



Cuticle expansion during feeding in the tick *Amblyomma hebraeum* (Acari: Ixodidae): The role of hydrostatic pressure



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ABSTRACT

Female *Amblyomma hebraeum* ticks (Acari: Ixodidae) increase their weight ~10-fold during a 'slow phase of engorgement' (7–9 days), and a further 10-fold during the 'rapid phase' (12–24 h). During the rapid phase, the cuticle thins by half, with a plastic (permanent) deformation of greater than 40% in two orthogonal directions. A stress of 2.5 MPa or higher is required to achieve this degree of deformation (Flynn and Kaufman, 2015). Using a dimensional analysis of the tick body and applying the Laplace equation, we calculated that the tick must achieve high internal hydrostatic pressures in order to engorge fully: greater than 55 kPa at a fed:unfed mass ratio of ~20:1, when cuticle thinning commences (Flynn and Kaufman, 2011). In this study we used a telemetric pressure transducer system to measure the internal hydrostatic pressure of ticks during feeding. Sustained periods of irregular high frequency (>20 Hz) pulsatile bursts of high pressure (>55 kPa) were observed in two ticks: they had been cannulated just prior to the rapid phase of engorgement, and given access to a host rabbit for completion of the feeding cycle. The pattern of periods of high pressure generation varied over the feeding cycle and between the two specimens. We believe that these pressures exceed those reported so far for any other animal.

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1. Introduction

The blood meal of female ticks (Acari: Ixodidae) is conventionally divided into three phases. In a preparatory phase, the tick cements itself to the skin and prepares the feeding lesion. The slow phase of engorgement lasts about 6–7 days, during which the fed: unfed mass ratio (hereafter called "mass ratio") reaches about 10; this is followed by the rapid phase (about 12–24 h) during which the mass ratio can approach 100 (Kaufman, 2007).

The mass of cuticle available to the unfed female is insufficient to contain the body volume at the end of engorgement. In order to meet this demand, the endocuticle increases about 40% in thickness during the slow feeding phase. Although the tick grows some additional endocuticle during the rapid feeding phase, because of rapid expansion at this time, the cuticle thins by about 50% (Flynn and Kaufman, 2011). Given that the width-to-length ratio of the tick remains constant (0.77) throughout the feeding cycle, this requires that the cuticle plastically (permanently) deform by more than 40% in each of two orthogonal directions (Flynn and Kaufman, 2011, 2015.) An analysis of deformation vs. stress

indicates that a stress level of 2.5 MPa must be reached in the cuticle before deformation of 40% or more can be achieved, and suggests that the neurotransmitter dopamine (DA) plays an enabling role, likely through a modification of cuticular pH (Flynn and Kaufman, 2015).

The Laplace equation relates internal pressure in a vessel to the stress in the wall. For a cylindrical shape, the pressure required to induce a given circumferential (hoop) stress is given by:

$$P = \sigma * t/r$$

where P is pressure (Pa), σ is circumferential wall stress (Pa), t is thickness (m), and r is the radius (m) (Vogel, 2003). For a spherical vessel, the pressure required to achieve a given wall stress level is twice that for circumferential stress in a cylinder.

We have previously modeled the tick as an ellipsoid (Flynn and Kaufman, 2011); the pressure/stretch relationship for such a shape would lie between that of a cylinder and a sphere. Fig. 1 shows the required pressure to achieve a wall stress of 2.5 MPa for a cylinder and sphere, based on dimensions for a feeding female *A. hebraeum* in the mass ratio of 20–100 (Flynn and Kaufman, 2011). Predicted pressures, in the range of 55–110 kPa at a mass ratio of 20, are well above those observed in mammalian circulatory systems, and in a previous study of the internal hydrostatic pressure of an argasid

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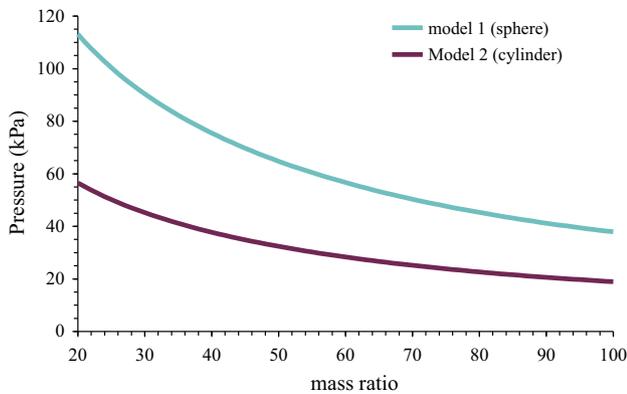


Fig. 1. Pressure (Y-axis) required to achieve a wall stress of 2.5 MPa in a cylinder and a sphere for ticks the size of *A. hebraeum*, as a function of mass ratio. A plot for the approximate ellipsoid shape of the tick would fall somewhere between these two curves.

tick during feeding (Kaufman et al., 1982). To confirm whether such high pressures could be generated within the feeding ixodid tick, we cannulated ticks and connected them to a telemetric pressure transducer, as described in Section 2.

2. Materials and methods

The tick colony was maintained in darkness at 26 °C at a RH exceeding 95%. For feeding ticks, a cloth-covered foam arena (~12 cm × 8 cm × 2.5 cm) was glued to the shaven back of a rabbit with a latex adhesive. Prior to cannulation, ticks were partially-fed to about 10× the unfed mass (about 6 days) on laboratory rabbits, as described by Kaufman and Phillips (1973). The use of rabbits was approved by the Biosciences Animal Policy and Welfare Committee, University of Alberta, which functions under guidelines established by the Canadian Council on Animal Care.

2.1. Cannulation procedure (Fig. 2)

For measuring internal hydrostatic pressure, partially fed ticks were removed from the host and cannulated with either

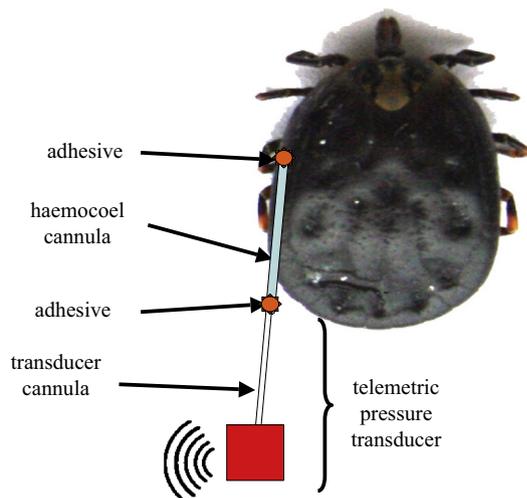


Fig. 2. The procedure for cannulating the *A. hebraeum* ticks to the telemetric pressure transducer. The tick shown is at the approximate mass ratio (~10) at which all the ticks were cannulated. The tip of the haemocoel cannula lodges approximately 5 mm beyond the point of penetrating the cuticle. The adhesive forms a seal to prevent leakage of haemolymph and to withstand the pressure generated. See Section 2 for further details.

microrenathane MRE-40 tubing (Braintree Scientific Inc., Braintree MA, USA) or polyvinyl V3/A tubing (Scientific Commodities Inc., Lake Havasu City, AZ, USA); the cannula had been pre-filled with a solution of 8 mg polyvinylpyrrolidone/10 ml heparinized saline. To insert the cannula, a puncture wound through the cuticle was made with a 20 g hypodermic needle at the lateral margin of the opisthosoma in the vicinity of the spiracle. The tip of the cannula was inserted through the puncture wound (the fit was snug) approximately 5 mm into the haemocoel, and was sealed in place with Silastic Medical Adhesive Silicone (Dow Corning, Midland, Michigan, USA). The end of the cannula distal to the tick was connected to a PA C-10 or C-40 telemetric pressure transducer (Data Sciences International, St. Paul, MN, USA). After allowing the adhesive to set for a few hours, the cannulated ticks were returned to the host rabbit, and the transducer was lightly taped to the rabbit's skin to inhibit it from rolling around as the rabbit moved. For pressure measurements, the rabbit was placed in a small pen (about 100 cm × 70 cm) made of radio-transparent walls. Pressure signals from the transducer were monitored using the PhysioTel system (Receiver model RPC-1) and recorded on a laptop computer using LabChart version 7.2.5 software (AD Instruments, Colorado Springs, CO, USA). Because of the limited transmission range of the transducer, two or three receivers were mounted on the walls of the pen so as to optimize signal reception. The maximum amplitude that could be recorded by the telemetric pressure transducer was ~57 kPa. MathWorks Matlab Fast Fourier Transform software was used to analyze the pressure trace of one of the ticks (Ah 266).

3. Results

For this study we cannulated 16 female *Amblyomma hebraeum* ticks. Two of the 16 never re-attached to the host. Three died on the host before meaningful data could be recorded. One was deemed invalid because of technical problems with the recording system that day. Three were ticks that had been killed by freezing and then thawed before cannulation; these ticks were to serve as a control to distinguish pressure generated by the tick as opposed to pressure generated on the transducer by movements of the host rabbit. In three cases the cannula disengaged from the tick at some point during the run. Four ticks did feed to engorgement. However, in two of these four (Ah 208 and 211), the cannula was probably blocked, perhaps because the cannula damaged the delicate gut wall causing a release of gut contents; the recording showed very little spontaneous activity, and when the tick was squeezed at the end of the run there was very little or no response by the transducer. Hence valid pressure recordings during rapid engorgement are available from two ticks: Ah 254 and 266. Note that the time of reattachment of the tick to the rabbit was uncertain for both ticks, so the exact relation of the time of pressure recording to the feeding cycle cannot be determined. The pressure traces can be thought of as a window into an interval of a longer process.

The control ticks showed infrequent small pressure “blips” that are likely related to the movement of the rabbit; one instance of 25 kPa was observed, all others were less than 12 kPa, and no repeated period of pressure generation was observed; i.e. the pressure blips were isolated events.

Pressure readings for Ah 266 were recorded at 1000 Hz, for 21.5 h. Repeated periods of high frequency high pressure (HFHP) pulses were observed; Fig. 3 shows three traces of pressure vs. time over a one second period. Note that 57.5 kPa was the maximum observable pressure from the experimental setup; it is likely from various instances of flat-lining of the peaks that internal pressure in the tick exceeded this value. The negative pressure values may reflect recoil of fluid in the tubing between the tick and the pressure transducer. Here we define a ‘high pressure spike’ as any pulse

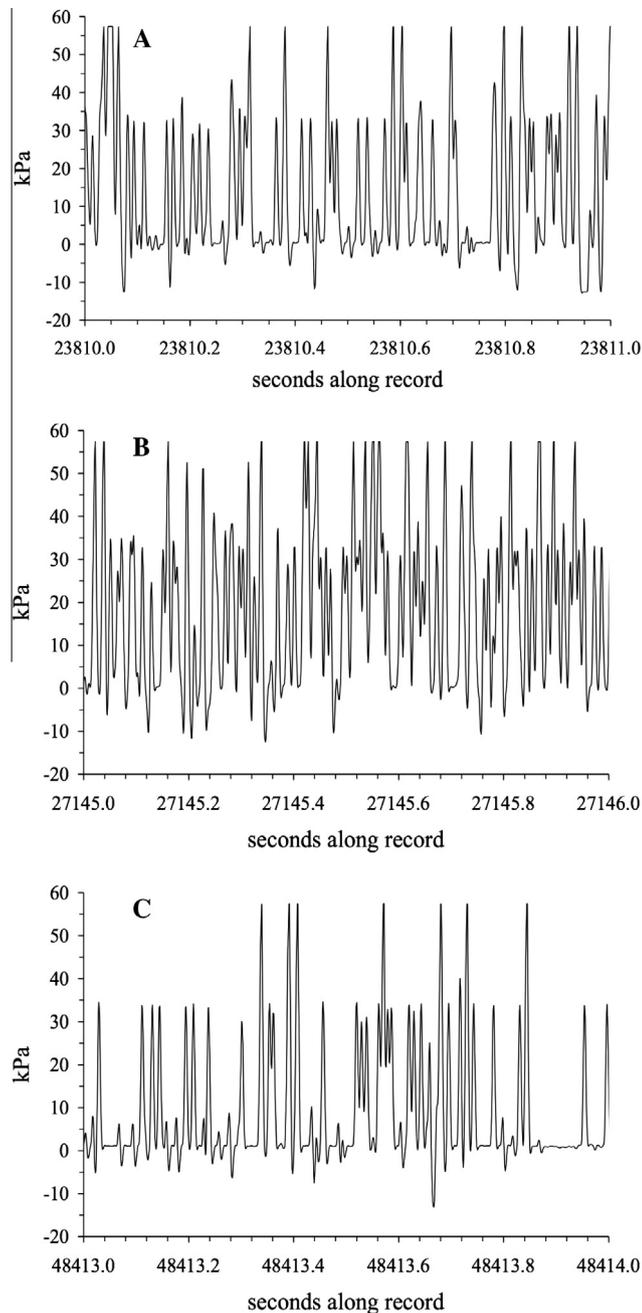


Fig. 3. Three 1-s fragments of pulsatile activity from the run for an *A. hebraeum* tick (Ah 266) recorded at 1000 Hz. (A) Record taken at time 6:56:51 (h:min:s); 49 spikes > 25 kPa. (B) Record taken at time 7:32:25; 72 spikes > 25 kPa. (C) Record taken at time 13:26:53; 35 spikes > 25 kPa.

over 25 kPa. We then analyzed 19 one-second intervals from Ah 266 taken from HFHP periods. The average high pressure spike frequency was 53 ± 4 Hz (range = 30–80 Hz).

Fig. 4 shows a 16-s pressure trace and its Fourier transform (FT) for a period of HFHP from Ah 266; the plot is representative of other segments. The spikes at ~ 60 and 120 Hz were likely noise from the AC electrical supply. With the exception of these two spikes, the FT shows no other predominant frequency: high pressure pulses were frequent but not regularly spaced, as is also evident from Fig. 3. The FT plot also suggests that no activity could be associated with a frequency greater than 200 Hz, and hence our recording at 1000 Hz conveys a full picture of the pressure behavior in the tick.

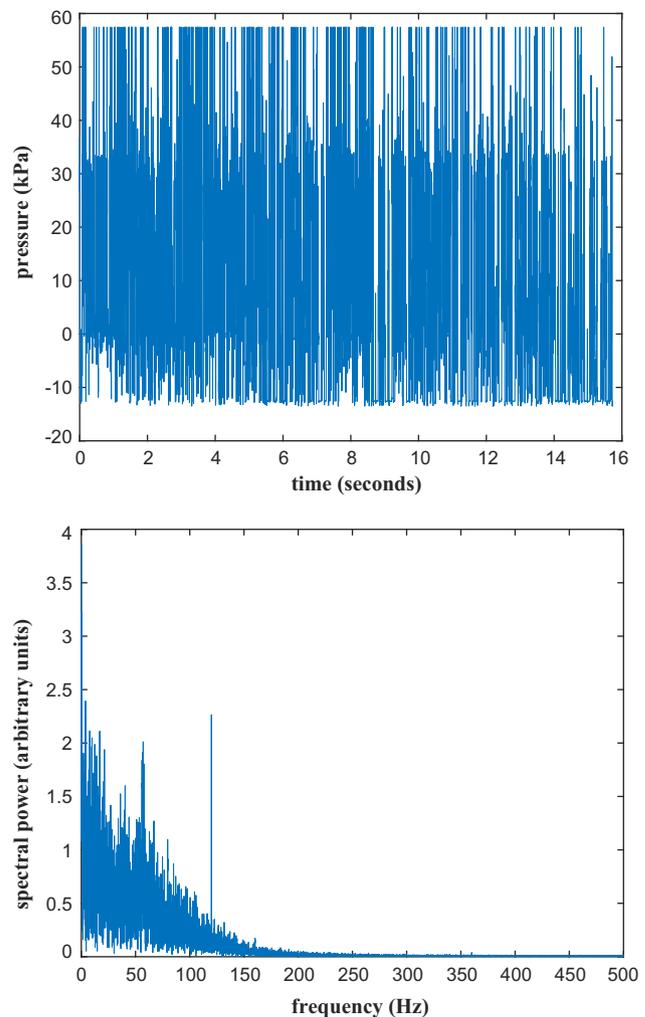


Fig. 4. A 16-s pressure trace and its FT for a period of HFHP from an *A. hebraeum* tick (Ah 266) sampled at 1000 Hz. The transform measures spectral power in arbitrary units, i.e. the contribution to the observed pressure trace attributed to a given frequency. Peaks at 60 and 120 Hz are likely related to noise from the 60 Hz electrical supply. Other than these peaks, no dominant frequencies are observed; pulsations are irregular. No significant contribution above 200 Hz was observed.

Pressure readings for Ah 254 (recorded before those of Ah 266) were recorded at only 4 Hz, for 18.25 h; given the high frequency of pulsation observed subsequently in Ah 266, we cannot use Ah 254 data to measure the frequency of high pressure spikes. However, data from both ticks can be analyzed for the duration of HFHP events and the fraction of time that HFHP occurs. Again, we observe an irregular pattern of generation of HFHP events. Pressure traces for 65,000 s were analyzed for each tick, with each time interval classified either as (a) HFHP (generating repeated pressure pulses greater than 25 kPa), (b) low frequency and/or low pressure, (c) rest periods or (d) out of range (no signal detected, for which the most likely cause was that the rabbit had moved to a point where the transmitter could not connect to any of the receivers). For our analyses we included only the times that the tick was in range of the receivers. Fig. 5 shows the wide range of duration of HFHP events. Fig. 6 shows the fraction of cumulative time of HFHP associated with each range of duration of pulses.

Finally, we observe that there was no consistent pattern between the two ticks. Fig. 7 shows the fraction of observed time in each of thirteen 5000 s intervals in which the tick was generating HFHP pulses. There is a clear difference: Ah 266 generated virtually continuous HFHP for 13,000 s, whereas Ah 254 showed a pattern of pulses interspersed with rest periods.

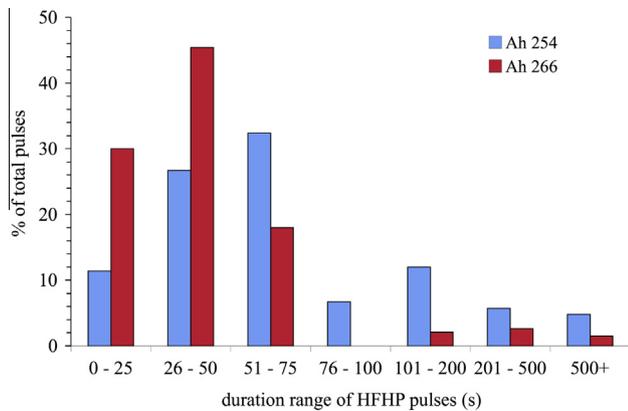


Fig. 5. Pressure events throughout each run (*A. hebraeum* ticks Ah 254 and Ah 266) were characterized by trains of HFHP pulses of varying duration.

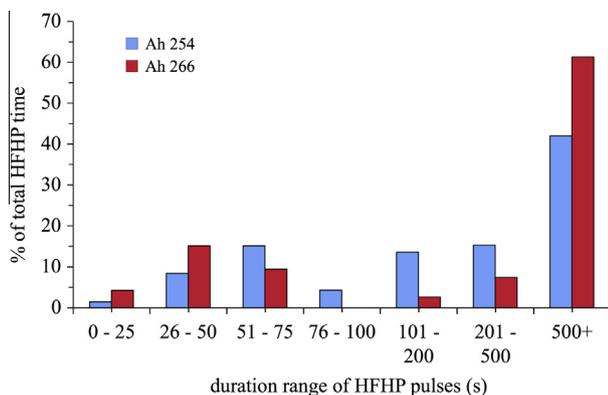


Fig. 6. The fraction of cumulative time of HFHP events associated with each range of duration of pulses in *A. hebraeum* ticks Ah 254 and Ah 266. Although longer duration pulses were less frequent (Fig. 5) they contribute the majority of cumulative time of exposure of the cuticle to high pressure.

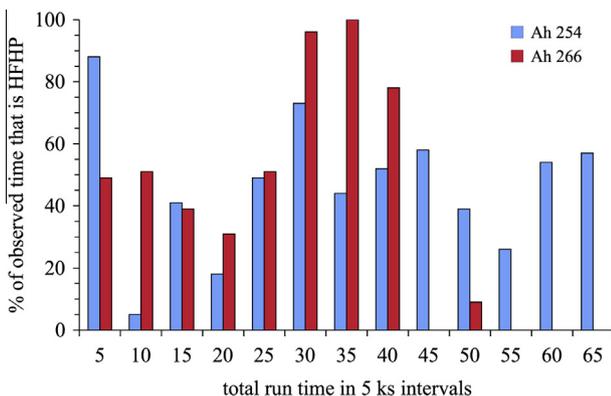


Fig. 7. Fraction of observed time, in 5 ks intervals, when *A. hebraeum* ticks (Ah 254 and Ah 266) were generating HFHP pulses. Ah 266 generated a continuous period of HFHP pulses in the period from 25.1 to 38.9 ks. Observed time excludes periods when the transducer and receivers were not in range of each other.

4. Discussion

Two striking observations emerge from this study. First, the engorging ticks generated high internal hydrostatic pressure (>55 kPa), much higher than values observed in other terrestrial arthropods. Second, instead of generating this pressure by tetanic contractions, as we anticipated, Ah 266 did so by means of pulsatile activity of very high frequency (30–80 Hz). The pattern

of pulsation was irregular at a micro scale (~1 s; Fig. 3); there was no steady pattern to the high frequency pulsation. The pattern was also irregular at a macro scale (~3600 s, Figs. 5–7); the ticks turned on and off HFHP with no uniformity in frequency, duration of pulsation, or duration of rest. Moreover, the pattern of HFHP and rest periods displayed by the two ticks differed substantially from each other.

Pressure values are available from one other study of ticks, and many of insects. Kaufman et al. (1982) measured haemolymph pressure in the argasid tick, *Ornithodoros moubata*, while it was excreting coxal fluid (the excess fluid of the blood meal). Coxal fluid excretion in *O. moubata* occurs by a filtration/resorption mechanism, and the haemolymph hydrostatic pressure approaches 14 kPa. This value is well within the range for those animals, both vertebrates and invertebrates, which produce urine by a filtration/resorption mechanism (Hill et al., 2008). In insects, haemolymph pressure has been measured in processes during ecdysis, including emergence and wing extension (reviewed by Reynolds, 1980, and Mullins, 1985); here we cite only the higher measured pressures. Bernays (1972) measured 8 kPa at the time of splitting of the exoskeleton during the first larval ecdysis of *Schistocerca gregaria*. Cottrell (1962) observed a maximum hydrostatic pressure of 16 kPa during the digging and expansion of newly eclosed blowflies (*Sarcophaga barbata*). Moreau (1974) measured 7 kPa during the wing extension of the moth *Bombyx mori*. All of these values are well below the mean pressure we record here for *A. hebraeum*. Reynolds (1980) notes, however, that localized pressure arising from peristaltic movement is thought to exceed the average haemolymph pressure. For *S. gregaria* the embryonic cuticle remained unsplit at 23 kPa, suggesting a role for localized pressure.

In mammals, the typical range of systolic blood pressure is 15–20 kPa (Hill et al., 2008). Only in the giraffe, an animal that has been studied because of the extreme variations in intravascular pressures encountered during postural changes, do the pressures approach the tick values reported here; observed values for the giraffe are in the range of 25–40 kPa (Goetz et al., 1960; Petersen et al., 2013), and the maximal value recorded is about 55 kPa (Hargens et al., 1987). Even in a very large arboreal snake such as the Burmese python, where blood pressure at the level of the heart is typically close to 13 kPa (Lillywhite, 1996), one would still not anticipate that hydrostatic pressure in the tail would exceed the levels observed in the current study. (Although lower extremity pressure has not been reported in these animals, it may be estimated from the vertical distance between the heart and the tail to be at most ~50 kPa.) The ostrich is the only other animal in which high pedal pressures might exist. Although there are no published studies on ostrich pedal pressure, one may again estimate that, standing as they do at about 2.7 m head height, hydrostatic pressure in the feet would probably only be in the range of that observed in humans.

The frequency of pulsation observed in Ah 266 is also high relative to values observed for other terrestrial arthropods. A pressure trace from *O. moubata* during excretion of coxal fluid (Kaufman et al., 1982) shows a pulse frequency of less than 1 Hz. Zheng et al. (2015) measured a pharyngeal frequency of 6–7 Hz in the feeding tick *Ornithodoros turicata*. Observed insect ecdysial pressure fluctuations occur at less than 1 Hz (Cottrell, 1962; Moreau, 1974). Pharyngeal pump frequency during feeding is less than 10 Hz in the bedbug *Cimex lectularius* (Araujo et al., 2009), about 7 Hz in *Rhodnius prolixus* (Smith, 1979), and 4 Hz in the mosquito *Aedes togoi* (Kim et al., 2011).

The question arises: is pulsatile pressure a more effective way to stretch out the cuticle compared to tetanic contraction? Viscoelastic behavior in materials conforms to the Boltzman superposition principle: the effects of the mechanical loading of a material are linearly additive (Ferry, 1980). The major component of defor-

mation of the cuticle of *A. hebraeum* is plastic (permanent deformation; Flynn and Kaufman, 2015), and hence the largest portion of the deformation arising from the individual pulses can be expected to be additive. An analysis of stretch/recoil data from a previous study showed that in the first second after stress is released from the cuticle of *A. hebraeum*, recoil was only $3.0 \pm 0.3\%$ (Flynn and Kaufman, 2015). Hence, at the molecular level, repeated deformation will not be “unwound” between pulses, and can be effective in thinning cuticle. High frequency pulsation may have the advantage of maintaining respiration by enabling air flow through spiracles at a time of high energy usage for muscular contraction to generate the pulses, whereas tetanic contraction could potentially compress the air spaces in large tracheae and thus hinder gas exchange.

Which muscles in the tick might be capable of producing pressures in the range of 60 kPa, and frequencies in the range of 30–80 Hz? It seems unlikely that the pharyngeal muscles used to suck in blood could produce such high pressures and contract at such high frequencies, given the tick and insect data cited above.

Ixodid ticks have a series of prominent dorso-ventral muscles (Sonenshine, 1991; Sonenshine and Roe, 2014). These seem to be the only muscles that might produce such high pressures as we observe here. The informal terms ‘soft ticks’ (*Argasidae*) and ‘hard ticks’ (*Ixodidae*) refer to the condition of the cuticle. Ixodid cuticle appears generally stiffer than argasid cuticle, even during the later period of feeding. It seems reasonable to suppose that the mechanical resistance of the stiffer cuticle of ixodids would enable higher hydrostatic pressures to be achieved in ixodids than in argasids.

The fact that the pulsatile frequency we observed here was so high (tens of Hz) raises the question: might the dorso-ventral muscles be in any way homologous to insect flight muscle, which also contracts at high frequency (Gillott, 2005). Although there seems to be some similarity between the tick’s dorso-ventral muscles and insect flight muscles, it is difficult to know whether they are homologous to each other; during embryological development of the tick (a chelicerate arthropod), there is considerable fusion of the ancestral arthropod segments that pertain to insects (a mandibulate arthropod). However, one can speculate that, in the latter, the ancestral thoracic wall muscles were adapted for strong contractions that either connected directly to the wings or deformed the body wall, in either case causing the wings to flap. The dorso-ventral muscles in ticks pulsate in a similar way. Possible functions of the dorso-ventral muscles other than that suggested here include generating hydrostatic pressure changes that compress and expand the midgut diverticulae, which enhances rapid dissemination of the incoming fluids during feeding (D.E. Sonenshine, personal communication, October 2015).

Author contribution

W.R. Kaufman oversaw all procedures with ticks, including initial feeding, cannulation, re-establishment of feeding, and recording of pressure data. He performed the analysis of pressure traces. S. Kaufman used extensive past experience with pressure recording from argasid ticks and mammals to supply equipment and develop the procedures for recording the pressure traces observed here. P.C. Flynn applied the Laplace analysis and material properties to predict the high pressures and performed data analysis on the outcomes to confirm the irregularity of pressure generation in the tick. All authors contributed to the completion of the manuscript.

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