

## A BIOASSAY TECHNIQUE FOR THE PHEROMONE EMITTED BY *AMBLYOMMA HEBRAEUM* MALES

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### ABSTRACT

RECHAV, Y., 1983. A bioassay technique for the pheromone emitted by *Amblyomma hebraeum* males. *Onderstepoort Journal of Veterinary Research*, 50, 133-135 (1983).

Attachment of *Amblyomma hebraeum* females, either around feeding males or in areas in which extracts of fed males had previously been placed, was studied. The percentage attachment of female ticks around feeding males was significantly higher than that inside extract-treated areas. It was also found that the percentage attachment around the males increases with time, but there is little or no increase in attachment with time inside the treated areas.

The amount of pheromone released by 1 feeding male was enough to stimulate attachment of females, although the rate of attachment accelerated as the number of males increased.

### INTRODUCTION

The well-known phenomenon of pheromone production by ticks was recently reviewed by Sonenshine, Silverstein & Rechav (in press). Three types of pheromones have been demonstrated in these parasites:

- (a) sex pheromones produced by the feeding females of hard ticks;
- (b) assembly pheromones for mate location away from the host (soft ticks and species of *Ixodes* and *Aponomma*), and
- (c) assembly pheromones produced by males while feeding on the host (several species of *Amblyomma*).

Apart from that of the sex pheromone (Type a), identified as 2,6 dichlorophenol, no information on the chemical structure of the other types is available. Thus the only "measurement" which could be used to evaluate the activity of the assembly pheromones (Type c) is "male equivalent extract" (number of males in 1 ml of extract) (Gladney, Ernst & Grabbe, 1974; Rechav, Parolis, Whitehead & Knight, 1977; Norval & Rechav, 1979).

The present work reports the attachment rate of *Amblyomma hebraeum* females either around feeding males or inside areas in which extracts of such males had previously been placed. Attempts were made to answer the following questions: (a) Do different numbers of males evoke the same response as extract from the equivalent number of males, i.e., is the percentage attachment dependent on the concentration, and (b) do the bioassay procedures used give a valid assessment of the pheromonal activity of chemicals?

### MATERIALS AND METHODS

#### Ticks

Ticks were originally collected from cattle on 2 farms in the Eastern Cape Province of South Africa and were then maintained at the Tick Research Unit as previously described (Rechav *et al.*, 1977). One-month-old adult ticks from these colonies were used in the experiments.

#### Preparation of extracts

*A. hebraeum* males which had been feeding for 8 d were removed from their hosts and washed with diethyl ether. This wash was first treated with anhydrous sodium sulphate to extract water derived from the males. It was then cooled to 0 °C and finally concentrated, using a Buchi rotary evaporator and a water pump, as previously described by Rechav *et al.* (1977).

#### Bioassay procedure

Four groups of rabbits, each containing 9 animals, were infested with 1, 3, 5 and 10 males respectively.

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Eight days later, 20 unfed female ticks were released on each rabbit under a cotton sleeve which had previously been secured around the animal (Rechav *et al.*, 1977).

Another 4 groups, each containing 9 rabbits, were used to test the effects of the extracts. Circular areas, each 3 cm in diameter, were demarcated on the backs of these rabbits and treated with 0.1 ml of extracts equivalent to 1, 3, 5 and 10 males, prior to the release of the female ticks.

The number of ticks that had attached either around the feeding males or inside the extract-treated areas was calculated at 15-minute intervals and presented as percentage attachment (mean  $\pm$  standard error of the mean). The T-test was used in analysing the results.

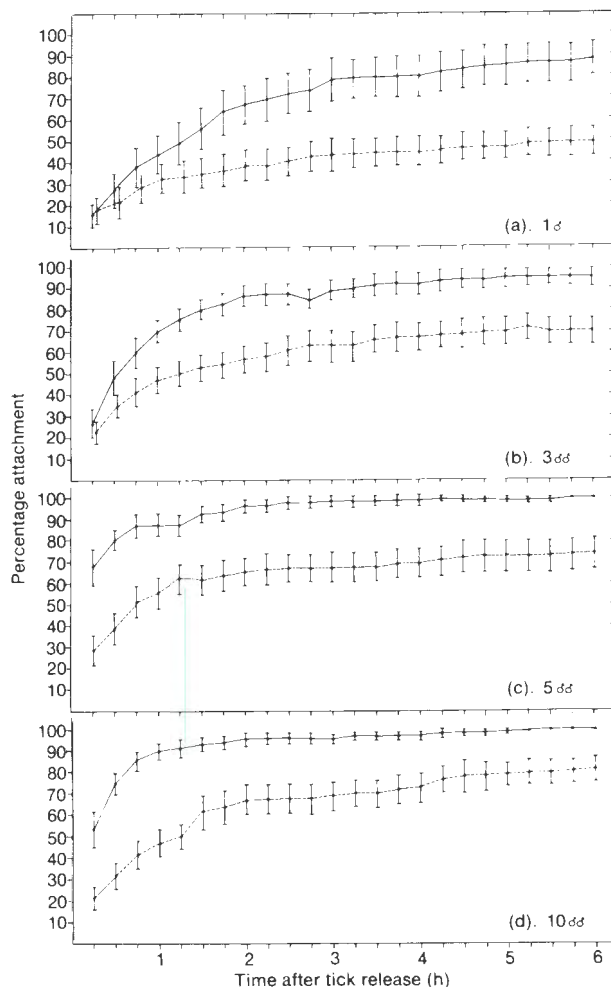


FIG. 1 Rate of attachment of *Amblyomma hebraeum* ♀♀ on the backs of rabbits, either in areas where 1 or more fed ♂♂ ticks were attached (●—●) or in areas which had been treated with extract equivalent (●---●). Vertical lines represent standard error of the mean. a) 1 ♂; b) 3 ♂; c) 5 ♂; d) 10 ♂

FIGURE LEGENDS

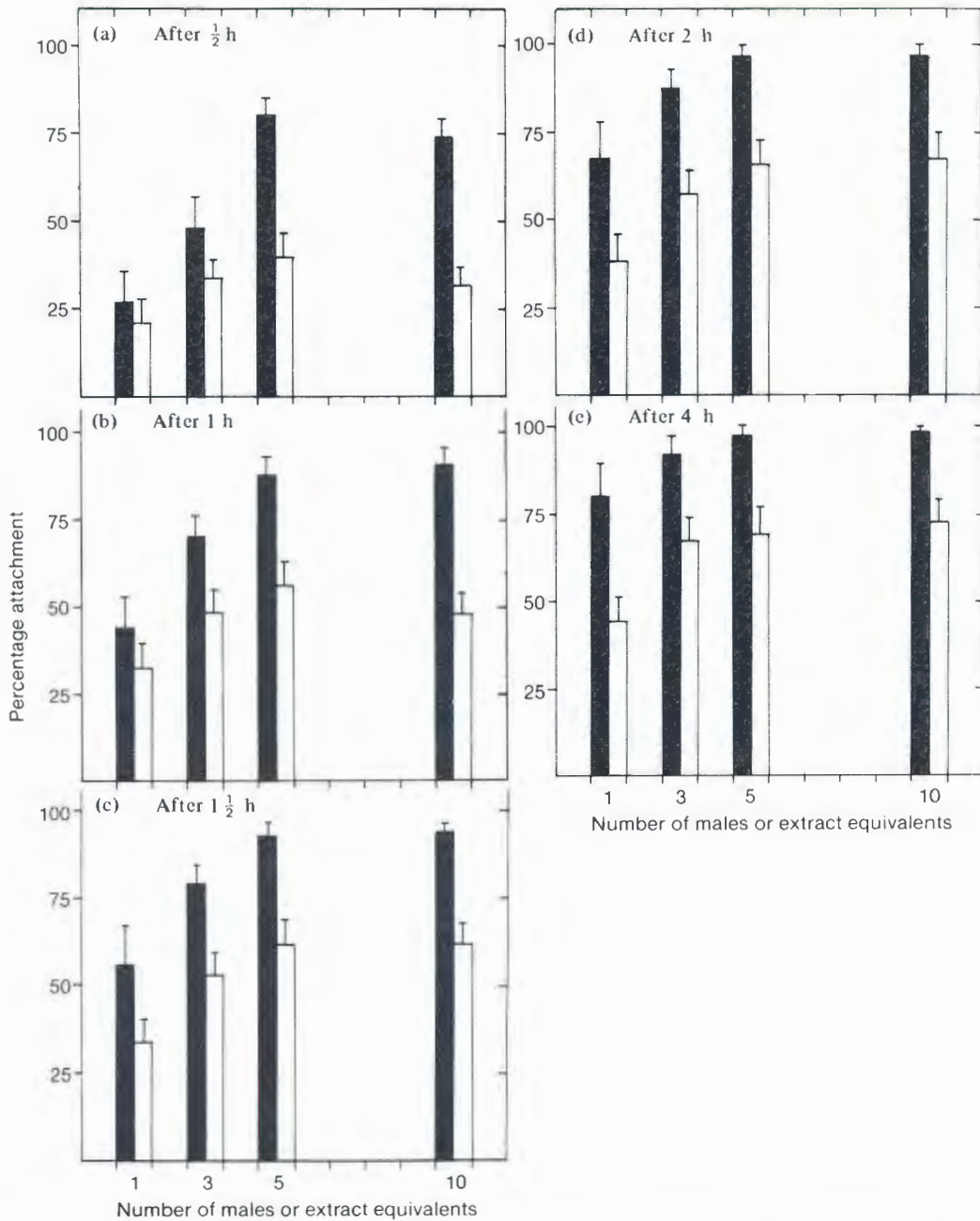


FIG. 2 Percentage attachment of *Amblyomma hebraeum* ♀♀, at specified times after their release, to areas on which 1, 3, 5 or 10 fed ♂♂ were present (■) or after the area had been treated with ♂♂ extract equivalent (□). Vertical lines represent standard error of the mean. a) After 1/2 h; b) after 1 h; c) after 1 1/2 h; d) after 2 h; e) after 4 h.

RESULTS

The rates of attachment (mean % ± SE) of female ticks around 1 fed male, or extract equivalent to 1 male, are presented in Fig. 1a. The percentage attachment around 1 fed male increased with time, while a very slight increase in attachment occurred inside the extract-treated areas. The difference became significant ( $P < 0,05$ ) 2 h after the release of the female ticks and highly significant ( $P < 0,01$ ) 3, 4 and 6 h later.

When 3 males were present on the rabbits, nearly 85% of the female ticks attached within the first 2 h of tick release, while only 60% did so inside the demarcated area which had been treated with the equivalent extract (Fig. 1b). As with 1 male, more females attached around

the males than in the extract-treated areas. These differences were significant after 45 min ( $P < 0,05$ ) and highly significant after 90 min ( $P < 0,01$ ).

A higher rate of attachment was also observed around 5 and 10 males than around the equivalent extracts (Fig. 1c & d). These differences were highly significant even within the first 15 min of tick release ( $P < 0,005$ ).

Comparisons between attachment values around various numbers of males and male extract equivalents at various time intervals after female tick release are presented in Fig. 2a-e. The data show that: (a) The percentage attachment was always significantly higher around males than around extract equivalents, except during a

short period after tick release, when the pheromone concentrations were low; then the difference was not significant ( $P < 0.3$ ). (b) Shortly after tick release the rate of attachment was markedly affected by the number of males on the rabbits. As time passed, however, this factor affected the attachment rate less and less, and no significant differences were observed 6 h after the release of the female ticks.

#### DISCUSSION

Basically, the rate of attachment of *A. hebraeum* females around various numbers of males was always higher than that in areas previously treated with extracts equivalent to the respective number of males. The differences in attachment were not significant shortly after the release of the tested female ticks, but they became highly significant 2 h later (Fig. 1a & b). These differences probably occurred because the feeding males released a pheromone, or pheromones\*, continuously, while the extract-treated groups probably lost some of the volatile pheromone applied initially. The possibility that the method of washing fed males does not extract all the pheromone(s) from them is ruled out by the fact that very similar attachment values around either 1 or 3 feeding males or their male extract equivalents were obtained immediately after the release of the female ticks.

Neither significant differences nor a positive correlation between female attachment and male numbers were observed 4 h after the release of the ticks (Fig. 2e).

\* It is not known if the males release one pheromone or more

This strongly suggests that the amount of pheromone released by 1 male is more than the minimum amount necessary to stimulate females to attach. Increasing the number of males did not increase the attachment values, although the rate of attachment accelerated shortly after application of the pheromone and the release of ticks. The lack of correlation between attachment values and the number of males 2, 4 and 6 h after the release of the females suggests that even a small amount of pheromone will stimulate the ticks to attach. Bioassays were proven to be valid for component and extract-equivalent assessment.

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