Short Communications

Pheromone-induced aggregation of ixodid ticks before host contact

M. Leahy[1], A. Kovacic, C. Mannion and L. Schulze

Biology Department, Mount St. Mary's College, 12001 Chalon Road, Los Angeles (California 90049, USA), December 13, 1982

Summary. The presence of a pre-feeding aggregation pheromone was demonstrated in the species *Dermacentor variabilis, Dermacentor andersoni, Dermacentor parumapertus, Amblyomma americanum* and *Haemaphysalis leporispalustris* by assay within a petri dish. However, *Amblyomma maculatum* and *Amblyomma cajennense* did not aggregate in the sector containing discs of presumed pheromone within the hour period. *D. andersoni* and *A. americanum* recognized each other's pheromone and *A. americanum* recognized that of *H. leporispalustris.* Preliminary experiments with guanine and hemin as possible aggregating factors have thus far given inconsistent results.

In 1980 we demonstrated that a metastriate species, *Hyalomma dromedarii* Koch produces a pheromone inducing aggregation before host contact[2]. The time of action of this water soluble agent is a major characteristic distinguishing it from other pheromones known in metastriate ticks, i.e. 1. pheromones produced by feeding males that attract and induce attachment of other males and females on the host[3] and 2. sex pheromones released by fed females attracting fed males for mating[4]. Properties of the aggregation pheromone of *H. dromedarii* are in striking contrast to that of its sex pheromone but are similar to those of argasid and prostriate ticks[5]. In the studies on assembly of unfed prostriate ticks[6-9] there has been no evidence of the organic sex pheromone released by fed metastriate ticks.

The finding of a pre-attachment assembly pheromone in a metastriate tick led us to investigate other ixodid species for evidence of such pheromone-controlled behavior before host contact. We followed the general procedure of pheromone harvest[10]. Secretions/excretions were collected from ticks on discs of filter paper (Whatman No. 1) in a shell vial (20 mm). A close-fitting disc of filter paper was placed in the bottom of the vial and then sufficient ticks to cover the disc. Over this layer of ticks we placed another close-fitting disc and then another layer of ticks, etc. In order to keep the ticks in place a cotton plug was pushed down onto the top disc. After 2 or more days we tested the discs for the presence of an aggregation factor.

The pheromone assay was conducted in a 145-mm petri dish marked off into 8 pie-shaped sectors each containing a disc of filter paper. One sector contained a disc from the harvest vial, all the 7 other sectors contained untreated discs. 10 ticks were placed in the center of the dish which was then covered and transferred to a dark incubator, 28 °C, 80% relative humidity. Distribution of ticks within each of the 8 sectors was recorded at the end of 1 h. 5 replicates were carried out for each experiment. For the 2 smallest species we replaced the 145-mm dishes divided into 8 sectors with 90-mm dishes divided into 4 sectors.

Table 1. Pre-feeding aggregation of ixodid ticks induced by homologous pheromone

Sex and species tested	Sex and species providing material for challenge disc	% ticks assemble in test sector	χ^2	p<
a) Test in 8-sector dish (df 7)				
D. variabilis	*D. variabilis*			
♂	♂	36	27.44	0.001
♂	♀	28	14.11	0.05
♀	♂	32	24.03	0.01
♀	♀	44	47.92	0.001
D. andersoni	*D. andersoni*			
♂	♂	62	122.72	0.001
♂	♀	32	31.39	0.001
♀	♂	54	81.95	0.001
♀	♀	40	60.40	0.001
A. americanum	*A. americanum*			
♂	♂	30	21.92	0.01
♂	♀	44	46.64	0.001
♀	♂	68	143.60	0.001
♀	♀	64	126.00	0.001
b) Test in 4-sector dish (df 3)				
H. leporispalustris	*H. leporispalustris*			
♂	♂	52	21.52	0.001
♂	♀	50	17.04	0.001
♀	♂	38	7.12	0.05
♀	♀	44	10.64	0.02
D. parumapertus	*D. parumapertus*			
♂	♂	30	4.24	0.17
♂	♀	30	5.84	0.12
♀	♂	52	19.60	0.001
♀	♀	42	7.76	0.06

Table 2. Pre-feeding aggregation of ixodid ticks induced by heterologous pheromone

Sex and species tested	Sex and species providing material for challenge disc	% ticks assemble in test sector	χ^2	p<
a) Test in 8-sector dish (df 7)				
A. americanum	*D. andersoni*			
♂	♂	32	26.80	0.001
♂	♀	20	13.92	0.06
♀	♂	32	27.44	0.001
♀	♀	50	68.19	0.001
D. andersoni	*A. americanum*			
♂	♂	36	51.63	0.001
♂	♀	28	30.43	0.001
♀	♂	56	86.64	0.001
♀	♀	38	33.42	0.001
b) Test in 4-sector dish (df 3)				
A. americanum	*H. leporispalustris*			
♂	♂	52	20.56	0.001
♂	♀	52	22.32	0.001
♀	♂	20	1.68	0.6
♀	♀	34	11.60	0.01

Intraspecific experiments demonstrated the presence of a pre-attachment pheromone in the species *Dermacentor variabilis* (Say), *Dermacentor andersoni* (Stiles), *Dermacentor parumapertus* (Neumann) and *Haemaphysalis leporispalustris* (Packard) (table 1). The species *Amblyomma maculatum* Koch and *Amblyomma cajennense* (Fabricius) tested in the same series did not aggregate within the 1-h period. Within the limits of our data it appears that when assembly behavior is present the ability to produce or respond to the pheromone is not sex-determined.

In a parallel control series (males and females tested in dishes with untreated discs only) no significant evidence of assembly appeared with the exception of the species *D. andersoni*. 'Clumping' of these ticks is so strong that aggregation occurred within the h but in various sectors. However, when pheromone was present in a specific sector aggregation invariably occurred in that sector.

Interspecific experiments demonstrated that *D. andersoni* and *A. americanum* recognized each other's pheromone and that *A. americanum* recognized that of *H. leporispalustris* (table 2). Our earlier studies indicated heterologous response between *H. dromedarii* and *Hyalomma asiaticum*[11].

To determine whether the tick's excretory products might function as aggregation factors we challenged *D. andersoni* males with guanine[12] in quantities of 10^{-5}, 10^{-4}, and 10^{-3} g and with hemin[13] in quantities of 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} g in 20 µl of 10% NH_4OH solution. Results were inconsistent but further work seems indicated.

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Influence of a meal on skin temperatures estimated from quantitative IR-thermography

M. J. Dauncey, C. Haseler, D. P. Page Thomas and G. Parr

Department of Applied Biology, ARC Institute of Animal Physiology, Babraham, Cambridge (England), and Rheumatology Research Unit, Addenbrooke's Hospital, Hills Road, Cambridge (England), January 24, 1983

Summary. In young men at 25 °C, quantitative IR-thermography showed that fasting values of skin temperatures over suspected areas of brown adipose tissue (BAT) were higher than where no BAT is thought to occur. However, at 30 min and again at 60–90 min after a meal of 2.5 MJ, the magnitude of the increase in skin temperature was similar in areas with or without suspected BAT. In conclusion, either thermography was unable to detect the activation of BAT, or the meal did not stimulate heat production in the sites of suspected BAT.

It has been shown previously in man that the sympathomimetic agent ephedrine causes an increase in skin temperature on the neck and upper back[1]. These locations correspond to the sites where brown adipose tissue has been found to exist in infants[2] and adults[3] and it was therefore suggested[1] that the findings could be interpreted as evidence that noradrenaline stimulates the production of heat by brown adipose tissue in adult man. Feeding causes an increase in both metabolic rate and the circulating concentration of noradrenaline in adult humans[4] and young pigs[5]. Since small amounts of brown adipose tissue occur in both these species[3,6], it is therefore possible that part of the extra heat production associated with feeding is due to the stimulation of this tissue. Further evidence which lends support to this idea is that the β-blocker propranolol can reduce the heat production caused by diet[1,7]. The aim of the present investigation was therefore to determine the extent to which any changes in skin temperature after a meal could be correlated with the probable presence of brown adipose tissue. Quantitative IR-thermography was used, since preliminary studies with temperature sensors attached to the skin had shown variations in recorded temperature which were probably due to differences in adhesion of the probes. These variations introduced errors which could have masked any small changes in skin temperature of the order of 0.5 °C.

Materials and methods. Five healthy men aged 21 ± 0.5 (SEM) years, volunteered to take part in the investigation. They were all familiarized with the procedures before measurements were made. Their mean ($\pm$ SEM) height and weight were 1.77 ± 0.013 m and 69.2 ± 0.35 kg. Skinfold thicknesses for triceps, biceps, subscapular and suprailiac sites were 6.0 ± 0.78, 3.5 ± 0.48, 8.6 ± 1.22 and 7.1 ± 0.70 mm respectively. The subjects were also well-matched for habitual physical activity; they were all students who climbed in their spare time. Dietary recall revealed that their food intakes were also similar.

On the day of experiment the subjects were transported to the unit by car and arrived at 09.00 h after an overnight fast. The measurement room was maintained at an ambient