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Regulation of feeding and ovipositional success of *Amblyomma americanum* ticks[1]

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Summary. Duration of the adult female *Amblyomma americanum* feeding period was found to be dependent upon male receptiveness to mating, which was in turn dependent upon when males were placed with females. Prolonging the time to mating by 5 days had no effect on female engorgement weight or length of the preovipositional period, but prolonging mating by 10 days substantially decreased the number of ovipositing females and ablated egg viability. In the absence of males, females were severely stunted in size and had to be forcibly removed from the host.

Regulation of tick activity in the environment, either daily or seasonally, is fairly well understood[2,3]. However, knowledge of the biology of ticks when on the host is only now beginning to be understood. It is known that some tick species have preferred attachment sites to the host[4], and aggregation of the sexes on the host results from chemical (pheromone) communication[5,6]. However, basic information pertaining to events leading to engorgement, a process dependent upon mating, are still poorly understood.

Materials and methods. Female albino Hartley guinea-pigs weighing 500–600 g were used as hosts. Adult *Amblyomma americanum* ticks were confined to hosts using the capsule technique[7]. Female ticks were placed on all hosts at day 0, and male ticks were added on day 0, 5 or 10. One group of guinea-pigs had only females. All ticks were individually marked with yellow (females) or red (males) acrylic paint on the dorsal surface to facilitate observations on migratory and pairing behavior while inside the capsules. Hosts were housed in plastic cages (43 × 20 × 18 cm) and given food

(guinea-pig chow) and water ad libitum. During the tick feeding period, ticks were observed daily for changes in attachment sites, initiation of, or termination of pairing behavior, and detachment. Detached female ticks were weighed and placed in individual vials held at 85% relative humidity, 25 °C and 12 h L/D, and observed daily for oviposition. Once oviposition was complete, egg massess were weighed and observed daily for larval eclosion.

For each experimental group, the mean ± SE of the female feeding and preoviposition period, and engorgement and egg mass weight was determined. In addition, the number of engorged females that oviposited and the viability of the eggs, as assessed by subsequent hatch, were recorded.

Results. When males were added to infested hosts by day 5 of the female feeding period, or earlier (groups A and B), all females oviposited and weighed in excess of 300 mg (table). Deposited egg massess had a mean weight of 136–179 mg and were viable. However, if males were added later than day 5 (group C), there was a substantial decrease (40%) in

Effect of prolonging mating behavior on the feeding success, ovipositional success and egg viability of female *Amblyomma americanum* ticks

Group	Combination (sex) n = 10	Feeding time (days)	Mean weight (mg)	Oviposition (%)	Mean time to oviposition (days)	Mean egg mass weight (mg)	Egg hatch
A	Female + male day 0	9.7 ± 2.3[a]	327 ± 14.5	100 (10/10)	13.3 ± 1.3	136 ± 10.8	+
B	Female + male day 5	13.2 ± 1.0	376 ± 75.2	100 (10/10)	16.2 ± 0.5	179 ± 44.5	+
C	Female + male day 10	19.8 ± 0.8	362 ± 76	60 (6/10)	14.7 ± 1.2	136 ± 19.2	−
D	Female only	20.0 ± 0[b]	93 ± 3.3	10 (1/10)	18.0[c]	18.0[c]	−

[a] Mean ± SE; [b] all ticks were removed on day 20; [c] only one tick oviposited.

ovipositional success and an ablation of egg viability. Interestingly, goup C engorged females weighed greater than 300 mg, similar to those individuals from groups A and B, but only 6 of the 10 females from this group oviposited. This finding indicates only some females were mated. but all had normal weights. When only females were present (group D), mean engorgement weight was significantly reduced (93±3.3 mg), and all ticks had to be forcibly removed by day 20. This process resulted in removal of considerable amount of host skin indicating abscess formation, a typical inflammatory response to non-feeding attached ticks. A single female from group D oviposited, but the egg mass weighed only 18 mg and was not viable.

During daily observations on tick behavior within the feeding capsules it was noticed that some unmated females detached and reattached after having fed for 4-5 days. While this is a unique finding for females, it is a well known behavior for males[8]. It was also noticed that males would mate with more than one female and would actively fight other males for mating position with a female.

Discussion. Female *Amblyomma americanum* ticks attach and feed for several days and then stop, but generally remain attached until they are mated and then continue to feed[9]. Upon engorging with blood, females drop from the host and enter the preovipositional phase leading to egg deposition. Therefore, the greater the disparity in time between initial attachment of the sexes to a host, the more prolonged the female feeding period becomes (table). A similar finding was reported for *Dermacentor variabilis*[10].

Since mating must occur before the 2nd phase of feeding commences, it appears that the act of mating and not necessarily the transfer of a spermatophore is sufficient stimulus to initiate refeeding. That is, since only 60% of the females from group C (males added on day 10) oviposited, it may be assumed that spermatophore transfer occurred in 6 of the 10 mating episodes. In any event, feeding by all 10 females commenced after the males left their ventral surface as indicated by the high mean tick weights. However, the eggs deposited by these females were not viable suggesting a) spermatophore transfer did not occur during any mating episode yielding unfertile eggs, suggesting the act of mating was a proper stimulus to resume feeding, or b) spermatophore transfer did occur, but prolonged attach-

ment ot the host prior to mating, was deleterious to some component critical to embryo development.

The observation that all females from groups A and B were mated, whereas only some individuals from group C were mated, indicates a difference in female attractiveness. The longevity of pheromone production by attached females is not known, but it is known that maximum production for *Amblyomma americanum* is 2 weeks post-eclosion[10]. The ticks used in this study were 4 weeks post-eclosion. However, since females from groups A and B were mated and obviously attractive to males, it is assumed that the lack of attractiveness by some females from group C was the result of prolonged attachment prior to exposure to males. Since mating is essential for fertility, it also seems reasonable that females could compete with other females for males by altering their pheromone in a way to make themselves more attractive. This theory has not yet been examined.

From this study, and others[5-10], it is apparent that male ticks are very important to the feeding success of female ticks, and ultimately responsible for the production of viable eggs. To impede the aggregation of the sexes by altering pheromone production, secretion or desemination is to severely diminish the reproductive capabilities of the females, a result that has significant implications on tick biology. This weak link in the tick life cycle would appear to be an excellent target site for tick control, either with the use of isecticides or artificial immunization.

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Anti-feedant activity of the polyacetylene, phenylheptatriyne (PHT), from the Asteraceae to *Euxoa messoria* (Lepidoptera:Noctuidae)

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Summary. Phenylheptatriyne (PHT), a polyacetylene from various species of Asteraceae reduced feeding and weight gain of larvae of the polyphagous insect *Euxoa messoria* when incorporated into an artificial diet at concentrations of 10-300 ppm. These results suggest a role as insect antifeedants for the widely distributed polyacetylenes of the Asteraceae.

In a recent article Eisner et al.[2] demonstrated the antifeedant properties to the predatory jumping spider *Phidippus sp.* of a polyacetylene found in soldier beetles (*Chauliognathus* supp.). Since polyacetylenes find their widest occurrence in nature in the plant family Asteraceae[3], Eisner et al.[2] have speculated that acetylenic compounds may also serve, in plants, as antifeedants that are effective against phytophagous insects.

We have been making a systematic study of the biological activity of polyacetylenes including their activity towards insects[4,5] and report, in this communication, the antifeedant

activity of the polyacetylene phenylheptatriyne (PHT) (fig. 1) to the dark-sided cutworm, *Euxoa messoria* (Lepidoptera: Noctiudae), polyphagous species. PHT is found in the leaves and stems of *Bidens pilosa* as well as numerous other composites where it can also be present in roots[3].

Material and methods. Isolation of PHT has been described previously[6], as have rearing conditions on artificial diets for *Euxoa*[7,8]. Groups of 8 larvae in their late instars were selected for uniform size (approximately 0.5 g) and were starved for 24 h before being offered a diet cube treated