SEXUAL BEHAVIOUR OF THE SHEEP TICK, *IXODES RICINUS* (L.) (ACARI, IXODIDAE)

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Abstract - The pattern of pre-copulatory and copulatory behaviour of the sheep tick, *Ixodes ricinus* (L.) was studied. Male attraction to female and subsequent copulation operate under the control of pheromones. Both engorged and unengorged (questing) females emit volatile substances which attract males, causing their pre-copulatory behaviour. About 90.6-97.6 % of active males are attracted to engorged females in a four-arm olfactometer, whereas 76.4-78.3 % in an Y-tube olfactometer. Unengorged females cause positive reaction in 55.6-62.5 % of males in the four-arm olfactometer and 57.3-70.9 % in the Y-tube. Reactions of ticks from our laboratory colony were less intensive than of those from nature. The attracting substance was trapped in an U-tube submerged in liquid nitrogen and then deliberated into an olfactometer. About 86,7 % of active males were attracted with such trapped and deliberated attractant. This attractant probably plays the role of the attractant sex pheromone (ASP) in the sheep tick. A high stimulatory effect of semi-engorged females on male sexual behaviour was observed. The following steps of mating were recognised in male behaviour: (1) excitation with ASP and localisation of the female as an emitting source, (2) contacting the female, (3) mounting onto her dorsal surface, (4) crawling over the female opisthosoma to the ventral surface, (5) location of the female gonopore, stretching out the palps and inserting chelicerae and hypostome into the gonopore, (6) repeated copulatory movements, (7) lifting of male opisthosoma, shifting the genital opening above the female gonopore and ejaculation of the spermatophore, (8) fixation of the spermatophore in the female gonopore.

Key words - Sexual pheromones, attractant, mating, copulation, ticks

INTRODUCTION

The sheep tick, *Ixodes ricinus* (L.), is the vector of Lyme borreliosis and the primary vector of tick-borne encephalitis. It is also notorious as vector of ehrlichiosis, Boutenneuse fever and tularemia in humans and several livestock diseases, especially bovine babesiosis, tick pyaemia and Louping ill. Traditional control methods using pesticides are enormously expensive, environmentally damaging and due to development of resistance against several pesticides, less effective. There is an urgent need to develop an efficient, environmentally safe method for the control of this tick. Perspective reveals to be two innovative methods: (a) The use of anti-tick vaccines and genetically tick-resistant animals (Brossard, 1998) and (b) the manipulation with tick pheromones (Dusbábek *et al.*, 1997; Allan *et al.*, 1998). In *I. ricinus,* the sexual pheromones (Graf, 1975, 1978a, b; Gray, 1987) seem to be the most perspective as the potential mating disruptants. An artificially overcrowded environment with female sexual pheromone may cause the disorientation of males and their inability to localise female and mate. The mating prevention may result in the reduction of the tick population density, especially in forest, suburban and urban populations of the sheep tick. For the development of this method of control, an exact knowledge on tick pre-copulation and copulatory behaviour is necessary as well as isolation and identification of the female sexual pheromone. Therefore, we present here the results of the first part of our studies, the observation of the pre-copulation and copulatory behaviour.

MATERIALS AND METHODS

Ticks

Ticks were collected from localities around České Budějovice from March 1997 to October 1998 and a pathogen-free laboratory colony was established at the Institute of Parasitology, Academy of Sciences

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Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

of the Czech Republic in České Budějovice. Ticks were kept at 20 ºC and 90-95% r.h. under a 14L:10D light/dark cycle and allowed to feed on guinea-pigs.

Apparatus

Two airflow olfactometers were used to study the male response to female sexual stimuli. The open system with an Y-tube (Figure 1) and a closed four-arm system according to Vet *et al.* (1983) (Figure 2). The air flow in the Y-tube system was 46 ml /min., and 300 ml/min. in the four-arm system.

A photomicroscope Wild equipped with a high resolution B&W CCD camera attached to a digital video recorder was used for studies of mating behaviour of ticks in a small glass arena (1 cm in diameter, height 0.3 cm). Videotaped sequences were analyzed for frequency and duration of individual behavioural events by means of The Observer, Video Tape Analysis System (Noldus Information Technology, 1994). Video frames with characteristic behaviour were captured by PC frame grabber and printed by means of digital photo printer. Mating activity of isolated pairs of ticks was also studied under constant laboratory conditions (14L:10D, 26 ºC).

Bioassay

Ticks from both the field and laboratory were used for bioassays. Field males were tested versus field ticks and laboratory males versus laboratory ticks. About 10 unengorged females or males and 5 engorged females , situated in the appropriate jar, served as the pheromone source. Males were placed on a starting point (beginning of the Y-tube or beginning of the starting tube in the four-arm olfactometer) and observed for 5 min. maximally. Reactions were evaluated as positive when males reached the end of the Y-tube branch with the attractant source or when males reached the orifice of that tube in the four-arm olfactometer. All experiments were done under artificial light and laboratory temperature.

Statistics

The χ^2-test and the G-test of goodness of fit according to Sokal and Rohlf (1981) were used for statistical assessment of results. The size of samples and the number of replications is apparent from Tables 1 - 3.

RESULTS

In the summer season all males of the sheep ticks, *I. ricinus*, originating from the field or laboratory, moved actively against the air flow in the Y-tube. In the four-arm olfactometer more than a half of males walked actively around the experimental arena (Table 1). During the winter period (October - February), the activity of males decreased, mostly in response to engorged females (Table 2).

The majority of active males were attracted to engorged and unengorged conspecific females and walked actively to the source of attractant, both in the four-arm olfactometer and Y-tube apparatus. However, they were rather repelled by other males (Table 3). In general, engorged females were more attractive for males than unengorged ones and the females from the field were more attractive than females from laboratory colony. Male reaction to engorged females was more conspicuous in the four-arm olfactometer than in the Y-tube. The attractant of engorged females was possible to trap in a U-tube submerged in liquid nitrogen and then deliberate it back into the four-arm olfactometer as a source of pheromone. About 86.7% of active males were attracted with such deliberated attractant.

A high stimulatory effect of semi-engorged females on male sexual behaviour was observed. The following steps of male activity in the mating process were recognised: (1) excitation and localisation of female as an source of sexual attractant, (2) contacting the female, (3) mounting onto her dorsal surface, (4) crawling over the female opisthosoma to the ventral surface, (5) location of female gonopore, stretching out the palps and inserting chelicerae and hypostome into the gonopore, (6) repeated forward, backward-, and side- copulatory movements, contorting the capitulum to perpendicular position to the female body up to turning it backwards under male body, broadly opening the female gonopore by

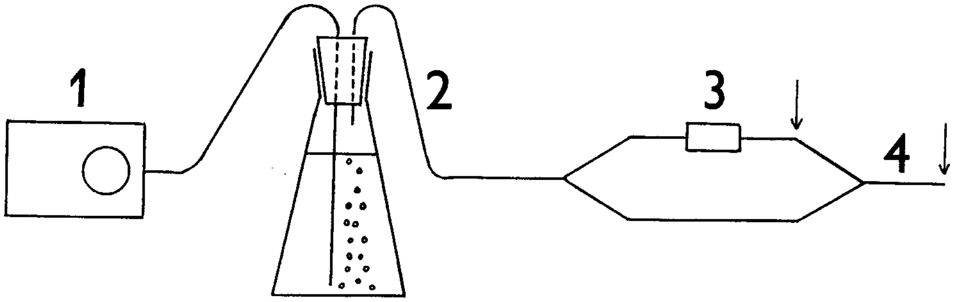

Figure 1. Scheme of the Y-tube olfactometer. 1 - aquarium air pump, 2 - water jar (humidificator), 3 - jar for source of pheromone, 4 - Y tube with starting and ending points marked with arrow-heads.

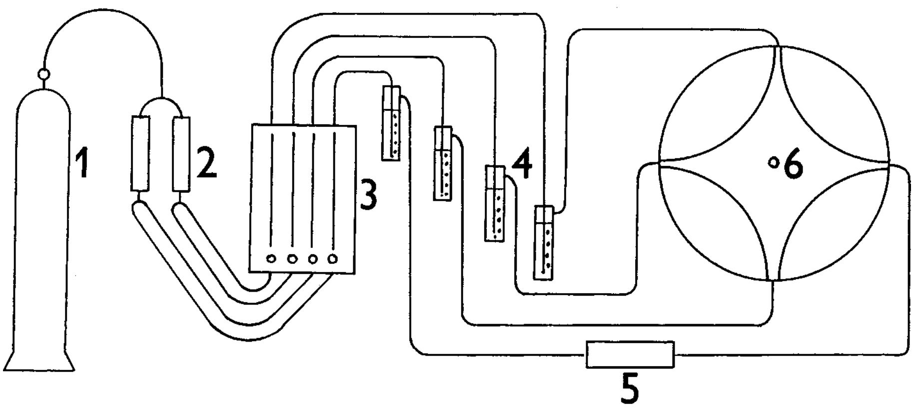

Figure 2. Scheme of the four-arm olfactometer. 1 - pressed air bomb, 2 - air filters, 3 - flowmeters, 4 - water jars (humidificators), 5 - jar for source of pheromone, 6 - exposure chamber with tick introduction tube in centre.

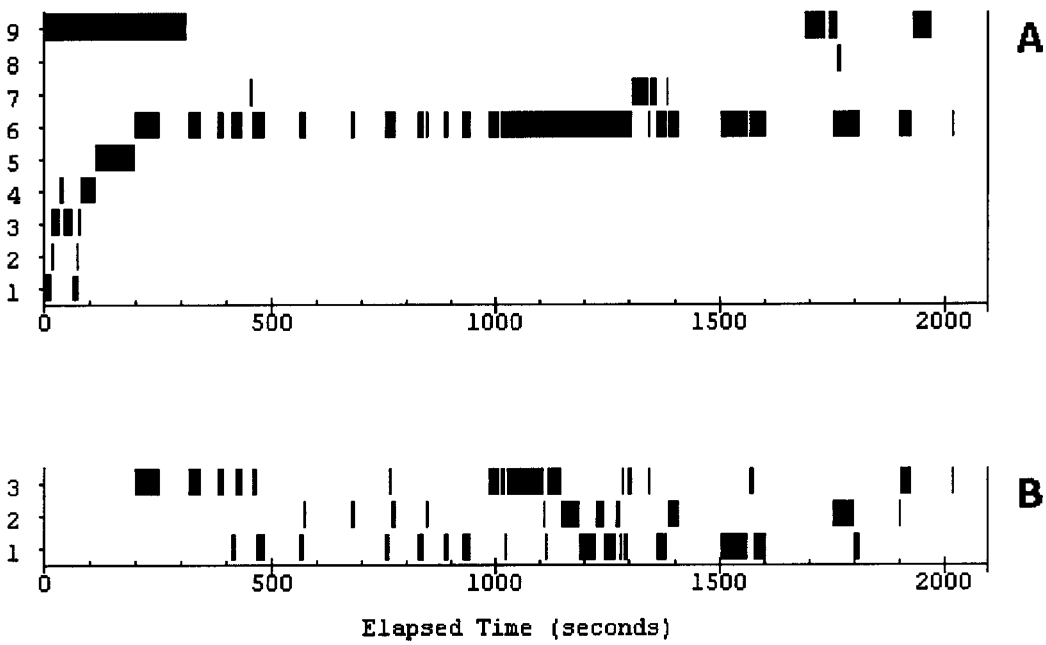

Figure 3. Time-event plot of mating behaviour pattern in an unexperienced male exposed to semi-engorged virgin female of *Ixodes ricinus*. **A:** The tracks 1-8 indicate the behaviour of male, 9 the behaviour of female, respectively : 1 and 9 - walking; 2 - contacting female; 3 - mounting onto female dorsal body surface; 4 - crawling to her ventral surface; 5 - location of the female gonopore and inserting chelicerae and hypostome into it; 6 - copulatory movements; 7 - lifting opisthosoma; 8 - fixation of the spermatophore in gonopore. **B:** The tracks 1, 2 and 3 indicate forward, backward and side copulatory movements of male, respectively.

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Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

Table 1. Activity of *Ixodes ricinus* males in summer period 1998

Males vs. engorged females	Four-arm olfactometer			Y-tube		
	N	active	%	N	active	%
Field ticks	97	67	69.1 a[1]	400	400	100 d
Laboratory ticks	87	52	59.8 a,b	800	800	100 d
Males vs. unengorged females						
Field ticks	98	58	59.2 a,b	452	452	100 d
Laboratory ticks	116	58	50.0 b	145	145	100 d
Males vs. unengorged males						
Field ticks	170	60	35.3 c	400	400	100 d
Laboratory ticks		not done		800	800	100 d

[1]) χ^2-test ($p<0.05$). Treatments not followed by the same letter are different ($p<0.05$) in the G-test of goodness of fit (Sokal and Rohlf, 1981).

Table 2. Activity of *Ixodes ricinus* males in the winter period 1997/1998.

Males vs. engorged females	Four-arm olfactometer			Y-tube		
	N	activity	%	N	activity	%
Field ticks	214	105	49.1 a	40	5	12.5 c
Laboratory ticks	297	132	44.4 a,b	40	16	40.0 b
Males vs. unengorged females						
Field ticks	103	63	61.2 d		not done	
Laboratory ticks	171	50	29.2 e		not done	
Males vs. unengorged males						
Field ticks	165	50	30.3 e		not done	
Laboratory ticks	116	58	50.0 a		not done	

[1]) χ^2-est ($p<0.05$). Treatments not followed by the same letter are different ($p<0.05$) in the G-test of goodness of fit (Sokal and Rohlf, 1981).

Table 3. Attractivity of females of *Ixodes ricinus* for conspecific males in summer period 1998.

Males vs. engorged females	Four-arm olfactometer			Y-tube		
	N	attraction	DP[1]	N	attraction	DP[1]
Field ticks	59	55	97.6 a[2]	398	313	78.3 d
Laboratory ticks	50	39	90.6 a	1200	917	76.4 d
Males vs. unengorged females						
Field ticks	51	24	62.5 b	1035	734	70.9 d
Laboratory ticks	56	24	55.6 b	286	164	57.3 b
Males vs. unengorged males						
Field ticks	51	16	27.1 c	425	161	37.9 c
Laboratory ticks		not done		800	266	33.3 c

[1]) DP - Degree of preference according to Otieno et al. (1985) = [1- (No. of ticks not attracted / 3) / (No. of ticks attracted)] . 100
[2]) χ^2-test ($p<0.05$). Treatments not followed by the same letter are different ($p<0.05$) in the G-test of goodness of fit (Sokal and Rohlf, 1981).

mouthparts as by lever, (7) lifting opisthosoma, shifting the male genital opening above the female gonopore and ejaculation of a spermatophore, (8) fixation of the spermatophore into the female gonopore (Figures 3 and 4). The copulatory phase (1) - (5) and (7) - (8) is very short, lasting only seconds or minutes. The phase of copulatory movements (6) last several hours (Figure 3).

The preliminary results indicate lower mating activity in unengorged females collected in the field (50% of pairs mated during the 1st day; average duration of mating during photophase was 4.1 hours) compared to semi-engorged laboratory females (60% of pairs mated during the 1st day; average duration of mating during photophase was 6.6 hours).

DISCUSSION

Male sheep ticks, *I. ricinus*, are attracted over distance to females by semiochemical stimuli and the sexual behaviour is therefore influenced by sexual pheromones, like as in other animals (Graf, 1975). Our results confirmed the volatile nature of this pheromone, which is emitted by both the engorged and unengorged females. This attractant in the sheep tick probably plays the role of an attractant sex pheromone (ASP), the pheromone described in Metatstriate ticks (Berger *et al.*, 1971) and identified as 2,6-dichlorophenol (Berger, 1972). However, the Prostriate tick *I. ricinus* does not respond to this substance (Graf, 1975).

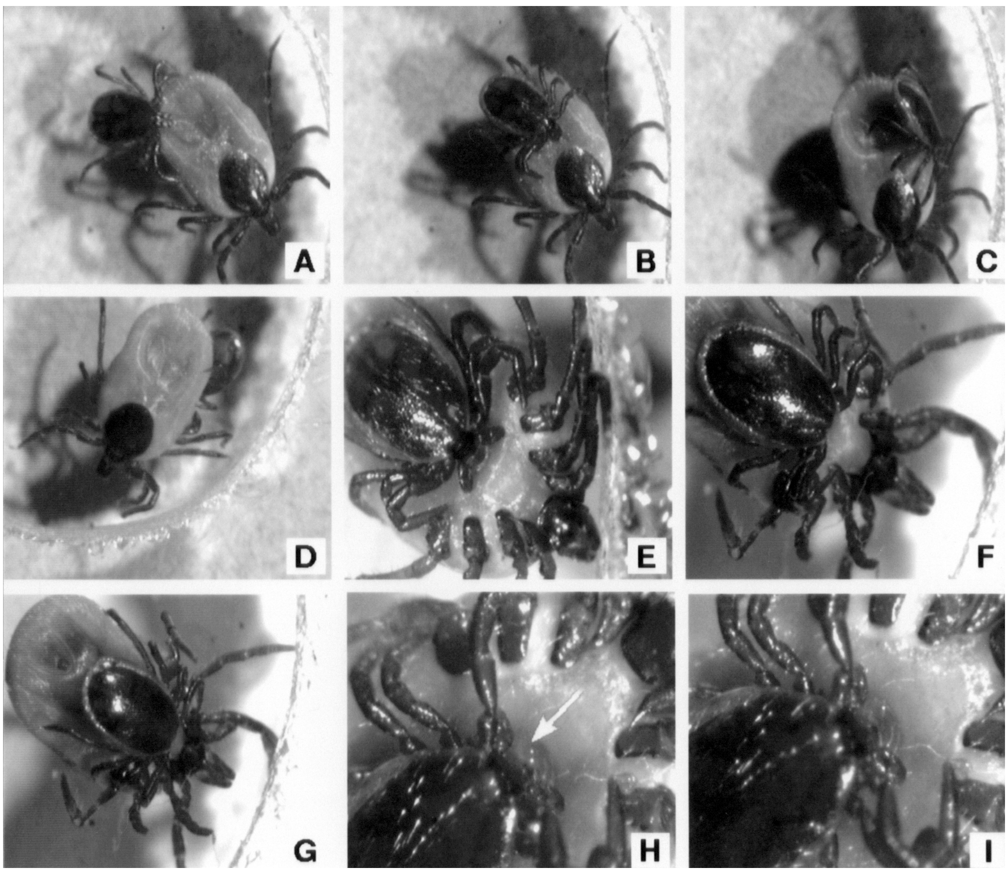

Figure 4. Sequence of mating behaviour of male *Ixodes ricinus.* A - contacting female; B - mounting onto her dorsal surface; C and D - crawling to her ventral surface; E - stretching out the palps and inserting chelicerae and hypostome into the gonopore; F - moving forward and contorting capitulum; G - lifting opisthosoma; H - and I - fixating the spermatophore in the gonopore (arrow indicates a drop of liquid).

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Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

Low activity of males in response to engorged females observed in the winter period, is probably connected with a behavioural diapause (Belozerov, 1982). As the male ticks do not show the behavioural diapause and are able to copulate with females also in winter months (Gray, 1987), the production of the mounting sex pheromone and of genital sex pheromone seems not to be limited. However, as the excitation of males on distance by female attractant is very low in winter period, the production of the attractant sex pheromone by females is probably lower or completely missing during that period. However, this hypothesis has to be confirmed by further studies.

ACKNOWLEDGEMENTS

This work was supported by the grant No. NI/4707-3 of the Internal Grant Agency of the Ministry of Public Health of the Czech Republic and by the grant No. 206/98/0575 of the Grant Agency of the Czech Republic. The authors thanks to Mrs. Marcela Šafaøíková from the Institute of Parasitology, Academy of Sciences of the Czech Republic for her valuable help during the experimental laboratory work and to Mr. Jan Erhart for keeping the tick laboratory colony.

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