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MATING ACTIVITY AND PHYSIOLOGICAL AGE OF FEMALES IN POPULATION OF *IXODES RICINUS* (L.) (ACARI: IXODIDAE)

ПОЛОВАЯ АКТИВНОСТЬ И ФИЗИОЛОГИЧЕСКИЙ ВОЗРАСТ САМОК В ПОПУЛЯЦИИ КЛЕЩЕЙ *IXODES RICINUS* (L.) (ACARI: IXODIDAE)

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ABSTRACT

Four categories of physiological age were distinguished in females of *Ixodes ricinus* (L.) according to the morphological characteristics: newly emerged, physiologically young, mature and old ones. No newly emerged females were collected in the field by flagging. Spring and summer cohorts of young females were recorded during the season. The peak occurrence of mature females followed the peaks for young females. Old females occurred mainly during the second half of the season. The mean weight of unfed young females was higher than that of mature and old females. From 25±5 females investigated each week, the number of mated ones started at 11.1% in March and reached 92.3% in June. A considerable decrease in the number of mated females during August and September up to 40% was followed by an increase to 65%) at the end of season. Only 6 of 25 (24%) mated laboratory females fully engorged without males in contrast to 23 (92%) females engorging with males. The mean weight of fully engorged females feeding together with males was significantly higher (0.30 g) than of females feeding without males (0.24 g). Male presence during feeding of mated female increased the success of female feeding.

РЕЗЮМЕ

По морфологическим характеристикам были дифференцированы четыре возрастные категории самок *Ixodes ricinus* (L.): нововылупившиеся, физиологически молодые, достигшие половой зрелости и старые. В полевых сборах клещей на флаг нововылупившиеся самки обнаружены не были. Вслед за пиками массового выхода молодых самок следовало появление максимального количества физиологически зрелых самок. Сезонная динамика числа самок указывает на наличие весенней и летней когорт молодых самок. Старые самки встречались в основном во второй половине сезона. Средний вес голодных молодых самок был больше, чем вес зрелых и старых самок. При еженедельном исследовании 25±5 самок обнаружено, что доля оплодотворенных особей возрастала с 11.1% в марте до 92.3% в июне. В период с августа по сентябрь количество оплодотворенных самок значительно уменьшалось (до 40%), а в конце сезона вновь увеличивалось (до 65%). В отсутствии самцов только 24% оплодотворенных лабораторных самок (6 из 25) полностью закончили насыщение, а в присутствии самцов количество насосавшихся самок возросло до 23 (94%). Средний вес самки, накормленной в присутствии самца, также был значительно больше (0.30 г), чем при его отсутствии (0.24 г). Таким образом, присутствие самцов при кормлении оплодотворенных самок положительно влияет на завершение процесса их насыщения.

INTRODUCTION

The tick *Ixodes ricinus* (L.) is widely distributed in all of Europe. In different part of the distribution area the tick inhabits different biotopes. In west Russia i.e. at the eastern part of distribution area, it occurs mostly on livestock pastures covered with alder, spruce, juniper and birch with swampy holes with bent-grass and sphagnum [Pomerantzev, 1935]. On the British Islands, at the western part of distribution area, the tick inhabits mostly rough pastures, woodlands and moorland grazings and places covered by ferns and heather. The main hosts are sheep [Milne, 1944, Varma, 1965, Gray, 1987]. In Central Europe, the same tick species inhabits dry oak forests, mixed forests (*Querceto-Carpinetum*), scree wood and flood-plain forests [Cerny, 1957]. The main hosts are wild mammals (rodents, insectivores, hares, small predators, roe deer), in less extend also livestock. The humid oceanic climate of British Islands differs sufficiently from the dryer Middle European continental climate and modifies tick biology, mostly in daily vertical migration and seasonal activity [Lees and Milne, 1951; Cerny, 1957; Dyk and Bouckova, 1968; Gray, 1991; Daniel and Dusbabek, 1994]. Estrada-Peña et al. [1996] found the differences between continental and British ticks also in the structure of cuticular hydrocarbons.

Feeding on a host is essential for tick development and reproduction. Females, however, are not able to finish feeding successfully without mating [Graf, 1974]. It has been long believed that *I. ricinus* copulation takes place predominantly on the host [Cerny, 1957]. However, Graf [1974], Babenko et al. [1979] and Gray [1987] confirmed that copulation in this species also takes place in the field before attachment to the host. These authors noticed that the number of mated females increases to 90 % at the end of the active tick season. Studies of mated female numbers and age structure of population during the year were done in the field only in Scotland, Ireland and Russia. It could be expected, that the Middle European Czech population could differ in these parameters due to different living conditions.

MATERIALS AND METHODS

Climate. The climate of the study area is typically continental. The year 2003 was extremely warm and dry with a mean temperature 9.4°C (+ 0.8°C deviation from thirty year average) and precipitation amounting to 488.8 mm (only 79% of thirty year precipitation average). The actual temperature and relative humidity at the ground surface level during tick collection were 11–38°C and 22–86%, respectively, measured by the BT-254 digital humidity & temperature monitor (Bionaire Europe B.V., The Netherlands).

Ticks origin and breeding. Ticks were collected in a non-cultivated meadow overgrown with willows, birches, hornbeams, aspens, oaks and solitary pine trees and situated near Ceske Budejovice, South Bohemia. During the whole active tick period (March-October, 2003), 25 (±5) females were randomly selected from a weekly sample of ticks collected by flagging during weeks 11–40th (with an exception of a rainy week 15 during which we failed to collect females). Collected females were kept separately at 4°C overnight and examined for physiological age statement and presence of spermiophores the next day.

Laboratory ticks originated from the line bred at the Institute of Parasitology, ASCR, Ceske Budejovice. They were kept at 25–26°C, 96–98% RH, at a photoperiod 14L:10D and fed on guinea pigs for 5–7 days up to natural dropping off (fully engorged females). A neoprene cell glued to the shaved back of a guinea pig with a topical skin adhesive (cyanocrylate adhesive, Henkel, Germany) was used. Altogether 25 females with or without males were put inside the neoprene cell, which was closed using fine meshed gauze. Guinea-pigs were kept in a climate-controlled room (22±2 °C, 60±10% RH, in a LD 14:10 h photocycle).

Virgin females and males were obtained from engorged nymphs kept separately in wells of an ELISA microplate. Each well was covered by a foam rubber plug and the microplates were kept under breeding conditions. Freshly moulted females and males were kept for 10–14 days under breeding conditions before being used for experiments. Ticks were weighed on digital balances R 180 D with precision 0.01 μg (Sartorius Gmbh, Göttingen, Germany).

Tick dissection. A series of five unengorged females was fixed with capitulum down on a paraffin filled Petri dish (5 cm in diameter) using a hot needle. They were covered by sterile saline (0.85% NaCl). The distal part of the alloscutum was cut off by microsurgery-scissors, the internal genital organs were removed using sharp watchmaker tweezers and they were placed on a microscopic slide. The organs were squeezed by a cover slide and observed by microscope for presence of spermiophores in seminal receptacles untreated or they were fixed by methanol and Giemsa stained.

Determination of the physiological age of females. Four features were applied to differentiate the female physiological age *in vivo* according to

Table 1
Criteria for determination of the physiological age of *Ixodes ricinus* females (according to Razumova, 2000, adapted)
Таблица 1
Признаки для определения физиологического возраста самок *Ixodes ricinus* (по Разумовой 2000, адаптировано)

	Colour of cuticle	Body volume	Shape of idiosoma	Midgut
1.Newly born	Brownish	thickened	convex	not visible
2.Young	Reddish	thickened	convex— slightly concave	not or hardly visible
3.Mature	Reddish	slightly thickened	concave — slightly convex	hardly visible or visible
4.Old	Reddish	flattened	concave	visible

Razumova [2000]: (**a**) colour of cuticle, (**b**) body volume, (**c**) shape of idiosoma, (**d**) midgut visibility (fullness). According to these characteristics in combination, four categories of physiological age were distinguished in *I. ricinus* females (Table1). However, this method is able to state the physiological age only of individuals studied, i.e. the grade of maturation or ageing of tick organism and not the real length of life. Therefore, the age categories in Tab.1 and in the text should be understand as physiologically young, physiologically mature and physiologically old females.

RESULTS

The number of mated (fertilized) females in the *Ixodes ricinus* population studied increased during the year from 11.1% on week 11 (March 10–16) to 92.3% on week 24 (June 9–15) and after small decrease once more to 92% on week 31 (July 28–August 3) (Fig. 1).

Three peaks in the seasonal occurrence of young questing females were recorded during the season (Fig. 2). After the first peak of young females (50%) on week 13 (March 24–30), the number of young females increased once more up to 73.1% on week 18 (April 28–May 4) and 76.9% on week 22 (May 26–31). After dropping down to 30.8% during week 25 (June 16–22), the number of young females increased again and culminated to 88% on week 28 (July 7–13). After this week, the occurrence of young females decreased and from week 30 oscillated between 50% and 53%, with the exceptions of weeks 33, 35 and 38 (August 11–31 and September 15–21), when the number of young females decreased to 20%, 20% and 28%, respectively.

Mature females occurred most frequently at the start of the season, reaching the maximum 84.6% at week 14 (March 31–April 6) (Fig. 2). The next peak of mature female occurrence appeared between weeks 21–26, following the peak of the occurrence of young females. The decrease to 12% and 11.5% at weeks 28 and 29 (July 7–20) respectively was followed by another increase beginning week 30 (July 21–27). The number of mature females then oscillated between 30% and 40%, with the exception of the week 38, when 48% mature females were recorded.

Only one old female (3.8%) was found at the start of the season at week 14 and regularly they were found from week 23 (June 2–8) when they reached the number 15.4%. The maximum number of old females was recorded in August (weeks 33 and 35), when 40% of those females were identified among collected questing females (Fig. 2).

The mean overall weight of young unfed females was significantly higher than the overall mean weight of mature females (1.612 mg versus 1.571 mg) which was higher than the overall weight of old females (1.394 mg) (t-test, T-value =1.7502, P= 0.0806; t-test, T= 5.6052, P= 3.7800e-8; t-test, T= 4.4410, P=1.2300e-5) (Table 2). The same relation was also observed between young, mature and old females if week average weights were compared.

Feral ticks of unknown mating history were able to attach to the host and fully engorge without males with varied success. Out of 25 females engorging in May and June, 17 (68%) and 25 (100%) attached, but only 12 (48%) and 11 (44%) females, respectively, fully engorged. In the second half of the season (July and August), 4 (16%) and 17 (68%) females attached and all engorged (Fig. 3). However, these data are not in correlation with the number of mated females recorded in these months.

In control experiments with laboratory ticks, mated females (with spermatophore) feeding with-

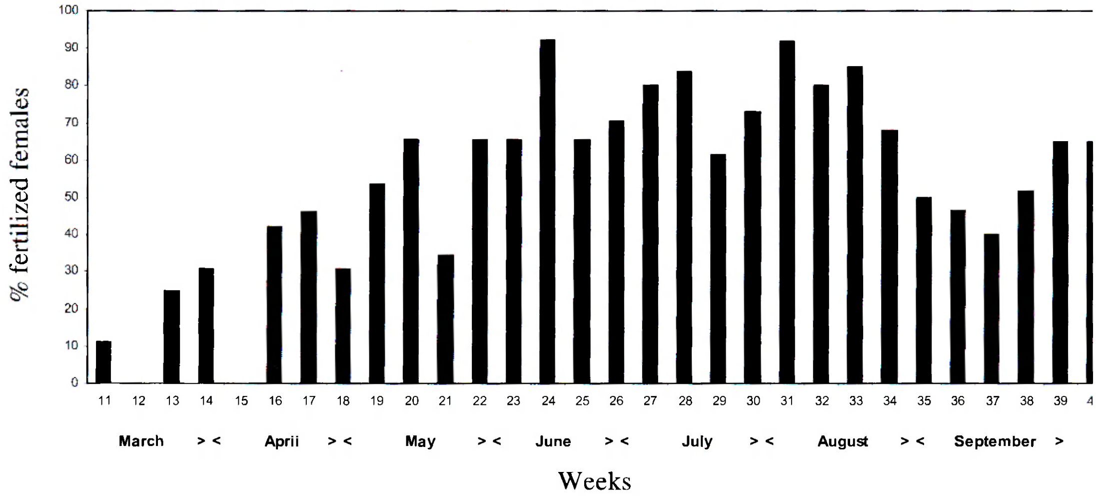

Fig. 1. The percentage of mated questing females in population of *Ixodes ricinus* during the season 2003 (n= 25±5 each week).
Рис. 1. Количество копулировавших активных самок в популяции *Ixodes ricinus* в течение сезона 2003 г. (25±5 ос. еженедельно).

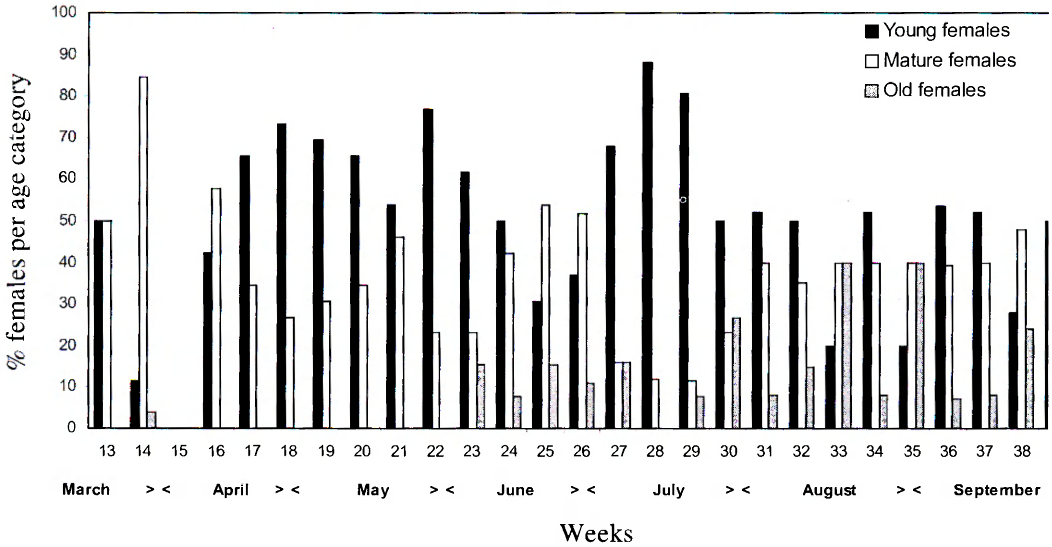

Fig. 2. Physiological age of questing females of *Ixodes ricinus* during the season 2003 (n= 25±5 each week).
Рис. 2. Физиологический возраст активных самок *Ixodes ricinus* в течение сезона 2003 г. (25±5 ос. еженедельно).

out males were less successful in attachment and engorgement than females feeding together with males. Only 13 (52%) of 25 females attached to the host without males and only 6 (24%) of them fully engorged. All females feeding together with males attached and 23 (92%) fully engorged (Fig. 3).

The mean weight of mated and fully engorged laboratory females feeding together with males was 0.30 g and significantly higher ($p<0.05$ of Wilcoxon Rank Sum Test, a=6, m=20, W=52) than the weight of mated and fully engorged laboratory females feeding without males (0.24 g) (Fig. 4). The mean weight of these females was not different from the weight of engorged feral ticks feeding without males (0.26–0.29 g) from May to July ($p>0.05$, Kruskal Wallis test, K=13.16). However, the mean weight of feral fully engorged females feeding in August without males was significantly higher (0.35 g) than that of engorged females feeding with males.

DISCUSSION

Uspensky [1995] characterised the physiological age of ticks as the state of nutritional reserves in tick body. Physiological age can be assessed from the accumulation of irreversible changes in the organism as a consequence of its normal life [Beklemishev, 1962]. It does not correspond with real age (length of life) because of each individual is exposed to different environmental stresses and these changes proceeds with different rate in different individuals. Balashov [1998] summed up the criteria for

Table 2

Mean weight of unfed questing females of *Ixodes ricinus*

Таблица 2

Средний вес голодных активных самок *Ixodes ricinus*

Females	n	Σ (mg)	*Mdn*	*M*	σ
Young	350	564.24	1.62	1.612 [a, b]	0.015
Mature	256	402.07	1.57	1.571 [a, c]	0.018
Old	70	97.60	1.43	1.394 [b, c]	0.039

[a] t-test, T-value =1.7502, P= 0.0806; [b] t-test, T= 5.6052, P= 3.7800e-8; [c] t-test, T= 4.4410, P= 1.2300e-5

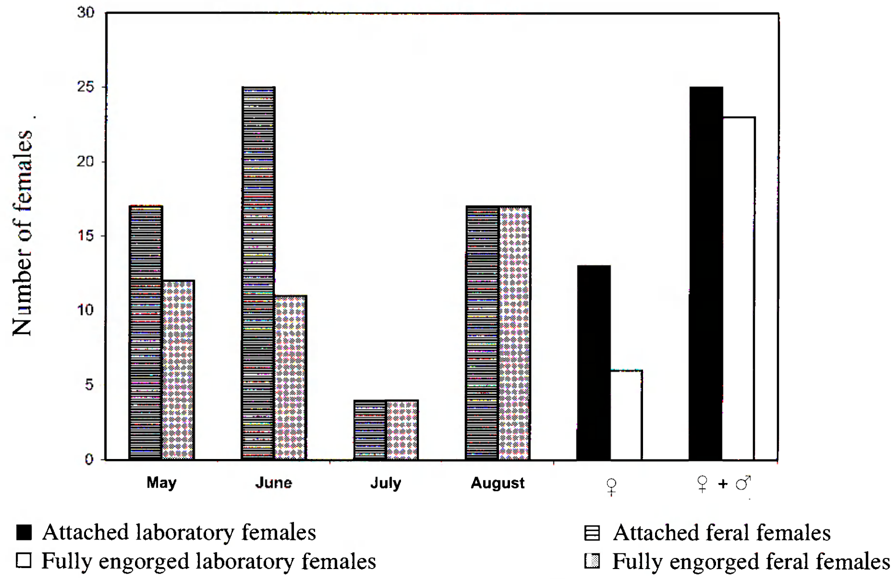

Fig. 3. Attachment and feeding success of feral females and mated laboratory females of *Ixodes ricinus* feeding with and without males (n=25 each).

Рис. 3. Успех присасывания и кормления самок из природы и копулировавших лабораторных самок *Ixodes ricinus*, в присутствии и в отсутствии самцов (каждая группа из 25 ос.).

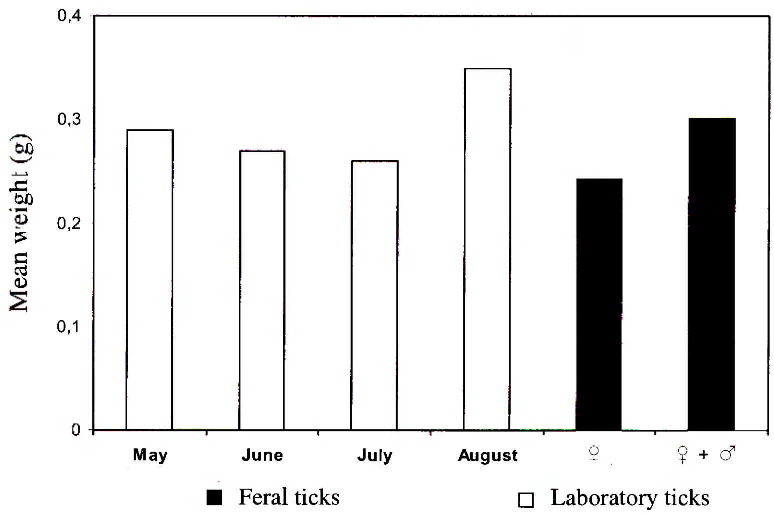

Fig. 4. Mean weight of fully engorged feral females feeding without males (white columns) and mated laboratory females of *Ixodes ricinus* feeding with and without males (black columns) (n= 25 each).

Рис. 4. Средний вес природных самок, накормленных в отсутствии самцов (белые колонки) и оплодотворенных лабораторных самок *Ixodes ricinus*, накормленных в присутствии и в отсутствии самцов (черные колонки) (каждая группа из 25 ос.).

physiological age statement including external and internal changes in tick body. Razumova [2000] respecting these criteria developed the visual method for determining the physiological age of ixodid ticks *in vivo,* which was accepted in our study.

Many females of *Ixodes* ticks mate before feeding during the whole season. Graf [1974], Babenko et al. [1979] and Gray [1987] recorded the maximal number of mated females in the second half of tick activity season. In our studies, the highest number of mated females was recorded in June and August, when two peaks of tick population density occur [Zapletal, 1955, Cerny, 1957, Bouckova and Dyk, 1967]. These two peaks correlate with the occurrence of young females in the population. The peaks of mated females (weeks 24 and 31) follow the peaks of young females (weeks 22 and 28). The maximum amount of young females on weeks 18 and 22 preceded the peaks of mature females on week 21 and 25 respectively. After a sharp decrease in weeks 27–30, the occurrence of mature females oscillated around 10 for the rest of season and the occurrence of old females increased. This is probably the consequence of regular population ageing. However, it must be taken into consideration that the questing mature and old females are only the rests of population of young females, which were not able to find an adequate host and remained still unengorged.

Young, mature and old questing females were collected by flagging. No newly emerged questing females were recorded using this method. According to Anderson et al. [1998] questing behaviour in *Amblyomma hebraeum* Koch increases over a 6 weeks period following moulting together with increase of the responsiveness to CO_2. In the laboratory reared females of *I. ricinus* in our experiments we observed the characteristics of newly emerged females only 2–3 days after moulting.

At the start of the season, predominantly mature females occurred. They probably were those hibernating as unengorged adults, similarly as small numbers of old females from week 14. The origin of young females from the week 13 and 14 is uncertain. They probably are a vanguard of the first cohort of young females. This cohort, representing the peak in May and June (weeks 18–22), originated from nymphs hibernating as engorged ones. A second cohort of young females, appearing in July (week 27–29), probably originated from nymphs overwintering in an unengorged state and engorging during spring [Chmela, 1969, Daniel et al., 1976, 1977, Perez and Rodhain, 1977].

Walker [2001] also observed in southwest Scotland bimodal curve of questing activity of *I. ricinus*. In his studies ticks were age graded by dissection of gut and Malpighian tubules and stained for lipid with Sudan red. Using laboratory ticks, he distinguished 5 age grades, the first being young ticks, the fifths the old one. In the field he recorded the peaks of young female occurrence in March and September and old females in July and October. The small differences of our observations are probably caused by different living conditions in both regions studied.

Young unfed females weighted more than mature and old females probably as a result of the loss of body fats and fluids in the latter groups. This finding correlates with the characteristics of the age groups in which young females are characterised by a thickened idiosoma, mature females by a slightly thickened idiosoma and old females by a flattened idiosoma [Razumova, 2000].

Our experiments confirmed that mated female ticks are able to feed and fully engorge without males. The feeding success of these females, however, is lower than in females feeding together with males. Although several mated females attach to the host without males, in many cases they are not able fully engorge. This behaviour is probably due to the absence of the phase of rapid feeding during the second half of feeding process [Graf, 1974]. According to Papas and Oliver [1972], in *Dermacentor variabilis* (Say) the stimulus for female rapid engorgement involves receiving the spermatophore and/or its content minus spermatids. The spermatophore content probably operates as a rapid engorgement stimulus or it may act to release or neutralise an engorgement inhibitor. Weiss and Kaufman [2004] described the engorgement factor (EF) in *Amblyomma hebraeum* engorged males. Extracts of the *testis/vas deferens* contain EF bioactivity when injected into hemocel of virgin females increasing their engorgement weight. These extracts also stimulate salivary gland degradation and partial development of ovary. The authors also prepared the *A. hebraeum* recombinant EF and proposed the name *voraxin* for the natural EF of ticks.

Feldman-Muhsam et al. [1970] mentioned salivary secretion of the male ticks during copulation in some metastriate ticks. Male saliva in some ticks contains immunoglobulin-binding proteins [Wang and Nuttall, 1994, Wang et al., 1998], that stimulate blood feeding in co-feeding females. One can speculate that if these proteins are present also in *I. ricinus* male saliva, they could penetrate during copulation to female body and help the female to feed, too. This process could also act as a rapid engorgement stimulus for female feeding.

The ability of females to fully engorge may also depend on the development of spermatids (capacitation) in the female reproductive tract [Oliver, 1982] and their survival. The destruction of spermatids in the female genital tract may occur during the time, as also confirmed by our observations (unpublished data). Therefore, females are probably able to fully engorge without the presence of males only for limited time after copulation.

Males of *I. ricinus* do not feed on the host and autogenous spermatogenesis must precede copulation [Kiszewski et al., 2001]. Therefore, attachment stimulation by aggregation-attachment pheromone [Gladney et al., 1974, Sonenshine, 1986, Norval et al., 1991] as described in some *Amblyomma* species is out of question.

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