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TICK REPRODUCTION IN A MALE TICK, *HAEMAPHYSALIS LONGICORNIS* (ACARI: IXODIDAE)

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Ticks are blood-sucking arthropods which inhabit all terrestrial regions of the earth and are important as pests affecting mankind, livestock and wildlife. A taxonomic group called "tick" in the subclass Acari including over 35,000 species (Oliver, 1989) is the Ixodoidea of the Metastigmata (Ixodida), which consists of approximately 850 species (Keirans, 1992). The superfamily Ixodoidea includes two major families: the Ixodidae called "hard tick" and Argasidae called "soft tick". The third family Nuttalliellidae is minor and consists of only a single species, and their reproduction is almost unknown. Furthermore, Ixodidae is often classified into Prostriata (Ixodidae: *Ixodes*) and Metastriata (Ixodidae except for *Ixodes*). *Haemaphysalis longicornis* Neumann, 1901 belonging to the Metastriata is characterized by having both the parthenogenetic and the bisexual races (Kitaoka, 1961).

Ticks cause severe toxic conditions, e. g. tick paralysis, various tick toxicoses, irritation, tick bite allergies, immune responses and economic losses due to blood sucking (Sonenshine, 1991a). Tick-borne protozoan, reckettsial, viral, and bacterial disease organisms continue to affect livestock and wildlife in the most area of the world. Heart water and cattle fever (babesiosis, theileriosis and anaplasmosis, etc.) are also caused by tick infestation (Bram, 1983; Bram & Gray, 1983). Furthermore, human tick-borne diseases are prevalent today (Lyme diseases, Rocky Mountain spotted fever, Russian spring-summer encephalitis, and human babesiosis) in addition to the diseases of veterinary and economical importance. *H. longicornis* is widely distributed in Australia, New Zealand, New Caledonia, the Fiji Islands, Japan, the Korean Peninsula and northeastern areas of both China and Russia. This species is known as a vector of the rickettsiae causing Q fever, viruses causing Russian spring-summer encephalitis, and protozoa causing theileriosis, respectively (Hoogstraal et al., 1968). *H. longicornis*, which is the most dominant tick in Japanese pastures, transmits theileriosis caused by *Theileria sergenti* / *buffeli* / *orientalis* group (Fujisaki et al., 1994) among grazing cattle (Ishihara, 1968). However, the relation between ticks and tick-borne disease organisms, especially the infection ways, are almost unknown, and then the intersexual infection of ticks during copulation is also supposed. Thus, examination of details of the tick reproduction and copulation is one of the most important fundamental studies in order to understand the cytological relation between ticks and tick-borne disease organisms.

In general, argasids and some prostriate ixodids are nidicolous, which live in or near the nests,

burrows, caves or other shelters used by hosts. Metastriates and most of prostriates, whereas, are non-nidicolous, which occupy open and exposed habitats and occur in forest, savannah, scrub, brush or meadow vegetation (Sonenshine, 1991b). The divergence of their habitats may greatly affect the probability of an encounter between a male and a female and/or a tick and a host. Thus, the variety of their reproductive systems is the diversity in adaptations to the divergence of their habitats. In argasids feeding and copulation occur at different sites, but they can meet hosts easily because they are nidicolous. The unfed Prostriata adults also copulate on the ground immediately after moulting without feeding as in argasids (Feldman-Muhsam & Borut, 1971; Fourie et al., 1988), so an encounter with the host is difficult in non-nidicolous species. On the other hand, Metastriata males must attach to the host for reproduction. The host will be infested by females during the host wanders wide area even if no females are discovered on the host. Thus, in Metastriata ticks the fact that both feeding and copulation occur on the host (at the same site) ensures that they are able to leave their offsprings whenever they can attach to the host, although an encounter with the host is not easy.

Newly emerged adult males of Argasidae and Prostriata are able to copulate, that is, their testes (spermatogenic cells) are developed by a feeding at the nymphal stage. Most Metastriata males, whereas, require a blood meal at the adult stage in order to copulate. Copulation of Metastriata ticks occurs between a completely fed male and a partially engorged female on the host. Metastriata males, which completed their feeding, search for a female on the host in order to copulate. Argasid ticks and many species of *Ixodes* (Prostriata) copulate off the host, whereas the Metastriata ticks copulate on the host. Therefore, it is considered that the copulatory behavior of argasids in the laboratory follows the same course as in nature and is easy to observe. It may be one of the reason why in *H. longicornis*, which need a relatively long feeding before mating and copulate on the host, studies on the male genital organs and spermatophore are not sufficient. Additionally, in Metastriata and Prostriata females there is an important difference, or the presence of the receptaculum seminis, which may directly correlates with their reproductive systems. But in male ticks the differences of the structure of the reproductive organs and the spermatophore almost never be important. The most important difference in male ticks is the timing of spermatogenesis and copulation.

We will describe here on reproduction of *H. longicornis*, especially in males: First, the process of the cell differentiation, the development of the testes during a feeding and the turnover of the spermatogenic cells will be discussed on the basis of observations of spermatogenic cells at each stage of spermatogenesis during feeding (Matsuo et al., 1997a) and degeneration of the spermatogenic cells after a completely feeding in the testes of adult males (Matsuo & Mori, 2000). Next, ultrastructure of the spermatophore, which play an important role in the transfer of elongated spermatids from a male to a female, and action of the spermatophore *in vitro* are observed, and then the structure and function of the spermatophore are discussed (Matsuo et al., 1998). Finally, the external shape and histological changes of the male accessory genital glands during a feeding and after a copulation are examined, and the derivation of the spermatophore components from the accessory glands is investigated histologically, histochemically and ultrastructurally (Matsuo, 2000; Matsuo et al., 1997b).

Spermatogenesis and Degeneration of spermatogenic cells in the testes.

Spermatogenesis

A male tick gives elongated spermatids to a female, that is, spermatogenesis within testes completes itself until elongated spermatids. The elongated spermatids are stored in the seminal vesicle of males, and then develop into spermatozoa after they enter into the female genital tract via the spermatophore. Ultrastructural observations of spermatogenic cells were made at each stage of spermatogenesis in male *H. longicornis* (Matsuo et al., 1997a). The testes, or spermatogenic cells, were developed gradually until the 5-day feeding period was complete (the complete feeding stage). Spermatogenic cells at all stages from spermatogonia to elongated spermatids in the testes of completely fed males: Spermatogonia and early primary spermatocytes in the anterior region of the testes, spermatocytes during their great growth phase in the middle large region of the testes, and from early spermatids just after the maturation division to complete elongated spermatids in the posterior region of the testes. Spermatogenic cells were packed in cysts. Elongated spermatids which had been completed at the posterior ends of the testes were made spermiation with the breakdown of the cyst cells. The spermatids were carried out of the testes through the central lumen. The cyst cells formed the lumen, and had some microvilli on their free surface.

One of the common characteristics of tick reproduction is that a male transfers elongated spermatids (prospermia) to a female. Spermatogenesis within the testes completes itself until the stage of elongated spermatids, and then these spermatids undergo "spermateleosis", or the process from elongated spermatids to mature spermatozoa after they enter into the female genital tract via the spermatophore. Terms in tick reproduction shows each process as follows:

- spermatogenesis; spermatogonia to spermatozoa
- spermiogenesis; rounded spermatids to spermatozoa
- spermateleosis; elongated spermatids to spermatozoa

The comparison of the processes of spermatogenesis between ticks and mammals is difficult, but the discrepancy in the application of these terms is very interesting. In mammals the intercellular bridges disappear as a result that spermatids shed off their cytoplasm as the residual bodies during spermiogenesis, thereafter capacitation occurs in spermatozoa after spermiation and ejaculation within the female genital tract. In ticks the intercellular bridges disappear at an early stage, or during the formation of the cisternal cavity in rounded spermatids, and this may be related that groups of spermatogenic cells packed in cysts develop and a whole spermatid transforms a spermatozoon without shedding off their cytoplasm. As the formation of the subplasmalemmal cisternae begins in primary spermatocytes, it is also capable in a broad sense that spermiogenesis occurs in tick primary spermatocytes. That is why the definition of spermiogenesis is the morphogenesis from simple cells (spermatids in mammals) to specialized spermatozoa. Moreover, spermateleosis were also called "capacitation" in ticks since elongated spermatids transform spermatozoa and then the preparation of fertilization is completed during spermateleosis within the female genital tract, but spermateleosis is possibly a part of spermiogenesis which is the morphogenesis of spermatogenic cells in order to be spermatozoa.

In Argasidae and Prostriata, whereas, an adult male must retain the developed testes (spermatogenic cells) until they meet a female since their copulation occurs mainly before feeding on the ground. Furthermore, in studies of reproduction in *Ornithodoros tholozani*, 30 % of first copulation of

starved males is successful matings. This percentage increases to about 82 % by the seventh copulation, but decreases again thereafter (Feldman-Muhsam, 1979). This suggests that *O. tholozani* males can form the spermatophores but spermatogenesis within the testes is not completed immediately after moulting. While, it seems that in all *Ixodes* species, which copulate at the same timing as argasids, the unfed male can always fertilize the female (Arthur, 1962; Balashov, 1972). *H. longicornis* males need a feeding for every copulation and degeneration of spermatogenic cells in testes without copulation after feeding.

Turnover of spermatogenic cells

As mentioned above, spermatogenesis advanced to slightly enlarged primary spermatocytes and early spermatids just after the maturation division in the testes of unfed and 3-day fed males, respectively. Spermatogenic cells at various stages of spermatogenesis were contained in the testes of completely fed males (5-day fed): Spermatogonia and early spermatocytes in the thin anterior region, spermatocytes during the great growth phase in the middle region, and from spermatids just after the maturation division to elongated spermatids in the thick posterior region. Furthermore, the degeneration of spermatogenic cells was observed in the posterior region of the testes after a completely feeding (Matsuo & Mori, 2000). The degeneration occurred in all the spermatogenic cells which had developed during feeding at the adult stage except in the elongated spermatids, that is, from the primary spermatocytes at the middle of their great growth phase to the spermatids just before their elongation is completed. The degeneration had already occurred at 2 days after a completely feeding. The degenerating area contracted gradually, and then the testes became thinner but their length remained the same. The complete elongated spermatids were normal morphologically until 10 days after a completely feeding. Some characteristics of the degenerating cells were as follows: The cells shrank, and ultimately became electron-dense dead cells. The shape of the nucleus became irregular, and the chromatin condensed into uniformly dense masses. Their plasma membrane had many blebs, but no loss of integrity. As these characteristics were similar to those of apoptotic cell death. Although the system causing the degeneration is unknown, the neuroendocrine system is believed to regulate the precise timing and their correlation with feeding of spermatogenesis with a relatively high degree of cellular differentiation (Sonenshine, 1991a). Thus, it is supposed that the degeneration is also regulated by the neuroendocrine system with the stimuli of the engorgement from the host and the stop of the nutrition supply.

If the males are not able to discover the females, an occurrence of rapid degeneration of the spermatogenic cells and the formation of new elongated spermatids are expected. It is known that male ticks are able to copulate many times, and male *H. longicornis* is also able to copulate at least six times by re-feeding (Yano et al., 1989). In the testes and seminal vesicle of a completely fed male there seems to be enough elongated spermatids to copulate a few times since these spermatids do not degenerate although their longevity is uncertain. Males may be able to copulate until elongated spermatids are dead even if the degeneration of the spermatogenic cells occurs. As *H. longicornis* (most Metastriata) copulate on the host, males can re-attach to the host soon if they can not meet a female. Therefore, it is expected that the developing spermatogenic cells degenerate rapidly, and their testes were developed again by re-feeding if necessary. Whereas, male Argasidae and Prostriata, which complete the spermatogenesis within testes at the engorged nymphal stage and mainly copulate off the host, may have to maintain their developed testes longer

time than Metastriata. As mentioned above, the degeneration and turnover of spermatogenic cells may play an important role in making the multi-copulation of a male tick possible in *H. longicornis*.

Spermatophore and Male Genital Accessory Glands

Structure of the Accessory Glands

The external shape and histological changes of the male accessory genital glands during feeding and after copulation were observed (Matsuo et al., 1997b). Histochemical properties between secretory granules of the accessory glands and components of the spermatophore were also compared. The accessory glands consisted of two single and five paired lobes opening into the collecting duct (CD) lying on the floor of the body cavity. The single dorso-median lobe (DML) opened to the posterior end of the CD and extended in an antero-dorsal direction. The single medio-ventral lobe (MVL) opened to the antero-ventral region of the CD. Five paired lobes were on both sides of the CD; the antero-ventral lobe (AVL), the latero-ventral lobe (LVL), the dorso-lateral lobe (DLL), the postero-lateral lobe (PLL) and the postero-ventral lobe (PVL) from anterior to posterior. Epithelia of the lobes showed remarkable histological changes synchronized with feeding and copulation: Secretory granules contained in the epithelia increased in number during feeding and were almost completely released after copulation. These histological changes suggest that the secretions of the accessory glands form the spermatophore. The number and shapes of lobes of the male accessory genital glands are different in Ixodidae and Argasidae (Balashov, 1972; Chinery, 1965; El Shoura, 1987; Mulmule & Thakare, 1985; Sonenshine, 1991a; Till, 1961). The external shape of the accessory glands in *H. longicornis* is similar to those of other ixodid tick species.

Although adult Prostriata and Argasidae males are able to copulate without feeding, adult Metastriata males require feeding to copulate. This also indicates that the latter accessory glands develop during feeding at the adult stage. In *H. longicornis*, the epithelial cells of each lobe of the accessory glands undergo various histological changes synchronized with feeding and copulation (Matsuo et al., 1997b). Particularly, dramatic histological changes occur during copulation (approximately for 10-15 min). These changes suggest that secretions of the accessory glands form the spermatophore. In addition, it is expected that *H. longicornis* males need a feeding especially for development of the accessory glands before every copulation because elongated spermatids are stored in the seminal vesicle after copulation or degeneration of spermatogenic cells in the testes but secretions of the accessory glands were empty after copulation.

Structure of spermatophore

It is known that the spermatophore of ticks is a pear-shaped duplex capsule made from secretions of the male accessory genital glands. Copulation in ticks is completed by the insertion of the spermatophore into the female genital aperture by a male. The endospermatophore, a cord-like structure and contents are packed in the ectospermatophore of the completed spermatophore. The endospermatophore extrudes just after insertion of the tip of the spermatophore. Only endospermatophore enters the female genital tract, and the ectospermatophore remains outside of the female body. The extrusion is observed *in vitro* in *H. longicornis* at various concentrations of NaCl solution: The process is accelerated in less concentrated solutions (Matsuo et al., 1998). The cord-like structure and the endospermatophore finally receive contents extruded from the ectospermatophore. The tip of the cord-like structure connects to the surface of the endospermatophore, and together form a loop after extrusion. Ultrastructural observations confirmed that the

ectospermatophore wall is composed of four layers, and the contents consist of spermatogenic cells and three types of secretions from the male accessory genital glands (Matsuo et al., 1998). As in other ticks the spermatogenic cells are elongated spermatids in spermatophores just after formation and extrusion. The secretions of the male accessory genital glands are also contained in the spermatophore with spermatogenic cells, and these granular contents are considered to trigger "spermateleosis" in ticks (Shepherd et al., 1982a, b), and to affect egg maturation and oviposition behavior in insects (Leopold, 1976). Adlerocysts described in other ticks are not found in the spermatophore of *H. longicornis* (Matsuo et al., 1998).

The spermatophore is a package for the transfer of male germ cells in many invertebrates, and also called sperm-bundle, spermatodesm, or spermatozeugmata. In most instances they consist of male accessory secretions, and may take the shape of a "thread", "tube", "worm", "seed", "pear", "balloon", "flask", "disk", and other forms. Similarly variable can be its size, ranging from the 0.15 mm long and 0.04 mm wide spermatophore of *Diarthrodes cystoecus* (a small copepod, less than 1 mm long) to 1 m long and 1 cm wide spermatophore of *Octopus dofleini* (the giant octopus, up to 50 kg in weight, in the Pacific) (Mann, 1984). In insects and mites transfer of the spermatophore from the male to the female may be performed with or without meeting of the sexes (Feldman-Muhsam, 1986). In ticks the spermatophore is inserted directly into the female genital aperture by a male, and then only the endospermatophore enters the female genital tract. The structure of the endospermatophore is dissimilar in Ixodidae and Argasidae, but the functional dissimilarity caused by the structural dissimilarity is unknown. If the spermatophore participates in the copulatory stimuli, the structural difference of the endospermatophore may be related to the property of the stimuli. In Metastriata females their engorgement is lead by the copulatory stimuli, therefore copulation is a more important event than in Argasidae and Prostriata.

It is also described that some of them are able to copulate many times without feeding: The number of copulation is 20 and 32 times in unfed and feeding male *O. savignyi*, respectively (Feldman-Muhsam, 1986). The male accessory glands of argasids and prostriates are able to form many spermatophores without feeding. Accordingly, it is easily expected that the ability of the male accessory glands in Argasidae and Prostriata is considerably different from that in Metastriata.

Derivation of Spermatophore Components

The male accessory genital glands of *H. longicornis* are a complex of glands consisting of the single DML and MVL and paired AVL, LVL, DLL, PLL, and PVL as mentioned above. The epithelia of each lobe produce various secretory granules during feeding and these secretions have important functions in the formation of the spermatophore. Some of the accessory gland secretions form the spermatophore as a bag of male germ cells and others are packed in the spermatophore. The fate of the accessory gland secretions was elucidated by comparison between the ultrastructure of the male accessory genital glands and the spermatophore, and the roles of these secretions are discussed (Matsuo, 2000). The characteristic fine structures of each lobe and 24 types of secretory granules were observed in the whole accessory glands. It was shown ultrastructurally that two layers of the ectospermatophore were derived from the AVL and PLL (= DLL) and three types of granular contents in the spermatophore from the PVL, LVL and MVL. The histochemical comparisons showed that the outer and inner layers of the ectospermatophore were, respectively, derived from the AVL and the DLL (PLL), and three types of granules in the spermatophore

were transferred from the MVL, the PVL and the LVL (Matsuo et al., 1997b). So, these findings support the results of histochemical observations, and more accurate information on the derivation of substances in the formation of the spermatophore were obtained.

According to histochemical studies on the spermatophore in ticks (Feldman-Muhsam, 1967a, b, 1986; Feldman-Muhsam & Borut, 1978, 1983, 1984; Oliver et al., 1974), the composition of the ectospermatophore is identical in ixodid and argasid ticks, that is, their ectospermatophores consist of three layers, an outermost thin acid mucopolysaccharide layer, a middle thick protein layer and an innermost thin polysaccharide layer. However, we were able to observe only two layers, i. e. the above-mentioned outermost and middle layers, by light microscopy. Histochemical results also showed that they are composed of acid mucopolysaccharides (the former) and basic proteins (the latter). Therefore, we made histochemical observations of the two distinguishable layers. On the other hand, it was confirmed that the wall of the ectospermatophore consists of four layers in *H. longicornis* by electron microscopy.

References

Arthur, D. R. 1962. Ticks and Disease, International Series of Monographs on Pure and Applied Biology, Vol. 9. Pergamon Press, Oxford.

Balashov, Y. S. 1972. Bloodsucking ticks (Ixodoidea) - Vectors of diseases of man and animals. Transl. by O. G. Strekalovski, *Misc. Publs. Ent. Soc. Am. 8*: 161-376.

Bram, R. A. 1983 Tick-borne livestock diseases and their vectors. 1. The global problem. pp. 7-11. *In* : FAO Animal Production and Health Papers. 36. Ticks and tick-borne diseases: selected articles from World Animal Review, FAO, Rome.

Bram, R. A. & Gray, J. H. 1983. Eradication - an alternative to tick and tick-borne disease control. pp. 54-59. *In*: FAO Animal Production and Health Papers. 36. Ticks and tick-borne diseases: selected articles from World Animal Review, FAO, Rome.

Chinery, W. A. 1965. Studies on the various glands of the tick *Haemaphysalis spinigera* Neumann 1897. *Acta Trop. 22*: 235-266.

El Shoura, S. M. 1987. Fine structure of the vasa deferentia, seminal vesicle, ejaculatory duct, and accessory gland of male *Ornithodoros* (*Pavlovskyella*) *erraticus* (Acari: Ixodoidea: Argasidae). *J. Med. Entomol., 24*: 235-242.

Feldman-Muhsam, B. 1967a. Spermatophore formation and sperm transfer in *Ornithodoros* ticks. *Science 156*: 1252-1253.

Feldman-Muhsam, B. 1967b. The components of the spermatophore, their formation and their biological functions in argasid ticks. *Proc. 2nd Int. Cong. Acarol.* 357-362

Feldman-Muhsam, B. 1979. Copulatory behaviour and fecundity of male *Ornithodoros* ticks. *In*: Recent Advances in Acarology, Vol. 2. pp. 159-166. Rodriguez, J. D. (ed), Academic Press, London.

Feldman-Muhsam, B. 1986. Observations on the mating behaviour of ticks. *In*: Morphology, Physiology, and Behavioral Biology of Ticks. pp. 217-232. Sauer, J. R. & Hair, J. A. (eds), Ellis Horwood Limited, Chichester.

Feldman-Muhsam, B. & Borut, S. 1971. Copulation in ixodid ticks. *J. Parasitol. 57*: 630-634.

Feldman-Muhsam, B. & Borut, S. 1978. Further observations on spermatophore formation in argasid ticks. *J. Insect Physiol. 24*: 693-697.

Feldman-Muhsam, B. & Borut, S. 1983. On the spermatophore of ixodid ticks. *J. Insect Physiol. 29*: 449-457.

Feldman-Muhsam, B. & Borut, S. 1984. Differences in the structure of the spermatophore between argasid and ixodid ticks. pp. 504-511. *In*: Acarology VI, Vol. 1. Griffiths, D. A. & Browman, C. E. (eds), Ellis Horwood Limited, Chichester.

Fourie L. J., de Jager, T. & Petney, T. N. 1988. Prolonged or repeated copulation and male longevity in the tick *Ixodes rubicundus*. *J. Parasitol. 74*: 609-612.

Fujisaki, K., Kawazu, S. & Kamio, T. 1992. The taxonomy of the bovine *Theileria* spp. Parasaitol. Today. *10*: 31-33.

Hoogstraal, H., Roberts, F. H. S., Kohls, G. M. & Tipton, V. J. 1968. Review of *Haemaphysalis* (*Kaiseriana*) *longicornis* Neumann (resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and northeastern China and USSR, and its parthenogenetic and bisexual populations (Ixodoidea, Ixodidae). *J. Parasitol. 54*: 1197-1213.

Ishihara, T. 1968. Bovine piroplasmosis in Japan. *Jpn. Agr. Res. Q. 3*: 23-31.

Keirans, J. E. 1992. Systematics of the Ixodida (Argasidae, Ixodidae, Nuttalliellidae); an overview and some problems. pp. 1-21. *In*: Tick Vector Biology, Medical, Veterinary Aspects. Fivaz, B. H. (ed), Springer-Verag, Berlin.

Kitaoka, S. 1961. Physiological and ecological studies on some ticks. VII. Parthenogenetic and bisexual races of *Haemaphysalis bispinosa* in Japan and experimental crossing between them. *Nat. Inst. Anim. Hlth Q. 1*: 142-149.

Leopold, R. A. 1976. The role of male accessory glands in insect reproduction. *Annu. Rev. Entomol. 21*: 199-221.

Mann, T. 1984. Thaddeus Mann Spermatophores., Spring-Verlag, Berlin.

Matsuo, T. 2000. Fate of the male accessory genital gland secretions in the formation of the spermatophore of a tick, *Haemaphysalis longicornis* (Acari: Ixodidae). *J Acarol. Soc. Jpn. 9*: 147-157.

Matsuo, T. & Mori, T. 2000. The significance of feeding for reproduction in a male Metastriata tick, *Haemaphysalis longicornis* (Acari: Ixodidae). *Acta Zool. 81*: 17-26.

Matsuo, T., Mori, T. & Shiraishi, S. 1997a. Ultrastructure observation on spermatogenesis within testes in *Haemaphysalis longicornis* (Acari: Ixodidae). *J. Fac Agr., Kyushu Univ. 41*: 179-188.

Matsuo, T., Mori, T. & Shiraishi, S. 1997b. Histological and histochemical studies on the male accessory genital glands and the derivations of the spermatophore components from the accessory glands in *Haemaphysalis longicornis* (Acari: Ixodidae). *J. Acarol. Soc. Jpn. 6*: 1-10.

Matsuo, T., Mori, T. & Shiraishi, S. 1998. Studies on *in vitro* extrusion and ultrastructure of the spermatophore in *Haemaphysalis longicornis* (Acari: Ixodidae). *Acta Zool. 79*: 69-74.

Mulmule, S. & Thakare, V. K. 1985. Cytology and cytochemistry of male accessory sex glands in the dog tick, *Rhipicephalus sanguineus* (L.)(Acarina: Ixodidae). *Z. mikrosk.-anat. Forsch. 99*: 25-36.

Oliver, J. H., Jr. 1989. Biology and systematics of ticks (Acari: Ixodida). *Ann. Rev. Ecol. Syst. 20*: 397-430.

Oliver, J. H., Jr., Al-Ahmadi, Z. & Osburn, R. L. 1974. Reproduction in ticks (Acari: Ixodoidea) . 3. Copulation in *Dermacentor occidentalis* Marx and *Haemaphysalis leporispalustris* (Packard)(Ixodidae). *J. Parasitol. 60*: 499-506.

Shepherd, J., Levine, S. & Hall, J. D. 1982a. Maturation of tick spermatozoa *in vitro*. *Int. J. Invertebr. Reprod. 4*: 311-321.

Shepherd, J., Oliver, J. H., Jr. & Hall, J. D. 1982b. A polypeptide from male accessory glands which triggers maturation of tick spermatozoa. *Int. J. Invertebr. Reprod. 5*: 129-137.

Sonenshine, D. E. 1991. Biology of Ticks, Vol. 2., Oxford Univ. Press, New York.

Till, W. M. 1961. A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus* Neumann. *Mem. Entomol. Soc. South. Afr. 6*: 1-124.

Yano, Y., Shiraishi, S. & Uchida, T. A. 1989. Feeding pattern, mating and oviposition in female *Haemaphysalis longicornis* Neumann (Acari: Ixodidae). *J. Fac. Agr., Kyushu Univ. 33*: 287-296.