurs within an hour or so, and significant cuticle growth during feeding does not occur. The engorged weight ratio of adult female *O. moubata* is about 5–12 (Diehl et al. 1982). Lees’ conclusions on *I. ricinus* were based on a small number of dried cuticle samples that showed a high degree of scatter. Andersen and Roepstorff (2005) supported Lees’ conclusions on *I. ricinus*, based on measurements of cuticle that were air dried overnight. Again, there was a high degree of scatter in the data.

A second approach to estimating cuticle weight is possible through a geometric analysis of ticks, because body dimensions and cuticle thickness are readily available data. The shape of the tick can reasonably be modeled as an ellipsoid, allowing both volume and surface area to be calculated. Combining weight and dimensional measurements allows tick density to be estimated. Cuticle density can be estimated from the density of unfed ticks. Combining these calculations, the volume and mass of cuticle can be estimated as a function of the mass of the tick. These steps allow an exploration of the following question: is the amount of cuticle present in a tick at the end of the slow phase of engorgement sufficient to cover the tick at the end of rapid engorgement without the formation of additional cuticle?

During recent experiments focusing on plasticization of the cuticle during feeding we accumulated a large body of data on tick weight, dimensions and cuticle thickness (Kaufman et al. 2010). An initial review of the data called into question whether the volume increase of the engorged female could indeed be accommodated by the amount of cuticle that had been laid down by the end of the slow phase of engorgement. We designed a two pronged approach to the question of whether *A. hebraeum* grows cuticle during the period of rapid engorgement: a dimensional analysis of data from a large sample of ticks and direct weight measurements of wet and dry weight of a smaller number of cuticle samples. In addition we performed a dimensional analysis of *I. ricinus* using data reported by Lees (1952) and Dillinger and Kesel (2002).

Materials and methods

Dimensional and weight data for female *A. hebraeum* ticks were recorded from two sources. What we call ‘data set 1’ were collected as part of an earlier study (Kaufman et al. 2010). Similar data came from a more recent batch of ticks (‘data set 2’) in order to extend the observations to higher body weights. Details on the feeding procedures for ticks are described in Kaufman et al. (2010).

Dimensional model of *Amblyomma hebraeum*

One stage of this work involved development of a geometric model of *A. hebraeum* from dimensional and weight data. Tick weight (W) was recorded on an electronic balance to the nearest 0.1 mg for ticks weighing below 1,000 mg, and to the nearest 1 mg for ticks weighing >1,000 mg. Body length (l), width (w) and thickness (t) data, necessary to calculate volume, density and surface area, were measured with calipers. Dimensional measures were drawn from two independent batches of *A. hebraeum*; the batches were from the same tick colony, but the ticks had moulted up to 2 years apart. The observed ratio of w to l remained constant at ~ 0.765 over the entire period of engorgement, whereas the body thickness relative to length increased as a function of weight (Fig. 1). Relative measurement error (dx/x) is high for small ticks, as seen in the high scatter for low tick weights in Fig. 1. The relationship of width to length and thickness to length used in the model was based on data set 2.

Our prior study on the mechanical properties of tick cuticle required the section of a loop of cuticle from the opisthosoma immediately behind the fourth coxae. Cuticle thickness (TC) was measured at the cut face of the carcass just anterior to where the loop had been cut. Multiple readings were recorded using a calibrated ocular micrometer under high power magnification of a dissecting microscope, and a single mean value used for calculations. For a complete description see Kaufman et al. (2010). In this study three correlation equations were used to predict cuticle thickness as a function of body weight at distinct weight ranges (Table 1); Fig. 2 shows the actual cuticle thickness values recorded in this study as a function of tick weight and the fitted equations used in the following tick model.

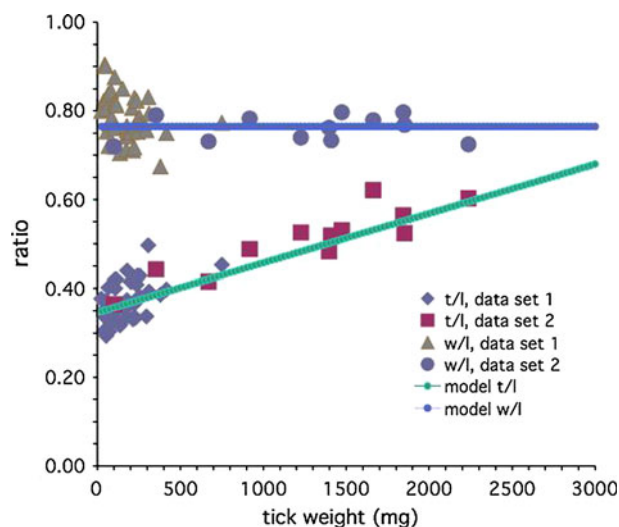
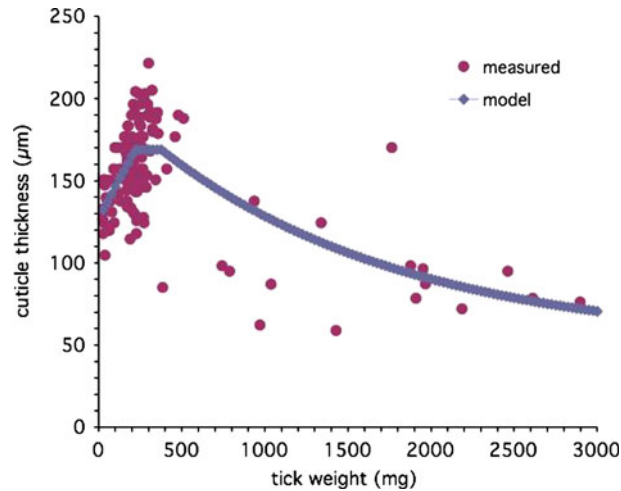


Fig. 1 Body width-to-length and body thickness-to-length ratios for female *Amblyomma hebraeum* as a function of tick weight. The ratio of width-to-length (w/l) stayed constant at a value of about 0.765 throughout the feeding period. The ratio of thickness-to-length (t/l) increased steadily throughout the feeding period; the relationship used in the model is $t/l = 0.00011114 \times \text{tick weight} + 0.34625$. Data are drawn from two sets of female *A. hebraeum* as described in “Materials and methods”

Table 1 Equations used in the model to calculate cuticle thickness for adult female *Amblyomma hebraeum*

Weight range of tick (mg)	Stage of cuticle development	Equation for modeling cuticle thickness, T (μm)
25–220	Thickening	$T = 0.1883 \times W + 127.68$
220–375	Plateau	$T = 169$
375–3,000	Thinning	$T = 50 + 119 \times \exp(-(W - 375)/1500)$

**Fig. 2** Cuticle thickness as a function of tick weight in *Amblyomma hebraeum*. Circles are measured values. The superimposed curve was generated from the three correlation equations shown in Table 1

Tick body shape was modeled as an ellipsoid, with volume (V) calculated by

$$V = 4/3 \times \pi \times (l/2 \times w/2 \times t/2) \quad (1)$$

The Knud Thomsen formula (Wikipedia 2010) was used to calculate surface area (A).

$$A = 4 \times \pi \times (((l/2)^{1.6075} \times (w/2)^{1.6075} + (l/2)^{1.6075} \times (t/2)^{1.6075} + (w/2)^{1.6075} \times (t/2)^{1.6075})/3)^{1/1.6075} \quad (2)$$

Relating tick weight to volume calculated from recorded dimensional data allowed a calculation of density (D) over the engorgement period. The data and the relationship used in the model are shown in Fig. 3. Because in this study density is based on a calculated volume, the higher relative measurement error in small ticks compounds, as seen in the high scatter at low tick body weights. However, an increase in tick density during engorgement is consistent with the feeding pattern of *A. hebraeum* (and female ixodid ticks in general) in that a significant portion of the blood meal fluid is secreted back into the host via the salivary glands (Kaufman 2007). In the model, density is assumed to increase up to a tick weight of 500 mg and remain constant at a value of 1130.5 kg/m^3 thereafter.

Cuticle volume (VC) was estimated as the product of area and thickness, in essence incorporating the assumption that cuticle thickness does not vary significantly throughout the alloscutum. A partial test of this assumption is available from comparing dorsal and

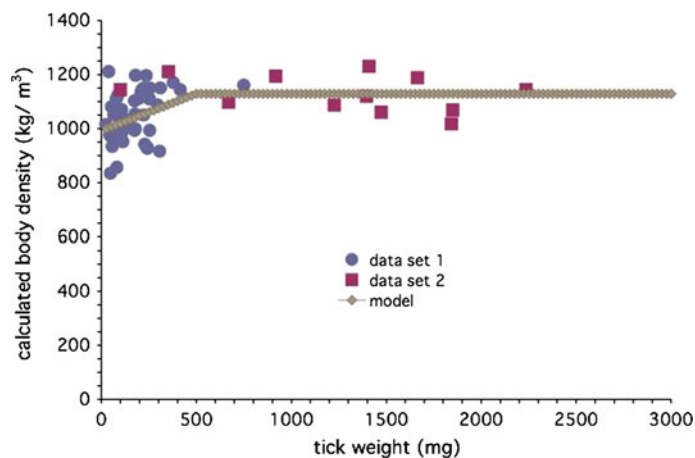


Fig. 3 Female *Amblyomma hebraeum* body density as a function of tick weight. Body volume was calculated from body length, width and thickness assuming an ellipsoidal model for body shape. Data are drawn from two sets of female *A. hebraeum* as described in “Materials and methods”. Relative measurement error is higher at small dimensions, resulting in high scatter at low body weights. The relationship used in the model was $D = 0.274 \times \text{tick weight} + 993.5$ for the range, 0–500 mg; $D = 1130.5$ for ticks weighing >500 mg

ventral cuticle thickness measurements. A sample of 70 ticks showed a ratio of measured dorsal-to-ventral thickness of 1.051 ± 0.134 (SD); the relatively high SD reflects the imprecision of measuring thickness with an ocular micrometer.

Cuticle weight (WC) was estimated based on an assumed constant density of cuticle (DC) of $1,010 \text{ kg/m}^3$. The assumption of constant cuticle density equates to the hypothesis that tick cuticle is isochoric, i.e. it maintains a constant volume when stretched and is incompressible. The validity of this assumption is discussed below. The further assumption of a density of $1,010 \text{ kg/m}^3$ is consistent with the density of unfed ticks; however, the precision of this second assumption has no significant impact on the conclusions of the study.

Using these correlations, a model of tick growth was developed through the following sequential calculation steps:

1. Set tick weight W in successive steps between 25 and 3,000 mg.
2. Calculate tick volume W/D .
3. Calculate tick dimensions l , w and t by solving Eq. 1 for l , using $w = 0.765l$ and the correlation for t from Fig. 1. Because thickness-to-length ratio is a function of tick weight, an iterative solution was performed using the Goal Seek function in Microsoft ExcelTM.
4. Calculate tick surface area A using Eq. 2.
5. Use the appropriate correlation equation from Table 1 to calculate cuticle thickness T .
6. Calculate cuticle volume and weight of tick cuticle, $VC = A \times T$, and $WC = VC \times DC$.

Measurement of tick cuticle

Lees (1952) reported measuring cuticle weight using all the cuticle of the tick. However, the ventral alloscutal cuticle is penetrated by the coxae, gonopore and rectal plate, and Lees made no mention of eliminating the legs, capitulum and spiracular plates from his cuticle

measurements. We considered it more accurate to measure alloscutal cuticle growth throughout feeding by sampling the dorsal alloscutal cuticle only. A miniature razor blade scalpel was used to cut around the lateral margin of the idiosoma mid-way between the dorsum and ventrum. On both sides of the anterior end, we sliced around the scutum/alloscutum margin. The severed dorsum was then removed by peeling it posteriorly. All soft tissue attached to the cuticle was removed by scraping with the blunt edge of the razor blade scalpel under liquid paraffin (light mineral oil; Fisher Scientific). After blotting the cuticle sample with tissue paper to remove the mineral oil each cuticle sample was transferred to a small, pre-weighed (nearest 0.01 mg) porcelain crucible, and wet weight was recorded on a microbalance to the nearest 0.01 mg. The sample was then dried in a 55°C oven for at least 2 h, a procedure that prior work had demonstrated reached a stable weight (Kaufman et al. 2010). The dry weight was then recorded to the nearest 0.01 mg.

Analysis of Lees' (1952) data

Lees (1952) reported cuticle thickness for *I. ricinus* of 105 μm at the end of the slow phase of engorgement, and of 50 μm at the end of the rapid phase. Thickness-to-width data at unfed, partial and full engorgement can be estimated from his drawings. Length values for unfed, partially engorged and fully engorged *I. ricinus* were also reported by Dillinger and Kesel (2002); width-to-length ratios can be measured from their photos. Table 2 shows the measured values. From these data the incremental mass of cuticle accumulating in the period between partial and full engorgement was estimated for *I. ricinus* using a volumetric calculation procedure similar to that outlined above for *A. hebraeum*.

Analysis of Andersen and Roepstorff's (2005) data

Andersen and Roepstorff (2005) concluded from a plot of cuticle weight measurements as a function of tick weight for *I. ricinus* that cuticle growth stops at the onset of rapid engorgement. We examined their estimated “best fit” plot of the data against their conclusion, and compared it to the alternative hypothesis that cuticle growth continues into the rapid phase of engorgement.

Results and discussion

Model and measured values of tick cuticle

Figure 4 shows the fit between measured length and thickness data for *A. hebraeum* and calculated values from the iterative solution for dimensions derived from the tick model.

Table 2 Body dimension and ratios for adult female *Ixodes ricinus*. Body length and width-to-length ratio were calculated from data provided by Dillinger and Kesel (2002). Thickness-to-width ratio was calculated from Lees (1952), from which cuticle thickness was also taken

Feeding stage	Body length (mm)	Width-to-length ratio	Thickness-to-width ratio	Cuticle thickness (μm)
Unfed	4	0.64	0.50	–
Partially engorged	7	0.65	0.48	105
Fully engorged	12	0.76	0.82	50

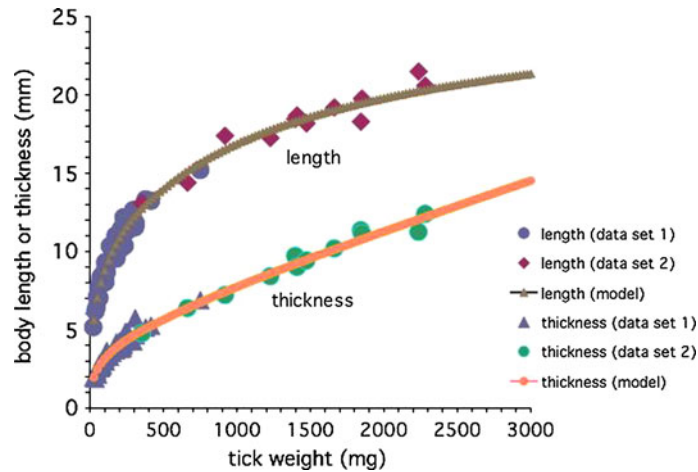


Fig. 4 Comparison of measured and model values for female *Amblyomma hebraeum* body length and thickness as a function of tick weight. Measured values are drawn from two sets of female *A. hebraeum* as described in “Materials and methods”

Figure 5 shows the calculated weight of tick cuticle from the dimensional model and the wet and dry weight of the dorsal segment of cuticle from dissected ticks. The average unfed weight of adult female unfed *A. hebraeum* ticks from a previous study (Kaufman et al. 2010) was 29.8 ± 6.4 (SD) mg ($n = 102$), so the transition from the slow to rapid phase of engorgement is in the range of 300–375 mg of body weight, as shown in Fig. 5. Measured mass of dorsal cuticle shows significant scatter, similar to the results of Lees (1952) and Andersen and Roepstorff (2005).

From both the model calculation of alloscutal cuticle wet weight and direct measurements of dorsal alloscutal cuticle wet and dry weights we observed that tick cuticle growth continues well into the rapid phase of engorgement. In *A. hebraeum* synthesis of tick cuticle appears to cease at a body weight of about 1,800 mg (a weight ratio of approximately 60). The physiological triggers and signaling mechanisms that trigger cuticle synthesis and subsequent shutdown are unknown.

From 375 mg body weight (the approximate upper range of average weight for the onset of rapid engorgement) to the end of rapid engorgement (at a tick weight of $\sim 2,800$ – $3,000$ mg), the cuticle weight predicted by the model increases by 46% (Fig. 5); of the total mass of cuticle at repletion, 32% has been formed during rapid engorgement. At 354 mg, the measured weight of dorsal alloscutal cuticle was 21.8 mg (wet) and 16.1 mg (dry). Average values for ticks of 1800 + mg increase by 75% (wet) and 58% (dry), equivalent to 37–43% of the total final mass of cuticle having been formed during the rapid phase of engorgement.

It is necessary to postulate continued growth of cuticle into the rapid phase of engorgement because the tick does not have enough material in its cuticle at the onset of this phase to equate to the amount of cuticle it has at the end of rapid engorgement. An inspection of dry versus wet weight of cuticle in Fig. 5 confirms that the observed tick cuticle weight increase during rapid engorgement cannot come simply from absorption of water, a result consistent with previously reported results indicating that the water content of *A. hebraeum* cuticle increases sharply at the onset of feeding (from $\sim 23\%$ for unfed

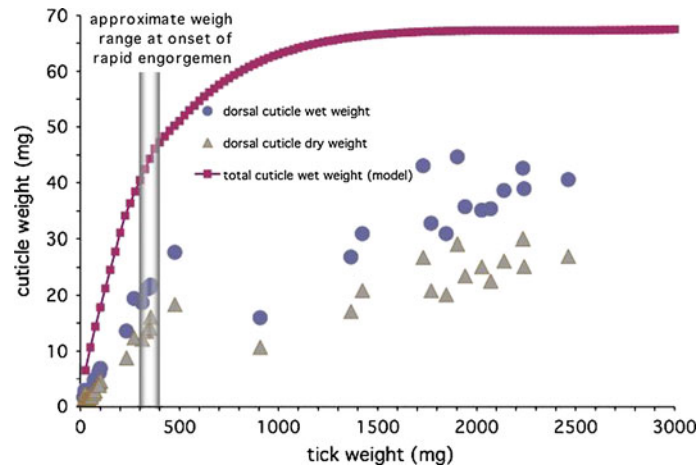


Fig. 5 Model calculation of total cuticle weight of female *Amblyomma hebraeum* as a function of tick weight, and measured wet and dry weights for dorsal cuticle samples

ticks to $\sim 34\%$ for ticks at a weight ratio of 3, and remains constant thereafter (Kaufman et al. 2010).

An analysis of Lees' (1952) data for cuticle thickness

Here we must point out a number of uncertainties regarding Lees' technique for obtaining cuticle samples. First, his method for cleaning the cuticle of soft tissue was: "...[ticks] were cut open and the body contents were washed out in a stream of water. The cuticles were then thoroughly dried in an oven over concentrated sulphuric acid" (Lees 1952, p. 763). We cannot imagine that such a procedure alone would remove all soft tissue, especially most of the skeletal muscle. Secondly, he does not mention removing sclerotized cuticle from his samples, such as appendages, the capitulum and scutum. We suspect that these two uncertainties would introduce a significant error in his absolute measurements. Finally, he mentions separating the inner and outer endocuticle layers from each other (but with no further detail as to how this was done), and measuring their dimensions separately. We cannot imagine how this can be done for a whole tick carcass. These points should be kept in mind when evaluating his data.

Lees (1952) reports measurements of *I. ricinus* cuticle dry weight; results for fully engorged ticks show a wide range from about 7.2–12.5 mg. He concluded from a pattern of cuticle weights over the engorgement cycle that no new cuticle was being formed during the rapid stage of engorgement. However, we were able to do a dimensional analysis of the changes in *I. ricinus* between the start and end of rapid engorgement based on reported thickness-to-width ratio and cuticle thickness data from Lees (1952), and width-to-length ratio and tick length data from Dillinger and Kesel (2002). Using the same calculation approach as above for *A. hebraeum* that assumes constant cuticle density, but with estimated dimensions from the two studies for *I. ricinus*, we calculate an increase in tick mass of 12.4 times during rapid engorgement. An increase in the mass of cuticle of 113% is required to achieve a 50 μm thick cuticle at the end of rapid engorgement, equivalent to 53% of the total final mass of cuticle having been formed during the rapid phase of engorgement.

Table 3 Calculated body and cuticle weight for adult female *Ixodes ricinus* calculated from the data presented in Table 2

Feeding stage	Length (mm)	Volume (mm ³)	Density (kg/m ³)	Weight (mg)	Surface area (mm ²)	Cuticle weight (mg)	% increase in cuticle weight from partially engorged (%)
Partially engorged	7	36.1	1090	39.4	63.6	6.74	–
Fully engorged	12	432	1130	488	285	14.4	113
Modified fully engorged ^a	11.2	348	1130	394	246	12.4	84

^a In the modified full engorged calculation, measured tick dimensions were reduced so that fully engorged calculated weight is ten times (not 12.4 times) that at pre-engorgement

The 12.4-fold calculated increase in body mass is higher than that reported by Lees (1952), so we also calculate the increase in cuticle mass for a ten-fold increase in body mass during rapid engorgement, by reducing the value of ultimate length of *I. ricinus* in the calculation procedure. Results for this adjusted case are also shown in Table 3 (“modified fully engorged”); we calculate an 84% required increase in cuticle mass for this case, equivalent to 46% of the total final mass of cuticle having been formed during the rapid phase of engorgement.

The dimensional measurements for *I. ricinus* are necessarily approximate and are drawn from two distinct sources. However, no reasonable assumption of dimensional error can support the conclusion that no new cuticle is formed during the rapid phase of engorgement. The calculated values for increase in *I. ricinus* cuticle mass are, like the results for *A. hebraeum*, in conflict with Lees’ conclusion from cuticle weight measurements that cuticle growth stops prior to rapid engorgement.

The data of Andersen and Roepstorff (2005)

Anderson and Roepstorff (2005) present a plot of actual measured cuticle weights as a function of tick body weight, and add their estimated “best fit” line for the data. Two concerns arise from their best fit line. First, the line is inconsistent with their conclusion of no cuticle growth in the rapid phase of engorgement, because it plateaus above 70 mg. of body weight, a point where *I. ricinus* is well into the rapid engorgement phase. Second, the line ignores the general trend of cuticle weight to increase past the point at which they suggest a plateau. Figure 6 reproduces the original Fig. 5 from Anderson and Roepstorff (2005), with two lines added by us. The lower dashed line is consistent with Lees’ original hypothesis and shows the plateau level required if cuticle growth truly stopped at the onset of rapid engorgement. Clearly, this line does not fit the data. The upper dashed line is consistent with the findings of this study for *A. hebraeum* that cuticle growth plateaus well into the rapid phase of engorgement, which we believe better fits the observed data. However, whether one uses the original line drawn by Andersen and Roepstorff or our upper dashed line, we believe that Andersen and Roepstorff’s (2005) data do not support Lees’ (1952) hypothesis, and instead are consistent with our own conclusion that ixodid ticks grow cuticle during the rapid phase of engorgement.

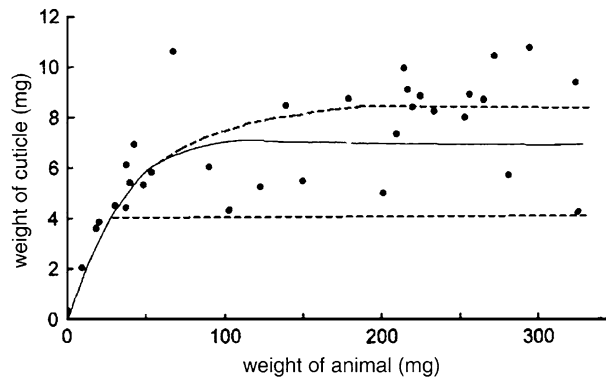


Fig. 6 Anderson and Roepstorff's (2005) plot (reprinted with permission from Elsevier) of cuticle weight versus tick weight (Fig. 5 in their paper), showing their estimated best fit line (solid line). The dashed lines are added by us. The lower dashed line is consistent with Lees' (1952) conclusion that the synthesis of cuticle stops at the onset of rapid engorgement, which occurs at a tick weight of about 30 mg. The upper dashed line and the line of Andersen and Roepstorff are consistent with the conclusion of this study that cuticle synthesis continues into the rapid phase of engorgement. The upper dashed curve appears to be the best fit for the observed cuticle weights and shows cuticle growth up to about 60% of final engorged body weight

The data of Cherry (1973) and Kitaoka and Yajima (1958)

Both Cherry (1973) and Kitaoka and Yajima (1958) report cuticle weight as a function of body weight for *Rhipicephalus (Boophilus) microplus*. The results of both are plotted in Fig. 2 of Cherry (1973); both show cuticle growth continuing well into the rapid phase of engorgement. The plot of cuticle weight versus body weight only begins to taper at a fed to unfed ratio of about 10 (Cherry 1973) to 45 (Kitaoka and Yajima 1958). The data of Cherry (1973) are consistent with the growth pattern predicted by the model in this work and illustrated in Fig. 5.

Is the isochoric assumption valid?

One key element of the dimensional model in this study is the assumption that the density of cuticle is not significantly altered during the growth of the tick. As noted above, this is equivalent to an assumption that tick cuticle is isochoric (that a given mass of cuticle maintains constant volume when deformed) and incompressible under pressure. If tick cuticle density decreased sufficiently while being stretched during the rapid phase of engorgement, for example by becoming porous, then Lees' (1952) hypothesis might be reconciled with the requirement for additional volume of cuticle (that emerges from the dimensional analysis) without the formation of additional cuticle mass.

The assumption that because of their high water content biological materials are incompressible and isochoric is common. For example, Lin et al. (2009) studied the mechanical properties of the cuticle of larval *Manduca sexta* (the tobacco hornworm), ultimately in order to better understand the mechanics of caterpillar crawling. They employed an isochoric assumption in developing a model of soft cuticle mechanical properties, citing experimental work by Humphrey (2002) on arteries and Franceschini et al. (2006) on brain tissue.

A second approach is to ask what reduction in density would be required to reconcile Lees' (1952) conclusion with the dimensional analysis. For *A. hebraeum* cuticle density would have to drop to 690 kg/m^3 for the volume of cuticle post engorgement to be consistent with the mass of cuticle at the onset of rapid engorgement. For the data reported in Lees (1952) for *I. ricinus*, the density of cuticle post engorgement would have to drop to 474 kg/m^3 . These values are clearly unreasonable: cuticle from unfed, partially engorged and fully engorged ticks all have a density greater than $1,000 \text{ kg/m}^3$, as is readily demonstrated by their sinking in water (our unpublished observations on pieces of cuticle from *A.hebraeum*).

An ellipsoidal model of *A. hebraeum* and *I. ricinus* is not an exact geometrical fit to the actual shape of the animal, and there may indeed be very small changes in the density of tick cuticle when stretched. However, the error from these approximations is not sufficiently large to negate the key observation of this study that cuticle growth continues in at least two ixodid tick species into the rapid phase of engorgement, and does not appear to cease until well into that phase of feeding.

For three ixodid ticks, *A. hebraeum*, *I. ricinus* and *R. microplus*, there is direct evidence that cuticle growth occurs in the rapid phase of engorgement. We believe this is likely true for ixodid ticks in general, since all undergo major body expansion, typically tenfold or more, during a rapid phase of engorgement. The challenge of this pattern of feeding is to have sufficient cuticle at the end of feeding to contain the expanded body. In the three species for which data are available this requires the addition of cuticle during rapid engorgement, which is likely characteristic of the family Ixodidae. This suggests that any tick control measure aimed at interference with cuticular growth could be effective well into the rapid phase of engorgement.

Conclusion

Lees' (1952) thesis that ixodid ticks grow cuticle only during the slow phase of engorgement and shut off cuticle growth at the onset of rapid engorgement is inconsistent with measurements of tick body and cuticle dimensions and with measured values of dorsal cuticle weight. *A. hebraeum* appears to synthesize cuticle during the first half of the rapid engorgement phase, shutting down cuticle growth at a weight ratio of about 60. This conclusion is also consistent with a dimensional analysis of Lees' (1952) data on cuticle thickness for *I. ricinus*, with a re-examination of the data on cuticle weight of Andersen and Roepstorff (2005), and with the findings of Cherry (1973) and Kitaoka and Yajima (1958) for *R. microplus*. It is likely characteristic of Ixodidae in general because of the shared characteristic of a rapid phase of engorgement producing tenfold or greater increases in body weight.

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