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TICK PHEROMONES

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ABSTRACT

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According to their activity profiles tick pheromones can be characterized as assembly pheromones, aggregation/attachment pheromones, pre-attachment or pre-feeding pheromones, attractant sex pheromones, contact sex pheromones, fecundity-reducing pheromone and various ungrouped pheromones. This paper gives a short review of the chemical characteristics, biological roles, and activity periods as well as the sites of production and release of tick pheromones.

INTRODUCTION

Pheromones are highly potent, intraspecifically active biosubstances, which are released on the skin or outer surface of an individual and are perceived by other individuals of the same species. These biosubstances act as chemical messengers or mediators by initiating and maintaining species-inherent response patterns and modes of behaviour.

According to their activity profiles tick pheromones are characterized as: (1) assembly pheromones, (2) aggregation/attachment pheromones, (3) pre-attachment or prefeeding pheromones, (4) attractant sex pheromones, (5) contact sex pheromones, (6) fecundity-reducing pheromone and (7) various ungrouped pheromones, which are not discussed in this paper. This classification system for tick pheromones, however, is probably arbitrary and is only provisional, because our knowledge of these biosubstances is still extremely limited.

1. ASSEMBLY PHEROMONES

These serve to concentrate off-host tick populations in optimal microenvironments. They occur mostly in argasid ticks, but probably also in ixodid species of the genera *Ixodes* and *Aponomma*, and possibly even in *Amblyomma, Hyalomma* and *Rhipicephalus*. The chemical nature of assembly pheromones, which exhibit a high degree of interspecific and interstadial activity, has not yet been elucidated. For *Argas (Persicargas) persicus, Argas (Persicargas) walkerae* and *Ornithodoros moubata* it has been shown that assembly pheromones are thermostable and non-volatile, with activity persisting for up to 3 months after storage at −25 °C. Their activity is retained only if the pheromone-impregnated filter paper discs are extracted with either physiological saline or water, but not with ether, benzene, acetone, pentane or methanol. A major component of these pheromones, at least for *A. (P). walkerae*, is guanine, which serves as a potent attractant for both engorged and unfed 1st and 2nd stage nymphs as well as for male and female imagines. Guanine, however, is certainly not the only constituent of the pheromone, because the response to it is lower than that to the naturally occurring pheromone (Gothe, 1983b; Gothe, Weck & Kraiss, 1984; Neitz & Gothe, 1984). The adaptive role of guanine as an assembly pheromone has also been demonstrated for *A. (P.) persicus, Amblyomma cohaerens* and *Rhipicephalus appendiculatus* (Otieno, Hassanali, Obenchain, Sternberg & Galun, 1985).

2. AGGREGATION/ATTACHMENT PHEROMONES

These are defined as inducers for regulating attachment to the host's body at sites where conspecific males have attached and fed. This pheromone-type is produced solely by male ticks, and in effective quantities only after completion of spermatogenesis. It attracts unfed males and females and even nymphs and thus results in aggregation of the sexes in close proximity to one another (Sonenshine, 1984, 1985). It is relatively species-specific and known so far only in *Amblyomma* spp. and in *Rhipicephalus evertsi evertsi*.

The pheromone-directed interactions in *Amblyomma hebraeum* and *Amblyomma variegatum*, the main vectors of *Cowdria ruminantium*, are governed primarily by male ticks which have fed for at least 5 days, with maximum activity taking place after infestation for 8 days (Gothe, 1983a; Sonenshine, 1984, 1985). The function of this pheromone, which is almost entirely specific and is attractive to unfed conspecific males and females, is to guarantee orientation on the host and to mark settling sites adjacent to fed males. This assists mating success and isolates different tick species, thus minimizing interspecific copulations. Copulation itself is regulated by a female pheromone which is emitted by female imagines which have been attached for 5 days and is attractive probably only for male ticks (Rechav, Parolis, Whitehead & Knight, 1977).

According to preliminary studies, the aggregation/attachment pheromone of *A. hebraeum*, and possibly also *A. variegatum*, is believed to consist of at least 2 active fractions. These trigger off 2 different behaviour patterns. One component is species-specific in its activity profile, regulating the attraction and orientation on the host, while the other is non-specific and designed to encourage and accelerate the process of attachment (Norval & Rechav, 1979; Rechav & Whitehead, 1979). The multi-component composition of this type of pheromone was confirmed for *A. variegatum* by demonstrating that the aggregation/attachment pheromone of 10-day-fed male ticks consists of 3 volatile compounds: O-nitrophenol, methyl salicylate, and pelargonic acid, in concentrations of 2,0; 1,0 and 8,0 μg per tick respectively. The isolation of individual components revealed that only O-nitrophenol induces orientation and dynamic aggregation without mounting and clasping, while methyl salicylate and pelargonic acid complete the action of O-nitrophenol by evoking mounting and clasping behaviour. The latter compounds induce no aggregation response when offered separately. A synthetic pheromone blend composed of all 3 components, of which the activity threshold was found to lie within a range of a few nanograms, evoked a complete aggregation response including mounting and clasping (Schöni, Hess, Blum & Ramstein, 1984).

From experimental data it can be deduced that fed *R. evertsi evertsi* males also liberate a volatile aggregation/attachment pheromone that is soluble in hexane and diethyl ether. This is emitted after an infestation period of at least 5 days via their foveae dorsales and is released in reaction-inducing quantities, sufficient to produce aggregation of unfed male ticks and to a lesser degree also of unfed females (Gothe & Neitz, 1985b; Neitz & Gothe, 1986).

3. PRE-ATTACHMENT OR PRE-FEEDING PHEROMONES

The aggregation/attachment pheromones probably form only one link in the chemical signal chain of male

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ticks. It has been demonstrated for *R. evertsi evertsi* and other metastriate tick species that various pre-attachment or pre-feeding pheromones are involved as inducers of an initial orientation of both sex partners in the pre-parasitic phase and their subsequent attachment adjacent to one another after finding a host. Probably the 1st link, as has been shown for *R. evertsi evertsi* (Gothe & Neitz, 1985b), is a volatile, water-soluble pheromone produced by unfed males. This is highly attractive for other unfed males and also females.

In addition to the volatile, water-soluble pheromone, unengorged males emit a non-volatile contact pheromone which is attractive only to female ticks. This contact pheromone, which is probably complementary to the volatile water-soluble male pheromone, is emitted even after the males have found a host. Thus newly arriving female ticks are further stimulated to attach near male sexual partners (Gothe & Neitz, 1985b). It has been shown for other metastriate tick species that female contact pheromones also participate in pheromonal communication during the pre-parasitic phase (Hájková, Bouchalová & Leahy, 1980; Leahy, Kovacic, Mannion & Schulze, 1983). However, the chemical characteristics of these pheromones are still unknown.

4. Attractant Sex Pheromones

After successful infestation, the intersexual and mating behaviour of metastriate ticks is initiated and directed by sex pheromones, which can be differentiated as attractant and contact sex pheromones. The possible participation of other pheromone types, however, is not excluded. Attractant sex pheromones are emitted only by attached feeding female ticks and serve to attract fed or feeding sexually competent males, initiating specific reactions. These reactions are manifested by interruption of feeding and excitation as well as recognition of and orientation towards females. This eventually guarantees sexual partnership with copulation.

The response to the attractant sex pheromone, perceived through chemoreceptors of Haller's organ on tarsus I, is quantitatively and qualitatively also dependent on the duration of infestation. This is because male ticks only react adequately to the pheromone stimulus from the 3rd day of infestation onwards.

The only sex attractant pheromone thus far clearly proved and experimentally confirmed in at least 13 species of the genera *Dermacentor, Amblyomma, Hyalomma, Haemaphysalis* and *Rhipicephalus* is 2,6-dichlorophenol, which is produced and stored in the paired foveal glands and released via the foveae dorsales. This compound is probably produced by the ticks through biosynthesis and is possibly combined with lipids in the foveal glands to mask its tissue toxicity until its secretion (Berger, 1974; Sonenshine, Silverstein & Homsher, 1979; Sonenshine, Gainsburg, Rosenthal & Silverstein, 1981; Sonenshine, 1984, 1985; McDowell & Waladde, 1986). It is probably catecholamine-mediated, because treatment of female *Dermacentor variabilis* with catecholamine-inhibiting agents such as reserpine or DL-α-methyl-m-tyrosine methyl ester hydrochloride reduced the activity and the concentration of this sex pheromone. In contrast, females injected with dopamine exhibited an elevated pheromone content (Sonenshine, Silverstein, West, Carson, Homsher, Bennet & Taylor, 1985). The increased concentration of ecdysteroids following eclosion of female *Hyalomma dromedarii* (Dees, Sonenshine & Breidling, 1985) as well as increased sex pheromone production after treatment with exogenous 20-OH ecdysone (Dees, Sonenshine & Breidling, 1984) may reflect an integrated role of these compounds in the stimulation of 2,6-dichlorophenol production.

Whether, and to what extent, other chemical substances are involved in the sexual intercommunication of metastriate ticks is still unknown. The pheromones responsible for this intercommunication in *Rhipicephalus sanguineus* and *Boophilus microplus* were described as slightly acid phenol compounds which are related to thymol (Berger, Dukes & Chow, 1971; Chow, Lu, Peng & Cheng, 1972). Phenol and p-cresol have been isolated from 2 *Rhipicephalus* spp. and from pre-infested female *R. appendiculatus* as well as *Rhipicephalus pulchellus* (Wood, Leahy, Galun, Prestwich, Meinwald, Purnell & Payne, 1975). Either on their own or in combination these compounds were exciting to male ticks. With p-cresol, strong excitation reactions were also induced in male *R. sanguineus* (Chow, Wang & Lin, 1975).

Investigations with *A. (P.) walkerae* have proved that female ticks emit volatile biosubstances, probably including a sex pheromone. Replete sexually active males reacted more strongly than other stages towards filter paper discs impregnated with volatile substances drawn from the extraintegumental spaces surrounding engorged female imagines. These females could emit pheromone through the genital aperture only and the volatile substances were collected in hexane or liquid air (Neitz & Gothe, 1984; Gothe & Neitz, 1985a).

As 2,6-dichlorophenol is a sex attractant pheromone in so many metastriate tick species specific stimuli must account for the high degree of reproductive isolation even in sympatric species. This species-isolating process may operate through differences in the natural concentration or emission of 2,6-dichlorophenol. Thus mate-seeking males respond to a very limited concentration range only, as has been demonstrated for *Hyalomma* spp. (Khalil, Nada & Sonenshine, 1981; Khalil, Sonenshine, Sallam & Homsher, 1983). Or the modus operandi may be associated with species-specific copulation-eliciting pheromone(s) (Sonenshine, Khalil, Homsher & Mason, 1982; Khalil *et al.*, 1983; Sonenshine, Homsher, Carson & Wang, 1984).

5. Contact (Mating Stimulant) Sex Pheromones

Research findings suggest that in *Dermacentor andersoni* and *D. variabilis* (Sonenshine *et al.*, 1982) as well as in *H. dromedarii* and *Hyalomma anatolicum excavatum* (Khalil *et al.*, 1983) probably 2 separate pheromones, designated as contact (mating stimulant) sex pheromones (Sonenshine, 1985), are responsible for eliciting male copulation. The 1st, a non-specific contact pheromone, guides the sexually active male to the female gonopore and the 2nd, also a contact pheromone, recognizes the conspecifity of the female partner and its sexual readiness.

Observations that males encountering females of other species probe their gonopores, but abort mating attempts, support the existence of the 1st pheromone. Evidence for the species-specific contact sex pheromone exists in the reduction, or even elimination, of copulation with spermatophore-transfer, after washing the genital area with hexane or acetone or after microsurgical severing of the vulva from the genital aperture. Perception of these contact sex pheromones in *Dermacentor* spp. is probably accomplished by chemoreceptors on the cheliceral digits (Sonenshine *et al.*, 1984; Sonenshine, Silverstein, Brossut, Davis, Taylor, Carson, Homsher & Wang, 1985). The chemical identity of these pheromones, however, is still unknown.

A similar excitatory sex pheromone, classified as a contact sex pheromone (Sonenshine, 1985) and also not yet identified, is believed to be released by females of *Ornithodoros erraticus* with their coxal fluid. This, even if it is of heterospecific origin to that of *O. moubata*

and *Ornithodoros tholozani*, attracts male imagines and stimulates copulation. The pheromonally evoked courting behaviour, however, is exhibited only in association with female ambulatory activity, thus proving that both pheromone and recognition of a behavioural pattern are essential components of the mating process (Schlein & Gunders, 1981).

6. Fecundity-reducing Pheromone

In argasid ticks, apart from assembly and sex pheromones, a 3rd type is described as a fecundity-reducing pheromone. This is produced in *Argas (Persicargas) arboreus* and is perceived only by fed adult ticks. It affects most, and possibly all, females in crowded conditions. It is considered to be a primer pheromone, probably causing alteration in normal vitellogenin synthesis and/or uptake and deposition in oocystes, thus resulting in reduced fecundity (Khalil, 1984). The source and nature of this pheromone has not yet been determined.

References

BERGER, R. S., DUKES, J. C. & CHOW, Y.-S., 1971. Demonstration of a sex pheromone in three species of hard ticks. *Journal of Medical Entomology*, 8, 84–86.

BERGER, R. S., 1974. Incorporation of chloride from $Na^{36}Cl$ into 2,6-dichlorophenol in lone star and Gulf coast ticks. *Annals of the Entomological Society of America*, 67, 961–963.

CHOW, Y.-S., LU, F.-M, PENG, C.-T. & CHENG, P.-C, 1972. Isolation of lipids and sex pheromone from hard ticks. *Bulletin of the Institute of Zoology, Academia Sinica*, 11, 1–8.

CHOW, Y.-S., WANG, C. B. & LIN, L. C., 1975. Identification of a sex pheromone of the female brown dog tick, *Rhipicephalus sanguineus*. *Annals of the Entomological Society of America*, 68, 485–488.

DEES, W. H., SONENSHINE, D. E. & BREIDLING, E., 1984. Ecdysteroids in *Hyalomma dromedarii* and *Dermacentor variabilis* and their effects on sex pheromone activity. p. 405–413. *In:* GRIFFITH, D. A., & BOWMAN, C. E. (eds). Acarology VI. Chichester: Ellis Horwood Ltd.

DEES, W. H., SONENSHINE, D. E. & BREIDLING, E., 1985. Ecdysteroids in the camel tick, *Hyalomma dromedarii* (Acari: Ixodidae), and comparison with sex pheromone activity. *Journal of Medical Entomology*, 22, 22–27.

GOTHE, R., 1983a. Pheromones in ixodid and argasid ticks. Part I: Ixodid ticks. *Veterinary Medical Review*, 1, 16–37.

GOTHE, R., 1983b. Pheromones in ixodid and argasid ticks. Part II: Argasid ticks. *Veterinary Medical Review*, 2, 157–171.

GOTHE, R., WECK, P. & KRAISS, A., 1984. Zur pheromonal induzierten Kommunikation von *Argas (Persicargas) walkerae* und biologisch-chemischen Bekämpfung durch kombinierten Einsatz eines Pheromons und Pheromon-Analogons mit Flumethrin. *Zentralblatt für Veterinärmedizin B*, 31, 161–179.

GOTHE, R. & NEITZ, A. W. H., 1985a. An investigation into the emission sites of the volatile pheromone produced by females of *Argas (Persicargas) walkerae*. *Onderstepoort Journal of Vetrinary Research*, 52, 31–33.

GOTHE, R., & NEITZ, A. W. H., 1985b. Investigation into the participation of male pheromones of *Rhipicephalus evertsi evertsi* during infestation. *Onderstepoort Journal of Veterinary Research*, 52, 67–70.

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

KHALIL, G. M., NADA, S. A. & SONENSHINE, D. E., 1981. Sex pheromone regulation of mating behaviour in the camel tick *Hyalomma dromedarii* (Ixodoidea: Ixodidae). *Journal of Parasitology*, 67, 70–76.

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.

KHALIL, G. M., 1984. Fecundity-reducing pheromone in *Argas (Persicargas) arboreus* (Ixodoidea: Argasidae). *Parasitology*, 88, 395–402.

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

MCDOWELL, P. G. & WALADDE, S. M., 1986. 2,6-dichlorophenol in the tick *Rhipicephalus appendiculatus* Neumann: a reappraisal. *Journal of Chemical Ecology*, 12, 69–81.

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.

NEITZ, A. W. H. & GOTHE, R., 1986. Investigations into the localization of pheromone emission of male *Rhipicephalus evertsi evertsi* during infestation. *Zentralblatt für Veterinärmedizin B*, 33, 269–273.

NORVAL, R. A. I. & RECHAV, Y., 1979. An assembly pheromone and its perception in the tick *Amblyomma variegatum* (Acarina: Ixodidae). *Journal of Medical Entomology*, 16, 507–511.

OTIENO, D. A., HASSANALI, A., OBENCHAIN, F. D., STERNBERG, A. & GALUN, R., 1985. Identification of guanine as an assembly pheromone of ticks. *Insect Science and its Application*, 6, 667–670.

RECHAV, Y., PAROLIS, H., WHITEHEAD, G. B. & KNIGHT, M. M., 1977. Evidence for an assembly pheromone(s) produced by males of the bont tick, *Amblyomma hebraeum* (Acarina: Ixodidae). *Journal of Medical Entomology*, 14, 71–78.

RECHAV, Y. & WHITEHEAD, G. B., 1979. Male produced pheromones of Ixodidae. *In:* RODRIGUEZ, J. G. (ed.): Recent advances in acarology, Vol. 2, 291–296. New York, San Francisco, London: Academic Press.

SCHLEIN, Y. & GUNDERS, A. E., 1981. Pheromone of *Ornithodoros* spp. (Argasidae) in the coxal fluid of female ticks. *Parasitology*, 82, 467–471.

SCHÖNI, R., HESS, E., BLUM, W. & RAMSTEIN, K., 1984. The aggregation-attachment pheromone of the tropical bont tick *Amblyomma variegatum* Fabricius (Acari: Ixodidae): Isolation, identification and action of its components. *Journal of Insect Physiology*, 30, 613–618.

SONENSHINE, D. E., SILVERSTEIN, R. M. & HOMSHER, P. J., 1979. Female produced pheromones of Ixodidae. *In:* RODRIGUEZ, J. G. (ed.): Recent advances in acarology, Vol. 2, 281–290. New York, San Francisco, London: Academic Press.

SONENSHINE, D. E., GAINSBURG, D. M., ROSENTHAL, M. D. & SILVERSTEIN, R. M., 1981. The sex pheromone glands of *Dermacentor variabilis* (Say) and *Dermacentor andersoni* Stiles. Sex pheromone stored in neutral lipid. *Journal of Chemical Ecology*, 7, 345–357.

SONENSHINE, 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.

SONENSHINE, D. E., 1984. Tick pheromones. *In:* HARRIS, K. F. (ed.), Current topics in pathogen-vector-host research, Vol. 2, 225–263. New York, NY: Praeger Press.

SONENSHINE, D. E., HOMSHER, P. J., CARSON, K. A. & WANG, V. D., 1984. Evidence of the role of the cheliceral digits in the perception of genital sex pheromones during mating in the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). *Journal of Medical Entomology*, 21, 296–306.

SONENSHINE, D. E., 1985. Pheromones and other semiochemicals of the Acari. *Annual Review of Entomology*, 30, 1–28.

SONENSHINE, D. E., SILVERSTEIN, R. M., WEST, J. R., CARSON, K. A., HOMSHER, P. J., BENNET, S. & TAYLOR, D., 1985. Studies on possible role of catecholamines in regulation of sex pheromone gland activity in American dog tick, *Dermacentor variabilis* (Say). *Journal of Chemical Ecology*, 11, 363–382.

SONENSHINE, D. E., SILVERSTEIN, R. M., BROSSUT, R., DAVIS, E. E., TAYLOR, D., CARSON, K. A., HOMSHER, P. J. & WANG, V. B., 1985. Genital sex pheromones of ixodid ticks. 1. Evidence of occurrence in anterior reproductive tract of American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae). *Journal of Chemical Ecology*, 11, 1 669–1 694.

WOOD, W. F., LEAHY, M. G., GALUN, R., PRESTWICH, G. D., MEINWALD, J., PURNELL, R. E. & PAYNE, R. C., 1975. Phenols as pheromones of ixodid ticks: a general phenomenon? *Journal of Chemical Ecology*, 1, 501–509.