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# PHEROMONE-REGULATED AGGREGATION IN LARVAE, NYMPHS AND ADULTS OF IXODES RICINUS (L.) (ACARINA: IXODIDAE)

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*DEDICATED TO ACADEMICIAN B. ROSICKÝ ON THE OCCASION OF HIS 60TH BIRTHDAY*

Abstract. In the species *Ixodes ricinus* (L.) aggregation of various developmental stages to their own pheromones and to those produced by other stages is demonstrated by the Petri-dish method. Male pheromone elicited the strongest response from larvae, nymphs, males and females. It is soluble in saline but not in ether, benzene or acetone and is relatively stable. Both sexes responded to pheromone from nymphal exuviae and in each sex the greater response was to exuviae of males rather than of females. Date of feeding experiments is considered in relation to aggregation pheromones and host location.

Graf (1975) was first to demonstrate pheromone activity in ticks of the genus *Ixodes*. He reported that female *I. ricinus* (L.) produces a sex pheromone to which males and females are attracted. Treverrow et al. (1977) found evidence of pheromone activity in *I. holocyclus* Neumann. They referred to the agent as an aggregation pheromone since the activity occurred not only between but within the sexes. Uspensky and Emelyanova (1980) demonstrated aggregation within and between the sexes of *I. ricinus* and *I. persulcatus* Schulze.

The few known characteristics of these pheromones of the genus *Ixodes* are more like those of argasid ticks than like the majority of pheromones reported for other ixodid ticks. The argasid pheromone is released by unfed adults, is relatively stable lasting for weeks and id aqueous soluble. The first two characters occur in the 3 species of *Ixodes* studied. Solubility was investigated only for the female pheromone of *I. ricinus* and it was aqueous.

On the other hand with only two exceptions (see Discussion) pheromones reported for other ixodids are released by feeding females (Berger et al. 1971, Sonenshine et al. 1974, Chow et al. 1975, Wood et al. 1975) or feeding males (Gladney et al. 1974, Rechav et al. 1976, 1977, Obenchain et al. 1977). These pheromones are organic soluble and the ones identified are phenolic compounds. Since the argasid assembly pheromones are produced not only by females but also by males our first interest was further investigation of male-induced assembly behaviour in *I. ricinus*. It has been reported recently that pheromone-regulated activity is present not only in the adult argasid tick *Argas persicus* Oken but also in its larvae and nymphs, and, in addition, between its various developmental stages (Leahy 1979). Hence our second aim was to extend the study of pheromone-regulated assembly to all stages of the life-cycle of *I. ricinus*.

## MATERIALS AND METHODS

Adult ticks were field collected in the area of Šárka (near Prague) or provided by Institut de Zoologie, Université de Neuchâtel, Suisse. Adults were fed in a sack on the back of a rabbit. Larvae and nymphs of the experiments were from the $F_1$ generation of these females and were fed on mice. Assembly tests were performed using the Petri-dish method (Leahy et al. 1973) with modifications for this tick which is quite minute and has extremely high humidity requirements. We harvested the presumed pheromone in centrifuge tubes 12 × 150 mm with water in the bottom. About 15—20 mm from the top of the tube was a cork with a hole in the centre and with fine nylon mesh on the top of it. A filtre paper disc (Whatman 1) was placed on the cork and sufficient number of ticks to cover it, (about 200 larvae, 50 nymphs, 30 males and 25 females unfed and 50 larvae, 30 nymphs and 2 females fed), then another disc and ticks, etc. Ticks for production and testing of pheromone were a month or more old and were maintained at 22 °C and 80—100 % RH. The assembly test dishes (60 mm) were divided into 4 sectors containing filtre paper discs (10 mm diameter). Strips of adhesive tape were wound around the outside wall of the bottom plate to prevent escape of ticks.

At bioassay we placed 10 ticks in the centre of the dish containing 3 clean discs and a challenge disc in the sector 4. We transferred the dishes to a dark incubator 22 °C and 80% RH and after 1 hr recorded tick distribution. In control tests we used clean discs in all 4 sectors or 3 clean discs and 1 with solvent. Each experimental and control test was performed with a minimum of 50 ticks (5 replicates with 10 ticks each) and we analyzed data by Chi square method. When we employed specific methods the details are given with the particular experiment.

## RESULTS

### 1. EFFECT OF FEEDING ON PHEROMONAL ACTIVITY

In order to decide whether to use as a standard source of pheromone hungry or fed ticks, we investigated the effect of feeding on pheromone activity. There are two factors which may be influenced by feeding: a) amounts of released pheromone and its composition may change after feeding, b) responsiveness of ticks to pheromone may change after feeding.

To test this in *I. ricinus*, unfed ticks were challenged with material from both fed and unfed ticks (Fig. 1). There is no question but that in these experiments with males, females and nymphs a significantly higher percentage aggregate near discs from fed than unfed ticks ($P < 0.01$). Whether there was quantitative or qualitative change in the pheromone on discs obtained from fed ticks is not known.

In the next experiment we tested the response of unfed and fed larvae and nymphs to material from fed ticks of the same stage in comparison to the expected distribution in a dish of equivalent sectors (Fig. 2). The unfed ticks of both stages responded significantly ($P < 0.01$) and the fed ones did not.

Thus the results of the release and response experiments determined our procedures in subsequent experiments. When harvesting pheromone we obtained it from fed ticks, whereas unfed ticks were used for testing. One exception to this procedure was introduced. We did not feed males before testing their pheromone production since it has been reported that they seldom feed in nature (Černý, pers. comm.).

### 2. MALE PHEROMONE

To determine whether males produce pheromone we held them in a harvest vial for 6 or more days and then challenged ticks with a disc from the vial. Both males and females responded to discs with male material ($P < 0.01$, Fig. 3). In addition both larvae and nymphs also responded to male pheromone ($P < 0.01$).

We investigated the solubility of the male pheromone by washing the active discs in 0.2 ml of ether, benzene, acetone and saline for 5 min and then transferred the washings to clean discs and air dried and tested the discs. Results were negative except for saline washings. In these tests males were challenged in dishes with 3 clean discs



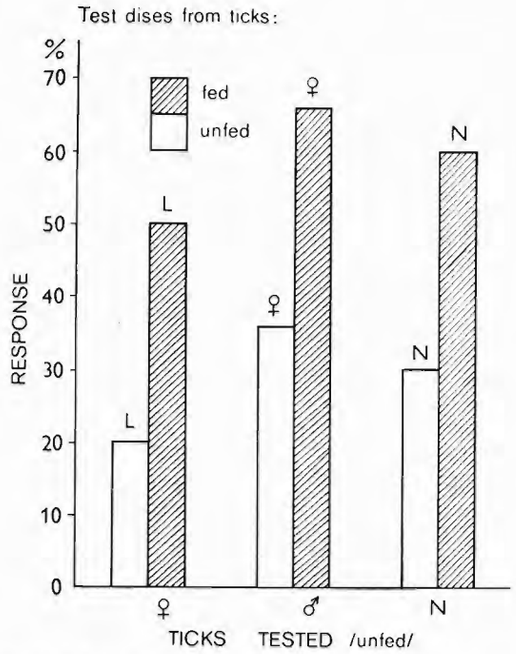


**Fig. 1.** Effect of feeding condition on pheromone release by larvae, nymphs and females of *I. ricinus*.

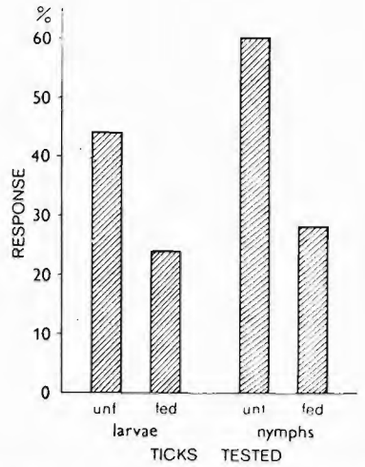


**Fig. 2.** Response of unfed and fed tick to challenge material from fed ticks of the same stage.

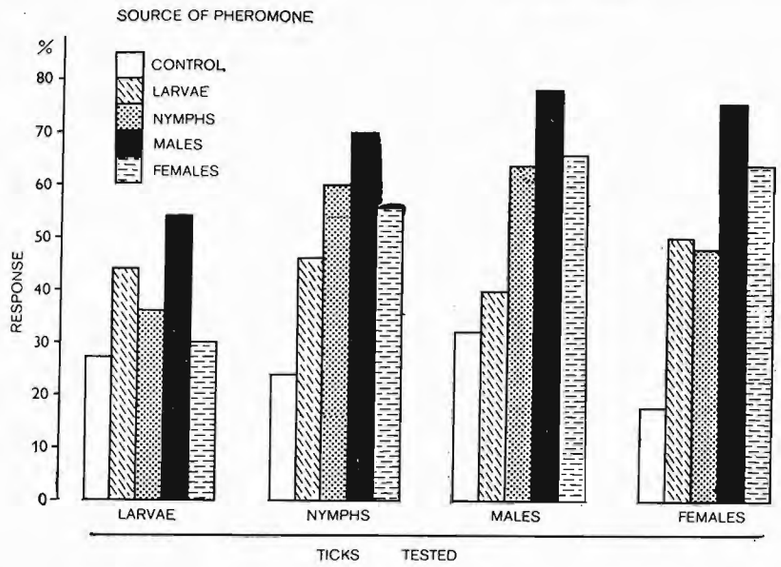


**Fig. 3.** Percentage response of *I. ricinus* ticks of each stage to their own pheromone and also to pheromone of other stages.

and 1 with saline washings. The control dishes had 3 clean discs and 1 with 0.2 ml saline. After 1 hr the response to saline was not significant whereas response to extract was highly significant ($P < 0.01$). These pheromone discs held at 4° C for 6 weeks retained activity.

### 3. PHEROMONE-REGULATED BEHAVIOUR IN LARVAE, NYMPHS AND ADULTS OF *I. RICINUS*

Observations of *I. ricinus* indicate the presence of pheromone-induced behaviour that is both homologous and heterologous in character. Homologous assembly is seen in Fig. 3 where larvae aggregate to larval material, nymphs to nymphal, males to male and females to female material, each at $P < 0.01$.

Heterologous assembly behaviour is demonstrated in most tests of larvae, nymphs and adults to pheromone material of other stages (Fig. 3). The most striking response observed in any stage is to male pheromone (larvae 54 %, nymphs 70 %, males 78 %, females 76 %).

Females, males and nymphs respond significantly ($P < 0.05$) in each heterologous test without exception. Larvae also respond heterologously but not to nymphal or female material within the hr period. In summary: 10 heterologous tests were significant, 2 were not.

### 4. RESPONSE OF ADULTS TO NYMPHAL EXUVIAE

To determine if pheromonal material is in some way connected with exuviae we challenged males and females with discs which had contacted nymphal exuviae. Since these exuviae are of 2 kinds i.e. those produced by the two sexes we tried to determine whether males respond more strongly to exuviae of the male or the female. To do this we put fed nymphs into individual vials and then after emergence of adults prepared harvest vials containing exuviae from females and from males. These containers did

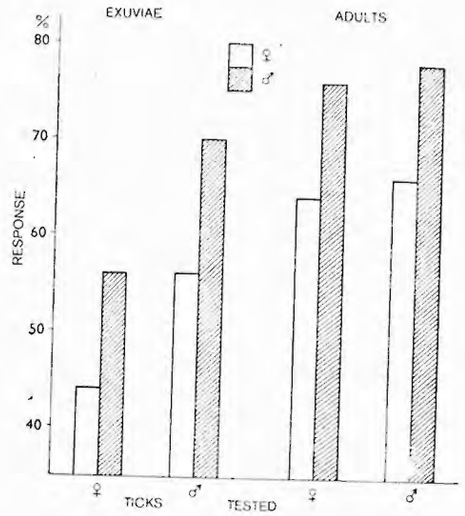


**Fig. 4.** Comparison of responses of *I. ricinus* to pheromones present on exuviae and pheromones collected from live adults of each sex.



not have water in them as in the case of the live ticks. This oversight did not prevent pheromone transfer to the discs since both unfed males and females responded significantly ($P < 0.01$) when challenged with discs from these vials (Fig. 4). However, the response is somewhat less to exuvial than to adult pheromones. It is interesting that both female and male response to exuvial pheromones is higher towards that of males than females and that the sex preference is the same as in the tests with adults.

## DISCUSSION

Where tested, pheromone release and response in *I. ricinus* was significantly affected by the feeding condition of the tick (Figs. 1, 2). These data are in agreement with earlier findings on argasid ticks,demonstrating that discs from fed ticks elicited a higher assembly response than did those from unfed ones, and that unfed ticks responded more rapidly than fed ones (Leahy et al. 1975). Our data indicated that this pattern of pheromone release and response in relation to feeding was present also in immature stages of *I. ricinus*. The only situation in which the effect of feeding was untested was in males and this was for two reasons. We already had evidence that pheromonal activity from unfed males was adequate to obtain significance in our tests and it seemed without value to attempt to feed them when feeding is not normal for them in nature.

In addition to the homologous assembly there were only 2/12 heterologous tests in which aggregation was not significant. (Fig. 3). Perhaps a longer test period or higher concentration of pheromonal material would reveal latent aggregation tendencies in these instances also.

The influence of the male pheromone of unfed *I. ricinus* is striking. It consistently dominates in tests with larvae, nymphs, males and females (Fig. 3). It does not seem related to the aggregation-attachment pheromones of some Amblyomminae (Gladney 1971, Rechav et al. 1976, Obenchain et al. 1977) which are produced by feeding males and are organic soluble, since in *I. ricinus* the pheromone is released by unfed males and it is water soluble. It may show some similarities with the aggregation pheromones of *Aponomma concolor* Neumann (Treverrow et al. 1977) and unfed *Hyalomma dromedarii* Koch (Hájková et al. 1980) both of which have water soluble pheromones. However, in neither of these species was male dominance demonstrated. Perhaps species differences or differences in the methods of pheromone harvest e.g. age of ticks, might account for the appearance or non-appearance of male dominance among ixodid pheromones.

Uspensky and Emelyanova (1980) report that under their experimental conditions significant production of male pheromone of *I. ricinus* appears before that of females. We found that the higher activity of male pheromone is already indicated in tests of the nymphal exuviae (Fig. 4). Although the male *I. ricinus* does not have significantly greater longevity than the female his dominant role commences earlier. When Treverrow et al. (1977) found nymphal cuticles of *A. concolor* were highly attractive they speculated that this would certainly intensify aggregation.

Aggregation might well have survival value for a species such as *I. ricinus* with a 2—3 year life cycle. In the complex interplay of survival factors, aggregation would affect such obvious needs as sexual recruitment and humidity. The results of our homologous and heterologous experiments suggest that the aggregation pheromones of *I. ricinus* have a role in its food location. It seems quite reasonable that after it feeds, the tick produces more material thus signalling successful host location and at the same time the tick becomes less active in response to pheromone stimulus. This tendency to aggregate and to do so specially when feeding would account for the fact that in nature the various stages of *I. ricinus* are usually found in the same area, i.e. the resting

place of hosts of adult ticks. However, there is some ambiguity here. On one hand, pheromones of fed larvae, nymphs and females induce a greater response than unfed ones. On the other hand, the strongest response is evoked by pheromone of unfed males. More precise information is needed on source, chemistry and quantitative aspects of pheromone(s) evoking aggregation response among the various age and sex groups of *I. ricinus*.

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## РЕГУЛИРОВАННОЕ ФЕРОМОНОМ СКОПЛЕНИЕ У ЛИЧИНОК, НИМФ И ВЗРОСЛЫХ КЛЕЩЕЙ *IXODES RICINUS* (L.) (ACARINA: IXODIDAE)

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**Резюме.** У вида *Ixodes ricinus* (L.) продемонстрировано с помощью метода чашки Петри скопление разных стадий развития, вызываемое собственным и производимым другими стадиями феромонами. Феромон самцов вызывал самую сильную реакцию у личинок, нимф, самцов и самок. Эта субстанция растворима в физиологическом растворе, но не в эфире, бензоле или ацетоне и относительно устойчива. Особи обоих полов реагировали на феромон от экзувии нимф и каждый пол более сильно реагировал на экзувии самцов чем на таковые самок. Период экспериментов с кормлением клещей рассматривается в связи с вызывающими скопление феромонами и местонахождением хозяина.

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