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Morphology of the Male Reproductive System and the Nature of Secretions of the Accessory Glands and Seminal Vesicles of Adult Atractomorpha lata Motschulsky (Orthoptera: Acrididae)【Research report】

雄長額負蝗（直翅目：蝗科）副腺和儲精囊之分泌物特性與其生殖系統之形態學【研究報告】

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Abstract

The male accessory gland complex of Atractomorpha lata (Motschulsky) consists of three pairs of long, coiled milky glands, one pair of white glands, one pair of club-shaped yellow glands, and eight pairs of short and long transparent glands of various lengths. The accessory gland types and seminal vesicles yielded various protein fractions of molecular weights ranging from 13 to 295 kDa on SDS - PAGE but each gland contained specific protein fractions which distinguished each of the tissues. The presence of several similar protein fractions in the seminal vesicles and accessory gland types has suggests that these tissues share a common origin. Detection of oviposition-stimulating fractions in white and yellow glands of A. lata further demonstrates the importance of male accessory glands in the reproductive physiology of female insects.

摘要

長額負蝗的雄性副腺包含三對長型的捲曲狀乳白色腺體、一對白色腺體、一對棒狀的黃色腺體和八對長短不一的透明腺體。這些腺體和儲精囊分別會生成各式各樣的蛋白質，以SDS-PAGE分析，這些蛋白質的分子量介於13Kda到295Kda之間，而每種腺體所生成的蛋白質都具有可以區分不同組織的特異性。由於儲精囊及副腺中存有相類似的蛋白質，顯示這些組織可能有共同的起源。而從長額負蝗的黃色和白色腺體偵測到能刺激排卵的蛋白質，更進一步證實雄性副腺對於雌性昆蟲生殖生理的重要性。

Key words: Morphology, accessory gland, seminal vesicle, Atractomorpha lata, densitogram.

關鍵詞: 形態學、副腺、儲精囊、長額負蝗、密度圖

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Morphology of the Male Reproductive System and the Nature of Secretions of the Accessory Glands and Seminal Vesicles of Adult *Atractomorpha lata* Motschulsky (Orthoptera: Acrididae)

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ABSTRACT

The male accessory gland complex of *Atractomorpha lata* (Motschulsky) consists of three pairs of long, coiled milky glands, one pair of white glands, one pair of club-shaped yellow glands, and eight pairs of short and long transparent glands of various lengths. The accessory gland types and seminal vesicles yielded various protein fractions of molecular weights ranging from 13 to 295 kDa on SDS – PAGE but each gland contained specific protein fractions which distinguished each of the tissues. The presence of several similar protein fractions in the seminal vesicles and accessory gland types has suggests that these tissues share a common origin. Detection of oviposition-stimulating fractions in white and yellow glands of *A. lata* further demonstrates the importance of male accessory glands in the reproductive physiology of female insects.

Key words: Morphology, accessory gland, seminal vesicle, *Atractomorpha lata,* densitogram

Introduction

The entire lives of insects depend on efficient reproductory capacities of the species, which in most insects, are regulated by accessory reproductive glands among other factors. Lange and Loughton (1984) demonstrated the presence of a variety of proteins with a wide range of molecular weights in the accessory glands of *Locusta migratoria migratoroids* (L). Gillott and Venkatesh (1985) resolved the extracts of long hyaline glands, white glands, short hyaline glands and seminal vesicles of *Melanoplus sanguinipes* (Fabr.) into more than 20 protein bands, each with different molecular masses of 19 to 100 kDa. A molecular weight fraction of 72 kDa from the long hyaline gland of *M. sanguinipes* was reported to constitute a major component of the viscous secretion for the formation of spermatophores (Cheeseman and Gillott, 1988). Gillott and Friedel (1977) reported that a fecundity-enhancing substance in the accessory glands of *M. sanguinipes* is a proteinous substance with a molecular weight of about 30 kDa. The reported proteins in the accessory gland of *Acheta*

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domesticus (L.) are a complex mixture, ranging in molecular weight of 10 to over 300 kDa; the gland tubule types also had different protein fractions with various molecular weights (Kaulenas, 1976).

Generally, studies on the nature of the components of accessory glands and spermatheca secretions in insects are rather scanty (Gillott, 1988; Kaulenas, 1992), in spite of the importance of these substances to insect reproduction. Accessory gland substances transferred to female *M. sanguinipes* have been shown to contain fecundity-enchancing and receptivity-inhibiting components which increase fecundity in mated females and prevent them from undergoing a second mating (Gillott and Friedel, 1977). There is scanty information on the reproductive behavior and physiology of *Atractomorpha lata* (Fujimori, 1990). Because of these we examined the protein components of the male accessory glands and seminal vesicles of male *A. lata* (Motschulsky) (Orthoptera: Acrididae), a male mounting grasshopper found on different grasses (Compositae, Convolvulaceae, Leguminosae, etc) and sweet-potato farms in many parts of Japan, Taiwan, northern China, and the Korean peninsula.

Materials and Methods

Atractomorpha lata nymphs at different instar stages were collected from the field in Moriyama area, Nagoya, Japan and maintained in a cage (45 x 29 x 30 cm) with a screen cover in the laboratory at 25 $\pm$ 5^{0}C using the grass *Artemisia* sp. as a food plant. Newly emerged males and females were removed and put in separate cages (32 x 18 x 24 cm) for adult maintenance on the same food plant. A 20-day-old unmated male insect was dissected to depict the morphology of its reproductive system. The insect was dissected under a dissecting microscope (x20). The entire reproductive system was removed and placed on a glass slide with fine forceps, and adhering tissues were removed as far as was possible. The reproductive system was then photographed and described in five replicates. Accessory glands were separated into four types based on the colors of their secretions observed under the dissecting microscope. The individual accessory gland tubule types from the five adults were measured with an ocular micrometer at x20 magnification, and their means (mm) were calculated.

Four accessory gland tubule types of white, milky, transparent, and yellow glands and seminal vesicles from whole accessory gland-seminal vesicle masses were isolated from five 20-day-old unmated males, put in labeled specimen vials, and kept at -25^{0}C. Sample extracts were prepared by puncturing each of the tissues with the pointed end of a micropipette to release their contents into 100 ml of Ringer's solution. The mixtures were centrifuged at 16,000 rpm in a centrifuge (model M150-IV, Sakura Co. Japan) at 4^{0}C for 10 min, and the supernatants were then collected. The extraction process was performed twice, and the supernatants were pooled together and dried to a powder in a vacuum concentrator (model EC-57, Sakura Co. Japan). The powdery extracts were dissolved in 30 µl of distilled water, and the protein content for each sample was measured using protein assay kit procedure (Bio-Rad, California). Thirty microliters of sample buffer (0.125 M Tris-HCL, pH 6.8; 20% glycerol; 4% SDS; 10% β-mercaptoethanol; 0.05% BPB) was added to each sample, and this was used for conducting Sodium Deodecyl Sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). Concentrations of acrylamide in the stacking and separation gels were 2.5% and 8.0%, respectively. The Tris-HCL buffer system was used in the stacking gel (0.25 M, pH 6.8, 0.1% SDS) and the

separation gel (0.375 M, pH 8.8, 0.1% SDS). Each sample containing 35 μg of protein was spotted in each cell of the slab and electrophoresed at a constant current of 20 mA per slab for about 5 h. After electrophoresis, the slab gels were fixed in a solution of 50% methanol and 10% acetic acid for 10 min and then stained with the Wako Silver Stain kit (Wako Pure Chemicals, Osaka, Japan). Stained slab gels were later photographed, and the photographic images were scanned with an Epson GT 6000 scanner. The electrophoretic patterns were analyzed by NIH Image ver 1.47 (PDS Soft, NIH, USA) to determine the molecular weights of each of the protein bands.

Results

Male reproductive system of *A. lata*

The male reproductive system of *A. lata* consists of a pair of testes which lie on either side of the midgut region (Fig. 1). Each testis comprised of numerous distinct, ovoid, transparent follicles bound together by a peritoneal sheath. The distal part of each follicle collectively joins the vas deferens. A pair of long slender ducts, the vasa deferentia, runs posteriorly from the two testes and individually dilate to form what is known as the seminal vesicles. The paired seminal vesicles are club-shaped and are whitish in appearance with a length of 3.49 ± 0.1 mm (mean ± SE) (n = 10). Adhering closely to the seminal vesicles are the accessory glands compose of two bilateral masses. Each is composed of 13 long slender, coiled accessory gland tubules of various lengths and colors. The types of accessory gland tubules were identified based on the appearance of their secretory products as follows:

Type I: Three pairs of long coiled tubules with a blunt end. They were filled with a milky secretion and had a length of 2.59 ± 0.15 mm (n = 15).

Type II: A pair of medium-sized tubules filled with a whitish secretion and with a length of 2.03 ± 0.09 mm (n = 10).

Type III: A pair of long tubules (3.44 ± 0.11 mm, n = 10) with club-shaped anterior end and filled with a yellowish secretion.

Type IV: Eight pairs of long, coiled tubules and short tubules with blunt ends. They had various lengths 2.62 ± 0.1 mm (n = 15), 2.36 ± 0.04 mm (n = 10) and 0.8 ± 0.1 mm (n = 15). They were filled with a transparent secretion.

The accessory glands that emerge as a forked-shaped projection at the anterior-end of the ejaculatory duct run posteriorly through the muscular wall of the phallus terminating in the gonopore. The phallus is armed with basal plates around the phallobase which protects the retractile aedeagus; this consists of a pair of spines at the tip of the gonopore.

Electrophoresis

The secretory proteins of the four accessory gland types and seminal vesicles of *A. lata* have been resolved as shown in Figs. 2 and 3.

There were 44 protein bands in the electropherogram of the milky accessory gland extract, including 16 prominent bands in the molecular mass range of 15 to 287 kDa. Protein bands of 15, 36 and 287 kDa were strongly stained. About 14 fractions of between 173 and 270 kDa were indistinct. The gel pattern comprising 50 bands with molecular weights ranging from 13 to 287 kDa was found in the white gland secretion. About 14 bands stained prominently in the region of 13 to 107 kDa. There were 19 less-distinct bands in the range of 114 to

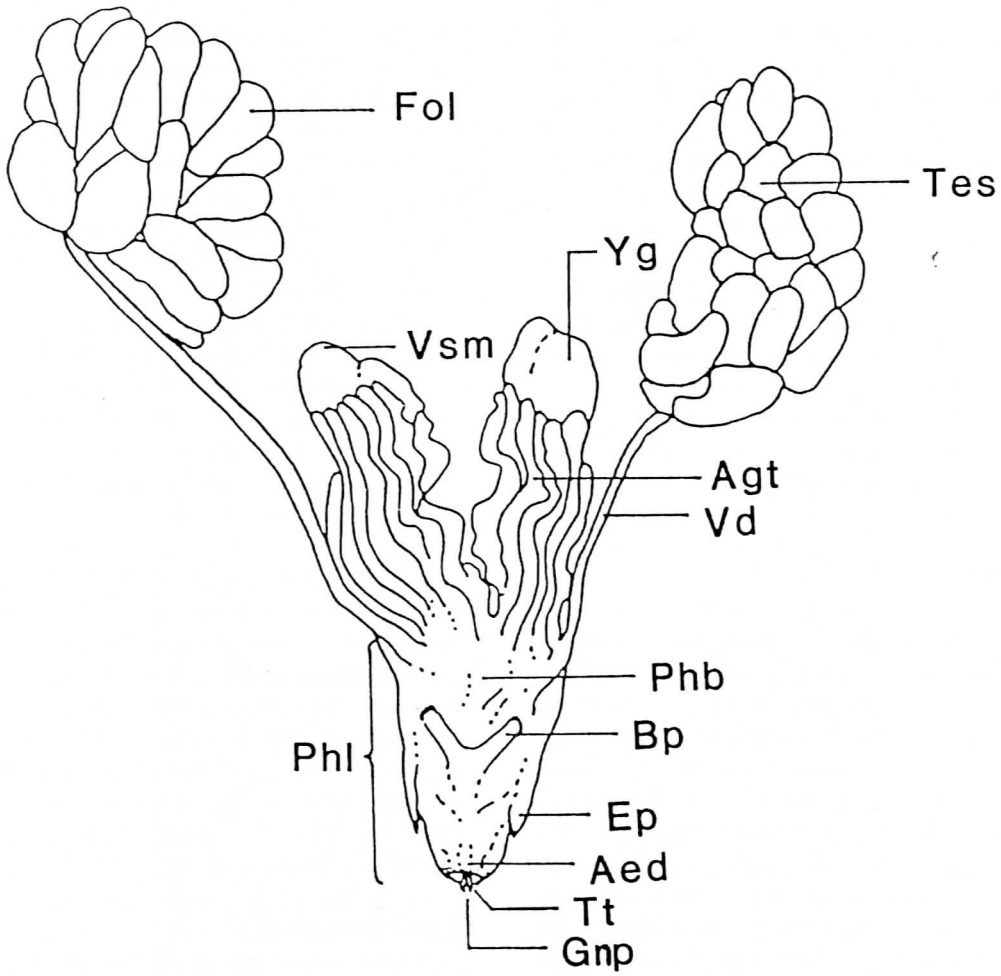


Fig.1A. Schematic male reproductive system of *Atractomorpha lata*. Aed: aedgus, Agt: accessory gland tubules, Bp: basl plate, Ep: epimere, Fol: follicle, Gnp: gonopore, Phb: Phallobase, Phl: Pphallus, Tes: testes, Tt: titilator, Vd: was deferens, Vsm: vesiculum seminalis, Yg: yellow gland.

287 kDa. Protein bands of 13, 15, 64 and 77 kDa were broad and prominent.

The yellow gland secretions separated into 56 protein bands with molecular mass ranging from 13 to 295 kDa. There were 20 prominent bands, 15 of which were in the range of 23 to 98 kDa. The protein band at 64 kDa was the most prominent one. Between 77 and 295 kDa, there were up to 27 indistinct protein bands.

The transparent glands were not separated into long and short glands. The transparent gland secretion analysis revealed the presence of 43 silver-stained bands. Protein bands at 15, 17, 28, 30, 75 and 287 kDa were strongly stained. Protein fractions of 17 and 75 kDa were broad and prominent.

The seminal vesicle secretion sepa-

Fig.1B. Light micrograph of Male reproductive system of *Atractomorpha lata*.

rated into 53 bands with 22 prominent fractions in the region of 17 to 87 kDa. There were 27 less-prominent and narrower bands between 92 and 270 kDa and a prominent one at 207 kDa. Protein bands at 32, 64, and 75 kDa were strongly stained.

The four accessory gland tubule types and seminal vesicles had 13 similar protein fractions ranging between 17.4 and 254.9 kDa. Protein fractions 43.4 and 66.7 kDa were specific for milky glands; 13.0, 30.0, 39.0, 41.4, and 189.2 kDa were specific for white glands; 18.5, 19.6, 45.3,

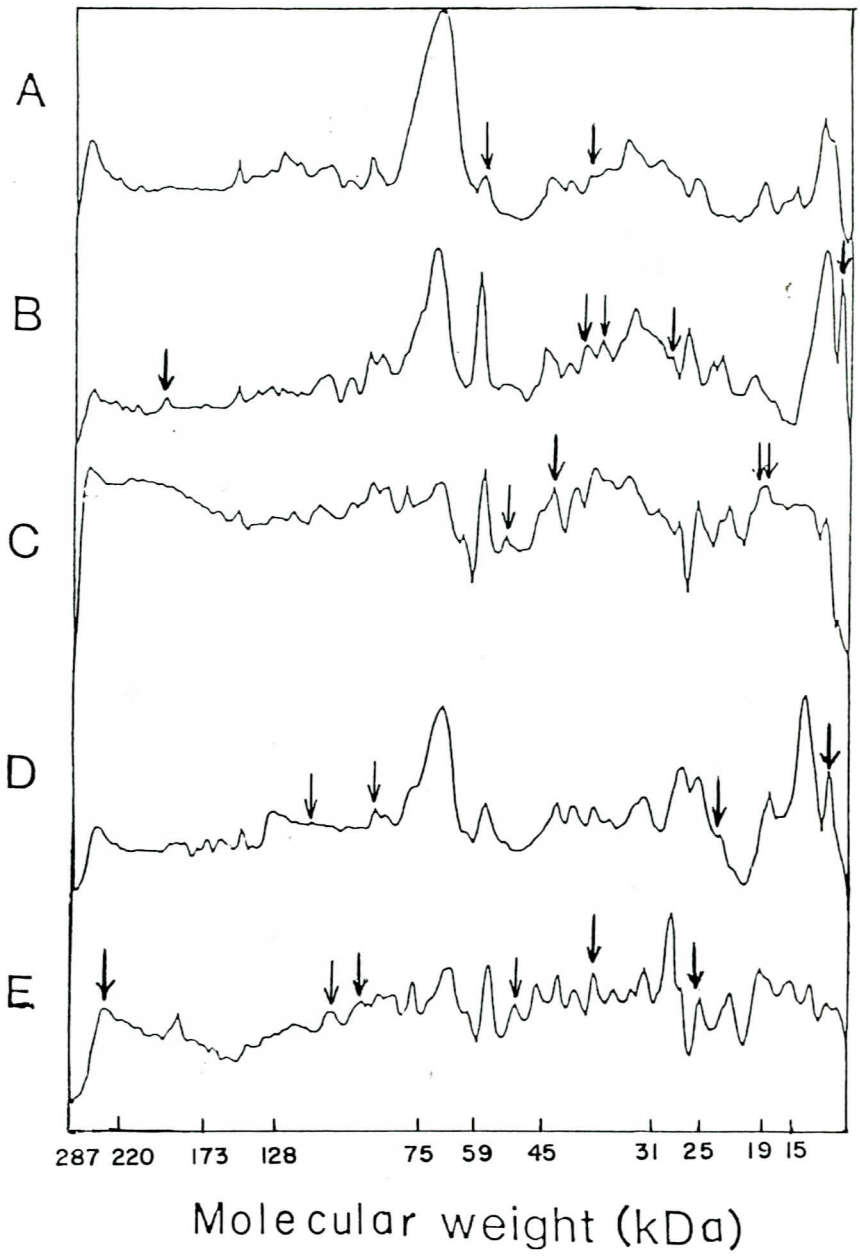
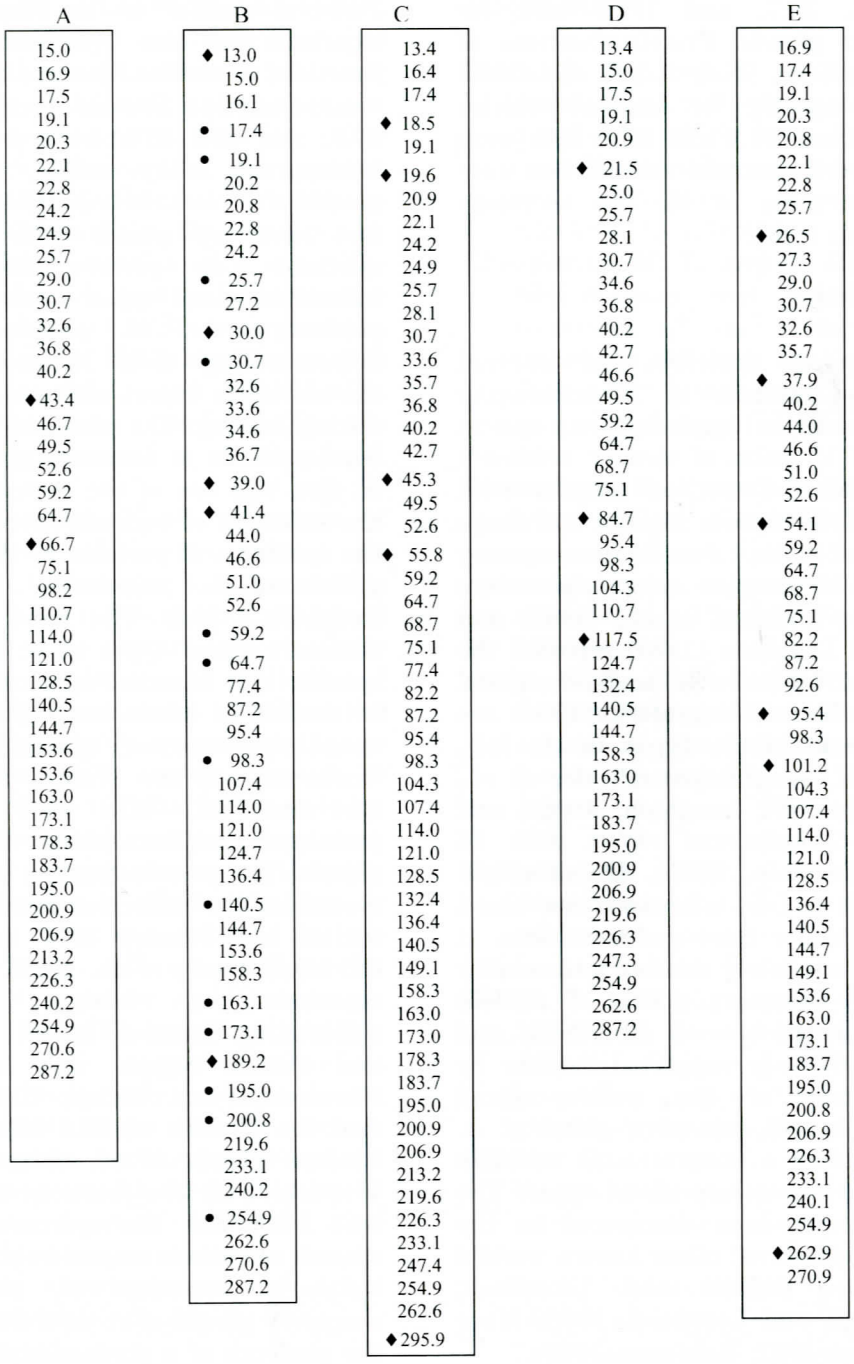


Fig.2. Densitogram of SDS-PAGE of accessory gland tubule types of seminal vesicles of *Atractomorpha lata*. A: milky glands, B: white glands, C: yellow glands, D: transparent glands, E: seminalis vesicles (arrows indicate specific protein fractions).



• Fractions common to accessory gland types and seminal vesicles

♦ Specific protein fractions

Fig.3. Accessory gland tubule types and seminal vesicle protein fractions. A: milky glands, B: white glands, C: yellow glands, D: transparent glands, E: seminal vesicles. Molecular weights of protein fractions are given in KDa.

55.8, and 295.9 kDa for yellow glands; 13.4, 21.5, 84.7, and 117.5 kDa for transparent glands. Protein fractions of 26.5, 37.9, 54.1, 95.4, 101.2, and 262.9 kDa were specific for seminal vesicles while fractions 24.2 and 36.8 kDa were not detected in seminal vesicles but were present in some or all the accessory gland tubule types.

Discussion

This paper describes the internal reproductive system of *Atractomorpha lata*. The male reproductive system consists of 13 pairs of slender accessory gland tubules of various lengths with considerable variations in color and shape. Hyaline and white colors in the accessory glands of *Melanoplus sanguinipes* were reported by Pickford *et al.*, (1969) and Lange and Loughton (1985) reported the presence of 16 pairs of accessory gland tubules in *Locusta migratoria*. There are four different tubule types in *A. lata,* three in *M. sanguinipes* (Pickfor *et al.*, 1969; Lange and Loughton, 1985), and eight in *Gomphocerus rufus* with 16 tubules (Hartman, 1970). Males of *M. sanguinipes* and *L. migratoria* are about 2.5 times larger than males *A. lata*. It seems that the lower number and smaller size of the accessory glands of *A. lata* may be due to species differences and evolution. This is supported further by the presence of the yellow gland contained in the accessory gland of *A. lata* which has a unique shape and size among other accessory gland types. The gland has not been discovered in the accessory glands of other known acridid grasshoppers (Gillott and Venkatesh, 1985; Lange and Loughton, 1985; Muse and Balogun 1991; Kaulenas, 1992).

Protein fractions of molecular weights 17.9, 22.8, 28.1, 68.7, 77.4, and 287.2 kDa of *A. lata* were distinguishable. Although Black and Happ (1985) implicated the fractions of 17.9 and 19.0 kDa from tubular accessory glands of *Tenebrio molitor* to be responsible for expulsion of the spermatophore, the functions of similar fractions from *A. lata* remain unclear. Protein fractions of 17.5, 17.9, and 19.1 kDa were prominent in transparent milky, white, and yellow glands of *A. lata*. It is possible to map *A. lata* accessory glands with respect to synthesis of protein fractions by individual gland types. Each gland type produces over 40 protein fractions, demonstrating their heterogeneity and the ability to export mixtures of proteins during mating. The presence of identical band patterns in accessory gland types of *A. lata* was one of the reasons given for the existence of regional specialization in the synthesis of proteins in the accessory glands of *L. migratoria* (Lange and Loughton, 1984). The band pattern of accessory gland types of *A. lata* having specific and identical molecular weights is similar to what was reported for *M. sanguinipes* accessory glands (Gillott and Venkatesh, 1985). Protein fractions at 28.1 and 30.7 kDa were broad and prominent in transparent and yellow glands. The protein fraction at 30.7 kDa resembles the molecular mass of 30 kDa (Gillott and Friedel, 1977) and matrone (60 kDa) (Fuchs *et al.*, 1969) which were reported as having oviposition-stimulating function in *M. sanguinipes* and *Aedes aegypti* (L.), respectively. Cheeseman and Gillott (1988) showed that the fraction at 30.8 kDa from long hyaline glands of *M. sanguinipes* was identical with the fractions at 32.2 and 30.5 kDa from the spermatophore and viscous secretions respectively. A regional synthetic capacity of proteins by accessory glands of *A. lata* was shown by the presence of a predominant fraction of 75.1 kDa in milky, transparent and yellow glands. The protein fraction of 71.5 kDa resembles the fraction of 72 kDa from the long hyaline gland of *M. sanguinipes,* which was shown to be

responsible for the maintenance of spermatophores (Cheeseman and Gillott, 1989). Cheeseman *et al.* (1990) identified soluble fractions at 21 and 22 kDa from white glands of *M. sanguinipes* as major structural spermatophore proteins. These fractions are similar to the distinct fraction at 21.5 and minor fraction at 84.7 kDa detected in the transparent accessory gland secretion of *A. lata.* The yellow gland of *A. lata* rather than the white gland of *M. sanguinipes* (Cheeseman *et al.,* 1990) also has protein fractions at 82.2 and 87.2 kDa, demonstrating some similarity in the protein components of *A. lata* and *M. sanguinipes.*

The accessory gland secretions of several species of insects contain an oviposition- strimulating substance. This was proven by implantation of the gland or injection of its extract into the body cavity of a virgin female (Gillott and Friedel, 1977). Fractions at 13 kDa in the opalescent gland of *L. migratoria* (Lange and Loughton, 1985) and fractions at 12 to 14 and 15 to 18 kDa in the tubular accessory glands of *T. molitor* (Happ *et al.,* 1977) were reported as oviposition stimulant factors. A similar fraction at 13 and 13.4 kDa was detected in the white gland as well as a 13.4 kDa fraction in the yellow and transparent glands of *A. lata.* This agrees with Gillott (1988) that the oviposition stimulant factor is exclusively produced in accessory glands rather than in seminar vesicles of acridid grasshoppers. Since the eggs of female grasshoppers mature in the absence of male substance, the accessory gland materials as in *A. lata* and other grasshoppers are what stimulate oviposition in this insect.

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雄長額負蝗（直翅目：蝗科）副腺和儲精囊之分泌物特性與其生殖系統之形態學

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摘　要

長額負蝗的雄性副腺包含三對長型的捲曲狀乳白色腺體、一對白色腺體、一對棒狀的黃色腺體和八對長短不一的透明腺體。這些腺體和儲精囊分別會生成各式各樣的蛋白質，以 SDS-PAGE 分析，這些蛋白質的分子量介於 13Kda 到 295Kda 之間，而每種腺體所生成的蛋白質都具有可以區分不同組織的特異性。由於儲精囊及副腺中存有相類似的蛋白質，顯示這些組織可能有共同的起源。而從長額負蝗的黃色和白色腺體偵測到能刺激排卵的蛋白質，更進一步證實雄性副腺對於雌性昆蟲生殖生理的重要性。

關鍵詞：形態學、副腺、儲精囊、長額負蝗、密度圖