Fate of the male accessory genital gland secretions in the formation of the spermatophore of a tick, *Haemaphysalis longicornis* (Acari: Ixodidae)

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ABSTRACT

The male accessory genital glands of *Haemaphysalis longicornis* are a complex of glands consisting of the single dorso-medial and medio-ventral lobes and paired antero-ventral, latero-ventral, dorso-lateral, postero-lateral, and postero-ventral lobes. 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 and then transferred into the female genital tract. 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. The characteristic fine structures of each lobe and 24 types of secretory granules were observed in the whole accessory glands. It was shown morphologically that two layers of the ectospermatophore were derived from the antero-ventral and postero-lateral (=dorso-lateral) lobes and three types of granular contents in the spermatophore from the postero-ventral, latero-ventral and medio-ventral lobes. These findings support the results of histochemical previous observations, and more accurate information on the derivation of substances in the formation of the spermatophore were obtained.

Key words: *Haemaphysalis longicornis*, tick, male accessory glands, ultrastructure, secretory granule, spermatophore formation

INTRODUCTION

The male accessory genital glands of ticks consist of many glands (=lobes) and contain various secretions after feeding. The spermatophore and its granular contents are made from secretions of the male accessory glands. There are many studies on the "sex peptide" from the male accessory glands in insects and it is known that the major functions of this peptide are reduction of receptivity and increased oviposition in mated females (Eberhard, 1996). It is described that important functions of accessory gland secretions (granular contents) include triggering spermateleosis (Borut and Feldman-Muhsam, 1976) within the female genital tract in ticks (Shepherd et al., 1982a, b) and providing some chemical copulatory stimuli for the female (Diehl et al., 1982), but the derivations, functions and mechanisms of the granular contents have not been completely clarified. Studies on the participation of the male accessory genital glands with the formation of the spermatophore were reported in *Argas persicus* (Tatchell, 1962), and ultrastructure of the accessory glands was observed only in *Ornithodoros erraticus* belonging into Argasidae (El Shoura, 1987). Although it is

known that the structure of the accessory glands and the spermatophore differs between Ixodidae and Argasidae (Balashov, 1972; Chinery, 1965; Feldman-Muhsam and Borut, 1984; Mulmule and Thakare, 1985; Sonenshine, 1991; Till, 1961), there are few histological studies on the glands of Ixodidae ticks.

To elucidate these problems, we first investigated the accessory glands of *Haemaphysalis longicornis* Neumann by scanning electron microscopic and histochemical techniques. The accessory glands consisted of seven types of lobes containing various secretions and the derivations of the spermatophore components were suggested (Matsuo et al., 1997). Following ultrastructural observations on the spermatophore revealed that it had four layers of the ectospermatophore and three types of granular contents (Matsuo et al., 1998). Based on these results, we observed the ultrastructure of the male accessory glands in *H. longicornis* and then attempted to reveal more accurately the fate of the accessory gland secretions.

MATERIALS AND METHODS

H. longicornis males and females used in the present study were collected by dragging on pastures in Kuju Highland, Oita Prefecture. They were fed on ears of laboratory rabbits until the males became potent (for 7 days) and females sexually receptive (for 5 days), and then some of the ticks copulated on the host.

The accessory genital glands of males (7-day fed and just after copulation) were removed. All the accessory glands were fixed with cold 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4), postfixed with 1% OsO₄ in the same buffer, dehydrated in an ethanol series, and embedded in epoxy resin. Semi-thin sections (about 1.5 µm in thickness) were cut on a Porter-Blum MT-1, using a glass knife, and stained with toluidine blue for light microscopic observation. Thin sections (about 60 nm in thickness) were cut on a Porter-Blum MT-2, using a diamond knife, and doubly stained with uranyl and lead acetate before examination in a Hitachi H-600A electron microscope.

RESULTS

The male accessory genital glands consist of five paired lobes on both sides of the collecting duct (CD), or 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, and two single lobes, or the dorso-median lobe (DML) opening to the posterior end of the CD and extending in an antero-dorsal direction and the medio-ventral lobe (MVL) opening to the antero-ventral region of the CD (Matsuo et al., 1997). The DLL and the PLL differ in external shape but are histologically identical. The findings on electron microscopic observations of the male accessory genital glands before and after copulation will be described. Different types of secretory granules (G) morphologically were distinguished by numbering consecutively.

The AVL was the smallest in the accessory glands, and G₁, G_{2,3} and G₄ were contained in the glandular cells at the proximal, middle and distal region, respectively (Fig. 1a-d). Secretory granules were almost never found just after copulation, but some showed features

Fig. 1. Light and electron micrographs showing the antero-ventral lobe (AVL) bearing four types of secretory granules (G_{1-4}). a, A cross section of the AVL in a fed male. proximal (*, b), middle (**, c), and distal (***, d) region. Both G_1 and G_4 are electron-lucent but only distal ones sometimes have an electron-dense core. G_2 and G_3 are contained in the middle region. e, Glandular cells just after copulation. Most of granules (G) are not found. f and g, Apical surface of the higher magnification of Fig. 1e showing the feature of exocytosis. CD, collecting duct; CT, connective tissue; L, lumen; N, nucleus. Bars = 50 μ m (a), 1 μ m (b, d, f, g), 3 μ m (c), 5 μ m (e).

of exocytosis (Fig. 1e-g).

The large lumen leading to the collecting duct was found in the proximal region of the LVL, and G_5 was observed in the distal region extending in a dorsal direction (Fig. 2a, b). G_6 and G_{7-8} were contained in glandular cells at the middle and proximal region around the lumen, respectively (Fig. 2c, d). Isolated mitochondria were also confirmed in the lumen just after copulation (Fig. 2e).

The DLL and PLL contained G_9 just after feeding (Fig. 3a, b). Wedge-like cells with

Fig. 2. Light and electron micrographs showing the latero-ventral lobe (LVL). a, A cross section of the LVL in a fed male. Asterisks show each region containing different granules. b, distal region (*). Irregular shaped G_5 are observed. c, middle region (**). G_6 contains fibrillar structures (arrow; inset). d, proximal region (***). Electron-dense and irregular shaped G_7 along the lumen (L) and electron-lucent G_8 at the basal portion are found. e, Apical region of glandular cells and the lumen just after copulation. The isolated mitochondria (MT) are also found in the lumen. CD, collecting duct; J, intercellular junction; MV, microvilli; N, nucleus; SV, seminal vesicle. Bars = 100 μm (a), 1 μm (b, c, e), 5 μm (d), 0.2 μm (inset).

homogenous cytoplasm were scattered among glandular cells and sometimes reached the lumen (Fig. 3a, c). The secretory granules were almost completely released just after copulation (Fig. 3d), and residual membrane winding like coils was confirmed at the apical region of the glandular cells (Fig. 3e).

The glandular cells of the PVL had pale and large nuclei and were filled with two types of granules; G_{10} was electron-dense irregularly shaped granules and G_{11} was relatively electron-lucent spherical granules having an annulate lamellae-like structure (Fig. 4) in fed males.

Fig. 3. Light and electron micrographs showing the postero-lateral lobe (PLL). a, A cross section of the PLL in a fed male. The slit-like lumen (L) are found. There are small number of pale cells between glandular cells (arrows). b, Glandular cells just after feeding. It contains huge G_g . c, Cells with homogeneity cytoplasm (indicated by arrows in Fig. 3a). d, Glandular cells just after copulation. Most of granules (G) have been released. e, Apical region of glandular cells just after copulation. The residual membrane winds like coils (arrows). MV, microvillus; N, nucleus; RER, rough endoplasmic reticulum. Bars= 100 μ m (a), 5 μ m (b, c), 10 μ m (d), 3 μ m (e).

The cross sections of the MVL appeared like a triangle (Fig. 5a), and the glandular cells at the lateral and bottom (ventral) region contained G_{12-13} and G_{14} , respectively (Fig. 5b-d). Small granules with a plasma membrane were found in the lumen by the lateral epithelium just after copulation only (Fig. 5e).

The DML was the largest lobe of the accessory glands, slightly divided into right and left at the anterior end, and had large protrusions on both sides at the posterior region. Electron-dense G_{15} broadly distributed in the dorsal side from the middle to posterior region and protrusions at the posterior region (Fig. 6a). The features of the G_{15} in these two regions

Fig. 4. Electron micrographs showing G_{10} and G_{11} in the postero-ventral lobe (PVL). inset: Electron-lucent G_{11} have an annulate lamellae-like structure (arrowhead). Bars = $1\ \mu\text{m}$, $0.1\ \mu\text{m}$ (inset).

were identical but in the former region entirely or partially electron-lucent granules were also observed on the basal side of the glandular cells. G_{16} with medium electron density were contained chiefly in the anterior region (Fig. 6b), and small number of G_{17-18} were found in the inner part of the anterior region (Fig. 6c, d). Electron-dense G_{19} with a core were contained in the tall glandular cells at the ventral side of the middle region (Fig. 6e). These granules became homogenous and their boundary was unclear at the apical portion. Electron-lucent G_{20} were also observed in the center of the cells in the same region, and sometimes these granules had a dense core (Fig. 6e). Small aggregations of pale G_{21} were scattered through the anterior region and the ventral side of the posterior region (Fig. 6f). Relatively electron-lucent and uneven G_{22} were confirmed in the narrow part between the G_{15} in the dorsal side and the protrusion at the posterior region (Fig. 6g). A small number of large granular structures appeared to contain some small G_{23} distributed in the dorso-lateral part of the middle region (Fig. 6h) and G_{24} in the outer part of the anterior region (Fig. 6i). Wedge-like cells with homogenous cytoplasm and no secretory granules were scattered between glandular cells in the anterior and middle region (Fig. 6j), and were highly similar to the cells in the DLL and PLL.

DISCUSSION

The following derivations of the spermatophore components were suggested by some histochemical methods in *H. longicornis* (Matsuo et al., 1997); (1) the outer layer of the ectospermatophore derives from granules in the AVL, (2) the inner layer of the ectospermatophore from granules in the DLL and the PLL, and (3) granules in the spermatophore from granules in the MVL, the PVL and the LVL. The derivations of the spermatophore components were discussed on the basis of the present observations as follows, and then refer Matsuo et al. (1998) for ultrastructure of the spermatophore (Table 1).

Fig. 5. Light and electron micrographs showing the medio-ventral lobe (MVL). a, A cross section of the MVL in a fed male. The lateral (*) and bottom (**) region. b and c, G_{12} and G_{13} in the lateral region. The latter appears to consist of some small granules. d, G_{14} at the bottom region. e, Glandular cells just after copulation. Granules (G) in the cytoplasm resembling the G_{13} . Small granules are found in the lumen (arrow). inset: The small granules with plasma membrane. CD, collecting duct; CT, connective tissue; L, lumen; N, nucleus. Bars = $100\text{ }\mu\text{m}$ (a), $2\text{ }\mu\text{m}$ (b-d), $5\text{ }\mu\text{m}$ (e), $0.2\text{ }\mu\text{m}$ (inset).

Derivation of the ectospermatophore: Substances forming the ectospermatophore may be released by exocytosis, so in order to decide their derivations not only morphological comparisons but also the style of secretion must be shown. The AVL showed features of exocytosis and the DLL and the PLL contained an identical type of huge electron-dense granules. The formation of a thick and electron-dense layer of ectospermatophore may require the largest quantity of secretion since an $80\text{ }\mu\text{m}$ -thick layer envelopes the whole spermatophore (about $800\text{ }\mu\text{m}$ long). In addition, the coil-like membrane in the lumen of the DLL and the PLL just after copulation may be shed from the G_9 after the apocrine-like

Fig. 6. Electron micrographs showing the dorso-medial lobe (DML). a, Electron-dense G₁₅ in the dorsal side from middle to posterior region and in protrusions on both sides of the posterior region. b, G₁₆ with medium electron density in the anterior region. c and d, G₁₇ and G₁₈ containing fine particle- or fibril-like structure in the inner part of the anterior region. e, Electron-dense G₁₉ with an electron-dense part and electron-lucent G₂₀ are found in tall glandular cells in the ventral side of middle region. The G₂₀ are found only at the center portion of glandular cells and some have an electron-dense core. f, Electron-lucent G₂₁ lie scattered in the anterior region and in the ventral side of the posterior region. g, G₂₂ with medium and uneven electron density are found in the narrow part between G₁₅ in the dorsal side and protrusions of the posterior region. h and i, Small number of large granular structures appeared to contain small G₂₃ are confirmed in the dorso-lateral part of middle region and G₂₄ in the outer part of the anterior region. The small granules in the G₂₄ are irregular in size and their profile is clearer than that in the G₂₃. j, Cells with homogeneity cytoplasm are similar closely to ones of the DLL and the PLL showing in Fig. 3c. N, nucleus. Bars = 5 μ m (a, e, j), 3 μ m (b, c, d, f-i).

Table 1. Derivations of the spermatophore components from the male genital accessory glands.

Spermatophore Components*	Origin	
	Accessory gland lobe	Granule number
Ectospermatophore		
Layer I (outermost)	?	?
Layer II	AVL	G2 and/or G3
Layer III	DLL and PLL	G9
Layer IV (innermost)	?	?
Endospermatophore		
Endospermatophore Sac	DML	G15
Cord-like Structure	DML	G19
Plug	DML	G16
Granular Contents		
Large size	PVL	G10 and G11
Intermediate size	LVL	G7
Small size	MVL	G13

*; Terminology after Matsuo et al. (1998)

secretion, since the huge granules must be released within a short time. Thus, we suppose that the two layers of the ectospermatophore (= the middle two layers on electron microscopic observations) consists of a thin outer layer derived from G₂ and/or G₃ in the AVL and a thick inner layer from G₉ in the DLL and PLL. Unfortunately the derivations of the outermost and innermost layers of the ectospermatophore were not ascertained in the present study. A gland enveloping the ejaculatory duct in *H. longicornis* (Matsuo, unpublished data), which has not been previously identified in ticks, was confirmed and its secretion may form the outermost layer as the lobular accessory gland coating eggs in female ticks. It was also described that the outermost layer corresponds to salivary secretions (Feldman-Muhsam et al. 1970) or mucin (Tatchell 1962), but the former speculation was contradicted by Matsuo et al. (1998) in *H. longicornis* because the male mouth parts did not touch the spermatophore in their observations.

Derivation of the endospermatophore: The DML was the largest lobe in the accessory glands and contained 10 types of granules ultrastructurally. The formation of the spermatophore occurs through formation of the ectospermatophore and injection of the male germ cells and the granular contents before the formation of the endospermatophore. Some different types of secretory granules appear to be randomly scattered in the DML, so it may be difficult for only a specific type of granules is secreted at specific times. The endospermatophore consists of the endospermatophore sac (EN), the cord-like structure (CS) and the plug (P) (Matsuo et al., 1998), and these may be derived from secretions of the DML. It is suggested by overall analysis of histochemical and ultrastructural results that the EN is derived from the G₁₅, the CS from the G₁₉ and the P from the G₁₆.

Derivation of the granular contents of the spermatophore: Findings of the granular contents of the spermatophore were also obtained by comparison with the ultrastructure of the spermatophore and then supported histochemical data. The accessory gland secretions resembling the granular contents were as follows; (1) two types of granules in the PVL (G₁₀ and G₁₁), which differed in their electron density and shape, as well as G₁₁ had an annulate lamellae-like structure, and (2) electron-dense and irregularly shaped granules were found in

the proximal region of the LVL (G_7), and isolated mitochondria were also found in the lumen just after copulation. Moreover, it was shown histochemically that the third granules in the spermatophore were derived from the MVL (Matsuo et al., 1997). Ultrastructurally, these were enveloped by a plasma membrane with mitochondria (Matsuo et al., 1998). Therefore, it was expected that apocrine occurs in the MVL. Small granules similar to the third granules were observed in the lumen of the MVL just after copulation only. Although the small granules may be derived from G_{13} , G_{24} in the DML is very similar to G_{13} . However, based on order of secretion in the DML described above, it is considered that the third granular content is derived from the MVL. It became possible to more accurately estimate the derivations of spermatophore components based on all the informations obtained.

In the present study, 24 types of granules were described but the fate of many other types of secretory granules which were not discussed above remained unclear. Some of these granules may be at different stages of the formation processes and it is unclear whether G_{13} , G_{23} and G_{24} are secretory granules or other structures such as a lysosome-like structure. Moreover, the G_1 and G_4 in the AVL and G_{13} in the MVL were very similar to G_{21} , G_{20} and G_{24} in the DML, respectively. Thus, some types of granules may be regarded as only one type in future and have other functions except for formation of the spermatophore, so these are problems to be solved hereafter.

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摘 要

フタトゲチマダニの精包形成における雄性生殖付属腺分泌物の行方 松尾智英(九大・農・動物)

フタトゲチマダニの雄性生殖付属腺は、単独の背中葉と中腹葉、および5種類の対をなす前腹葉、側腹葉、背側葉、後側葉と後腹葉から構成される腺の複合体である。各葉の上皮細胞は吸血の間に多様な分泌顆粒を生産し、これらの分泌物は精包形成に大きな役割を果たしている。これらは生殖細胞の輸送容器としての精包を形成するだけでなく、精包内にパックされて雌の生殖道内に入るものもある。本種の雄付属腺分泌物と精包との微細構造の比較によって、その行方を明らかにし、役割を考察した。その結果、各葉の特徴的な微細構造と形態学的に合計24種類の分泌顆粒の存在が確認され、光顕によっても確認される外精包の中二層が前腹葉および後側葉に、雌体内に移送される3種類の物質が後腹葉、側腹葉、および中腹葉に由来することが形態学的に明らかになった。これは組織化学的手法による報告をほぼ裏付けるものであり、精包形成における物質の由来に関してより正確なデータが提示された。