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Salivary gland degeneration and vitellogenesis in the ixodid tick *Amblyomma hebraeum*: Surpassing a critical weight is the prerequisite and detachment from the host is the trigger

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ABSTRACT

The normal engorged body weight of female ixodid ticks (Acari: Ixodidae) is about 100× the unfed weight. Virgin female *Amblyomma hebraeum* normally do not feed beyond 10× the unfed weight. However, about 10–20% of a population of virgins will feed to perhaps 20× the unfed weight, but not much beyond that. In *A. hebraeum*, when females surpass about 10× the unfed weight, the following changes in physiology occur if they are removed from the host: (a) they will not reattach if given the opportunity, (b) their salivary glands (SGs) will undergo autolysis within 4 days if they are mated or 8 days if they are virgin, and (c) egg maturation and oviposition will occur in due course. Mated or virgin female ticks removed from the host below about 10× the unfed weight do not experience the latter changes (Kaufman, W.R., Lomas, L., 1996. 'Male Factors' in ticks: their role in feeding and egg development. Invertebrate Reproduction and Development 30, 191–198). In 1984 we named this transitional weight, the 'critical weight' (CW). Its absolute value is probably a species-specific characteristic (Kaufman, W.R., 2007. Gluttony and sex in female ixodid ticks: how do they compare to other blood-sucking arthropods? Journal of Insect Physiology 53, 264–273). Although mated females tend to engorge within a day of surpassing the CW, virgin females surpassing the CW can remain attached to the host for at least several weeks more. It is not known whether the physiological changes in the SGs and ovaries listed above occur in those large virgins that remain attached, although we suppose that this would be maladaptive. Instead, we hypothesize in this study that surpassing the CW is only a prerequisite for inducing these changes, and that detachment is the actual trigger. We support our hypothesis by demonstrating that large virgins, remaining attached to a host for 8 days, did not undergo SG degeneration nor complete egg maturation during the attachment period. Those changes occurred only within 8 days following detachment. So some type of sensory information associated with attachment to the host, and still undefined, inhibits expression of the physiological changes hitherto associated merely with surpassing the CW.

1. Introduction

The taking of a blood meal in female ixodid ticks is conventionally divided into three phases (Balashov, 1972). (1) During a 'preparatory phase' (about 1 day) the female establishes a feeding lesion. (2) During the 'slow phase' (7–9 days) it grows to about 10× its unfed body weight. (3) During the 'rapid phase' (about 1 day) it increases its weight a further 10-fold and detaches from the host. In our laboratory colony of the ixodid tick, *Amblyomma hebraeum* (Koch), engorgement of the female normally occurs within about 10 days of attachment.

It has been known for a long time that the salivary glands (SGs) of ticks undergo substantial development during feeding, and then are resorbed within a number of days post-engorgement (Till, 1961). Haemolymph ecdysteroid titre in *A. hebraeum* rises substantially during the first week post-engorgement (Kaufman, 1991; Friesen and Kaufman, 2002). This rise in ecdysteroids triggers SG autolysis (Harris and Kaufman, 1984, 1985; Kaufman, 1991; Chang and Kaufman, 2005). Ecdysteroids also stimulate synthesis of the main yolk protein, vitellogenin (Vg) in both ixodid ticks [*A. hebraeum* (Friesen and Kaufman, 2002, 2004) and *Dermacentor variabilis* (Sankhon et al., 1999; Thompson et al., 2005)] and argasid ticks [*Ornithodoros moubata* (Ogihara et al., 2007; Horigane et al., 2007)].

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In most ixodid species, mating occurs on the host. Copulation facilitates the female to enter the rapid phase of engorgement and to feed past a 'critical weight' (CW) that is characterized by several behavioral and physiological changes. For example, if a female is removed from the host prior to reaching the CW, it will actively seek a new host in order to complete the blood meal. But if it is removed after exceeding the CW, it will no longer seek a host, nor will it attach to one if given the opportunity. In addition, the SGs of females above the CW will undergo autolysis, and ovarian development will proceed to full maturation. The CW for mated female *A. hebraeum* is approximately 10× the unfed body weight (Harris and Kaufman, 1984; Kaufman and Lomas, 1996), although a more rigorous experimental design has shown that the exact value (between 10× and 13× the unfed weight) depends on which physiological parameter is being measured (Weiss and Kaufman, 2001).

Under normal circumstances, the female engorges and detaches from the host within 24 h of surpassing the CW. Although most virgin *A. hebraeum* do not feed beyond the CW, perhaps up to 20% do, though rarely do they feed beyond 20× the unfed weight, and they do not engorge to repletion (Kaufman, 2007). Virgins heavier than the CW can remain attached to the host for at least several weeks (Kaufman and Lomas, 1996; Lomas and Kaufman, 1999). Moreover, if such large virgins are forcibly removed from the host, their SGs degenerate within 8 days (Lomas and Kaufman, 1992a,b) and complete ovarian development and oviposition occur, although the resulting eggs rarely hatch (Kaufman, 1991). The question arises: If large virgins can remain attached to the host for several weeks without engorging to repletion, do their SGs undergo autolysis and does complete ovarian development occur during this time? Because it would be maladaptive for SG degeneration to occur while a tick is still attached to its host, perhaps SG degeneration and complete ovarian development are triggered by the act of detachment, and not by merely exceeding the CW? The present study was designed to answer this question. Our results indicate that although surpassing the CW is a prerequisite for SG autolysis and complete ovarian development, detachment from the host constitutes the trigger. Until now the act of detachment has not been appreciated as a crucial part of the signaling pathway to SG degeneration and vitellogenesis in *A. hebraeum*.

2. Materials and methods

2.1. Ticks and tick feeding

All host animals used in this study were cared for according to the guidelines mandated by the Canadian Council of Animal Care. Our colony of *A. hebraeum* was kept in darkness, at 27 °C and at >85% relative humidity. Ticks were fed on rabbits as described by Kaufman and Phillips (1973). Prior to feeding, individual females were weighed and marked by gluing (cyanoacrylate glue) a short length of colored thread to a leg. Female *A. hebraeum* are reluctant to attach in the absence of males. Hence, a number of male ticks were first allowed to attach to the back of the host rabbit. The males were confined within a cloth bag that permitted their attachment to the host but prevented copulation. The next day, unfed females were added to the same rabbit. When females were removed at the appropriate stage for experiments, they were rinsed with water, dried with tissue paper, weighed, and stored individually in gauze-covered glass vials until their tissues were collected (see Section 2.4).

2.2. Estimating tick body weight while they are attached to the host

This study required that we determine the day on which an individual female surpassed the CW, but without removing the tick from the host to weigh it. We did this by measuring body volume of feeding ticks as follows. Using digital calipers, we measured (1) the width of the body at its apparently widest part (approximately at the site of the posterior pair of legs; dimension *X*), (2) the anterior-to-posterior length of the body along the midline (dimension *Y*), and (3) the dorso-ventral thickness of the body (dimension *Z*). For these measurements the calipers could be read to the nearest 0.01 mm. We calculated tick volume from the formula:

$$\text{Volume} = \frac{4}{3\pi} * 0.5 * (XYZ)$$

This formula approximates the geometry of a fed tick (Patriquin, 1991). In a preliminary trial, we prepared a standard curve as follows for converting volume to weight: Body volume of ticks of diverse sizes (from about 2× to about 30× the unfed weight) was measured on-host and the ticks were then forcibly removed, washed and weighed to the nearest 0.1 mg. Body volume was re-measured off-host. Off-host measurement was considered to be intuitively more accurate because of greater ease to align the ticks with the calipers. Standard curves were drawn plotting volume as a function of body weight using Microsoft Excel software. Fig. 1 shows the standard curves for on-host and off-host volume measurements. The linear standard curves corresponded to each other almost exactly.

2.3. Experimental procedure

The day on which each tick was deemed from its volume to have surpassed the CW was designated as 'day 0'. Experimental ticks were assigned to one of five groups. Group 1 consisted of ticks above the CW removed from the host on day 0. Henceforth this group is labeled '>CW day 0'. Salivary fluid secretory competence was measured as described in Section 2.5, and ovarian development was determined as described in Section 2.6. Group 2 consisted of ticks above the CW that were allowed to remain attached to the host for an additional 8 days before removal and measurement of salivary fluid secretory competence and ovarian development. Henceforth this group is labeled '>CW day 8'. Group 3 consisted of ticks that were similar to Group 2, but were kept off-host in the colony incubator for an additional 8 days before salivary secretion and ovarian development were measured. Henceforth this group is labeled '>CW day 8 + 8'. Group 4 was a control group, similar to Group 1, except that these were ticks that had not yet reached their CW. Henceforth this group is labeled '<CW day 0'. Group 5, a second control group of below-CW ticks, were treated similarly to Group 3. Henceforth this group is labeled '<CW day + 8'.

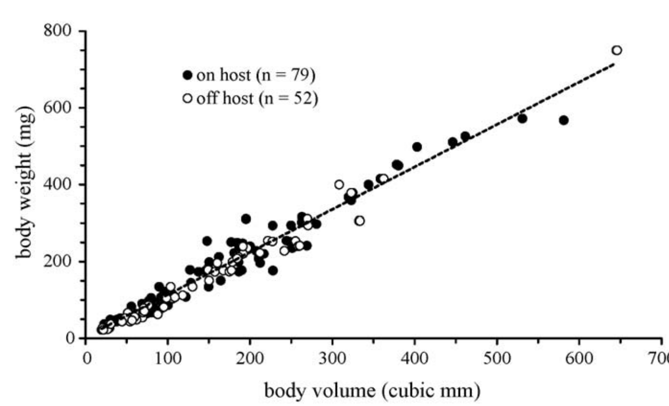

Fig. 1. Calculation of tick body weight from body volume measurements (see Section 2.2). The linear regression curve for off-host ticks ($Y = 1.142X - 10.287$, $R^2 = 0.976$; $n = 52$) was virtually identical to that for on-host ticks ($Y = 1.106X + 3.32$, $R^2 = 0.960$; $n = 79$).