ROLE OF BURSA COPULATRIX IN OOSORPTION IN *CADRA CAUTELLA* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Calco Red incorporated into the diet of the almond moth larvae , *Cadra cautella* Walker, stained developing oocytes in virgin females. During oocyte degeneration stained contents from oocytes were traced to the bursa copulatrix. Females of different ages showed a direct correlation between the intensity of stain in the bursa copulatrix and the number of oocytes that were digested. The results indicate that, in some Lepidoptera, the bursa copulatrix is involved in the storage and distribution of metabolites during oosorption.

Key Words: *Cadra cautella*, bursa copulatrix, oosorption, reproduction.

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INTRODUCTION

Oocyte degeneration in the bulla seminalis of the female moth, *Cadra cautella* Walker, begins ca 24 hr post eclosion and continues for the lifetime of the adult (Lum 1979, 1983). The process involves the movement of the primary follicles from the ovarioles into the bulla seminalis where contractions of the bulla break the chorion and release the contents of the oocytes. Virgin females that lack the oviposition stimulus tend to retain their oocytes and as many as 30 oocytes are degenerated over a period of 7-10 days. Although the shells of the degenerated oocytes form a pellet that remains in the bulla seminalis, the destination of the yolk material from the oocytes has not been determined. If oocyte degeneration is a form of oosorption, the contents of the oocytes must be metabolized and distributed. Because the bulla seminalis is a slender muscular duct that connects the bursa copulatrix and the common oviduct (Fig. 1), the yolk material that is released from the degenerated oocytes in the bulla has only 2 possible outlets. Visual identification of the yolk material is sometimes difficult because of the colorless nature and small quantity, especially that of a single oocyte, but there is evidence that yolk from the oocyte may be deposited and metabolized in the bursa copulatrix. First, the role of the bursa copulatrix in Lepidoptera to process metabolites has been established. During mating, the bursa accepts the spermatophore from the male and within a few hours proceeds to digest the spermatophore, releasing the spermatozoa for fertilization (Norris, 1932). In addition, the bursa also passes metobolites from the male fluid into the hemolymph (Greenfield, 1982). Second, examinations of the bursae in virgin female *C. cautella* of different ages have shown that in the newly eclosed female, the bursa copulatrix is empty, but in older females, the bursa contains a colorless, granular material resembling the yolk of the oocytes (Lum, unpublished information). The appearance of the material in the bursa coincides with onset of oocyte degeneration in the bulla seminalis which begins ca 24 hr after eclosion (Lum, 1979).

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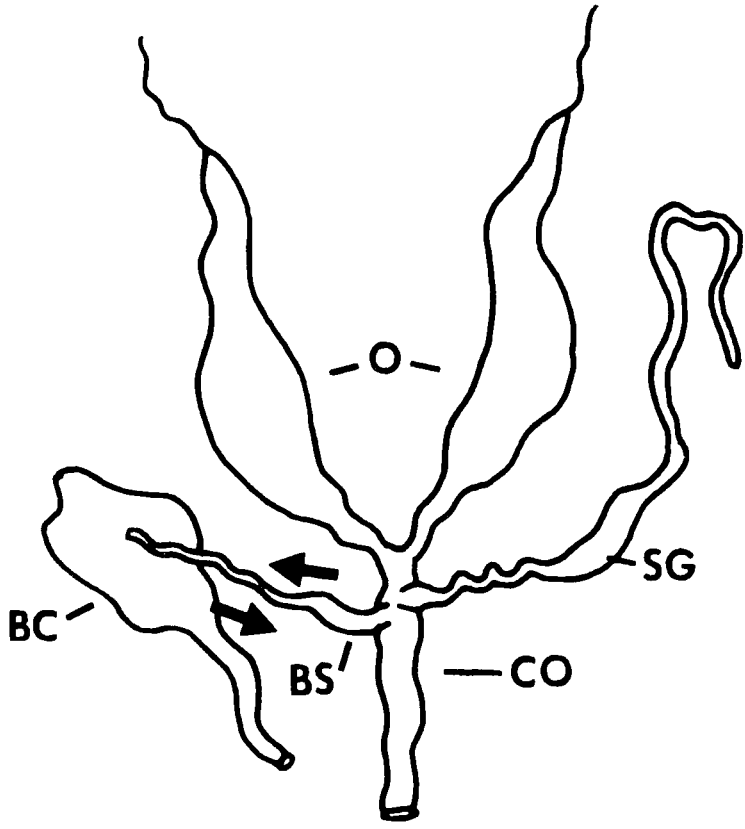

Fig. 1 Diagrammatic sketch of female reproductive tract in the Almond moth (not drawn to scale): 0 = ovaries; CO = common oviduct; BS = bulla seminalis; BC = bursa copulatrix; SG = spermatheca. During oocyte degeneration oocytes from ovaries are shunted through common oviduct to the bulla seminalis. Contractions of the bulla break the chorions of the oocytes, releasing yolk material which is translocated to the bursa copulatrix. Flow of material also occurs in opposite direction when sperm from spermatophore that is deposited in bursa copulatrix is pumped to the spermatheca through the bulla seminalis.

Thus, it appears that the bursa copulatrix may be the site for the metabolism of yolk material for oosorption in addition to that of digesting the spermatophore. Confirming the nature of the material in the bursa of the virgin female is therefore necessary to understanding oocyte degeneration, oosorption, and determining the full role of the bursa copulatrix. Hendricks and Graham (1970) successfully marked the tissues of the adult tobacco budworm, *Heliothis virescens* (F.), by incorporating an oil-soluble dye, Calco Red (WP-American Cyanamide) into the larval diet. They found that the oocytes and fat bodies in the female adult carried the stain for several days post eclosion and that the stain was also detectable in deposited eggs. Calco red thus provides a means to trace the movement of yolk material

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from degenerated oocytes. The following study was conducted to determine whether stained contents of the oocytes could be traced to the bursa copulatrix.

MATERIALS AND METHODS

The oil soluble dye, Calco Red (WP-American Cyanamid) which marked adult tissues of the tobacco budworm (Hendricks and Graham, 1970) was incorporated into the diet of almond moth larvae. The dye was added to standard media (Boles and Marzke 1966) by dissolving 5 gm of dye in 5 ml of refined cottonseed oil, and blending it into 500 gm of media. Cultures consisting of 500 eggs/500 gm of media were maintained at 30°C, 60% RH, and in alternating 12 hr light and dark periods. Twenty-two days later female adults were collected at eclosion and isolated to prevent mating.

To determine whether the dye stained the oocytes and was detectable for at least 7 days post eclosion, virgin females were examined when they were 0-6 hrs old and also when they were 7 days old. Each chain of follicles in the ovaries was examined for a reddish or pinkish color imparted by the dye. The bursa copulatrix in the newly eclosed female was also examined to determine whether it was stained. Adult females from diets containing the dye were compared to those from normal diets. Detection of yolk material in the bursa copulatrix was based on the presence and intensity of the stain in the bursa after oocyte degeneration began in the bulla seminalis. Each bursa from a female was examined with a light microscope at a magnification of 60X. The intensity of the stain was scored on a scale from 0-5. Three values were assigned to obvious differences: 0 = no color; 3 = pinkish tint; and 5 = reddish tint. The number of degenerated oocytes in the bulla seminalis was recorded in addition to the intensity of the stain in the bursa. Samples consisted of 10 females per group and each sample was replicated 3 times.

RESULTS AND DISCUSSION

Calco Red, that was added to the larval diet, stained specific tissues of the reproductive tracts of both male and female almond moths. The ovaries, fat bodies, testes, and vasa deferentiae, all stained an intense red. In the newly eclosed female, the bursa copulatrix and bulla seminalis showed no signs of the dye and were similar to that of the normal unstained female. However, in the 24-36 hr-old female, the bursa copulatrix in the stained virgin female showed a light pinkish trace of the dye. The stain was incorporated into a transparent liquid resembling a lipid droplet which when placed into a drop of water, floated to the surface and broke into tiny droplets. The first detectable sign of the dye in the bursa coincided with the degeneration of 2-3 oocytes. The stain could not be detected in the bursa of females containing only a single degenerated oocyte. This was probably due to the small quantity of stain from a single oocyte.

A comparison of the bursa copulatrixes from females of different ages showed distinct differences in the intensity of the stain in the glands. Based on the three obvious differences in the coloration of the bursa copulatrix, there was a direct correlation between intensity and the number of degenerated oocytes in the bulla seminalis (Table 1). These results indicate that material from oocytes are deposited in the bursa copulatrix when the oocytes are broken down by the bulla seminalis.

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Table 1. Relationship of oocyte degeneration in the bulla seminalis and intensity of Calco Red Stain in the bursa copulatrix.

Age of female (Days)	Mean ± S. E.*	
	No. oocytes in Bulla Seminalis	Itensity of stain in bursa (Scale of 0-5)
0.5	0.2 ± 0.1	0
1.5	0.9 ± 0.4	0
7.0	7.3 ± 0.6	4.0 ± 0.4
10.0	10.5 ± 0.8	4.7 ± 0.3

* Mean ± S. E. of 3 replicates; 10 female/replicate.

The results of this study help to explain the observations by others concerning substances found in the bursa copulatrix of virgin female moths. Musgrave (1937) described a granular secretion in the bursa of *Ephestia kuehniella* Zeller, and Khalifa (1950) found a lipid-like substance in the bursa of *Galleria mellonella* (L). In both cases the exact origin and function of the secretion in both species were not determined. Also, both Khalifa (1950) and Musgrave (1937) discovered oocytes in the bulla seminalis of the female moth but neither associated oosorption with the presence of oocytes in the bulla. It is likely that the substance that they described in the bursa copulatrix was material from degenerated oocytes. Other accounts of oocytes in the bulla seminalis of various Lepidoptera (Norris 1932; Stitz 1901; and Peterson 1900) are probably more than coincidental observations. The evidence is strong that in a number of lepidopteran species, oosorption is initiated by the mechanical or physical breakdown of oocytes by muscular contractions of the bulla seminalis. This is in contrast to the chemical digestion of oocytes that is characteristic of other species (Bell and Bohm, 1975).

Thus, in addition to digesting the spermatophore for the translocation of sperm, which is essential for reproduction, the bursa copulatrix in several species of lepidoptera performs another role in the distribution of metabolites during oosorption. The exchange of material in either direction between the bursa and bulla (Fig. 1) shows the versatility of the two muscular organs and their functions in reproduction.

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