Experimental and Applied Acarology
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Flynn et al. A comparison of the cuticular properties of Argasid and Ixodid ticks: *Ornithodoros moubata* (Argasidae) vs. *Amblyomma hebraeum* and *Ixodes pacificus* (Ixodidae)

Methodology

Sources:

The methodology used in this study was used in two previous studies. In addition to the discussion of methodology in the manuscript, we draw on comments from three previous publications:

Kaufman, W.R., Flynn, P.C., and Reynolds, S.E. (2010). Cuticular plasticization in the tick, *Amblyomma hebraeum* (Acari: Ixodidae): possible roles of monoamines and cuticular pH. J. Exp. Biol. 213: 2820-2831.

Flynn, P.C. and Kaufman, W.R. (2015). Mechanical properties of the cuticle of the tick *Amblyomma hebraeum* (Acari: Ixodidae). J. Exp. Biol. 218: 2806-2814.

Kaufman, W.R. and Flynn, P.C. (2018). A comparison of the cuticular properties of the female ticks Ixodes pacificus and Amblyomma hebraeum (Acari: Ixodidae) throughout the feeding period, Exp. Appl. Acarol. 76, 3: 365-380.

Preparation of Samples

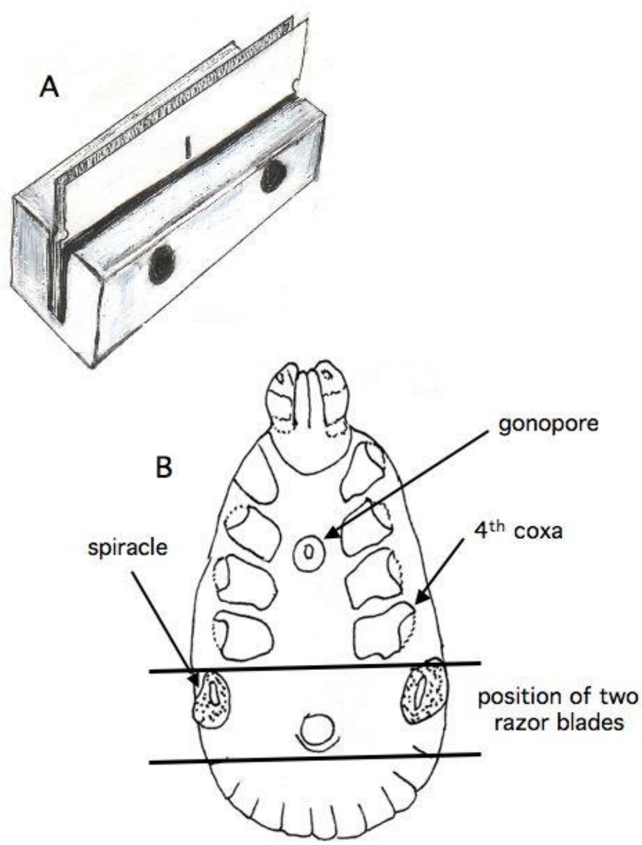
Results draw on the measurement of tick cuticle dimensions and analysis of the mechanical properties based on the analysis of time dependent stretch and recoil of a loop of cuticle. Loops are cut from ticks using a two blade device illustrated below. The cuticle was cut all the way around the lateral margin of the tick, and then the cut followed the contour between the scutum and alloscutum as shown in Fig 7. The dissected cuticle was submerged in Hank's saline and all the soft tissue on the inner surface was scraped away gently using the blunt surface of the scalpel so as not to scrape away endocuticle.

Loop length and thickness was measured with an optical microscope: To measure cuticle thickness, the tick carcass was mounted on a piece of modelling clay (Plasticine®) and placed under a dissecting microscope fitted with an ocular micrometer, so that the cut face of the cuticle was perpendicular to the line of sight. Loop length and width were measured under a dissecting microscope. The loop was flattened and the length recorded was the average of the two cut surfaces of the loop. Multiple readings of the width (dorsal and ventral surfaces) were taken from one end of the loop to the other. The average dorsal and ventral widths were recorded; for calculating the stress for each loop, the mean of the pooled dorsal and ventral readings was used. Tick overall body dimensions were measured with a Vernier caliper.

Measurement of Stretch and Recoil

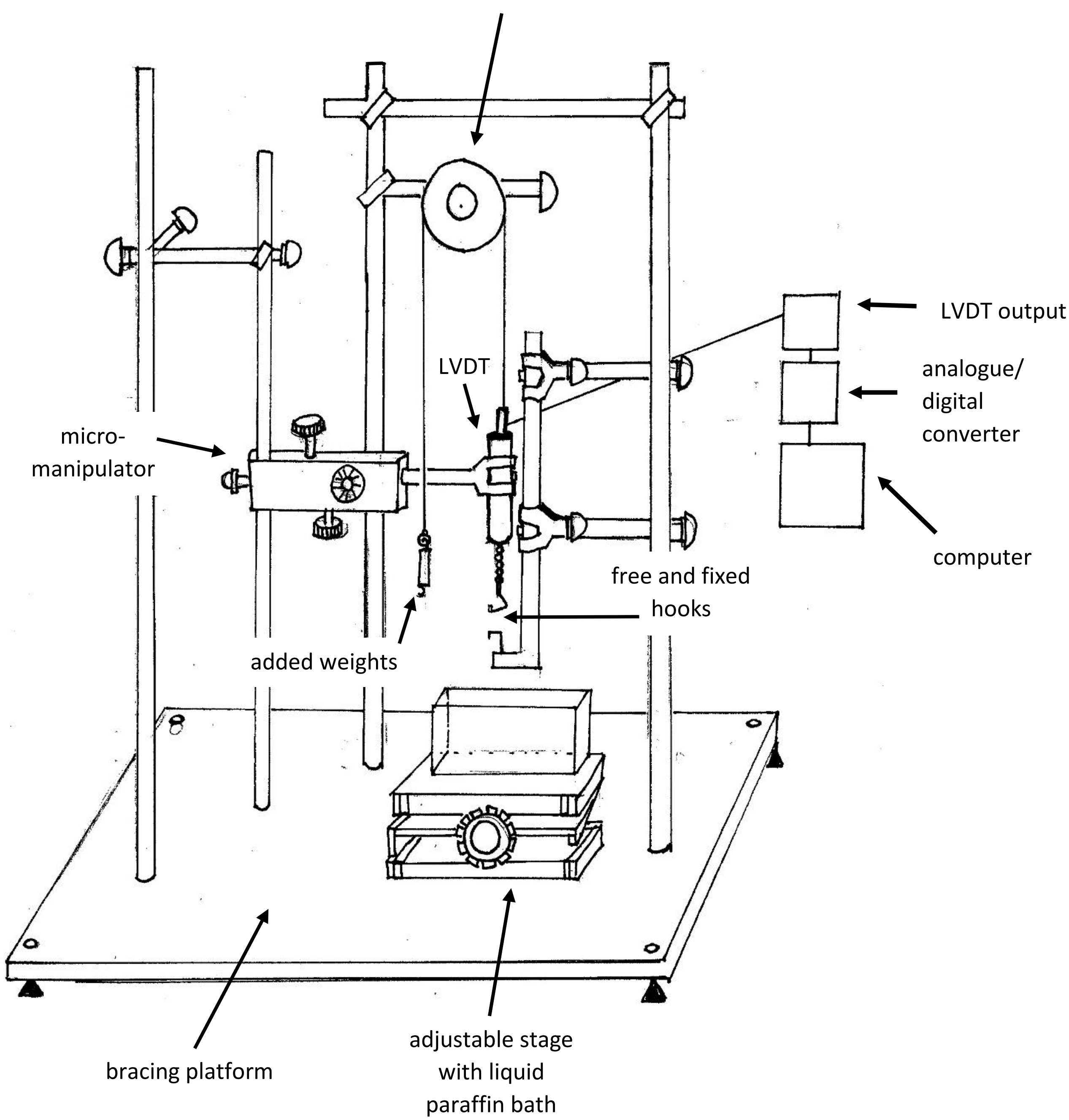
Stretch and recoil data was collected by a linear variable differential transformer (LVDT; Macro Sensors, Pennsauken, NJ,, USA); two models were used, (DC 750 125 010, DC 750 050 010) depending on the expected amount of cuticle distension. Three improvements were made to the earlier apparatus described in Kaufman et al. (2010): (a) greater rigidity in the apparatus, (b) a more accurate and linear LVDT and (c) improved data collection through an analog to digital converter (National Instruments, model USB-6008). Apparatus stretch was measured before each experiment by applying the experimental weight with the clips or hooks attached (without cuticle); measured cuticle stretch and recoil was corrected to remove the contribution of apparatus stretch and recoil. In most cases apparatus stretch was not more than ~10% of the loop stretch. All tests were conducted at room temperature. The apparatus is illustrated below.

Figure: Cutting the Cuticle Loop



Kaufman, Flynn & Reynolds fig. 1

Figure: Apparatus for Strech and Recoil



Mounting frame and instrumentation for fixed stress testing of compliance. Apparatus stretch, which is small relative to loop stretch, was first measured by loading weights with the two hooks connected; this was subtracted from the subsequent measured loop stretch. The loop was then placed across the hooks and the mineral oil bath was raised to cover the loop to prevent loss of moisture during the stretch. LVDT, linear variable differential transformer. (from Fig 8, Flynn & Kaufman 2015. J. Exptl. Biol. 218: 2806 – 2814.)

For some experiments we ran two successive stretch/recoil cycles, with two loop treatments prior to the second stretch-recoil, to determine ESp: restretch after one hour, and incubation overnight in 1.2% NaCl, pH 6.5 and a control at pH 7.2.

Analyzing Stretch and Recoil

A typical pattern of stretch and recoil of cuticle is shown in the manuscript: engineering strain, ES, is a function of time. (The compliance curve has an identical shape: engineering strain is compliance times the initial stress.) The permanent deformation (ESp) is the difference between displacement during stretch and recoil; recoverable displacement (viscoelastic engineering strain: ESv) is the recoil. ESp does not contribute to ultimate stiffness of the material (resistance to breakage), because there is no residual stress associated with that displacement; ESp does absorb energy during the stretch. Young's Modulus, a measure of the stiffness of the material, defined as stress/strain, is determined from the applied stress per unit ESv at low values of stress.

Over the course of a period of stretch, cuticle stiffness and viscosity increase. To gain insight into the material properties of the cuticle we perform two distinct analyses of the compliance curve. First, we use a five-element Kelvin-Voight model to model the compliance curve. Kelvin-Voigt elements are modeled as a spring (stiffness) and dashpot (viscosity) in parallel, with one element having zero viscosity. The properties of each KV element (modulus and viscosity) are determined by a stepwise best fit to the actual compliance curve. This analysis generates a replicate of the observed compliance, as an instantaneous deformation under load plus four exponential functions describing the subsequent time-dependent stretch. The four time-dependent elements can be thought of as a "spectrum" of the polymeric matrix that reflects the molecular processes involved in stretching, e.g. uncoiling, disentanglement and slippage. The absolute values of modulus and viscosity associated with the steps in the KV analysis are not of significance in this work; rather, the merit is in comparing interspecies and inter-treatment impact. We also calculate an instantaneous value of viscosity, which we label as Maxwell viscosity, from the inverse of the instantaneous slope of the compliance curve (estimated from a best fit linear regression over an interval of the curve). As with KV values, the merit of Maxwell viscosities at fixed points in the stretch-recoil cycle is for comparing interspecies and inter-treatment impact. The calculation methodology is described in greater detail in Flynn and Kaufman (2015). Note that some stretch and recoil continues to take place over a very long time frame.

Excel files showing the analysis of stretch and recoil are available at http://www.biology.ualberta.ca/faculty/reuben_kaufman/index.php; equations used in the analysis are embedded in the worksheets.

Results

Data was collected in a "Master File", which is available at http://www.biology.ualberta.ca/faculty/reuben_kaufman/index.php