

**THE ACCESSORY GLANDS OF THE FEMALE GENITAL TRACT
IN *AESHNA JUNCEA* (L.) AND *A. GRANDIS* (L.)
(ANISOPTERA: AESHNIDAE)**

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Received June 11, 2004 / Revised and Accepted December 2, 2004

The micro-anatomy of the ♀ accessory glands in adult *A. juncea* and *A. grandis* is similar but the size of the *A. grandis* glands is clearly larger than that of *A. juncea*. The secretory cells constitute a simple columnar epithelium surrounding a cuticle-lined lumen. The glandular epithelium is provided with a peculiar system of deep, narrow, intercellular crypts bordered with microvillar cell membranes. Lipids released to the crypt lumen are presumably forced into the central gland lumen by contractions of the muscular network attached to the outside of the gland. The efferent duct of each gland that opens to the distal part of the vagina has a complicated muscular apparatus, probably serving as a pump. The secreted substances accumulate in the central gland cavity mainly during the pre-reproductive phase, which the dragonflies spend away from water. The secretion contains substances with wax-like properties and becomes darkened by osmication. Secretory cells appear to possess a limited life span; scattered cells in process of dying occur already during the early reproductive phase. In the late reproductive phase most of the glandular epithelium presents a disintegrated appearance. There is no cell renewal in the gland in the course of adult life. The pattern of cell death indicates a decomposition by apoptosis. Besides contributing to investment of the eggs, the glands presumably intervene also in other aspects of the reproductive processes.

INTRODUCTION

In the dragonfly *Aeshna juncea* (L.), a pair of conspicuous accessory glands make up an integral part of the female genital tract together with sperm-storing organs (ÅBRO, 2004a). However, these glands do not seem to have received any particular attention from former workers concerned with dragonfly anatomy (TILLYARD, 1917; WHEDON, 1919; WINKELMANN, 1973). The aim of the present study has been to provide further information on the structure and function of these neglected glands.

Preliminary observations have disclosed that the female accessory glands of *Odonata* might vary considerably between different species. The two aeshnid species *A. juncea* and *A. grandis* are presumed to be closely related. The accessory glands of both were examined in order to reveal any dissimilarities in their micro-anatomy.

MATERIAL AND METHODS

Ovipositing females of *Aeshna juncea* (L.) and *A. grandis* (L.) were captured at a breeding site near Bergen, western Norway. Also, at clearings in surrounding woodland, a few specimens were collected during their pre-reproductive phase in the early flight season (late June - early July). Most specimens were fixed immediately in the field by injecting cold fixative into the posterior abdomen with a fine hypodermic needle. The female genital tract was next dissected in a larger volume of the same fixative. As a control, fresh tissues of some specimens, anaesthetized in carbon dioxide, were dissected.

The fixative was made up of 3% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) with 0.17 M sucrose added; postfixation took place in a 1% solution of osmium tetroxide in the same buffer. Specimens were dehydrated through a graded series of ethanol and cleared in propylene oxide, embedded in epoxy resin, and sectioned with a diamond knife. Semi-thin plastic sections for light microscopy, from material processed for electron microscopy, were stained in a 1% solution of toluidine blue. Ultrathin sections for electron microscopy were contrasted with uranyl acetate and lead citrate.

RESULTS

From the gross anatomy of the female genital tract it is scarcely possible to distinguish between *Aeshna juncea* and *A. grandis*, except for the accessory glands which in *A. grandis* are clearly larger than those of *A. juncea*. However, their micro-anatomy is similar. The paired accessory glands of the *Aeshna* female are situated ventrally in the ninth and tenth abdominal segments. In fresh material the length of each gland from the duct proximally to the tip of the tail measures, for *A. grandis* in the range 2.9-4.0 mm, for *A. juncea* 1.9-2.7 mm.

The position in situ of the accessory glands of *A. juncea* has been drawn in outline from several dissections of the adult female genital tract (Fig. 1). In some individuals the two glands are of uneven size.

Each gland is flattened with folds and clefts. When sectioned they appear lobulated and made up of microscopic subunits recognized as sac-

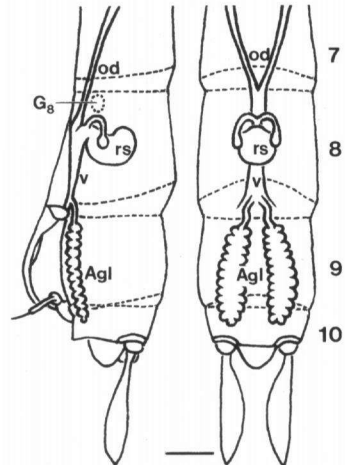
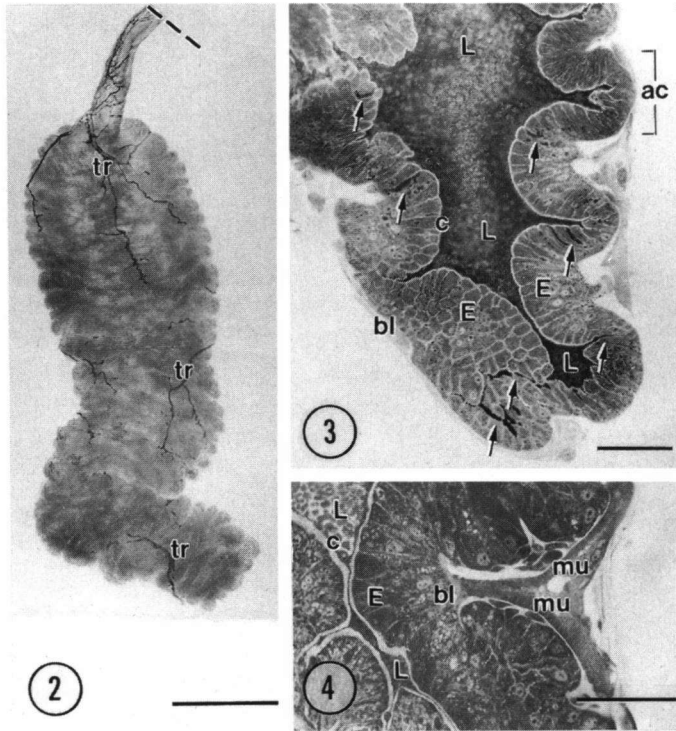


Fig. 1. *Aeshna juncea*: distal region of the adult female genital tract showing the posteriorly situated accessory glands (Agl); lateral (left) and dorsal (right) views. Based on dissections of several specimens, fixed and stored in buffered glutaraldehyde solution. Surrounding musculature and nerves are omitted. G_8 : position of the eighth abdominal ganglion; od: oviduct; rs: seminal receptacle with accessory sacs; v: vaginal canal; 7-10: abdominal segments. [Scale bar 2.0 mm].

cular out-pocketings called acini or alveoli (Fig. 2). The gland units are formed by a single layer of columnar epithelial cells that surround a central lumen, lined with a thin cuticle that is not in contact with the cell apices, giving rise to a subcuticular space (Figs 3, 4). The central glandular cavity exhibits narrow branches and is connected to a short efferent duct opening to the distal part of the vagina. The cavity system, carrying the glandular secretion, is in its entirety cuticle-lined. The female accessory glands of aeshnid dragonflies are of ectodermal origin, having developed by invagination of the integument.

In fresh material, the gland cavity contains a granulated substance dispersed in a liquid. Fixed glands exhibit an osmiophilic content. Components of the secretion indicate wax-like properties. Electron micrographs show a fine granular pre-



Figs 2-4. The female accessory gland: (2) *Aeshna juncea*: the gland with the efferent duct cut off near its outlet to the vagina (broken line), tr, remaining tracheal trees. Tissue fixed in buffered glutaraldehyde solution; unstained whole-mount in cacodylate buffer. Viewed in transmitted light, bright field. [Scale bar 400 μ m]; - (3) *A. juncea*: view of a gland lobule; pre-reproductive phase. The lumen (L) contains expelled secretion. Note deep intercellular crypts with secreted material. (arrows). ac: glandular acinus; bl: basal lamina; c: lining cuticle; E: glandular epithelium. Toluidine blue. [Scale bar 25 μ m]; - (4) *A. grandis*: part of gland (reproductive phase) showing two muscle fibres (mu) connected with a common insertion to the basal lamina (bl) between adjacent acini. c: cuticle; E: glandular epithelium; L: lumen. Toluidine blue. [Scale bar 25 μ m].

cipitate on the cuticle (Fig.5).

The columnar gland epithelium, mostly with ovoid nuclei, rests on a prominent basal lamina. In electron micrographs the cells exhibit an apical surface with small, intermittent, poorly-developed microvilli. The microvillar border extends into deep intercellular crypts, lined by microvillar cell membranes (Fig. 5) reaching close to the basal plasma membrane. In early adult life, the glandular epithelial cells contain

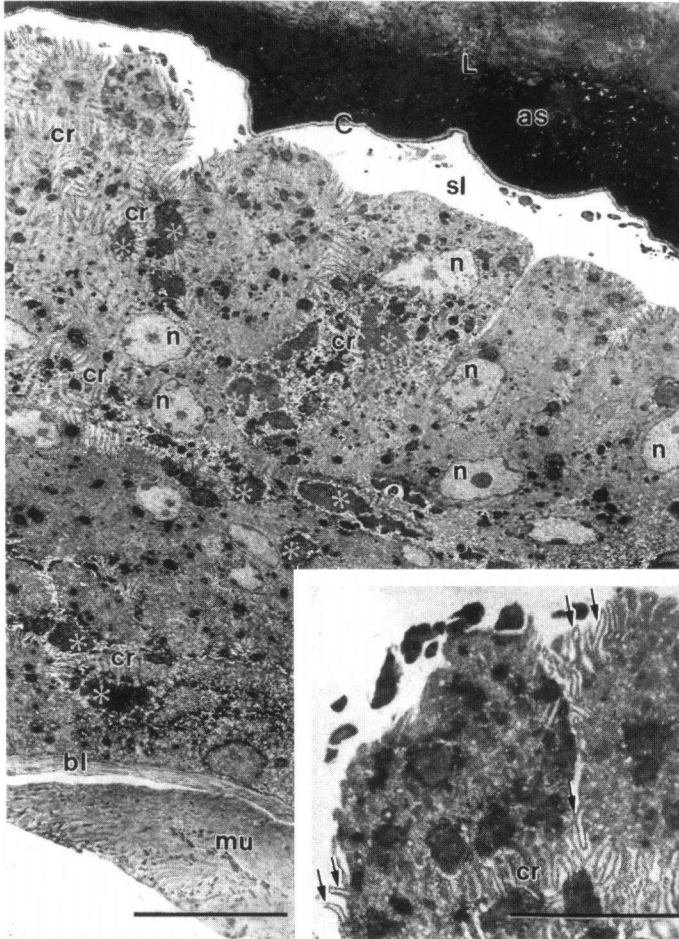
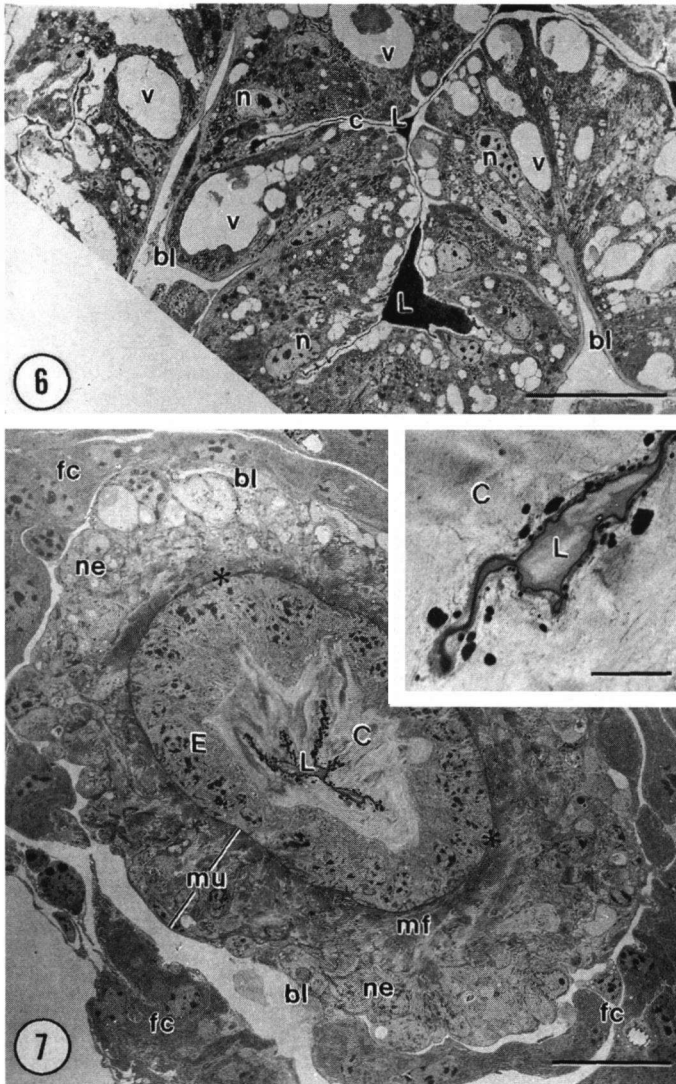


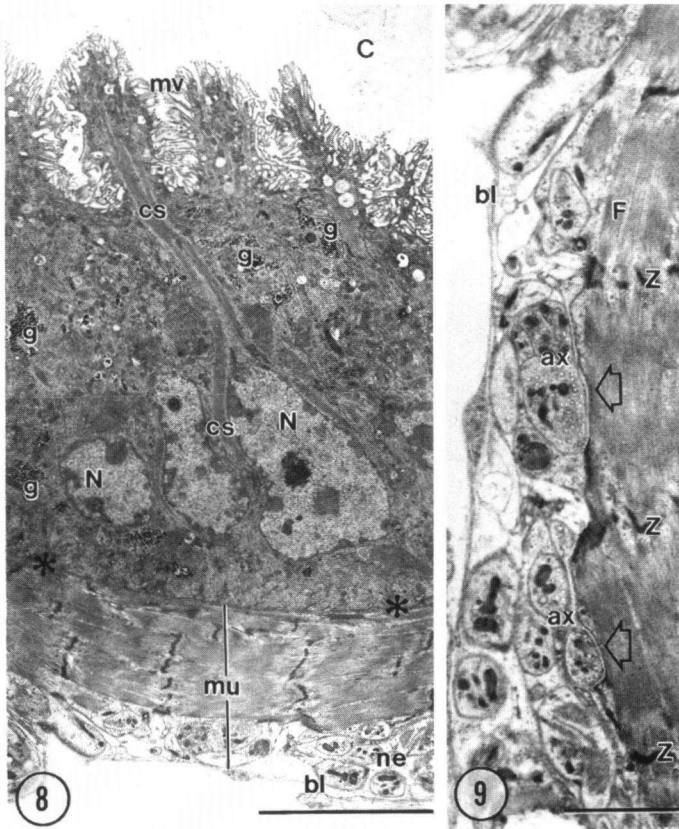
Fig. 5. *Aeshna juncea*: fine structure of the glandular epithelium; pre-reproductive phase. Survey view of the epithelium with its intercellular crypts (cr) lined with microvilli, from the same specimen as in Fig. 3. Intraplasmic lipid is preserved as spherical globules stained black, presumably owing to constituents of unsaturated fatty acids. In the crypt lumen, accumulations of exocytosed lipids (white asterisks). as: accumulated secretion; bl: basal lamina; C: cuticle; L: glandular cavity with secretion; mu: muscle fascicle; n: epithelial nucleus; sl: subcuticular lumen. [Scale bar 10 μ m]. *Inset*: Enlargement showing microvillar borders. Each villus holds a tubule of smooth endoplasmic reticulum (arrows). [Scale bar 5 μ m].



Figs 6-7. *Aeshna juncea*: (6) Electron micrograph of strongly vacuolated glandular epithelium; reproductive phase. Some intraplasmatic darkened lipids are still present. In the gland cavity there is some residual secretion (L). bl: basal lamina; c: cuticle; n: epithelial nucleus; v: vacuole. [Scale bar 20 μ m]; – (7) The efferent duct, transversely cut, displaying tissue coats around the folded, cuticle-lined lumen (L). The border between the epitheloid cells (E) and the muscular tunic (mu) is indicated by asterisks. bl: basal lamina; C: cuticle; fc: fat cell; mf: muscle fibres; ne: cortical nervous tissue. [Scale bar 20 μ m]. – I n s e t: Enlargement showing cuticular intima next to some residual secretion in the duct lumen (L). [Scale bar 5 μ m].

organized arrays of granular and smooth endoplasmic reticulum, Golgi complexes, numerous mitochondria and membrane-bound lipid inclusions (Fig. 5). Tubular elements of agranular endoplasmic reticulum can be seen extending into the microvilli almost to the tip (Fig. 5, inset). Minor lipid inclusions gather in the glandular cytoplasm near the crypts; large lipid deposits can be seen in the nearby crypt lumen (Fig. 5). Secreted lipid in the crypts and also in the subcuticular space is likely to traverse the cuticle and accumulate in the main cavity.

Surrounding the glandular units are muscular fascicles, forming a network with insertions on the basal lamina between adjacent acini (Fig. 4). In addition, adven-



Figs 8-9. *Aeshna juncea*: electron micrographs: (8) The wall of the efferent duct showing epitheloid cells with cytoskeleton (cs) and glycogen deposits (g). Cell apices furnished with microvilli (mv) against cuticle (C); each villus contains a tubule of smooth endoplasmic reticulum. The border between epitheloid and muscular (mu) layers is indicated by asterisks. bl: basal lamina; N: nucleus of epitheloid cell; ne: cortical nerve tissue. [Scale bar 5 μ m]; - (9) The wall of the efferent duct showing part of a muscle fibre with nerve terminals (open arrows). Within the axon terminals (ax) are seen numerous small (synaptic ?) vesicles. F: muscle fibril; Z: muscular Z-band. [Scale bar 2 μ m].

titious cells carry tracheoles. Differences occur in the histological appearance of glands from various individuals, depending on whether they are captured during pre-reproductive or reproductive phase. Secreted substances seem to accumulate in the central glandular lumen mainly during the pre-reproductive phase of adult life. In early reproductive phase, glandular cells of functional decline could be recognized as local atrophy. Later on there occurs cytoplasmic vacuolation (Fig. 6) and/or chromatin condensation of scattered nuclei in the glandular parenchyma, indicative of dying by apoptosis. The presence of apoptotic cells is characterized by nuclei and cytoplasm broken up into several compact fragments and a number of small membrane-bound globules.

The efferent duct of the gland is built up of concentric tissue coats around a cuticle-lined, folded-up lumen. The cuticle is rather thick, fibrous and with an electron-dense cuticular intima (Fig. 7). Beneath the cuticle is a simple layer of tall epitheloid cells, bordering a muscular tunic (Fig. 8). The epitheloid cells have interdigitated lateral borders and a folded apical membrane with microvilli, each containing a tubule of smooth endoplasmic reticulum (Fig. 8). In the cells are intraplasmatic deposits of glycogen and large, branched bundles of closely spaced parallel microtubules and interspersed microfilaments that run from the cell base upward through the cell body to end at the apex, apparently serving as cytoskeleton (Fig. 8). The layer of muscle fibres appears thickest around the proximal part of the duct. Muscle fibres and epitheloid cells are closely attached without a basal lamina inbetween but there is a border of tightly-woven texture with some short branching fingers on the epitheloid side of the border (Fig. 7). Muscle fibres are seen concentrated nearest to the epitheloid layer. A cortical sheath of nerve tissue can be seen close to the basal lamina: axons accompanied along their course by neuroglial cells and also neuromuscular junctions (Figs 7-9). Outside the muscular tunic is an irregular investment of fat cells and tracheolar cells (Fig. 7).

DISCUSSION

The accessory glands of the female genital tract are typical exocrine glands and are presumed to have multiple functions in the reproductive processes (ÅBRO, 2004a). The glands are thought to secrete the bulk of their production early in adult life and store it in the central cavity; later they secrete continuously at a low rate. Under certain conditions they might be stimulated to secrete larger volumes of their products. However, there are no morphological criteria by which it is possible to determine whether a given gland is stimulated. The branching cavity system of the gland and its efferent duct carry the secretion to the distal vaginal canal. The lipids are predominantly released to the intercellular crypts by exocytosis. From there they are likely to be squeezed out to the subcuticular space and then through pores in the cuticle to the common gland lumen by contractions of the muscular reticulum surrounding the glandular lobules/acini and the muscular apparatus of the ef-

ferent duct. Blackening of the secretion by osmium fixatives indicates the presence of unsaturated lipids. The *Aeshna* glands exhibit several morphological features in common with the egg-waxing glands of ixodid ticks (BOOTH, 1989). Thus they possess similar crypt systems in their glandular epithelia, the cells of which bear microvilli containing tubular elements of smooth endoplasmic reticulum. Smooth reticulum is known to be involved in lipid metabolism (FAWCETT, 1981). The efferent ducts of *A. juncea* and *A. grandis* glands exhibit structural similarity to that of the damselfly *Pyrrhosoma nymphula* (ÅBRO, 2004b) but are of greater size, so that each investing tissue contain more cellular elements. The many nerve terminals to the muscle fibres, apparently with polyneuronal innervation, indicate an intricate nervous control. The duct probably functions as a pump (ÅBRO, 2004b). Like the glandular epithelial cells, the epitheloid cells of the efferent duct are provided with microvilli containing tubules of smooth endoplasmic reticulum, which indicates that both cell types are of common origin.

The average longevity of the adult *Aeshna* female is not known but will certainly not extend beyond a few weeks. As to the activity of the gland, the present study indicates that most of the produced substances accumulate in the glandular lumen during the pre-reproductive phase, which the dragonflies spend away from the water. Secretory gland cells seem to possess a limited life span of a few days, followed by a functional and structural decline. Thus decomposition of scattered single cells in the gland parenchyma starts early in the reproductive phase. This becomes an accelerating trend in the course of the reproductive phase. In the late reproductive phase most of the gland exhibits disintegrating tissues. Renewal of secretory cells in the adult gland appears not to occur. The short adult lifetime presumably does not need proliferation of undifferentiated renewal cells. Degradation within the glandular epithelium appears to affect individual cells rather than tracts of contiguous cells dying spontaneously by apoptosis. Cell death in the *Aeshna* accessory glands looks like a stochastic process with different probabilities of dying.

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