Corresponding Author: Prof Reuben Kaufman, PhD

Corresponding Author's Institution: U. Alberta

First Author: Reuben Kaufman, PhD

Order of Authors: Reuben Kaufman, PhD

Abstract: The central issue dealt with here is the role of copulation in the control of feeding behaviour in ticks and some haematophagous insects. Female ticks of the family Ixodidae normally engorge to ~100 X their unfed weight, and then drop from the host, produce and lay eggs, and die. Virgins, on the other hand, normally do not exceed 5-40% (depending on species) of the normal engorged weight. But instead of detaching voluntarily at that point, virgins remain fixed to the host for extended periods, waiting for males to find them so they can complete engorgement. Virgin haematophagous insects, and virgin ticks of the family Argasidae display little, if any, reduction in blood meal size compared to mated females, at least not during the first ovarian cycle. During subsequent ovarian cycles, meal size in some virgin insects may be somewhat reduced depending on how many eggs are retained in the reproductive tract, but the reduction is never to the same extent as that observed for virgin ixodid females. The stimulatory effect of copulation on engorgement in the latter is caused by a pair of proteins (voraxin α and β) produced in the testis and transferred to the female with the spermatophore. Here I propose why it might be adaptive for an ixodid female to remain small until mated. The hypothesis is suggested from the facts that ixodid ticks remain attached to the host for days (rather than minutes), and that virgin ticks, above a certain critical weight, lose

all opportunity for producing viable offspring should they be groomed off the host prematurely, or should the host die.

MS 06-SR-00027 (Kaufman)

Referees' comments are in normal text,

- **Response to referees are in bold text, bulleted.)**

Referee 1

Page 4 line 10 up. Strictly speaking, there is a Syntax problem here at the beginning of the list "including: to nourish Ö.". The structure of that sentence needs changing.

- **I understand and agree with the syntax problem. I have modified the structure of the sentence to remove the offense (p. 5, 3rd sentence under "2. Mating factors", in revised MS. But the sentence is complex; perhaps the editor would like to check it himself.**

Page 13 line 9-10 up. It might be best to clarify that 'prothoracic glands of many Lepidoptera release 3-dehydroecdysone'

- **This was mentioned just a few lines further down. I've examined the paragraph again and, with respect to the referee, I feel that it is more coherent to leave it as is. I will defer to the editor, of course. See p. 17, 1st paragraph of "Concluding Remarks", in revised MS.**

'It is also not known whether the epidermal cells release ecdysone itself or, ÖÖ. 3-dehydroecdysone'. Actually, Lomas et al. (1997) did not detect 3-dehydroecdysteroids as products of in vitro incubations of integument. This strongly suggests that the products of the latter are 3<beta>-hydroxy-ecdysteroids.

- **I have now added a sentence attesting to that (p. 15, last 3 lines in revised MS.**

Referee 2

This is a most informative and useful article. Dr. Kaufman has presented an interesting and insightful exposition of the importance of copulation as the controlling factor regulating the completion of engorgement in ixodid ticks. In contrast, as he notes in this review, it has little if any role in determining the size of the blood meals in argasid ticks and haematophagous insects. Although the role of the inseminating male in triggering the switch to full engorgement in ixodid ticks has long been known, it was only recently that the specific peptidic stimuli, namely, voraxin <alpha> and <beta> were identified. This was done by Dr. Kaufman's group and represents one of the most important contributions to our understanding of reproductive biology in this medically important group. Unfortunately, at this point, the manuscript delves into speculations about the advantages of small feeding size until an opportunity to mate occurs, while ignoring the equally fascinating

questions concerning how the introduction of these two peptides initiates the process of engorgement to repletion. Do the peptides, voraxin <alpha> and <beta>, act directly on the tick's digestive tract to signal rapid blood uptake and, if so, how? Since they are introduced into the female reproductive tract, how do they act on the pharynx, midgut and body

musculature to activate the massive uptake of huge quantities of blood in little over 24 hours? Or, if they do not act directly, what else occurs? Do they trigger upregulation of additional, unknown hormones that signal the target organs? And how does the introduction of the voraxins initiate the process of vitellogenesis, now known to be stimulated by rapidly increasing high concentrations of 20-hydroxyecdysone? This is noted only briefly on P. 15. Although much remains unknown about these issues, further discussion should be included in the overall review along with suggestions for future research.

- **The referee is correct that I did not comment further on the obvious questions surrounding the site(s) and mode of action of voraxin. This is because none of the required experiments has yet been done. However, I have now added some text (constituting a brief second paragraph of "Concluding remarks" in the revised MS) to spell out some of the possibilities; these do not include, however, a few of the referees' specific suggestions (see p. 18, paragraph beginning "Although voraxin enters…." and continuing through the next paragraph in the revised MS). Intuitively, it seems more likely that voraxin stimulates feeding behaviour by stimulating neural pathways in the CNS rather than by stimulating a suite of pharyngeal muscles, and other tissues (s)he mentions, independently. It's hard to see how feeding would be coordinated if voraxin acted directly at multiple sites.**

Several other minor comments should also be considered:

P. 3. line 6. I suggest changing "for a matter of days" to "for a period of several days"

- **I've changed it to "a number of days" (p. 3,2nd paragraph in revised MS).**

P. 3. lines 23 - 24. insert "female body" so as to read, "between 200 - 300 X the unfed female body weight.

- **Good point; done (p. 3 last line in revised MS) !**

 P. 4. Line 17. I'm confused about the comment about inhibiting ticks below the CW from reattaching. I understood from reading previous papers on this subject that ticks that had not reached their CW would reattach readily if given the opportunity. Normally, high titers of 20E would not be encountered; it would have to be administered artificially. Is this what is meant? Perhaps some clarification is needed here.

- **Good point. I have now clarified that the data refer to injections of ecdysteroid (p. 4, last 3 lines in revised MS). But I have also linked this to the natural situation: rising haemolymph ecdysteroid titres occurring in ticks removed from the host above the "critical weight" may well be the reason why such ticks do not reattach to a host when given the opportunity (p. 5, first 6 lines in revised MS).**

P. 6. Line 8. Substitute "outstanding" for "astounding."

- **Done (p. 7, 1st line under "*Ticks*").**

P. 13. Lines 11 - 14. In nature, females of A. hebraeum and its close relative, A. variegatum, normally will not attach to a host unless fed males are present and releasing large amounts of male aggregation and attachment pheromone. This strategy for attracting and aggregating

the sexes is quite different than in most other ixodid ticks, where males and females attach independent of one another. Some additional discussion of the distinctions in these different strategies would be useful here.

- **Good point. The multiple pheromones that ticks use to ensure aggregation followed by mounting and conspecific mating is a fascinating story. Although it is peripheral to the story I'm telling here, the referee is correct that the reader should at least be made aware that we know a great deal about these pheromones. So I've added a section at this point in the MS and refer to an excellent recent review (p. 16, 2nd paragraph beginning "What accounts for…).**

- **Finally, an independent colleague has suggested that voraxin should be referred to as a "two proteins" rather than a "two peptides", because the molecular weight of each exceeds 10 kDa (the conventional cutoff between peptide and protein being the order of a few kDa). So in the revised MS, I have changed "peptide" to "protein" whenever referring to voraxin.**

- **I've also taken the opportunity to make other minor editorial changes throughout the MS.**

Gluttony and sex in female ixodid ticks: How do they compare to other blood-sucking arthropods?

W. Reuben Kaufman, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

reuben.kaufman@ualberta.ca

Abstract

The central issue dealt with here is the role of copulation in the control of feeding behaviour in ticks and some haematophagous insects. Female ticks of the family Ixodidae normally engorge to ~100 X their unfed weight, and then drop from the host, produce and lay eggs, and die. Virgins, on the other hand, normally do not exceed 5-40% (depending on species) of the normal engorged weight. But instead of detaching voluntarily at that point, virgins remain fixed to the host for extended periods, waiting for males to find them so they can complete engorgement. Virgin haematophagous insects, and virgin ticks of the family Argasidae display little, if any, reduction in blood meal size compared to mated females, at least not during the first ovarian cycle. During subsequent ovarian cycles, meal size in some virgin insects may be somewhat reduced depending on how many eggs are retained in the reproductive tract, but the reduction is never to the same extent as that observed for virgin ixodid females. The stimulatory effect of copulation on engorgement in the latter is caused by a pair of proteins (voraxin α and β) produced in the testis and transferred to the female with the spermatophore. Here I propose why it might be adaptive for an ixodid female to remain small until mated. The hypothesis is suggested from the facts that ixodid ticks remain attached to the host for days (rather than minutes), and that virgin ticks, above a certain critical weight, lose all opportunity for producing viable offspring should they be groomed off the host prematurely, or should the host die.

1. Introduction

Ixodid ticks (Arachnida: Acari: Ixodidae) are obligatory haematophagous arthropods during the three phases of their life cycle — a single larva, a single nymph and adult. Similar to blood-sucking insects, each meal is followed by a moult (larva and nymph) or by spermiogenesis or oogenesis/oviposition. But they differ from blood-sucking insects in at least four important respects.

First, they remain on the host for a number of days (4-14 depending on stage and species) rather than minutes.

Second, although the male takes a relatively small meal (perhaps up to 50% of the unfed weight), the female is truly gluttonous, increasing her weight approximately 100-fold (Figure 1). This is at least an order of magnitude more than the blood-sucking bug *Rhodnius prolixus* and adult ticks of the family Argasidae, about which more will follow.

Third, the final engorged weight actually very much underestimates the true meal size. Adult female *Rhodnius* take the meal (about 3-4 X the unfed weight) within about 10 minutes, during which time diuresis begins. The main period of diuresis occurs over the subsequent few hours, eventually resulting in a weight reduction to about twice that of the unfed insect (Maimets, 1985). Although female ixodid ticks likewise excrete the excess fluid of the blood meal, this does not occur during an uninterrupted bout of diuresis following the meal. Instead, interspersed with bouts of blood-sucking, the female secretes an enormous volume of hyposmotic saliva back into the host's circulation throughout the feeding period. The blood meal is thus concentrated approximately 2-3 fold while the tick is still attached to the host, and osmoregulation and haemolymph volume regulation are achieved (Kaufman and Phillips, 1973; Koch et al., 1974; Koch and Sauer, 1984). Hence the total volume of whole blood removed from the host during a meal is actually somewhere between 200-300 X the unfed female body weight!

Finally, and as will be elaborated further on, the difference in meal size between virgin and mated females is substantially greater than that reported for any insect.

Although the total meal size of ixodid females can be >200 X their unfed body weight, their feeding progress is not of the slow and steady variety. After *Amblyomma hebraeum* cements itself to the skin and forms the feeding lesion (about a day), it feeds gradually over the next 7-8 days, by which time it will have increased its weight ~10-fold; this is the 'slow

phase' of engorgement. During the subsequent day (if it has copulated) it increases its weight a further 10-fold ('rapid phase' of engorgement). The transition between the slow and rapid phases (at ~10 X the unfed weight) has been called the 'critical weight' (CW) because, as reviewed in the next section, important physiological changes occur once the CW is surpassed (Kaufman and Lomas, 1996; Lomas and Kaufman, 1999; Weiss and Kaufman, 2001). With the exception of prostriate ticks (comprising the single genus *Ixodes*), which can copulate before or during the blood meal, copulation in all other ixodid genera (metastriate ticks) occurs only during the feeding period. This is because both sexes among the metastriates require a blood meal to complete gonad maturation (Sonenshine, 1991).

Following engorgement, the salivary glands undergo autolysis within about 4 days, and vitellogenesis proceeds. Vitellogenin uptake in *A. hebraeum* begins at around 3-4 days post engorgement, and oviposition begins at around day 10 or 11, and continues for a month or so, after which the spent female dies (Friesen and Kaufman, 2002). An ecdysteroid hormone, probably 20-hydroxyecdysone (20E), triggers both salivary gland autolysis (Harris and Kaufman, 1985; Mao and Kaufman, 1998, 1999) and vitellogenesis (Friesen and Kaufman, 2002, 2004). Injecting high doses of 20E into the haemocoel also inhibits ticks that have been removed from the host below the CW from reattaching (Weiss and Kaufman, 2001). When injected into feeding females, 20E also slows their feeding rate (Thompson et al., 2005). These observations are consistent with earlier data on ticks that are forcibly removed from the host after exceeding the CW. In such ticks haemolymph ecdysteroid titre normally rises substantially within a few days, and such large, partially fed ticks generally will not attach to a host if given the opportunity (reviewed by Kaufman and Lomas, 1996 and by Lomas and Kaufman, 1999). It seems likely that this reluctance by large partially fed ticks to reattach and resume feeding is caused by the naturally elevated haemolymph ecdysteroid titre.

2. Mating factors

Seminal fluid is a complex mixture of secretions from the testis and male accessory glands (MAGs). Gillott (2003) reminds us that the MAG secretions in insects perform numerous functions. MAG secretions are used (1) to nourish/protect the spermatozoa within the male tract, (2) as components of the spermatophore, (3) to enhance fecundity and attenuate female receptivity, (4) as components of the mating plug, (5) as antimicrobial and

antifungal agents to protect the gametes during their sojourn in the female reproductive tract, and (6) as a nutritional supplement to the female. Finally, (7) some MAG secretions extend the duration of sperm viability within the female genital tract. In ticks there are at least four known proteins from the male gonad that are functionally equivalent to MAG proteins (Kaufman, 2004): a sperm capacitation factor (argasid and ixodid ticks) that activates the spermiophores (= spermatozoa) once they are within the seminal receptacle; a vitellogenesis stimulating factor (argasid ticks); a 'male factor' (ixodid ticks), that hastens the onset of salivary gland degeneration and ovarian development; and an 'engorgement factor' (ixodid ticks) that stimulates the female to engorge. The last two merit further attention here.

As mentioned above, the salivary glands of female *A. hebraeum* degenerate within 4 days post-engorgement, a process triggered by 20E. But mated ticks removed from the host at any size above the CW also undergo salivary gland degeneration within 4 days (Harris and Kaufman, 1984), whereas weight-matched virgins above the CW require 8 days (Lomas and Kaufman, 1992a). A protein from the testis, originally named 'male factor' by Harris and Kaufman (1984), is the substance responsible for hastening the rate of salivary gland degeneration from 8 to 4 days, and it achieves this effect by hastening the release of 20E into the haemolymph by 3-4 days (Lomas and Kaufman, 1992b). Vitellogenesis is also triggered by 20E (Friesen and Kaufman, 2002, 2004), so this process is also hastened by 'male factor'. The engorgement factor will be discussed in the next section.

3. Control of feeding behaviour and the effect of mating on feeding

Insects

There is a large body of literature relating to the many environmental and physiological factors influencing insect feeding behaviour (Chapman and de Boer, 1995). Not surprisingly, many studies have noted increased feeding rates associated with each ovarian cycle. Females displaying discrete ovarian cycles feed at a high rate during the early part of each cycle, and then feeding rate drops to low levels prior to ovulation (Barton Browne, 1995). Inasmuch as any egg mass produced by virgins is generally smaller than that produced by mated females, it seems inevitable that mated females will feed at a higher rate. Thus in the milkweed bug (*Oncopeltus fasciatus*) and in the housefly (*Musca domestica*) the oviposition rate of virgins is 30-40% that of mated females, and food intake is less by a comparable

amount. Although fewer studies have been conducted on males, a single spermatophore can account for up to 10% of a male's body weight, and in the male cockroach (*Blatella germanica*), food intake increases as a function of copulation frequency (Barton Browne, 1995).

Until recently, however, it has not been easy to resolve a specific mechanism linking copulation to increased food intake in insects. It is equally surprising that copulation does not seem to enhance feeding in the majority of blood-sucking insects where it has been specifically looked for (see citations in Table 2). What might such discrepancies teach us about the control of feeding behaviour in vector arthropods? There is now one definitive study on how copulation increases feeding rate in a non-vector arthropod: *Drosophila*. Carvalho et al. (2006) demonstrated that mated female *Drosophila* consume over twice as much food within 24 h than do age-matched virgins. Most interesting is that the effect of copulation is mediated specifically by a single MAG peptide ('sex peptide' or Acp70A); females mated with males that do not transfer the sex peptide during copulation for one reason or another consume no more than do virgins.

Ticks

The most outstanding instance of meal size being influenced by mating status is that of female ixodid ticks. As outlined in the Introduction, they normally imbibe >200 X their own unfed weight in host blood over the 7-14 day feeding period, the CW marking the transition between the slow and rapid phases of engorgement. But this occurs only if the female has copulated. Most virgin *A. hebraeum* do not feed beyond the CW, and can remain attached to the host for weeks waiting for a male to find her. If a male eventually copulates with her, she proceeds to engorge normally; the longer it takes for a male to appear, the more rapidly does the female complete subsequent engorgement (Snow, 1969). Otherwise she remains attached until immunological responses by the host may eventually result in her rejection or death (Brossard and Wikel, 2004). A small percentage of virgin *A. hebraeum* do manage to feed beyond the CW, but only rarely beyond 20% of the engorged weight (Kaufman and Lomas, 1996).

If a tick is removed from the host prematurely, her subsequent behaviour is determined by two circumstances: (a) whether she is above or below the CW and (b) if above the CW, whether she is virgin or has copulated. Females that are detached from the host

below the CW (mated or virgin) maintain a low haemolymph ecdysteroid titre (< 20 ng ml^{-1}), do not undergo salivary gland degeneration, or initiate vitellogenesis, and will reattach to a host and continue feeding if given the opportunity. On the other hand, females that are detached from the host above the CW display a rising haemolymph ecdysteroid titre (eventually achieving 300-500 ng ml^{-1}; Kaufman, 1991; Lunke and Kaufman, 1992; Lomas and Kaufman, 1992b), do undergo salivary gland degeneration, do complete vitellogenesis (and ultimately oviposition), and will not reattach to a host and continue feeding if given the opportunity (Kaufman and Lomas, 1996); in all the latter, detached virgins above the CW proceed more slowly than mated females. Finally, in common with many blood-sucking insects, ticks are able to sense an elevated CO_2 concentration in a plume of air — one signal that a potential host is nearby; but detached ticks that have exceeded the CW are no longer responsive to elevated CO_2 (Anderson et al., 1998).

The concept of a CW was established originally over 20 years ago (Harris and Kaufman, 1984). Then, and in subsequent studies, we assessed the CW by any of the several measures just mentioned above. In these studies, we could establish only an approximate value for the CW, because the unfed ticks used, although chosen to be within a fairly narrow weight range, were not individually marked. Weiss and Kaufman (2001), in contrast, marked individual unfed ticks, so that a specific fed-to-unfed weight ratio could be recorded for each individual subsequently removed from the host. In this way we expected to establish a reasonably precise value for the CW. Instead, we discovered that the absolute value of the CW differed depending on which parameter was used to measure it. The CW as defined by reluctance to re-attach to the host was 9 X the unfed weight, while that for elevated haemolymph ecdysteroid titre and triggering salivary gland degeneration was 10 X, for increased ovary weight and oocyte length it was 12 X, and for increased oocyte vitellin content it was 13 X the unfed weight. The biological meaning of these modest (but significant) differences remains obscure, although it may well relate to a differential sensitivity of each parameter to haemolymph ecdysteroid titre.

What is the mating signal that triggers rapid engorgement in female ixodid ticks? A body of literature, primarily from the 1960s, considered the possibility of mechanical stimuli associated with copulation. However, none of the evidence suggested that a mechanical stimulus (mimicked by inserting inert beads into the seminal receptacle, for example) might be the trigger for rapid engorgement (reviewed by Diehl et al., 1982). Pappas and Oliver

(1972) were the first to demonstrate that the male (*Dermacentor variabilis*) produces a specific 'engorgement factor' that is transmitted to the female via the spermatophore. [The following sentence contains sexually graphic language; reader discretion is advised!] Females exposed to males whose genital apertures were sealed, did not fully engorge even though they received normal precopulatory stimuli — male mounting behaviour and the male inserting his mouthparts into her gonopore to secrete a droplet of lubricant saliva. Thus neither mechanical stimulation from the mouthparts, nor a putative signaling molecule from the saliva (eg: prostaglandins; Shemesh et al., 1979; Bowman et al., 1996, Aljamali et al., 2002), constitute the engorgement stimulus. In a second experiment, males were exposed to 2.5 kilorads of gamma radiation to kill the spermatids. Females were fed in the company of these irradiated males, and those receiving a spermatophore engorged and oviposited normally; as expected, no embryonic development occurred. Thus viable spermiophores are not required for full engorgement, but something else in the spermatophore is (Pappas and Oliver, 1972).

Weiss et al. (2002) produced 28 recombinant proteins from genes that were upregulated in the testes of fed male *A. hebraeum*. Two of these proteins together were necessary and sufficient for stimulating complete engorgement in virgin females: we named them voraxin α and β (Weiss and Kaufman, 2004). In insects, all of the known male gonad signaling peptides are produced in the MAGs (Wolfner, 2002; Gillott, 2003); voraxin, in contrast, is produced in the testis/vas deferens (Weiss and Kaufman, 2004). Voraxin has not yet been isolated from other tick species, although the following circumstantial evidence suggests that homologous proteins do occur among the Ixodidae: In all species examined to date, virgins feed much less than do mated females, although the unfed-to-fed weight ratio at which virgins stop gaining net weight is variable, ranging from a low of 0.05 for *A. americanum* to a high of 0.35 – 0.40 for *D. andersoni*, *D. variabilis* and *H. anatolicum* (Table 1; see also Aeschlimann and Grandjean, 1973; Falk-Vairant et al., 1994). Diehl et al. (1982) state that cross-matings between the following species resulted in complete engorgement: *A. americanum* with *A. maculatum*; *Boophilus annulatus* with *B. microplus*; *Rhipicephalus pulchellus* with *R. appendiculatus*; and "… all twelve possible hybrid matings among four species of Kenyan *Amblyomma* ticks (*A. variegatum, A. gemma, A. eburneum* and *A. cohaerens*)." Whether the recombinant voraxin isolated from *A. hebraeum* is able to stimulate engorgement in other ixodid species remains to be tested, however. Although the concept of

a CW (i.e. a weight above which ticks can lay at least some eggs) has been defined in *A. hebraeum*, other species have not been examined as carefully. An interesting difference between *Amblyomma* and the other ticks shown in Table 1 is that, in the former, the mean weight at which virgins stop gaining weight is below the threshold for egg-laying. Virgin *Dermacentor* and *Rhipicephalus*, on the other hand, do feed up to a weight at which they lay batches of (infertile) eggs following removal from the host (Pappas and Oliver (1972), and unpublished observations from my laboratory).

4. Comparing ixodid ticks to other vector arthropods

Although the difference in meal size between virgin and mated ixodid female ticks is substantial, this is not the case for other blood feeding arthropods. Potential reasons for this discrepancy will be considered in Section 5.

Ticks of the family Argasidae

Argasid ticks (vectors for several *Borrelia* species that cause relapsing fever; Sonenshine, 1993) display a lifestyle and reproductive strategy vastly different from that of ixodid ticks. Argasids pass through one larval and up to five nymphal instars, followed by the adult. Males are frequently smaller than females because they emerge from an earlier instar than do females. In *Argas (Persicargas) arboreus*, females usually arise from 3rd or 4th instar nymphs, whereas males usually arise from 2nd instar nymphs (Khalil, 1969). In *Ornithodoros moubata*, males usually emerge from 4th instar nymphs and females from 5th instar nymphs (personal communications from Dr. DeMar Taylor, University of Tsukuba, Japan, and Prof. A. Neitz, University of Pretoria, South Africa). *Ornithodoros* nymphs and adults feed much more rapidly (30-60 minutes) than ixodid ticks, though somewhat slower than blood-sucking insects such as *Rhodnius prolixus* (~10 minutes; Maddrell, 1964) or most mosquitoes [as little as 1 min for some anopheline mosquitoes (Chadee and Beier, 1995) or 2-5 minutes for *Aedes* (Chadee et al., 2002)]. The fed weight of adult female *O. moubata* is about 5-12 X their unfed weight (Diehl et al., 1982). As in insects, excess fluid of the blood meal is excreted to the exterior, although unlike insects, excretion occurs via a specialized coxal organ rather than the Malpighian tubules (Kaufman et al., 1981, 1982). Argasid females can complete a gonotrophic cycle following each meal, laying a modest number of eggs each time. In many of the latter respects, argasid ticks are rather similar to vector insects. Most unlike ixodid ticks (and similar to insects), copulation in *O. moubata*, does not appear to influence meal size

much if at all, although it increases rate of digestion and vitellogenesis (Table 2; Aeschlimann and Grandjean, 1973; Germond and Aeschlimann, 1977; Diehl et al., 1982).

Rhodnius prolixus

During the first gonotrophic cycle of this hemipteran, virgin and mated females feed to a similar extent. However, virgin females do lay fewer eggs, thus leaving less room for a subsequent blood meal (Davey, 1997). Consequently, averaged over four cycles (fed every 10 days), virgin females consume 12-22% less blood than mated females (Davey, 1967). Moreover, virgin females use 41-44% more blood to produce a given weight of eggs than do mated females, perhaps because virgins use more of their nutrients in metabolic activity searching for a mate. A far more complete examination of feeding and mating in *Rhodnius* is presented by Davey in this Special Issue (Davey, 2006).

Glossina

As is the case for many blood-sucking insects, the absolute meal size imbibed by tsetse flies depends on age and on the frequency of feeding (among other factors). In one study using *Glossina palpalis*, Adlington et al. (1996) could detect no significant difference between mated and virgin females fed under the same conditions (Table 2). Males, however, imbibe only about half the amount as virgin or mated females (Loke and Randolph, 1995). These data are from laboratory studies, and probably bear little relevance to what occurs in the field, where copulation normally occurs soon after emergence (Dr. S. Randolph, Oxford University, personal communication). The same likely applies to many insects and argasid ticks that are able to mate prior to taking a blood meal.

Mosquitoes

Adlakha and Pillai (1976) reported that virgin female *Aedes aegypti* imbibe ~20% less blood than do mated females, and that virgin *Culex pipiens fatigans* imbibe 8-16% less blood (Table 2). However, these data were based on gravimetric methods, which are subject to errors because they do not take into account urine losses occurring immediately after the meal. Klowden (1979), measuring haemoglobin content in fed mosquitoes spectrophotometrically (a technique not affected by the extent of diuresis), demonstrated that copulation in *Aedes aegypti* has no effect on feeding rate (Table 2), although mating apparently does increase the rate of blood meal utilization.

Cimex lectularius

As in *Rhodnius*, adult bed bugs take in relatively smaller meals than the larvae (1.5 X the unfed weight for the male and 2.2 X for the female; Ussinger, 1966). The blood meal is taken in about 5-10 minutes, and diuresis begins soon after feeding, half the blood meal volume being excreted within ~5 hours. When fed every 12 days, oviposition occurs in a peak/trough cycle, but if fed semi-weekly, egg production becomes essentially continuous, the female putting out more than 5 eggs per week for 4-5 months (Ussinger, 1966). I have found no indication in the literature that mating influences meal size (Table 2); in any case, bed bugs tends to mate after rather than before taking the blood meal (Dr. Ted Morrow, Uppsala University, personal communication).

5. Why does mating have such a profound effect on meal size in female ixodid ticks?

Although the number of vector arthropods referred to in the foregoing is limited, ixodid ticks display significant differences in lifestyle from all of them. It is tempting to hypothesize that these differences — the necessity for the tick to feed as an adult in order to complete spermiogenesis (with the exception of prostriate ticks), the very long sojourn on the host, the enormous size of the blood meal, and non-nidiculous behaviour (see immediately below) — together may account for ixodid females curtailing full engorgement until mating occurs.

Insects that fly probably encounter a suitable host frequently. Argasid ticks are wingless, but most of them are nidiculous, feeding on the host within the cave, burrow, nest or human-made shelter (Sonenshine, 1993), and so they also have frequent opportunities to feed. In all these cases, it seems to be advantageous to consume numerous but relatively small meals, each triggering a gonotrophic cycle in which relatively few eggs are laid close to home. Because ixodid ticks remain on the host for an extended duration however, one might expect them to drop from the host, remote from home, more frequently. Hence finding the next meal would be much less certain, and from this one could imagine how imbibing a huge meal, and laying a single, very large batch of eggs (thousands as opposed to tens) might be adaptive. But as outlined just below, the true story is more complicated, because oviposition sites of at least some ixodid ticks can be close to home.

Ixodid ticks are believed to have evolved from an argasid tick ancestor (Black and Piesman, 1994; Klompen et al., 1996), and some prostriate ticks display nidiculous behaviour (Sonenshine, 1993). The circumstances that might have led the metastriate ixodids to

abandon nidiculous behaviour are not known, of course. In this context, however, it's relevant to note that most ixodid ticks tend to complete their engorgement and drop from the host during the scotophase (Sonenshine, 1993), a behaviour that should increase the likelihood for eggs to be laid, and larvae to hatch, near home. A telling exception that supports this rule is the rabbit tick, *Heamaphysalis leporispalustris*, which tend to engorge and detach during the day, when their host is confined to the warren (George, 1971).

Traditionally, the evolutionary pressures on a parasite have been viewed as deriving primarily from the host, but today, other pressures (eg, habitat/ecological specificity) have gained some currency. For example, many ticks parasitize phylogenetically distinct hosts that share a similar ecological niche. Klompen et al. (1996) reviewed the topic thoroughly and offer the example of *Ornithodoros turicata*, which is found in the burrows of gopher tortoises in the southeastern US. This tick will feed successfully on a number of amphibia, reptiles, birds and mammals that also inhabit these burrows. Some nidiculous ixodid ticks also tend to be distributed near the host's nest. For example, cliff swallows (*Hirundo pyrrhonota*) are known to be parasitized by both the argasid *O. concanensis* and the ixodid *I. baergi* (Sonenshine, 1993). Likewise, *I. uriae* are normally associated with seabird colonies along North Atlantic coastlines, and all these ticks are most often found in cliff faces and soil near the bird nests (Sonenshine, 1993).

The extended sojourn on the host exposes ixodid ticks to some potential hazards, the two major ones being increased grooming by the host, and activation of the immune system against numerous saliva proteins injected into the host (Brossard and Wikel, 2004; Willadsen, 2004). During the slow phase of engorgement, the tick undergoes significant development and growth in numerous structures — salivary gland (Fawcett et al., 1982), cuticle (Lees and Beament, 1948; Hackman and Filshie, 1982), and reproductive tissues (Diehl et al., 1982) — but relatively modest increase in size (~10 X). All this is in preparation for the rapid feeding phase. Remaining small during the slow phase of engorgement might be advantageous because it would seem to be more difficult for a host to dislodge a small tick by grooming. Moreover, because the smaller the tick, the tougher the cuticle, grooming behaviour by the host would also be much less likely to tear open the cuticle and thus kill the tick. These conjectures remain to be formally tested, however.

The final consideration for remaining small as a virgin relates to the facts that parthenogenesis is almost unknown in ixodid ticks (Sonenshine, 1991), and that attaining the

CW represents such an important physiological and behavioural milestone. Recall from Section 3 that if ticks are removed from the host prematurely, they are only able to reattach and resume feeding if they are below the CW, and they are only able to lay any eggs at all if they are above the CW (Kaufman and Lomas, 1996; Lomas and Kaufman, 1999; Weiss and Kaufman, 2001). The two main circumstances that would lead to detachment in the wild are grooming by the host or death of the host (the latter eventually resulting in the ticks detaching voluntarily). Hence, it is only if the virgin remains below the CW that she would have some chance of finding another host (hopefully infested with some feeding males) and completing the gonotrophic cycle. Once mated, however, ticks removed from the host at any point above the CW are able to lay at least some fertile eggs, the absolute mass being a direct function of the meal size to that point. Such ticks do not have to find another host to complete the gonotrophic cycle. Taken together, it makes sense that virgins should curtail feeding as they approach the CW. If males are not present at that time, they remain attached, but do not feed further. Should they be groomed off the host, or if the host should die, they retain the ability to seek another host. Once males appear and copulate with them, however, they enter the rapid feeding phase (Snow, 1969) and so are now capable of laying at least some fertile eggs if removed prematurely. This hyper-dependence on males for full engorgement has rendered the females of some *Amblyomma* species (*A. hebraeum* is one) reluctant even to attach to an animal that doesn't already harbour feeding males. In our laboratory, for example, we routinely place males on the host at least a day or two before introducing females, otherwise only a minority of the females will attach within a week.

What accounts for the reluctance of some female *Amblyomma* species to attach in the absence of males? A great deal is now known about the pheromones responsible for aggregation and courtship behaviour in ticks. The reader is referred to Sonenshine (2004) for many further details on the following very brief account. At least four pheromone mixtures lead to courtship in metastriate ixodid ticks, although the details vary enormously among species: (1) An attraction-aggregation-attachment pheromone (AAAP), secreted by some male *Amblyomma* species, stimulates females to congregate and attach near the males. (2) In most other metastriate ticks, feeding females secrete an attractant sex pheromone, 2,6-dichlorophenol that excites feeding males to detach and search for the attractant source. (3) Feeding females also secrete a non-volatile mounting sex pheromone (cholesteryl oleate in *Dermacentor variabilis*; mixtures of different cholesteryl esters in other species) that induces

males to probe the females and ultimately leads them to the female's gonopore. (3) A genital sex pheromone (a mixture of long-chain fatty acids and ecdysteroids) is secreted by the vestibular vagina. When the male detects it, he probes the female's genital pore with his mouthparts and ultimately inserts a spermatophore. The genital sex pheromone is the most species-specific of the three, and helps minimize interspecific copulation when several tick species infest the same host.

It is the absence of AAAP that is responsible for some female *Amblyomma* species not attaching to the host unless feeding males are present. (Other metastriate female ticks will attach readily in the absence of males). AAAP's range of action is the order of a few metres, and its effect is enhanced by carbon dioxide. AAAP is a mixture of primarily three organic volatiles (*0*-nitrophenol, methylsalicylate and nonanoic acid). The concentration ratio among the three substances, and the presence or absence of additional volatile components, vary among species. This variability probably accounts for some species-specific aggregation when multiple species are feeding on the same host, even though each pheromone component may attract all species to some extent. AAAP is secreted by large dermal glands located on the ventral surface of the male (Sonenshine, 2004).

6. Concluding remarks

Ticks do not possess a prothoracic gland, the major source of ecdysone in insects. Instead, in both argasid and ixodid ticks, ecdysone is released from epidermal cells under the control of a neuropeptide (Zhu et al., 1991; Lomas et al., 1997), although it is worth noting that the prothoracic gland is also of ectodermal origin (Klowden, 2002). In some Lepidoptera, the prothoracic gland releases 3-dehydroecdysone, which in turn is converted to ecdysone in the haemolymph by a 3β-reductase enzyme (Warren et al., 1988; Kiriishi et al., 1990). Although 3β-reductase activity has been detected in the tick salivary gland, it is not found in the haemolymph (Lomas et al., 1998). This is consistent with an earlier observation by Lomas et al. (1997) who did not detect 3-dehydroecdysteroids as secreted products from fragments of integument incubated in vitro; this observation also suggests that the epidermis releases ecdysone directly. Whether ecdysone is released from the epidermis in general, or from a specific population of epidermal cells, has not been established.

Although voraxin enters the female via the gonopore, we do not know its site of action. The fact that testis homogenates or recombinant voraxin injected into the haemocoel

stimulates engorgement in virgin females suggests that voraxin's target is not in the wall of the seminal receptacle. A direct demonstration that voraxin is transported from the seminal receptacle to the haemocoel has to await, however, the development of an ELISA for voraxin. The simplest hypothesis to explain an effect on feeding behaviour is that voraxin stimulates neurons in the synganglion (a single neural mass that comprises the complete CNS in ticks) that innervate the pharyngeal muscles (directly or indirectly). But voraxin and 'male factor' are probably the same proteins, because the two proteins (α and β) constituting voraxin are also necessary and sufficient for hastening salivary gland degeneration and ovarian development in *A. hebraeum* (Weiss and Kaufman, 2004).

An apparent anomaly arises from three additional observations: (1) If partially fed female *A. hebraeum*, below the CW, are forcibly removed from the host, and 20E is injected into the haemocoel before returning them to the host, re-attachment is inhibited for a time; the duration of inhibition is a function of the dose of 20E (Weiss and Kaufman, 2001). (2) Also, when injected into virgin *D. variabilis* ticks while they are attached to the host, exogenous 20E reduces the subsequent rate of blood intake by 21–37% (depending on dose) that of controls (Thompson et al., 2005). (3) Furthermore, the haemolymph ecdysteroid titre of *A. hebraeum* rises significantly during the rapid phase of engorgement (15 ng ml^{-1} in unfed ticks, 52 ng ml^{-1} during the rapid phase, falling back to 22 ng ml^{-1} on the day of engorgement; Mao and Kaufman, 1999). So the highest natural ecdysteroid titre during the attachment period occurs at the very time that feeding rate is most rapid[*].

One might be able to reconcile these apparently contradictory observations as follows. The natural haemolymph ecdysteroid titre during the rapid phase of feeding is still relatively low compared to the exogenous amounts shown to inhibit tick attachment in *A. hebraeum*, and the exogenous amounts shown to inhibit feeding rate in *D. variabilis*. When 20E is injected into the haemolymph, the concentration is initially high, but falls rapidly, presumably because of two processes: movement from the haemolymph space to the rest of the body (the haemolymph volume of partially fed *A. hebraeum* is approximately 20-25% of body weight; Kaufman et al., 1980) and rapid excretion and/or metabolism (most of the injected ecdysteroid disappears from the haemolymph within 4-7 hours; Weiss and Kaufman, 2001). It is thus very difficult to attribute any physiological effect to a unique haemolymph

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[*] Following engorgement, however, haemolymph ecdysteroid rises much higher, achieving a titre in the range of 300-500 ng ml^{-1} (Kaufman, 1991; Lunke et al., 1992; Lomas and Kaufman, 1992b).

concentration. However, if one uses the initial concentration in the haemolymph following injection, the concentrations at which re-attachment was inhibited were 7.4 to 384 times the haemolymph concentration that prevails during the rapid phase of engorgement (Weiss and Kaufman, 2001). If one assumes that the haemolymph concentration during the rapid phase of engorgement of *D. variabilis* is similar to that of *A. hebraeum*, the ecdysteroid concentrations at which feeding rate of virgin *D. variabilis* was inhibited were 47 to 2365 times the haemolymph concentration during the rapid phase of engorgement (calculated from data presented by Thompson et al., 2005). Taken together, the small rise in ecdysteroid titre occurring naturally during the rapid phase of engorgement would seem to be insufficient to influence feeding rate. The functional significance of the haemolymph ecdysteroid peak during the rapid phase of engorgement, however, is still unknown, although some hypotheses were presented by Lomas et al. (1998). Consequently, voraxin/male factor may have distinct sites of action at the synganglion: one site at which it stimulates engorgement and another at which it hastens the release of the neuropeptide that stimulates ecdysone synthesis in the epidermis. Or indeed, voraxin might act directly on the epidermis to stimulate ecdysone release. Designing experiments to address these questions should not be difficult.

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Figure Legend

Figure 1. Unfed and fully engorged female *Amblyomma variegatum*, demonstrating the astounding size increase (~100 fold). Note that this represents the weight after salivation has disposed of the excess fluid of the blood meal. Hence the total meal volume would be 2-3 times the apparent size of the engorged female in this photo (Koch et al., 1974; Koch and Sauer, 1984). Photo courtesy of Prof. Frans Jongejan, University of Utrecht, The Netherlands, and University of Pretoria, Republic of South Africa, and reproduced with his kind permission.

Table 1. Mated and virgin fed weights for a number of ixodid tick species.

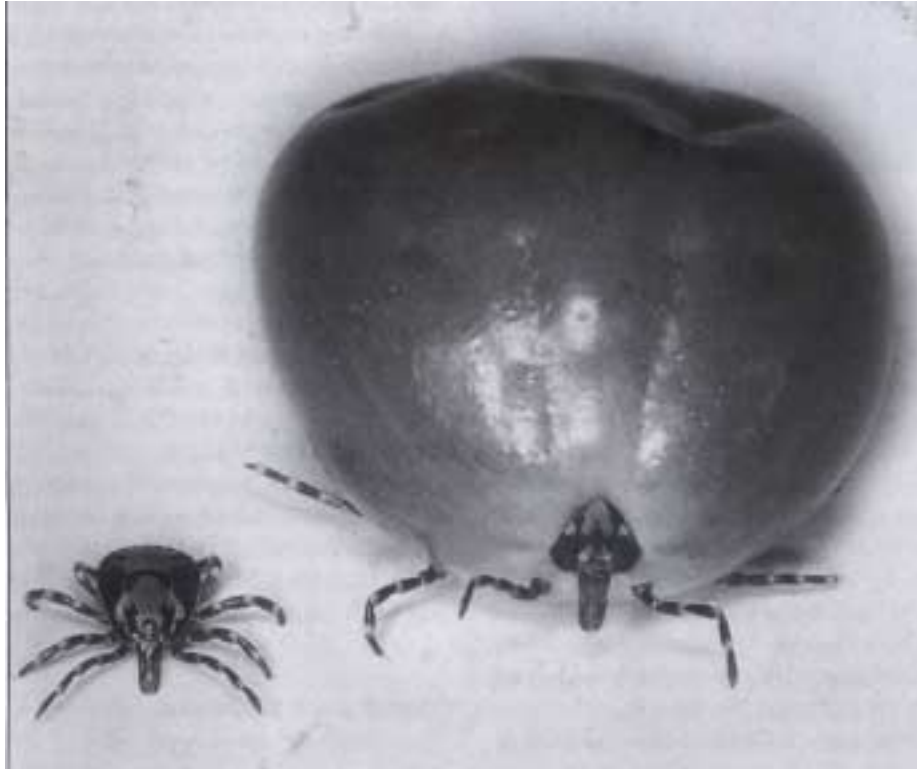| Species	Engorged weight (mg) mated			Maximun weight (mg) of virgin			Virgin-to-mated weight ratio
	Mean	SEM	N	Mean	SEM	N	
Amblyomma americanum	**682**	33	9	**31.3**	6.8	13	0.05
Dermacentor andersoni	**791**	44	14	**267**	33	15	0.34
Rhipicephalus sanguineus	**212**	30	13	**36.9**	5.4	10	0.17
D. variabilis (from Pappas and Oliver, 1972)*	**459**	-	-	**160**	27	6	0.35
Hyalomma anatolicum (from Snow, 1969)* For day 7 ticks: For day 11 ticks: For day 14 ticks:	**375** **440** **415**	- - -	- - -	**60** **120** **160**	- - -	- - -	0.16 0.27 0.39

* For Pappas and Oliver (1972) the mean engorged weight (459) was stated without SEM or N. For Snow (1969), I have estimated the values quoted by inspecting his Figure 1. In this case, the maximum virgin weight rose linearly with days on the host, whereas the engorged weights on various days appeared not to be significantly different. Thus the virgin-to-mated weight ratio increased steadily.

Methods: For each species (*A. americanum*, *D. andersoni*, and *R. sanguineus*), ticks were fed on the shaved backs of rabbits in a divided, foam rubber, cloth-covered arena glued to the back (Kaufman and Phillips, 1973). In one part of the divided arena males were absent (virgin female group), and in the other, an equal number of males was provided (mated female group). Ticks were fed for up to 28 days. All of the mated females engorged (i.e. detached spontaneously) within 12 days (*D. andersoni* and *A. americanum*) or 16 days (*R. sanguineus*). Few virgins ever detached spontaneously; they were removed and weighed after 18 days (*D. andersoni*), after 28 days (*A. americanum*) or after 16 days (*R. sanguineus*). *Ixodes ricinius* and *I. scapularis* were also tested, but most specimens were reluctant to attach to the rabbit and died.

Table(s)

Table 2. Average meal sizes in several vector arthropods: A comparison of mated and virgin females				
Arthropod	Average meal size (mg)[1]			
	Mated females	Virgin females	Virgin to mated ratio	Comments and reference
Tsetse fly (*Glossina palpalis*)	29.9	32.1	1.07 NS[2]	Meal 3, females fed every 2 days (Adlington et al., 1996)
Tsetse fly (*Glossina palpalis*)	34.8	32.6	0.94 NS[2]	Meal 4, females fed every 2 days (Adlington et al., 1996)
Kissing bug (*Rhodnius prolixus*)	-	-	~1	No difference during first meal, but virgins feed 12-22% less than mated females during subsequent meals (Davey, 1967)
Mosquito (*Culex pipiens*)	1.94 to 3.77	1.78 to 3.17	0.92 to 0.84 $p < 0.05$	Virgins feed 8-16% less than mated (gravimetric measurement; Adlakha & Pillai, 1976)
Mosquito (*Aedes aegypti*)	1.44 to 1.59	1.15 to 1.29	0.80 to 0.81 $p < 0.05$	Virgins feed 19-20% less than mated (gravimetric measurement; Adlakha & Pillai, 1976)
Mosquito (*Aedes aegypti*)	4.3	4.3	1.0	No difference between virgin and mated (spectrophotometric measurement; Klowden, 1979)
Bed bug (*Cimex lectularius*)	-	-	~1	No difference between virgin and mated (Ussinger, 1966)
Argasid tick (*Ornithodoros moubata*)	-	-	~1	No difference between virgin and mated (Aeschlimann & Grandjean, 1973; Germond & Aeschlimann, 1977; Diehl et al., 1982).

[1] Absolute meal sizes quoted only when data available for mated and virgin females in the same study.

[2] NS: not statistically significant

Kaufman, Figure 1