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Morphology and Histology of the Accessory Glands of Male Variegated Grasshopper *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae) at different Ages of the Adult

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ABSTRACT

The morphology and histology of the accessory glands of adult male *Zonocerus variegatus* (Linnaeus) at different ages were described. The accessory glands consist of eleven pairs of slender tubules and five accessory gland types were identified. The glands comprised the basement membrane and an epithelial layer. As the insect aged, the cells of the epithelial layer decreased in height and the diameter of its lumen increased simultaneously suggesting an apocrine mode of secretion.

INTRODUCTION

Studies on the morphology and ultrastructure of the accessory glands of several male insects have been carried out by several workers (Chen, 1984). However, very little work has been done on the accessory glands of male Orthoptera, although both the agricultural importance of the taxa and the effect of the accessory gland secretory protein on the physiology of the female insect are well known. For instance, Kaulenas *et al.* (1975) reported that the accessory gland of the cricket, *Acheta domesticus* L. consists of several hundred delicate, blind-end tubules of varying size, each comprised by a layer of secretory cells surrounding a central cavity.

Zonocerus variegatus is found in West and Central Africa as a pest of cassava which provides carbohydrates to millions of people in the region. The aim of the present study was to elucidate the morphology and histology of the accessory gland of *Z. variegatus* at different ages of the adult.

MATERIALS AND METHODS

Nymphal stages of *Zonocerus variegatus* were maintained on Siam weed, *Chromolaena odorata* L. (King), and cassava (Manihot esculenta Cranz) shoots in an illuminated cage in the insectary at 27° ± 2°C. The insectary measured 3 x 3 x 5m, it was aerated and provided with light and an entrance. After adult eclosion, the insects

were separated according to sex and kept in illuminated cages. They were maintained on *M. esculenta* shoots. Adult insects were immobilized in a deep freezer at -25°C for 25 minutes (Yang and Davies, 1967).

A pair of accessory gland-seminal vesicle bundles of a mature male insect (21-day-old adult) were used for morphological studies. The paired bundles were placed on a glass slide. The accessory gland tubules were carefully dissected from seminal vesicles in order to discard the latter. A permanent whole mount of the accessory glands was prepared using standard staining technique (Junqueira et al., 1977). Photograph of the preparation was obtained using Nikkon 135 Camera (55mmμ. length).

To prepare slides for histological studies, the pair of accessory gland-seminal vesi e bundles was dissected in saline solution (58g NaCl, 1.6g KCl, 1.2g CaC12 per litre) (Barker and Davey, 1982) under illuminated dissecting microscope from one male adult insect each at ages 0 (newly emerged), 7, 14, 21, 28, 35, 49 and 56 days owing to the relatively slow development of the insect. The accessory gland-seminal vesicle bundles from insects at different ages were separated in the region of the expanded portion of the ejaculatory duct. They were immersed in Bouin's fixative, dehydrated in graded alcohol, infiltrated in xylene and each separately embedded in paraffin wax. The accessory gland sections (7um thick) were stained with Erlich's haematoxylin and eosin. The acessory gland sections were photographed using Olympus camera PM-C attached to the Olympus BHB 03 miroscope at 40X and 400X magnifications for the cross-sections of cessory gland-seminal vesicle bun individual accessory glands, resp The basement membrane, epitheli and the lumen of each of the eleve sory glands at the ages of the adult were measured with an ocular mic at 100X magnification on a cor microscope. The means and standar of the means were calculated.

RESULTS

Morphology of the accessory glands

The accessory glands of adult n *variegatus* are shown in Plate 1. The pairs of accessory glands were long, rc and slender tubules of varying length blunt and slightly bulbous anterior The posterior end of each tubule o separately into the expanded portion ejaculatory duct. Five types of tubules identified as summarised below:

Type I: Three pairs of long and sl ly curved tubules filled milky secretion

Type II: A pair of relatively thin short tubules filled with g nular whitish material.

Type III: Four pairs of long and sligh curved tubules filled wi transparent secretion.

Type IV: Two pairs of short and straigl tubules filled with transparer secretion.

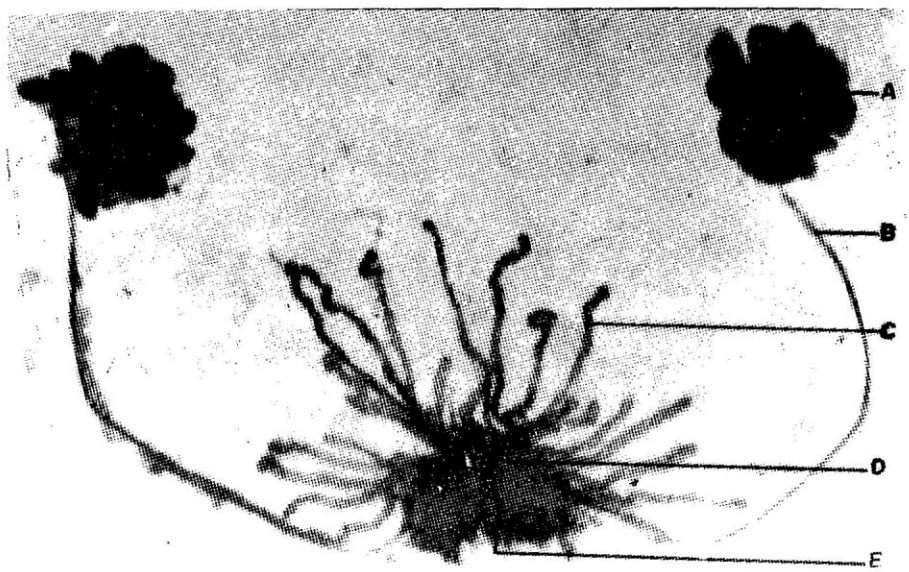

Plate 1: Male accessory reproductive glands of Z. Variegatus with paired testes. A testis, B. Vas deferens; C. Accessory glands; D. Expanded portion of ejaculatory duct; E Ejaculatory duct. X8.

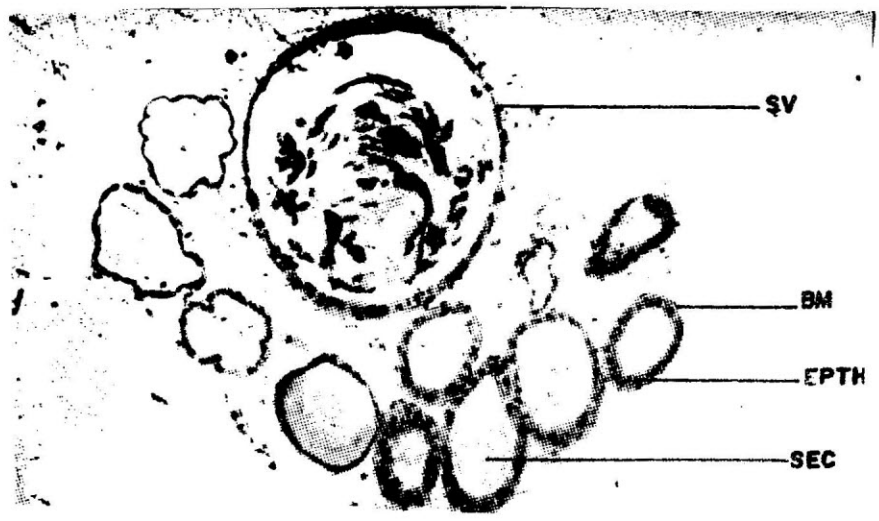

Plate 2: Cross-section of an accessory gland-seminal Vesicles bundles BM, Basement membrane; EPTH Epithelium; SEC, Secretion in lumen; SV Seminal Vesicle. X40.

Type V: A pair of long and extensively coiled tubules filled with dark brown secretion.

A typical cross-section of an accessory gland-seminal vesicle bundle showing the basement membrane, epithelium, secretion in the lumen and the centrally located seminal vesicle in shown in Plate 2.

Plates 3 and 4 show the cross sections of one of the eleven accessory glands from 0- (newly emerged). 7-, 14-, 21-, 28-, 35, 42-, 49-, and 56-day-old adults, respectively.

Plate 3A: Newly emerged adult: The lumen diameter measured 0.39 ± 0.03mm and contained very little secretion. The epithelial layer which was 0.37 ± 0.03mm at its widest portion contained round, large, granular and darkly stained nuclei. The nucleated basement membrane was 0.05 ± 0.005mm in width and surrounded the epithelium (Table 1).

Plate 3B: 7-day-old adult: The lumen had increased diameter (1.04+0.10mm) and contained secretory products. The columnar cells of the epithelial layer contained a darkly-stained row of nuclei. The basement membrane was 0.04 ± 0.004mm in width.

Plate 3C: 14-day-old adult: There was a further increase in the diameter of the lumen which was filled with secretion. The width of the lumen (1.34 ± 0.13mm) was more than thrice that of the newly emerged adult. There was concomitant decrease in the width of the epithelium which measured 0.22 ± 0.02mm. The single row of nuclei in the columnar epithelium became more darkly stained. The nucleated basemen membrane remained unchanged in widt 0.04 0.003mm).

Plate 3D: 21-day-old adult: The acc sory gland lumen showed little increase diameter over that of the 14-day-old ad (1.47%). The diameter measured 1.36 0.14mm and was filled with secretion. T width of the epithelium decreased to 0.24 0.03mm resulting in the granular nuclei c cupying most part of the columnar cells. Tl width of the basement membrane remain unchanged and the nuclei maintained th roundish shape as in plate 3C.

Plate 3E: 28-day-old adult: The epith lial layer measured 0.22 ± 0.04mm in widtl The cells gradually became cuboidal i shape and contained slightly elongate darkly-stained nuclei. The lumen further in creased in diameter (1.54 ± 0.09mm) and was over 70% that of the newly emerged adult. The basement membrane was 0.04 ± 0.004mm wide and contained round as well as rod-like nuclei.

Plate 4A: 35-day-old adult: The lumen had increased in diameter more than five times in comparison with the accessory gland lumen of freshly emerged adult (2.22 ± 0.13mm). It was filled with a dense secretion. The width of the epithelium (0.11± 0.04mm) had further reduced by 50% and contained cells with darkly-stained elongated nuclei. The basement membrane was greatly reduced in width (0.02 ± 0.002mm) with rod-like nuclei which were similar to those found in the accessory gland of the 28-day-old adult.

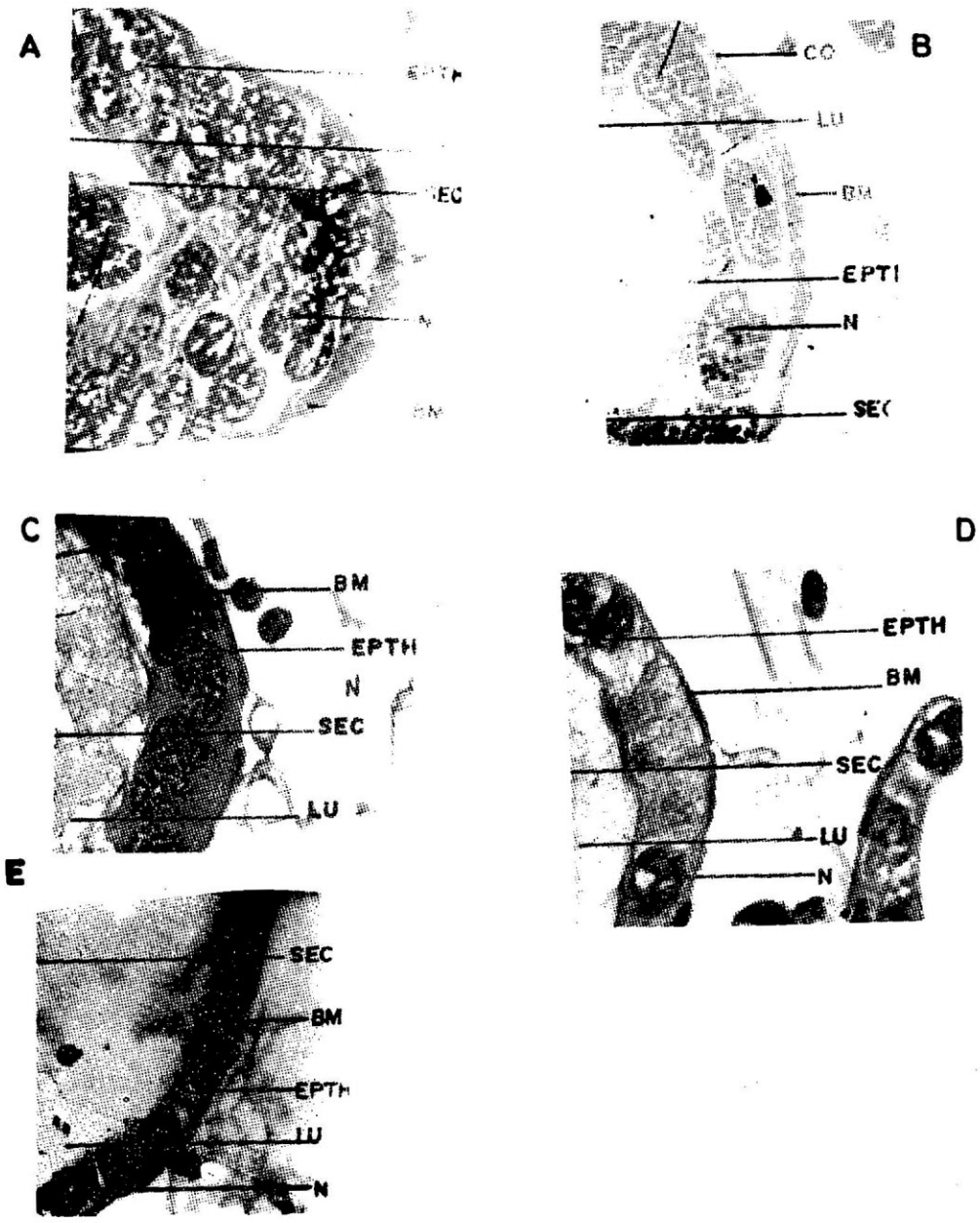

Plate 3: A: Cross-section of an accessory gland of 0-day-old insect B:7day-old insect C:14-day-old insect D:21-day-old insect E:28-day-old insect Abbreviation:BM-basement membrane EPTH-epithelium, SEC-Secretion LU-lumen N-Nucleus. X400.

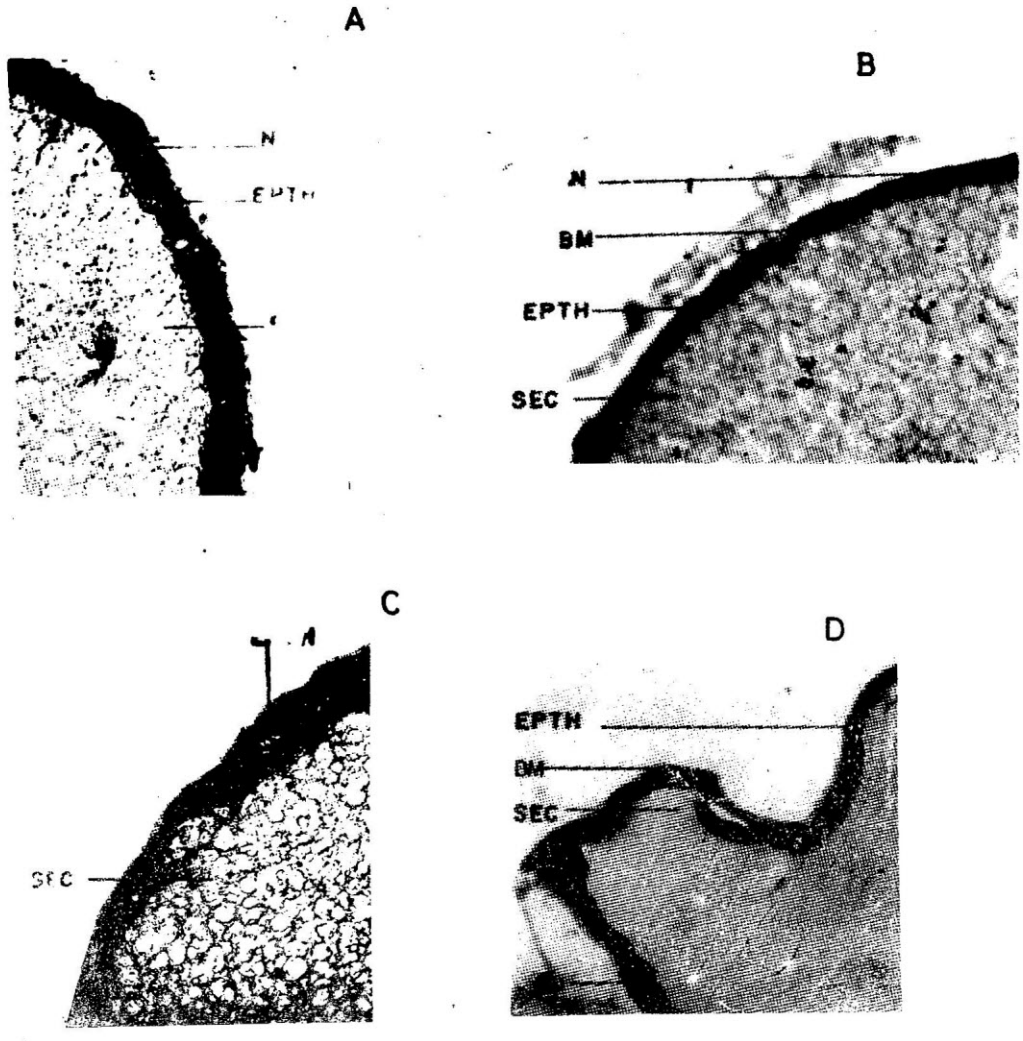

Plate 4. A: Cross section of an accessory gland of 35 day old insect, B: 42 day-old insect, C: 49-day old insect, D: 56-day-old insect, Abbreviation: BM-basement, EPTH epithelium, SEC- secretion, N-Nucleus X400

Plate 4B: 42-day-old adult: The accessory gland lumen was filled with a dense secretion and measured 2.11 + 0.14mm in diameter. The epithelial layer measured 0.13 ± 0.01mm in width, and the cells were indistinct with the nuclei elongated. The basement membrane consisted of thin rod-like nuclei and measured 0.01 ± 0.002mm in thickness.

Plate 4C: 49-day-old adult: The diameter of the lumen of the accessory gland was 2.90 ± 0.20mm and was lined with secretion. The epithelial layer which measured 0.11± 0.009mm in width had been strongly compressed and only the rod-like nuclei were visible. The basement membrane with sparse rod-like nuclei was 0.02 ± 0.005mm wide.

Plate 4D: 56-day-old adult: The lumen which was 2.25 ± 0.13mm in its widest part, formed the largest part of the accessory gland. The epithelial layer was 0.10 ± 0.004mm wide and the cells were not discernible. It was occupied by rod-like and darkly stained nuclei. The basement membrane measured 0.02 ± 0.002mm in width and contained thin rod-like nuclei which had become sparse.

Changes in the sizes of the epithelial layer and the lumen in the accessory glands of the variously-aged adult insects are depicted in Figure 1. The mean diameter of the accessory gland lumen progressively increased from day 0 to day 35 but fluctuated subsequently. The epithelium on the other hand, progressively decreased from day 0 to day 35 and thereafter more or less levelled off. Analysis of variance test carried out on the two variables showed that there was significant differnce ($P<0.01$) in the mean diameter of the lumen and the width of the epithelium of the accessory glands at various ages between day 0 to day 56 adult insects. Duncan's multiple range test for the mean widths of the epithelial layer of the accessory glands showed that the mean values for days 0 to 28 adult insects were significantly different from those of days 35, 49 and 56-day-old adult insects. Duncan's multiple range test for the mean lumen diameter showed that the mean values for days 14 and 21, and 35, 42 and 56-day-old adult insects were not significantly different from each other.

DISCUSSION

The present study revealed that the accessory glands of mature male *Zonocerus variegatus* consisted of eleven pairs of tubules that closely adhered to the paired club-shaped seminal vesicles. Both structures arose from the expanded portion of the ejaculatory duct. Five accessory gland types were also identified from the eleven pairs of accessory gland tubules found in each specimen. Kaulenas (1976) reported six types of glands in the several hundred accessory glands of male *Acheta domesticus*. Variable numbers of pairs of accessory gland tubules have been recorded for many species of insects (Pollock, 1974; Dailey *et al.*, 1980). The presence of 15 pairs of accessory glands in male *Melanoplus sanguinipes* (Pickford et al., 1969) and 16 in *Schistocerca gregaria* (Odhiambo, 1969 quoted by Dhadialla *et al.*, 1986) suggests that there is variation in the number of male accessory reproductive glands among Orthopteran insects. The ac-

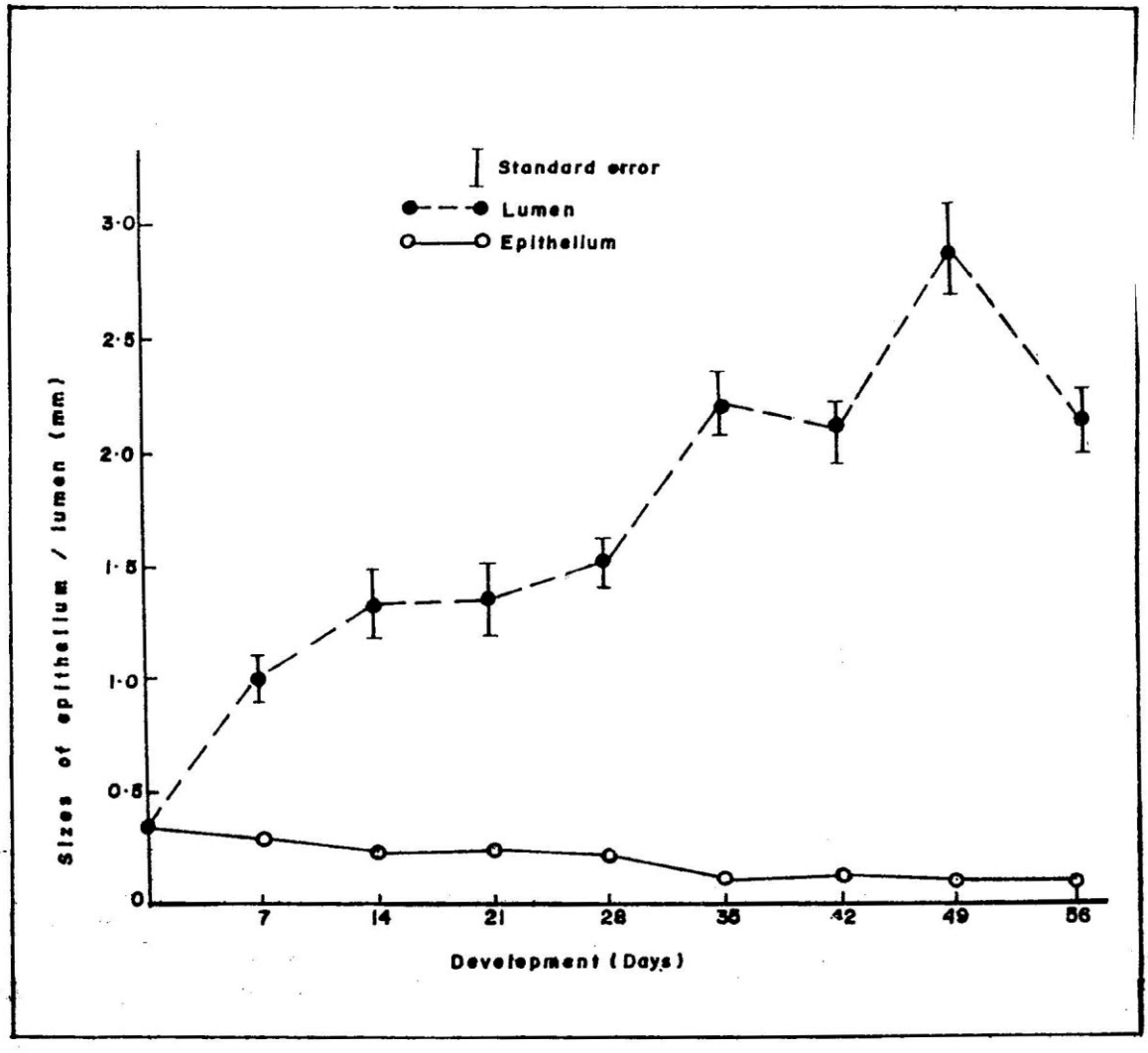

Fig. 1: Relative changes in the sizes of the epithelium and lumen in the accessory glands of unmated maturing adult male Zonocerus variegatus

Table 1

Mean measurements (mm) of lumen diameter, widths of the epithelium and basement membrane, in the accessory glands of 0 to 56 day old adult Zonocerus variegatus.

Age of adult insect	Width of basement membrane (± S.E.)	Width of Epithelium (±S.E.)	Lumen diameter (± S.E.)
0.	0.05± 0.005	0.37± 0.03 a	0.39 ± 0.03 a
7	0.04 ±0.004	0.29 ± 0.04 a	1.04 ± 0.10 b
14	0.04 ± 0.003	0.22 ± 0.02 b	1.34 ± 0.13 c
21	0.04 ± 0.003	0.24 ± 0.03 b	1.36 ± 0.14 c
28	0.04 ± 0.004	0.22 ± 0.04 b	1.54 ± 0.09 e
35	0.02 ± 0.002	0.11± 0.04 c	2.22 ± 0.13 d
42	0.01 ± 0.002	0.13 ± 0.01 c	2.11 ± 0.14 d
49	0.02 ± 0.005	0.11 ± 0.009 c	2.90 ± 0.20 f
56	0.02 ± 0.002	0.10 ± 0.04 c	2.25 ± 0.17 d

Mean values with the same alphabets are not signficantly different from each other according to Duncan's Multiple Range (DMR) test.

cessory gland tubules of *Z. variegatus* are morphologically distinct in the newly emerged adult insects. Happ (1984) contended that primary organogenesis of the accessory glands occurs in pre-adult stages; it is however not known whether it commences in the last nymphal stages of *Z. variegatus* since only adult insects were studied.

The accessory glands of *Z. variegatus* consists essentially of a basement membrane and an epithelial layer. Ultrastructural studies on the accessory glands of several species of insects have revealed that they comprise a basement membrane and an epithelial layer (Dapples *et al;* 1974. Ramalingham and Craig, 1978). Histological study of the accessory glands of *Z. variegatus* shows that the epithelial layer does not have a chitinous intima. The absence of chitinous intima on the epithelium was reported in the accessory glands of *Heliothis zea* (Callahan and Casciom 1963). Brits (1978) made a similar report on the accessory glands of the moth, *Phthorimaea operculela* (Zeller). The absence of chitinous intima on the epithelial layer of the accessory glands of *Z. variegatus* suggests that the glands are mesodermally derived and supports the observation of Szopa (1982) that accessory glands of Acrididae are mesodermal in origin

As the accessary glands of *Z. variegatus* develop, the lumen is filled with secretory materials and the epithelial layer decreases progressively in height. Riemann and Thorson (1979) have shown that the epithelium of the accessory glands of *Anagasta kueniella* has the ultrastructural characteristics appropriate to the synthesis and secretion of protein. Baker and Davey (1982) have demonstrated that the accessory glands of *Rhodnius prolixus* produce secretory protein *de novo*. The results of the present study suggest that the accessory gland epithelium of *Z. variegatus* produces a secretion that fills the lumen.

The manner of secretion of protein b the epithelium is not known but the gradu decrease in height of the accessory glar epithelial layer of *Z. variegatus* as the inse matures suggests that the columnar cells the gland shed their apical portion and e port secretory material by apocrine secr tion as suggested for *Tenebrio molit* (Happ, 1984). This suggestion is reinforce by the earlier report of Gadzama *et a* (1977) that the apical portion of the colun nar secretory cell of tubular accesso glands of *T. molitor* is filled with secreto vesicles in maturing glands.

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