International Journal of Tropical Insect Science Vol. 26, No. 4, pp. 266–272, 2006 DOI: 10.1017/S1742758406668458

Virus particles in male accessory reproductive glands of tsetse, *Glossina morsitans morsitans* (Diptera: Glossinidae) and associated tissue changes

Elizabeth D. Kokwaro*

Department of Zoological Sciences, Kenyatta University, Nairobi,
PO Box 43844-00100, Kenya

(Accepted 14 September 2006)

Abstract. The present study was undertaken to determine the occurrence of virus particles in male accessory reproductive glands and to describe the changes in the affected tissues. Using electron microscopy techniques, it was possible to identify rod-shaped virus-like particles in accessory reproductive glands of male tsetse, *Glossina morsitans morsitans* Westwood. The viruses occurred intracellularly within the epithelial cells and in the lumen of the glands. Cell degeneration characterized by abundant clear vacuoles, membrane-bound vesicles, disorganization and elimination of cell organelles typified the infection. The inference, therefore, is that virus infection may be primarily responsible for the necrotic changes identified in the gland cells. It is suggested that the lesions caused in the gland epithelium by the infection would disturb the glandular cells and disrupt synthesis of the secretion. This may eventually destroy the male accessory reproductive glands leading to inability of the male flies to form spermatophores for transferring spermatozoa to the female tsetse. Lack of sperm transfer would consequently result in no egg fertilization.

Key words: virus particles, tsetse, *Glossina morsitans morsitans*, male accessory reproductive glands, electron microscopy

Résumé. Cette étude a été entreprise afin de déterminer la fréquence des particules virales dans les glandes accessoires mâles et décrire les modifications dans les tissus infestés. A l'aide de la microscopie électronique, nous avons pu identifier des particules virales en forme de baguette dans les glandes accessoires de mâles de la mouche tsetse, *Glossina morsitans morsitans* Westwood. Les virus sont présents à l'intérieur des cellules, dans les cellules épithéliales et dans le lumen des glandes. La dégénérescence des cellules, caractérisée par la présence de nombreuses vacuoles claires, des vésicules bordées par une membrane, la désorganisation et la disparition des organites cellulaires, caractérise l'infection. On en conclut que l'infection virale des cellules se traduit en premier par les changements nécrotiques observés dans les cellules de la glande. Il est vraisemblable que les lésions observées dans l'épithélium de la glande, suite à l'infection, perturberont les cellules glandulaires et affecteront la synthèse de la sécrétion. Cela devrait aboutir à la destruction des glandes accessoires mâles et se traduire par l'impossibilité pour les mouches mâles de former des spermatophores et de transmettre des spermatozoïdes aux mouches tsetse femelles et ainsi, empêcher la fertilisation des œufs.

*E-mail: elizkokwaro@yahoo.com

Mots clés: particules virales, tsetse, *Glossina morsitans morsitans*, glandes accessoires mâles, microscopie électronique

Introduction

The accessory reproductive glands (ARGs) in male insects produce secretions with multifunctional roles in reproduction (Chen, 1971; De Wilde and De Loof, 1973). According to Gillott (2003), specific components have a variety of functions that collectively serve to improve the likelihood that the male will sire a significant proportion of a female's offspring. The ARG components exert their effects at all phases of the reproductive biology of the mated female, from mating to egg laying.

Processes affected may include sperm protection (Lung *et al.*, 2001), storage (Bertram *et al.*, 1996; Neubaum and Wolfner, 1999), activation (Viscuso *et al.*, 2001) and sperm competition effects on rival males' sperm (Price *et al.*, 1999). The accessory gland secretions may affect female behaviour, notably induction of refractoriness (Fuchs *et al.*, 1968, 1969; Fuchs and Hiss, 1970), reduction in attractiveness (Hartmann and Loher, 1996, 1999), fecundity, ovulation, oviposition (Yi and Gillot, 2000) and protection of laid eggs (Blum and Hilker, 2002; Eisner *et al.*, 2002; Hilker and Meiners, 2002).

In tsetse, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae), ARG secretory components form one or more sac-like structures, spermatophores, by which sperm is conveyed and deposited in the female uterus before migrating to the paired spermatheca (Kokwaro and Odhiambo, 1981; Kokwaro *et al.*, 1981).

According to Jaenson (1978), male *Glossina pallidipes* Austen with virus-infected hypertrophied salivary glands often transferred empty spermatophores. So far, virus-like rods have been described in the cytoplasm of normal-sized salivary glands in adult *G. morsitans* (Jenni, 1973; Jenni and Steiger, 1974) and from the nuclei of midgut epithelial cells of *Glossina fuscipes* Newstead (Jenni and Steiger, 1974). According to these authors, the presence of virus-like particles in their laboratory-bred *G. fuscipes* could account for the high mortality rate of the flies. In the case of *G. pallidipes* and *G. morsitans*, elongated but naked DNA-containing baculovirus-related viruses have been found intra- and extracellularly within enlarged hypertrophied salivary glands, where they cause cellular proliferation and cytoplasmic vacuolation (Jaenson, 1978; Otieno *et al.*, 1980; Kokwaro *et al.*, 1990). Similar particles were also observed in the germarial cells of *G. pallidipes*, where they caused severe necrosis (Jura *et al.*, 1988).

Sang *et al.* (1999) studied ARGs of tsetse *G. morsitans centralis*, experimentally infected with microinjected DNA virus and did not observe virus particles in the affected cells. They suggested that the pathological lesions in ARG were due to the presence of a subviral entity or that the virus could affect the endocrine system, leading to impaired development of the reproductive system.

Apart from these circumstantial deductions relating to the ARG of male *Glossina* with salivary gland hyperplasia, the natural occurrence and distribution of virus particles in accessory reproductive gland of male insects has not been previously demonstrated. The present study aimed at determining the occurrence and distribution of virus particles and describing pathological changes in infected ARGs.

Materials and methods

In this study, unmated 7-day-old male adult tsetse, *G. m. morsitans* were fed on rabbit ears daily and reared in an insectary at a temperature of 25 ± 1°C and 65–70% relative humidity and a 12 h light:12 h dark cycle. The flies were dissected and examined for enlarged salivary glands. The ARGs of males with enlarged salivary glands were dissected out into the fixative consisting of 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer at pH 7.4 containing 5% sucrose. The tissues were kept in this fixative and allowed to fix for 2 h at room temperature, rinsed in the cacodylate buffer at pH 7.4. The fixed tissues were then dehydrated in 50, 70 and 90% ethanol (10 min in each concentration). They were finally dehydrated in three changes of 100% ethanol (10 min in each change) and then cleared in propylene oxide. Cleared tissues were infiltrated in a 1:1 propylene oxide:araldite mixture overnight, in araldite for 24 h at room temperature and then embedded in araldite at 60 °C for 72 h.

Thin sections (500–700 nm) were cut with glass knives on an LKB ultramicrotome and mounted on uncoated copper grids. The sections were stained in uranyl acetate followed by lead citrate and examined under a Phillips 201 transmission electron microscope. Similar treatment was given to ARGs obtained from healthy (with normal salivary gland) *G. m. morsitans.*

Results

The male ARGs of *G. m. morsitans* consisted of long tubular paired organs, which empty into the ejaculatory duct, which in turn opens into the aedeagus. From the electron microscope studies, the male ARGs of *G. m. morsitans* were found to be infected with rod-shaped virus-like particles. The viruses seemed to have caused extensive vesiculation and vacuolation within the cytoplasm. Degenerative changes in the infected gland cells were characterized by detachment of the epithelium from the basement membrane. Rod-shaped virus-like particles were observed in the degenerated cytoplasm occasionally associated with isolated mitochondria and vesicles (Fig. 1).

Lysosomal-like bodies appear lodged within the degenerated cytoplasm of infected cells (Fig. 2). These cells lacked Golgi complexes (GC), endoplasmic reticulum (RER) and secretory vesicles. These observations contrasted with the ultrastructure of uninfected ARGs, in which the rough RER, numerous free ribosomes, polyribosomes and mitochondria were abundant (Figs 3 and 4). There were also GC with adjacent secretory vesicles, which contained several types of secretions (Figs 3 and 4). The apical surface of uninfected ARGs formed a series of long microvilli, which projected into the lumen (Fig. 3) but that of infected ARGs had no microvilli (Fig. 2). Muscle layer was discernible at the basal plasma membrane surface in both infected (Fig. 1) and uninfected ARGs (Fig. 4). The nuclei (Fig. 5) and nucleoli (Fig. 6) had numerous small rod-shaped virus-like particles and clumped electron-dense chromatin material appeared scattered irregularly within the nucleoplasm (Fig. 5). The rod-shaped virus-like particles penetrated some of the clumped chromatin material (Figs 5 and 6). Other virus particles were scattered in the electron-lucent parts of the nucleoplasm (Fig. 5). In contrast, the uninfected ARGs had well-defined nucleus, nucleolus and chromatin material all enclosed with a double membrane nuclear envelope (Fig. 7).

Within the lumen of virus-infected ARGs, the most common types of secretory material included loose matrix, dense aggregates of material (Figs 2 and

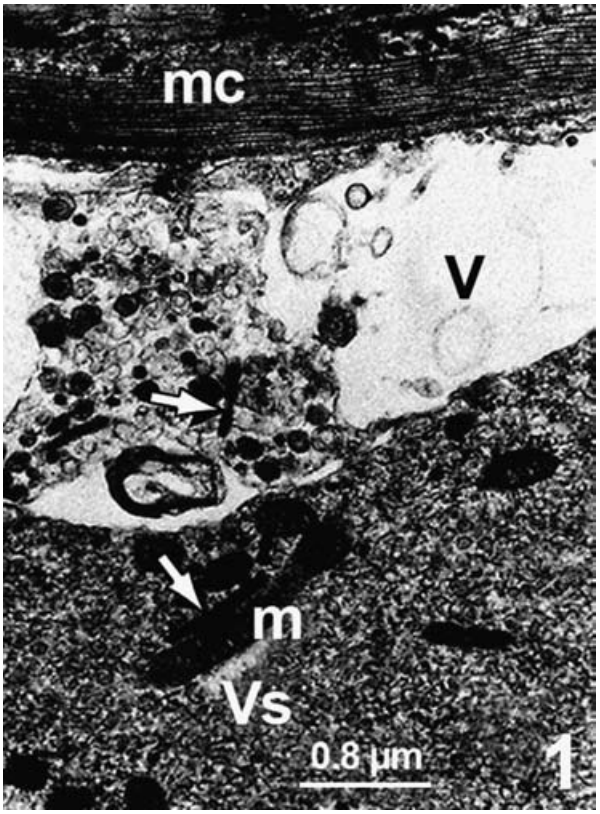

Fig. 1. Electron micrograph of a portion of virus-infected accessory reproductive gland showing degenerating epithelium and part of a muscular coat (mc). Large vacuoles (V) are depicted at the basal surface. Numerous membrane-bound vesicles (Vs) and a few mitochondria (m) are associated with virus-like particles (arrows). Magnification × 22,500

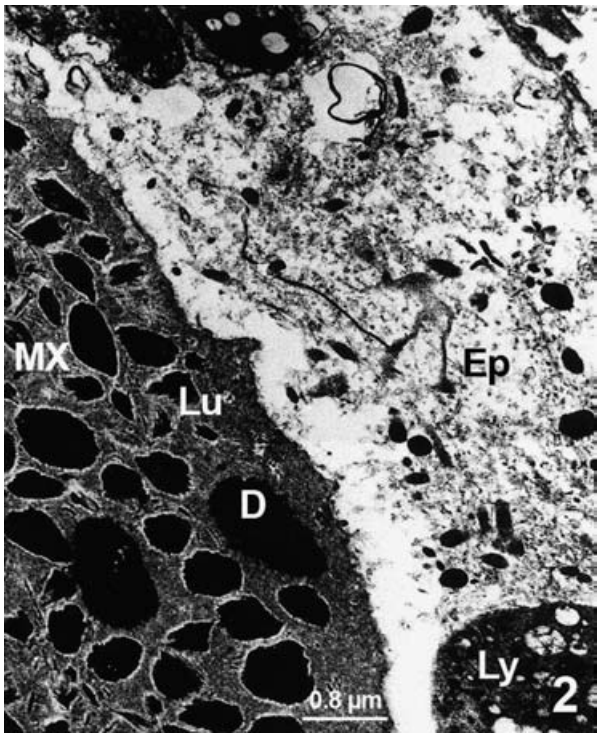

Fig. 2. Electron micrograph of infected accessory reproductive gland showing the relationship between the lumen (Lu) and degenerated epithelium (Ep). Cytoplasm contains lysosome-like bodies (Ly) and lumen contains electron-dense aggregates of material (D) and matrix (mx). Magnification × 22,500

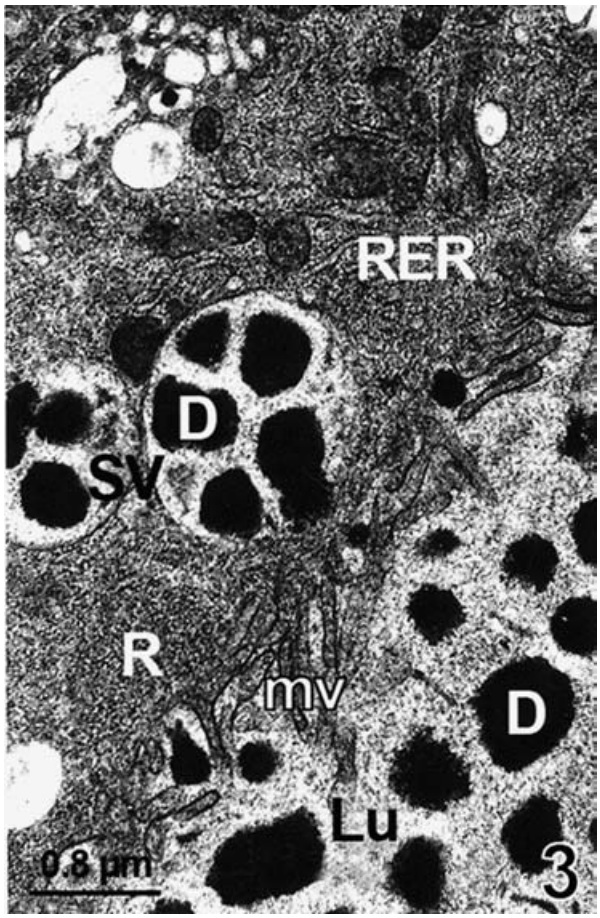

Fig. 3. Parts of epithelial cells of uninfected accessory reproductive gland with its characteristic apical microvilli (mv). Cytoplasm contains rough endoplasmic reticulum (RER), free ribosomes (R) secretory vesicles (sv), electron-dense aggregates of material (D) contained in vesicles. Lumen (Lu) contains secretion. Magnification × 22,500

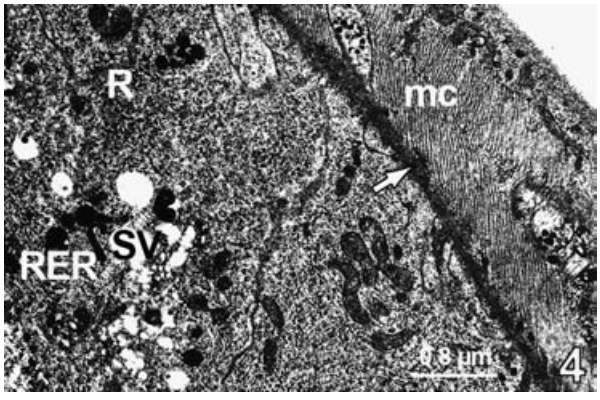

Fig. 4. Cell from uninfected accessory reproductive gland showing secretory vesicles (sv), rough endoplasmic reticulum (RER) and ribosomes (R). A part of a muscular coat (mc) is seen overlying the basement membrane (arrow). Magnification × 22,500

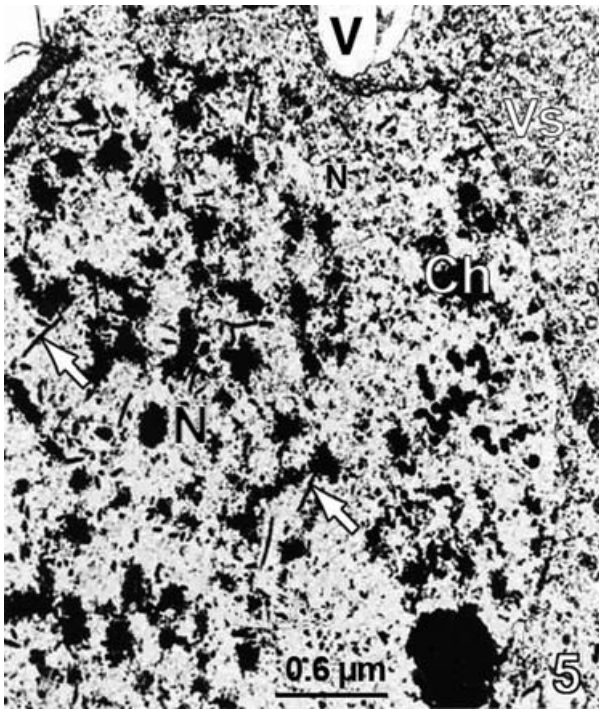

Fig. 5. Electron micrograph of nucleus (N) of virus-infected accessory reproductive gland showing clumping of nuclear chromatin (Ch), rod-shaped virus-like particles (arrows), cytoplasmic vacuoles (V) and vesicles (vs). Magnification × 30,000

8) and electron-dense clusters of granules (Fig. 8). These components seemed to undergo necrotic changes and coalesced into masses of electron-dense granules associated with rod-shaped virus-like particles (Fig. 8). Although the uninfected glands had similar secretory products to those observed in the lumen of infected ARGs, there were no virus particles associated with these materials (Fig. 9).

Discussion

This electron microscopy study demonstrated the presence of rod-shaped virus-like particles in ARGs of male tsetse, *G. m. morsitans*. Although the infected tissues showed severe necrosis, the lumen contained at least three characteristic secretory types similar to those previously described by Odhiambo *et al.* (1983) in normal ARGs of *G. m. morsitans*.

The secretions of male accessory glands contain a variety of bioactive molecules. When transferred during mating, these products exert wide-ranging effects on female reproductive activity and they improve the male's chances of siring a significant proportion of the females' offspring (Gillott, 2003). In *G. m. morsitans*, the ARG secretions of normal ARG have been implicated in providing

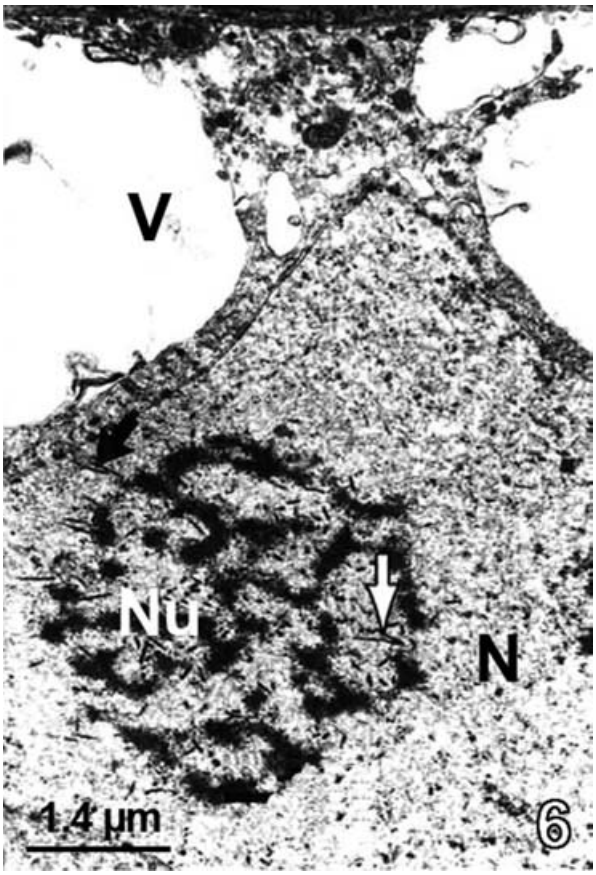

Fig. 6. Cell from virus-infected accessory reproductive gland showing vacuoles (V), nucleus (N) and disintegrated nucleolus (Nu) containing numerous, small rod-shaped virus-like particles (arrows). Magnification × 15,000

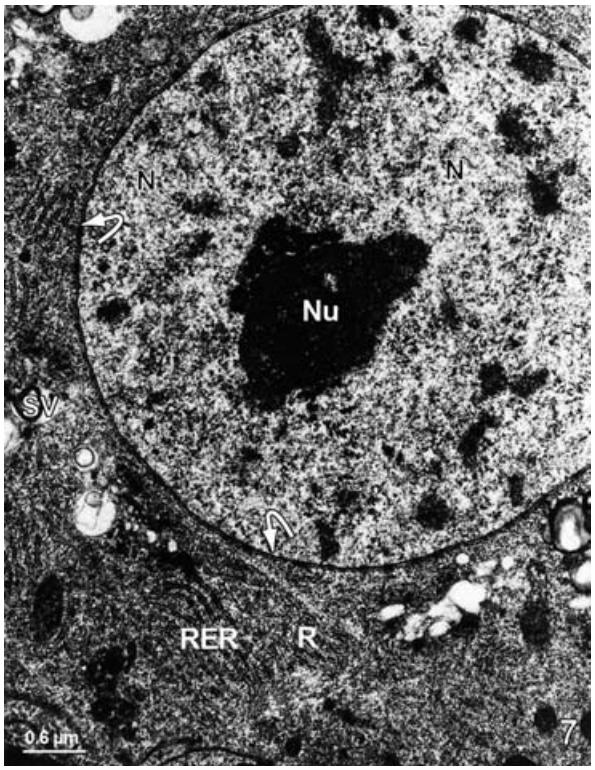

Fig. 7. Electron micrograph of uninfected accessory reporductive gland showing a prominent nucleus (N). A nuclear envelope (arrows) surrounds the nucleus containing a nucleolus (Nu). The surrounding cytoplasm contains rough endoplasmic reticulum (RER), secretory vesicles (sv) and free ribosomes (R). Magnification × 37,500

protein–carbohydrate secretions required for the assembly of the spermatophore (Kokwaro and Odhiambo, 1981; Odhiambo *et al.*, 1983; Kokwaro *et al.*, 1987) to effect successful transfer of spermatozoa to the female. The presence of virus-like particles within the lumina of *G. morsitans* alongside the secretory products suggests that if such male flies mate with female flies, virus particles can be transferred with ARG material into the female uterus and that this might be the basis of horizontal transmission (Jura *et al.*, 1988). However, the infection seemed to have altered the normal morphology of these secretions, which appeared degenerated and coalesced (Figs 2 and 8). In this state of abnormal organization of the secretion, efficient transfer of the secretion into the female during mating becomes quite uncertain, thus leading to the inability of infected males to construct complete spermatophores as reported by Sang *et al.* (1999). This also could account for the reproductive abnormalities and male sterility reported by Jaenson (1978).

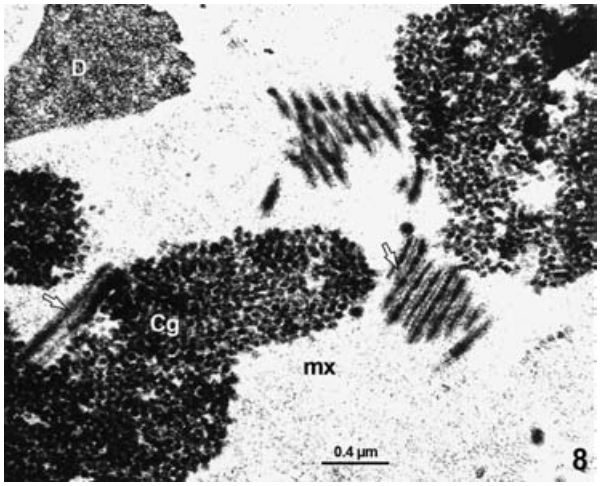

Fig. 8. Small portion of the lumen of virus-infected accessory reproductive gland showing coalesced electron-dense clusters of granules (Cg) closely associated with rod-shaped virus-like particles (arrows). Degenerated matrix (mx) and electron-dense aggregates of material (D) are evident. Magnification × 45,000

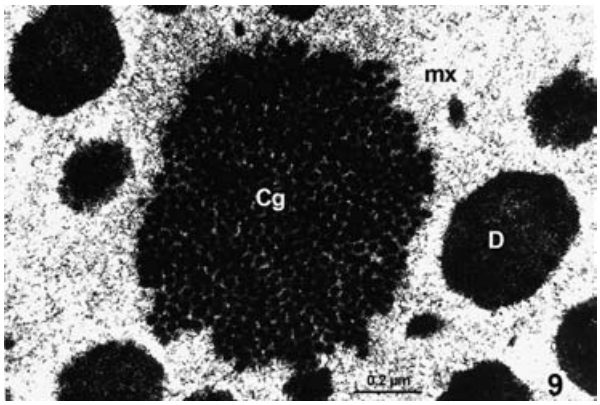

Fig. 9. Secretions that occur throughout the lumen of uninfected accessory reproductive glands consist of electron-dense aggregates of material (D), matrix (mx) and electron-dense clusters of granules (Cg). Magnification × 67,500

The infected ARGs lacked extensive RER, GC and ribosomes, all of which are important in protein synthesis and secretion. The absence of secretion and GC in the cytoplasm of infected cells, lack of RER, presence of clear vacuoles and loss of microvilli are pathological indications of impairment in some of their secretory and transport mechanisms

The nuclei seemed to be the major sites for virus lodgement and possibly multiplication as evidenced by numerous small rod-shaped virus-like particles. It is suggested that the successive infection of ARG cells requires movement of infected materials and virus particles from diseased nuclei into the cytoplasm, where they exert their pathogenicity to the cell. Presumably, this could happen by contaminative infections of nucleic acids, movements of virus particles through nuclear pores or massive spread of virus particles after disintegration of the nuclear membranes.

The clumped electron-dense materials in the nucleoplasm may represent an abundance of the inactive heterochromatin material and/or disintegrated nucleoli resulting from the infection. These morphological changes could probably interfere with the normal synthesis of ribosomal RNA, hence altering metabolic functions of these glands. The severe degradation would eventually destroy the male ARGs.

Acknowledgements

The author wishes to express her gratitude to Drs R. Mukabana and B. Tengecho and Mr Kautie for critical reading of the manuscript and for their helpful discussions.

References

Bertram M. J., Neubaum D. M. and Wolfner M. F. (1996) Localization of the *Drosophila* male accessory gland protein Acp 36 DE in the mated female suggests a role in sperm storage. *Insect Biochemistry and Molecular Biology* 26, 971–980.

Blum M. S. and Hilker M. (2002) Chemical protection of insect eggs, pp. 61–90. In *Chemoecology of Insect Eggs and Egg Deposition* (Edited by Hilker M. and Meiners J.). Blackwell, Berlin.

Chen P. S. (1971) *Biochemical Aspects of Insect Development*. Karger, Basel. 230 pp.

De Wilde J. and De Loof A. (1973) *Reproduction – The Physiology of Insecta*. 2nd edn. Academic Press Inc., London. pp. 11–95.

Eisner T., Rossini C., González A., Lyengar V. K., Siegler M. V. S. and Smedley S. R. (2002) *Paternal Investment in Egg Defence*. Blackwell, Berlin. pp. 91–116.

Fuchs M. S. and Hiss E. A. (1970) The partial purification and separation of the protein components of matrone from *Aedes aegypti*. *Journal of Insect Physiology* 16, 931–939.

Fuchs M. S., Craig G. B. Jr and Hiss E. A. (1968) The biochemical basis of monogamy in mosquitoes. I. Extraction of the active principle from *Aedes aegypti*. *Life Science* 7, 835–839.

Fuchs M. S., Craig G. B. Jr and Despommier D. D. (1969) The protein nature of the substance inducing female monogamy in *Aedes aegypti*. *Journal of Insect Physiology* 15, 701–709.

Gillott C. (2003) Male accessory gland secretions: modulators of female reproductive physiology and behaviour. *Annual Review of Entomology* 48, 163–184.

Hartmann R. and Loher W. (1996) Control mechanisms of the behaviour 'secondary defense' in the grasshopper *Gomphocerus rufus* L. (Gomophocerinae: Orthoptera). *Journal of Comparative Physiology A* 178, 329–336.

Hartmann R. and Loher W. (1999) Post-mating effects in the grasshopper, *Gomphocerus rufus* L. mediated by the spermatheca. *Journal of Comparative Physiology A* 184, 325–332.

Hilker M. and Meiners J. (Eds) (2002) *Chemoecology of Insect Eggs and Egg Deposition*. Blackwell, Berlin, 416 pp.

Jaenson T. G. T. (1978) Virus-like rods associated with salivary gland hyperplasia in tsetse, *Glossina pallidipes*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 72, 234–238.

Jenni L. (1973) Virus-like particles in a strain of *G. morsitans centralis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 67, 295.

Jenni L. and Steiger R. (1974) Virus-like particles of *Glossina fuscipes fuscipes* Newst. *Acta Tropica* 31, 177–180.

Jura W. G. Z. O., Odhiambo T. R., Otieno L. H. and Tabu N. O. (1988) Gonadal lesions in virus-infected male and female tsetse, *Glossina pallidipes* (Diptera: Glossinidae). *Journal of Invertebrate Pathology* 52, 1–8.

Kokwaro E. D. and Odhiambo T. R. (1981) Spermatophore of tsetse *Glossina morsitans morsitans* Westwood: an ultrastructural study. *Insect Science and its Application* 1, 185–190.

Kokwaro E. D., Odhiambo T. R. and Murithi J. K. (1981) Ultrastructural and histochemical study of the spermatheca of the tsetse *Glossina morsitans morsitans* Westwood. *Insect Science and Its Application* 2, 135–143.

Kokwaro E. D., Okot-Kotber M., Odhiambo T. R. and Murithi J. K. (1987) Biochemical evidence for the origin of spermatophore material in *Glossina morsitans morsitans* Westwood. *Experientia* 43, 448–451.

Kokwaro E. D., Nyindo M. and Chimtawi M. (1990) Ultrastructural changes in salivary glands of tsetse, *Glossina morsitans morsitans* infected with virus and rickettsia-like organisms. *Journal of Invertebrate Pathology* 56, 337–346.

Lung O., Kuo L. and Wolfner M. F. (2001) *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. *Journal of Insect Physiology* 47, 617–622.

Neubaum D. M. and Wolfner M. F. (1999) Mated *Drosophila melanogaster* females require a seminal fluid protein, Acp 36 DE, to store sperm efficiently. *Genetics* 153, 845–857.

Odhiambo T. R., Kokwaro E. D. and Sequeira L. M. (1983) Histochemical and ultrastructural studies of the male accessory reproductive glands and the spermatophore of the tsetse, *G. morsitans morsitans*. *Insect Science and Its Application* 4, 227–236.

Otieno L. H., Kokwaro E. D., Chimtawi M. and Onyango P. (1980) Prevalence of enlarged salivary glands in wild population of *Glossina pallidipes* in Kenya, with a note on the ultrastructure of the affected organ. *Journal of Invertebrate Pathology* 36, 113–118.

Price C. S. C., Dyer K. A. and Coyne J. A. (1999) Sperm competition between *Drosophila* males involves both displacement and incapacitation. *Nature* 400, 449–452.

Sang C. S., Jura W. G. Z. O., Otieno L. H., Mwangi R. W. and Ogaja P. (1999) The effects of a tsetse DNA virus infection on the functions of the male accessory reproductive gland in the host fly *Glossina morsitans centralis* (Diptera: Glossinidae). *Current Microbiology* 38, 349–354.

Viscuso R., Narcisi L., Sottile L. and Violetta B. M. (2001) Role of the male accessory glands in spermatodesm reorganization in Orthoptera: Tettigonioidea. *Tissue and Cell* 33, 33–39.

Yi S. X. and Gillott C. (2000) Effects of tissue extracts on oviduct contraction in the migratory grasshopper, *Melanoplus sanguinipes*. *Journal of Insect Physiology* 46, 519–525.