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Original article

Salivary gland degeneration and ovary development in the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles (Acari: Ixodidae). II. Determination of the 'critical weight'

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ABSTRACT

The feeding cycle of female ixodid ticks is divided into preparatory, slow, and rapid feeding phases. When a female *Amblyomma hebraeum* is removed from the host after achieving a minimum size of about 10–13× the unfed weight, various physiological and behavioural changes occur: (a) haemolymph ecdysteroid concentration rises dramatically, (b) the tick does not reattach to the host when given the opportunity, (c) its salivary glands degenerate within about 4 days (if mated) or 8 days (if virgin), and (d) oocyte maturation and oviposition occur (Kaufman and Lomas, 1996; Invert. Repr. Devel. 30: 191–198). None of these changes occur if the tick is removed from the host at smaller sizes. This transition, which occurs when the tick enters the rapid phase of engorgement, has been named the 'critical weight'. To date, the critical weight has been determined for *A. hebraeum* only. The present study established that, in both mated and virgin *D. andersoni*, the critical weight is similar to that of *A. hebraeum*. Although a small percentage of virgin *A. hebraeum* do exceed the critical weight, achieving perhaps 20× the unfed weight, virgin *D. andersoni* regularly fed well beyond their CW (>50× the unfed weight) and occasionally engorged completely (100× the unfed weight), although they did not detach spontaneously from the host within 21 days of attachment.

Introduction

Female ixodid ticks ingest a huge blood meal (approaching 100× the unfed weight) during 3 distinct feeding phases. The feeding lesion is created during a brief preparatory phase (~24–36 h). During the slow feeding phase (anywhere between 5 and 10 days, depending on species or laboratory strain), the female feeds to about 10× its unfed weight, and during the rapid feeding phase (the final 12–24 h) the female increases its weight a further 10-fold and detaches from the host (Kaufman and Lomas, 1996). Normally, the female must be inseminated in order to achieve full engorgement. In most species, copulation occurs only on the host following at least a few days of feeding, but in the genus *Ixodes*, copulation may also occur between unfed ticks (Snow, 1969; Kiszewski et al., 2001).

Virgin *Amblyomma hebraeum* females remain attached to the host indefinitely, most of them not exceeding ~10× their unfed weight (Kaufman and Lomas, 1996). But once fed males are introduced and copulation occurs, the female feeds rapidly to engorgement. Although a minority of laboratory-reared virgin *A. hebraeum* may achieve ~20× the unfed weight, they do not engorge fully (Kaufman and Lomas, 1996).

If female *A. hebraeum* are removed from an experimental host prior to engorgement, their subsequent behaviour will depend on at least 2 factors: (i) the amount of blood they have ingested up to that point, and (ii) whether or not they have mated (Lomas and Kaufman, 1992; Weiss and Kaufman, 2001). If a virgin or a mated female *A. hebraeum* is forcibly removed from the host before it achieves ~10× its unfed weight, it will reattach and continue to feed if given the opportunity, but not if it exceeds ~10× the unfed weight (Lomas and Kaufman, 1999).

The weight at which a partially fed *A. hebraeum* removed from the host switches from the so-called 'feeding strategy' (up to ~10× the unfed weight) to an 'egg development strategy' (beyond ~10× the unfed weight) was termed the 'critical weight' (CW)

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by Harris and Kaufman (1984). The following physiological and behavioural changes occur once a tick exceeds the CW: (i) the tick does not reattach if given the opportunity, (ii) the salivary glands (SGs) degenerate, (iii) ovarian development accelerates, and (iv) haemolymph ecdysteroid titre increases dramatically (Lomas and Kaufman, 1999). Although the CW can be determined from any of the latter parameters, the absolute value differs slightly depending on the parameter measured. The CW as measured by failure to reattach, by SG degeneration, by haemolymph ecdysteroid titre, and by ovary maturation is 9×, 10×, 10×, and 12–13× the unfed weight, respectively (Weiss and Kaufman, 2001).

So far, the concept of CW has been established only for *A. hebraeum*. But there are substantial differences in feeding behaviour between *A. hebraeum* and several *Dermacentor* spp. (e.g. *D. andersoni* and *D. variabilis*). For example, unlike *A. hebraeum*, *Dermacentor* females attach and feed readily in the absence of males. Moreover, virgin female *Dermacentor* normally feed well beyond 20× the unfed weight. So, the major question arises: When a virgin *Dermacentor* female feeds well beyond 10–13× its unfed weight, is it feeding beyond its CW, or is its CW much higher than that of *A. hebraeum*? This study was designed to address that question.

Materials and methods

Ticks

The adult *D. andersoni* ticks used in this study were provided by Dr. Tym Lysyk (Agriculture and Agri-Food Canada, Lethbridge Research Centre, Canada), mean female unfed weight of 6.47 ± 0.13 mg (n = 260), and by Dr. Glen Scoles (Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington and ARS, USDA), mean female unfed weight of 4.67 ± 0.06 mg (n = 328). As we frequently compare the results of this study with those done previously using *A. hebraeum*, the mean unfed weight for a large sample of the *A. hebraeum* females from our recent colony was 30.0 ± 0.6 mg (n = 192).

Details on tick rearing and feeding are presented in Ullah and Kaufman (2014). In brief, ticks were maintained at 7–9 °C until the day before putting them on a rabbit, when they were transferred to a humid 27 °C incubator. In order to determine the ultimate fed/unfed weight ratio of each experimental tick, prior to feeding, the ticks were coded by glueing a coloured silk thread to a leg and then weighed.

Equal numbers of males and females were introduced together to the backpack for experiments requiring mated females; only females were added for experiments requiring virgin females. Ticks were removed from the rabbit at various stages of feeding as required for each experiment. The maximum duration that each rabbit was exposed to ticks was 21 days. This animal-use protocol was approved by the Biosciences Animal Policy and Welfare Committee of the University of Alberta. This committee functions according to the current guidelines established by the Canadian Council on Animal Care.

The partially fed ticks in this study were divided into 3 groups based on their fed/unfed weight ratio. The 4–9× group comprised individuals that (for *A. hebraeum*) would represent ticks below the CW; the 13–19× group would represent individuals at and slightly above the CW, and the 19–50× individuals, if still attached to the host, would represent large-partially fed ticks above the CW. Ticks that exceeded about 50× and which, in the case of mated females, detached spontaneously, were defined as "fully engorged". The 4 latter groups were further characterized as either mated or virgin. In our laboratory, we define 'full engorgement' for virgins to be a weight beyond 50× the unfed weight, even though such virgins did not detach spontaneously.

Assay for salivary fluid secretory competence

The technique used was based on that described by Harris and Kaufman (1984), and details are presented in Ullah and Kaufman (2014). Briefly, the SGs (and ovary) were dissected out under a modified Hank's saline and then transferred to TC medium 199 buffered to pH 7.2 with morpholinopropanesulphonic acid (MOPS). The organs were then dissected free of extraneous tissue. The main salivary duct was ligated with a fine strand of silk thread. Each SG was gently blotted with filter paper to remove extraglandular fluid. The gland was then weighed to the nearest 10 μg on a 5-place electronic balance and immediately transferred to TC medium 199 containing freshly prepared dopamine. The bathing medium was slowly agitated with a magnetic stir-bar during the 15-min incubation period. The increase in wet weight at 15 min was used as an index of salivary fluid secretory competence (hereafter abbreviated to 'secretory competence') (Harris and Kaufman, 1984).

Ovary maturation assays

The assay details have been presented in Ullah and Kaufman (2014). In brief, the ovary was submerged in TC medium 199, and the lengths of the 10 apparently largest oocytes were measured. The ovary was then gently blotted, weighed to the nearest 10 μg, homogenized in 1 ml milliQ water, frozen quickly on dry ice, and stored at −20 °C in small microfuge tubes. For the spectrophotometric analysis of vitellin content, ovary homogenates were treated as described by Ullah and Kaufman (2014). For the final analysis, samples and blank were thawed and the absorbance at 400 nm (specific for the haem moiety of vitellin) and 500 nm (non-specific for haem) were measured on a spectrophotometer. The difference between the 2 absorbance readings was recorded as a measure of vitellin content and was normalized to ovary weight (expressed as 400 nm Δ 500 nm per gram ovary; Kaufman et al., 1986; Seixas et al., 2008).

Transmission electron microscopy (TEM) of salivary glands

The technique used was presented in Ullah and Kaufman (2014) and consisted of relatively standard TEM methods. Tissues were fixed in glutaraldehyde/paraformaldehyde. After fixation and post-fixation in OsO_4, the tissue was dehydrated through a graded ethanol series and then mounted in spur resin. Tissue sections were stained with 4% uranyl acetate and counterstained with lead citrate prior to observation under a TEM.

Reattachment assay

Partially fed females (both mated and virgin) were removed from the host and weighed. They were then kept in individual plastic vials in the colony incubator for ∼24 h. The females were returned to the host, thus giving them the opportunity to reattach. Reattachment was monitored over several hours on the first day, and then at 24 h intervals, to a maximum of 4 days.

Statistics

Unless otherwise stated, all data are reported as mean ± SEM (N), using Microsoft Excel software (Microsoft Office 2007). Statistical significance was determined by Student's t-test using STATA 10.0 (StataCorp, Texas, USA) on a Macintosh Computer. When analyzing the effect of time post-removal or post-engorgement for any weight group, the comparator group was always day 0. Other comparisons will be specified within the text or the figure legends.