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INVESTIGATION INTO THE PARTICIPATION OF MALE PHEROMONES OF *RHIPICEPHALUS EVERTSI EVERTSI* DURING INFESTATION

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ABSTRACT

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It was shown in this study that *Rhipicephalus evertsi evertsi* males emit pheromones in the preparasitic as well as in the parasitic phases. Conspecific unengorged male and female images exhibited a high response to water-soluble volatiles collected from unengorged males. A vigorous assembly of unfed female *R. evertsi evertsi* occurred on filter paper discs which had been in contact with either unfed or 5- and 9-day fed males. Progressively increasing percentages of conspecific unengorged males reacted to organic soluble volatiles obtained from 5- and 9-day fed males as well as to hexane washings of intact males which had fed for 9 days.

It is concluded that *R. evertsi evertsi* males participate not only as pheromone receptive, but also as pheromone emitting, partners in the intraspecific communication of this tick species.

INTRODUCTION

The search for, location, exploration and exploitation of a host is an essential part in the biology of all ticks and thus the basis of their damaging effect on their hosts. The reaction sequence in this ecophase, which occupies only 2–6 % of the life-span of an individual tick, is basically identical for the 800 described tick species, and may be presented as a 10-step series of events. It starts with the sensation of hunger by the ticks and is followed by appetence behaviour, pelage contact, exploration of the infestation site, penetration of the skin, attachment, food ingestion, engorgement and withdrawal of the mouthparts; it ends with the ticks leaving the host (Waladde & Rice, 1982).

It can be inferred that these successive activities, before, during and after infestation, are phases of a total process. Furthermore, it is anticipated that not merely a single entity but rather a series of stimulating agents, determine the course of events. These agents, however, have been only partially investigated for some tick species. With respect to the function of pheromones of metastriate species, studies have been confined mainly to their influence on the initiation of a sexual partnership. Consequently, female and male pheromones have been differentiated qualitatively and very schematically according to which sex of a particular metastriate tick species acts either as the primary or the exclusive pheromone receptor or as the predominant pheromone emitter (Gothe, 1983).

This rigid classification of either 1 sex or the other as a pheromone-emitting or a pheromone-reacting partner is, however, unfounded. It is not to be understood as an expression of a species-typical adaptation mechanism in the very complex interrelations between individual metastriate tick species and their hosts. This is also true for *R. evertsi evertsi*, for which the females have been described as predominantly the pheromone-emitting partners (Schniewind & Gothe, 1982). However, it has been shown (Gothe & Burkhardt, 1981) that an analogous cellular organization of the foveal glands, which are vital for the production of pheromone, exists in male as well as in female ticks and that the morphological integrity of this organ is preserved in males only, even after very long periods of infestation.

Male metastriate imagines remain sexually active even after long periods of being attached to the host and are capable of multiple inseminations (Pervomaisky, 1954; Rechav & Oppenheim, 1969; Gladney & Drummond, 1970; Oliver, 1974; Norval, 1974; Londt, 1976; Thompson, Davy, Osburn & Cruz, 1980; Jordaan & Baker, 1981; Davey, Osburn & Castillo, 1983). Because the morphological integrity of the foveal glands persists, they can possibly emit pheromonal stimulating substances over a long period. Favourable attachment areas are probably marked by this means, so that newly-arriving ticks are immediately attracted to them. Experimental proof of the action of male pheromones in the infestation process of *R. evertsi evertsi*, however, is lacking.

The present study was therefore undertaken to investigate whether *R. evertsi evertsi* males can produce and emit pheromones and to determine the reactions of both female and male imagines towards conspecific exposure to male pheromones. An analysis of pheromone volatility was also undertaken.

MATERIALS AND METHODS

Origin and rearing of ticks.

Laboratory colonies of *R. evertsi evertsi*, originally established from ticks collected in Nelspruit, Republic of South Africa, in 1971, were maintained and reared in an incubator at 27 °C and 90–95 % R.H.

Collection of male R. evertsi evertsi *pheromones*

Pheromones were collected from unengorged *R. evertsi evertsi* males and from males that had fed for either 5 or 9 days on sheep. Pheromones were obtained by (a) volatilization from effluent air drawn over ticks, (b) contacting ticks with filter paper discs, and (c) washing intact ticks with hexane.

(a) *Collection by volatilization.* Two methods were employed, both of which were performed at 24 °C and 70–80 % R.H. In the 1st method (Sonenshine, Silverstein, Collins, Saunders, Flynt & Homsher, 1977; Neitz & Gothe, 1984), 100 male ticks were placed in a cylindrical container of 40 cm^3 capacity with an inlet 10 mm in diameter and an outlet of 3 mm diameter. To prevent the ticks escaping both openings were closed by thin cotton wool plugs. Air was drawn through the container at a flow rate of 40 cm^3/min by means of a vacuum pump connected to two 25 cm^3 capacity glass vials, each containing 5 cm^3 hexane and 5 cm^3 0,5 M NaOH. To minimize evaporation, the vials were cooled with ice. After a collection period of 4 hours, the hexane-NaOH mixtures were combined and neutralized with 0,5 M HCl. After vigorous shaking, the 2 phases were separated and each was bioassayed for the presence of volatile pheromones. A control collection was performed as described above, omitting the ticks.

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In the 2nd method (Browne, Birch & Wood, 1974; Neitz & Gothe, 1984), air was similarly drawn over 100 male ticks, but the effluent air was liquefied and trapped in a U-tube (total length 16 cm and internal diameter 10 mm), immersed in liquid nitrogen. The collection time was 6 hours, after which the liquid air was carefully evaporated. When the ice had thawed, the U-tube contents were salted with excess sodium chloride and extracted with diethyl ether. Control collections were performed in the absence of ticks.

Aliquots of all the phases were bioassayed for the presence of volatile pheromones. Sterile filter paper discs 1,8 cm in diameter were separately impregnated with 0,1 cm^3 of each phase and each disc was then placed in a 10 cm diameter sterile Petri dish, 0,5 cm from the rim. Three clean discs were added as controls and arranged symmetrically in each dish. Ten unengorged male or female ticks of *R. evertsi evertsi* were placed in the centre of the dishes. The dishes were then closed and incubated at 25 °C and 70–80 % R.H. The locations of the ticks were observed at hourly intervals over an initial period of 5 hours and again after 24 hours.

(b) *Contact collection.* One hundred male ticks were placed between two 3 cm filter paper discs in a small beaker. The top disc was pressed down firmly by means of a cotton wool plug to ensure direct contact between the ticks and the discs. After incubation for 5 days at 27 °C and 90–95 % R.H. in an incubator, the discs were tested for the presence of pheromones. Each disc was placed in a 13,5 cm Petri dish, 0,5 cm from the rim. Three clean discs of similar size, as controls, were also arranged symmetrically in each dish. Fifteen unengorged male or female *R. evertsi evertsi* were placed in the centre of each dish. The closed dishes were then incubated at 25 °C and 70–80 % R.H. The locations of the ticks were recorded at hourly intervals during the first 5 hours and again after 24 hours.

(c) *Collection by washing intact ticks with hexane.* One hundred male ticks were placed in a small glass-stoppered flask and 5 cm^3 of hexane was added. The flask was periodically gently shaken by hand for 1 hour, then the hexane was decanted. All operations were performed at 24 °C and 70–80 % R.H. To test for the presence of pheromones, 1,8 cm filter paper discs were impregnated with 0,1 cm^3 of the hexane wash fluid and placed in 10 cm Petri dishes, 0,5 cm from the rim. As controls, 3 similar-sized discs were arranged symmetrically in each dish. Additional controls were performed with hexane-impregnated filter paper discs. Ten unengorged *R. evertsi evertsi* males or females were then placed in the centre of each dish. The behaviour of the ticks was recorded as described above.

RESULTS

Response of conspecific unengorged male and female imagines to pheromones of male R. evertsi evertsi

Responses to pheromones collected by volatilization. Conspecific unengorged male and female imagines showed either no or only weak aggregation, the maximum being 25 %, to biosubstances in the hexane and ether phases collected from unfed male *R. evertsi evertsi*. Male ticks, however, reacted with progressively increasing percentages of 65 % and 70 %, and 50 % and 75 %, to pheromones trapped from 5- to 9-day fed male ticks in the hexane and ether phases, respectively. On the other hand a maximum of 40 % of the female ticks aggregated to the organic phases obtained from 5- and 9-day fed male ticks (Table 1).

TABLE 1 Aggregation (%) of unengorged male and female *Rhipicephalus evertsi evertsi* on filter paper discs impregnated with various pheromone isolates from conspecific male ticks fed for 0, for 5 or for 9 days

Pheromone isolation		No. of ticks tested	Aggregation (%)	
			Males	Females
Hexane-NaOH:				
Hexane phase	0 (days)	40	0	10
	5	40	65	15
	9	40	70	25
Aqueous phase	0	40	85	75
	5	40	15	35
	9	40	0	0
Liquid air:				
Ether phase	0	40	5	25
	5	40	50	40
	9	40	75	25
Aqueous phase	0	40	45	25
	5	40	25	20
	9	40	0	0
Contacted filter paper	0	15	7	73
	5	15	7	73
	9	15	53	87
Intact ticks washed with hexane	0	40	15	0
	5	40	20	20
	9	40	80	60

Apart from the high rates of 85 % and 75 % for male and female ticks elicited respectively by the aqueous phase of the hexane-NaOH collection, all other aqueous phases induced either no or only weak aggregation of either males or females (Table 1). In control trials, material collected by volatilization in the absence of ticks evoked no reaction from the test ticks.

Responses to pheromones on contacted filter paper discs. A vigorous assembly by females, of 73 %, 73 % and 87 %, was observed on filter paper discs contacted by males which were either unengorged or had fed for 5 and 9 days, respectively. Male ticks responded only weakly to analogous pheromone sources, with 7 %, 7 % and 53 % aggregation respectively (Table 1).

Responses to pheromones in hexane washings of intact ticks. Stimulation rates of 80 % for males and 60 % for females were induced by hexane washings obtained from intact ticks which had fed for 9 days. Either no or only poor reactions were observed with washings from unengorged or 5-day fed ticks (Table 1) and in control trials.

DISCUSSION

It can be deduced, from experimental data, that male *R. evertsi evertsi* act not only as pheromone recipients (Schniewind & Gothe, 1982), but also as pheromone donors in intraspecific communication. Furthermore, male ticks emit pheromones during the preparasitic as well as the parasitic phases. From the different pheromone isolation methods it can be concluded, however, that distinct male pheromones with different biological modes of action and chemical structure are involved.

The first link in the chemical signal chain may be characterized as a volatile, water-soluble pheromone produced by unfed *R. evertsi evertsi* males which is highly attractive, with a stimulation effectivity of 85 % and 75 % respectively for other unfed males and also females. This pheromone may possibly be considered analogously to that produced by *Hyalomma dromedarii* (Hajkova, Bouchalova & Leahy, 1980) and other metastriate tick species (Leahy, Kovacic, Mannion & Schulze, 1983), as inducing an initial orientation of both sex partners in the preparasitic phase and their subsequent adjacent attachment after finding a host. An argument in support of this function lies in the definite subsi-

dence and finally the total arrest of emission of the pheromone in 5- and 9-day preinfested male ticks.

In addition to the volatile water soluble pheromone, unengorged male imagines also emit a non-volatile pheromone which is attractive only to the female ticks. Initially this helps the 2 sexes to make contact off the host and then stimulates them to attach close to each other on the same host. The emission of this contact pheromone persists even after the males have found a host. This is clearly shown by the high aggregation rates of 73 %, 73 % and 87 % for female ticks on paper discs contact-impregnated by unfed as well as 5- and 9-day preinfested males. Thus newly arriving female ticks are further stimulated to attach near their male sexual partners.

The pheromone liberated by 9- day preinfested male ticks, however, attracts unengorged males as well as females. It is probably complementary to the volatile water-soluble male pheromone. Because of its extended action on both sexes, this biosubstance is thought to be a mixture of at least 2 pheromones, of which the intrasexually active component is presumably emitted by male ticks only after an infestation period of more than 5 days.

The hexane and diethyl ether soluble, volatile, male pheromones, however, form a fundamental part of the pheromonally controlled regulation mechanism of male ticks during infestation of the host. These pheromones are emitted during the period of maximal hypertrophy and hyperplasia and highest granular concentration of the foveal glands (Gothe & Burkhardt, 1981). They are released after an infestation period of at least 5 days in reaction-inducing quantities, sufficient to produce aggregation of male ticks only. It can be postulated that, because of the long period of sexual activity of male ticks and their capability for multiple inseminations (Pervomaisky, 1954; Rechav & Oppenheim, 1969; Gladney & Drummond, 1970; Oliver, 1974; Norval, 1974; Londt, 1976; Thompson *et al.*, 1980; Jordaan & Baker, 1981; Davey *et al.*, 1983), the function of this pheromone is to guarantee orientation on the host and mark the most suitable settling sites for newly arriving male ticks. Simultaneously, but to a lesser degree, female ticks are also attracted. These newly attached male imagines subsequently liberate the water soluble, volatile pheromones, to which high percentages of both male and female imagines respond. The contact pheromone may also be involved in this process. This favours the orientation of the next adult population and the attachment of female ticks in the vicinity of sexually competent partners.

It may be concluded from these investigations that the pheromonally directed interrelations between *R. evertsi evertsi* and its host are extremely complex. Furthermore, both sexual partners of this tick species are involved in pheromone reception as well as pheromone emission during the establishment of their reproductive association. The whole course of their biological activities up to copulation and fertilization of the female ticks is thus regulated by changing pheromonal stimulants.

A volatile, water soluble male pheromone and, by analogy with other metastriate tick species, most probably also a female pheromone (Hajkova *et al.*, 1980; Leahy *et al.*, 1983), initially determine the behaviour of the adult ticks. The 2 sexes are thus brought together while they are still in the preparasitic phase. A further non-volatile pheromone enhances this effect. After successful infestation, the intersexual behaviour of *R. evertsi evertsi* is initiated and directed by a female pheromone. This is in conformity with other metastriate tick species but contrary to the situation seen in the *Amblyomma* species, *maculatum, hebraeum, variegatum, gemma, lepidum, eburneum* and *cohaerens* (Sonenshine, Silverstein & Rechav, 1982; Berger, 1983; Leahy & Booth, 1983; Rechav, 1983a,b; Shoeni, Hess, Blum & Ramstein, 1983). This female pheromone is emitted during repletion only and, even with sexually still inactive male imagines, it initiates specific reactions. These reactions are manifested by interruption of feeding and excitation as well as recognition of an orientation towards females; this eventually guarantees sexual partnership with copulation (Gothe, Schniewind & Burkhardt, 1982; Schniewind & Gothe, 1982).

This response to the stimulus is quantitatively and qualitatively also dependent on the duration of the infestation, because male ticks can only adequately or optimally perceive the pheromone stimulus from the 3rd day of infestation onwards. The pheromone is perceived mainly, or possibly exclusively, through the chemoreceptors of Haller's organ or tarsus I; male *R. evertsi evertsi* react satisfactorily only if their functional and/or morpholigcal integrity is maintained. The pedipalps, on the other hand, cannot recognize the pheromonal stimuli. The production and emission of this pheromone is morphologically associated with the foveal glands and the fovea dorsales respectively. If these are selectively blocked on potentially attractive females, the response to stimuli by sexually competent male imagines is either partially or fully depressed (Gothe *et al.*, 1982; Schniewind & Gothe, 1982).

During the infestation phase there may be, by analogy with *Dermacentor* spp. (Sonenshine, Khalil, Homsher & Mason, 1982) and *Hyalomma* spp. (Khalil, Sonenshine, Sallam & Homsher, 1983), additional pheromones involved in *R. evertsi evertsi* which are emitted through the genital aperture. These pheromones guide the sexually active male imagines to the female gonopore. They may either trigger intraspecific copulation with implantation of the spermatophore and/or prevent interspecific mating.

References

BERGER, R. S., 1983. Occurrence of 2,6-dichlorophenol in *Dermacentor albipictus* and *Haemaphysalis leporispalustris* (Acari: Ixodidae). *Journal of Medical Entomology*, 20, p. 103.

BROWNE, L. E., BIRCH, M. C. & WOOD, D. L., 1974. Novel trapping and delivery systems for airborne insect pheromones. *Journal of Insect Physiology*, 20, 183–193.

DAVEY, R. B., OSBURN, R. L. & CASTILLO, C., 1983. Longevity in males and parthenogenesis in females in hybridized *Boophilus* ticks (Acari: Ixodidae). *Journal of Medical Entomology*, 20, 614–617.

GLADNEY, W. J. & DRUMMOND, R. O., 1970. Migration of male lone star ticks on the host in relation to mating. *Journal of Economic Entomology*, 63, 1214–1216.

GOTHE, R. & BURKHARDT, E., 1981. Zur Histologie der Fovealdrüsen bei *Rhipicephalus evertsi evertsi* Neumann, 1897. *Berliner und Münchner Tierärztliche Wochenschrift*, 94, 492–496.

GOTHE, R., SCHNIEWIND, A. & BURKHARDT, E., 1982. Zur Morphologie und Funktion der Fovealdrüsen bei *Rhipicephalus evertsi evertsi*. *Kurzreferate, 10 Tagung der Deutschen Gesellschaft für Parasitologie Hohenheim.*

GOTHE, R., 1983. Pheromones in ixodid and argasid ticks. *Veterinary Medical Review*, No. 1, 16–37.

HAJKOVA, Z., BOUCHALOVA, J. & LEAHY, M. G., 1980. A preattachment pheromone in the adult metastriate tick *Hyalomma dromedarii* Koch (Acari: Ixodidae) *Folia Parasitologica (Praha)*, 27, 367–372.

JORDAAN, J. O. & BAKER, J. A. F., 1981. Survival rate on the host and mating capacity of *Amblyomma hebraeum* (Koch) male ticks. *Proceedings of the International Conference on Tick Biology and Control, 27–29 January, Grahamstown, South Africa*, 115–117.

KHALIL, G. M., SONENSHINE, D. E., SALLAM, O. A & HOMSHER, P. J., 1983. Mating regulation and reproductive isolation in the ticks *Hyalomma dromedarii* and *H. anatolicum excavatum* (Acari: Ixodoidea: Ixodidae). *Journal of Medical Entomology*, 20, 136–145.

LEAHY, M. G. & BOOTH, K. S., 1983. Attraction of metastriate ticks (Acari: Ixodidae) to the sex pheromone 2,6-dichlorophenol and to substituted phenols. *Journal of Medical Entomology,* 20, 104–105.

LEAHY, M., KOVACIC, A., MANNION, C. & SCHULZE, L., 1983. Pheromone-induced aggregation of ixodid ticks before host contact. *Experientia,* 39, 859–860.

LONDT, J. G. H., 1976. Fertilization capacity of *Boophilus docoloratus* (Koch 1844) (Acarina: Ixodidae). *Onderstepoort Journal of Veterinary Research,* 43, 143–146.

NEITZ, A. W. H. & GOTHE, R., 1984. Investigations into the volatility of female pheromones and the aggregation-inducing property of guanine in *Argas (Persicargas) walkerae*. *Onderstepoort Journal of Veterinary Research,* 51, 197–201.

NORVAL, R. A. I., 1974. The life cycle of *Amblyomma hebraeum* Koch, 1844 (Acarina: Ixodidae). *Journal of the Entomological Society of Southern Africa*, 37, 357–367.

OLIVER, J. H., 1974. Symposium on reproduction of arthropods of medical and veterinary importance. IV. Reproduction in ticks (Ixodoidea). *Journal of Medical Entomology,* 11, 26–34.

PERVOMAISKY, G. S., 1954. Variation in pasture ticks (Acarina: Ixodidae) and its significance for systematics. *Trudy Vsesoyuznogo Entomologicheskogo Obshchestva,* 44, 62–64 (In Russian).

RECHAV, Y. & OPPENHEIM, J., 1969. Feeding and fertilizing capacity in male ticks of the species *Hyalomma excavatum* (Kock 1844). *Refuah Veterinarith,* 26, 71–74.

RECHAV, Y., 1983a. A bioassay technique for the pheromones emitted by *Amblyomma hebraeum* males. *Onderstepoort Journal of Veterinary Research,* 50, 133–135.

RECHAV, Y., 1983b. Evidence for the existence of a sex pheromone in *Hyalomma marginatum rufipes* (Acarina: Ixodidae). *Journal of Medical Entomology,* 20, p. 342.

SCHNIEWIND, A. & GOTHE, R., 1982. Investigations on the pheromone mediated intersexual relations and on the localization of pheromone-emission and -perception in *Rhipicephalus evertsi evertsi* Neumann, 1897. *Berliner und Münchner Tierärztliche Wochenschrift,* 95, 307–312.

SCHOENI, R., HESS, E., BLUM, W. & RAMSTEIN, K., 1983. The assembly pheromone of the tropical bont tick *Amblyomma variegatum* Fabricius (Acari: Ixodidae). Isolation and identification of an active component. *Zentralblatt für Backteriologie, Mikrobiologie und Hygiene*. I. *Abteilung Originale* A, 256, p. 263.

SONENSHINE, D. E., SILVERSTEIN, R. M., COLLINS, L. A., SAUNDERS, M., FLYNT, C. & HOMSHER, P. J., 1977. Foveal glands, source of sex pheromone production in the ixodid tick *Dermacentor andersoni* Stiles. *Journal of Chemical Ecology,* 3, 695–706.

SONENSHINE, D. E., SILVERSTEIN, R. M. & RECHAV, Y., 1982. Tick pheromone mechanisms. *In:* OBENCHAIN, F. D. & GALUN, R. (eds). Physiology of ticks, 439–468. Oxford: Pergamon Press.

SONENSINE, D. E., KHALIL, G. M., HOMSHER, P. J. & MASON, S. N., 1982. *Dermacentor variabilis* and *Dermacentor andersoni:* genital sex pheromones. *Experimental Parasitology,* 54, 317–330.

THOMPSON, G. D., DAVEY, R. B., OSBURN, R. L. & CRUZ, D., 1980. Longevity and fertilization capacity of males and parthenogenesis in females of *Boophilus annulatus* and *B. microplus*. *Journal of Economic Entomology,* 73, 378–380.

WALADDE, S. M. & RICE, M. J., 1982. The sensory basis of tick feeding behaviour. *In:* OBENCHAIN, F. D. & GALUN, R. (eds). Physiology of ticks, 71–118. Oxford: pergamon Press.

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