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Some Morphological Features of the Rectal Sac of the Silkworm (Bombyx mori: Bombycidae)

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Some Morphological Features of the Rectal Sac of the Silkworm (*Bombyx mori*: Bombycidae)

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Abstract: The silkworm moth escapes from the pupal-protection case by utilizing cocoon-digesting enzymes. The rectal sac was examined in relation to the behaviour of the animal. Pupae and pharate adults were obtained from commercial races and were reared in the laboratory. The ball-shaped rectal sac, easily distinguishable within the abdomen of the silkworm, was found to contain two-phased creamy fluid. The occurance and the shape of the organ and the usage of its fluid suggest its role in perforation of the cocoon.

Key Words: Silkworm, Rectal Sac, Pupae

İpekböceği (*Bombyx mori*: Bombicidae) Rektal Kesesinin Bazı Morfolojik Özellikleri

Özet: İpekböceği erginleri özel enzimik salgılar kullanarak kozadan çıkarlar. Rektal kese, hayvanın bu davranışı ile ilgili olarak incelemeğe alınmıştır. Ticari ırk ipekböcekleri laboratuvarda yetiştirilerek pupa ve genç erginler elde edilmiştir. İpekböceğinin abdomeninde kolaylıkla farkedilen top şeklindeki rektal kese iki ayrı fazda yoğun kıvamda bir sıvı içermektedir. Organın bulunuş yeri, şekli ve içeriğinin boşaltılma durumu kozanın delinmesinde bir rolü olduğunu düşündürmektedir.

Anahtar Sözcükler: İpekböceği, Rektal Kese, Pupa

Introduction

In insects the rectum is a common part of the digestive and excretory systems. It is a constituent of the hindgut where the malpighian tubes discharge their contents (1). They operate in conjunction with a specialized gland which is very important to maintain a constant internal environment of the body by elimination of unwanted substances and reabsorbtion of constituents, such as water, necessary to the organism (2).

Because the silkworm passes its pupal period in a silken cocoon, the moth uses some proteases which are known to be secreted by several organs, to free itself. However, it has been pointed out that the crop, maxillae and midgut are responsible for the production of such secretions (3, 4), the rectal gland was considered for investigation in relation to evasive behaviour, because of the place of occurence and the time of its distinctness during pupal-adult development.

Materials and Methods

The strains of silkworm, *Bombyx mori*, used in the present study were the F_1 hybrids of *C-124 x N-124* cross of commercial races. The animals were reared on fresh mulberry leaves in laboratory conditions at 25°C and 70% humidity. Four days after the onset of cocoon shell formation they were stored in special boxes. Rectal development was followed morphologically by making an incision on the ventral of the abdomen at various stages of pupal-adult evolution. Fully developed sacs were fixed in 30 % formaldehyde solution for further investigation of their contents.

Results

Within the abdominal region of pharate-pupae there was a light coloured area as a continuation of the midgut. This region became more prominent with pupal development reaching its greatest size in the late pupal stage. This structure, the rectal gland, then could be distinguished as a well-shaped organ extending out from the intestine and filling almost one third of the abdominal cavity in either sex. The gland was visible having a sac-like appearance among the ovarioles within the female body (Fig. 1), and among the testis, vas deferens and accessory glands in males (Fig. 2).

Prior to the transitional development into adults, pupal weight was measured with and without rectal sac. The values of the body weight and the calculated weight of the rectal sac are shown in a range diagram where female pupae were found to be heavier than the male (Fig. 3), while the rectal sac was found to have close mean values in both sexes (Fig. 4).

The rectal gland was found to contain a thick-creamy fluid which appeared in two separate phases, recognizable by their coloration (Fig. 5). When the ball-shaped organ was held in an upright position, the darker fluid always formed the top layer while the lighter was in the lower half. The fine-grained denser fluid showed gravitation when tilting the gland and sank to the bottom. This was also observed while the sac was still in situ (Fig. 6). The difference in density was also indicated by the presence of spherical structures. While the lighter phase was made up

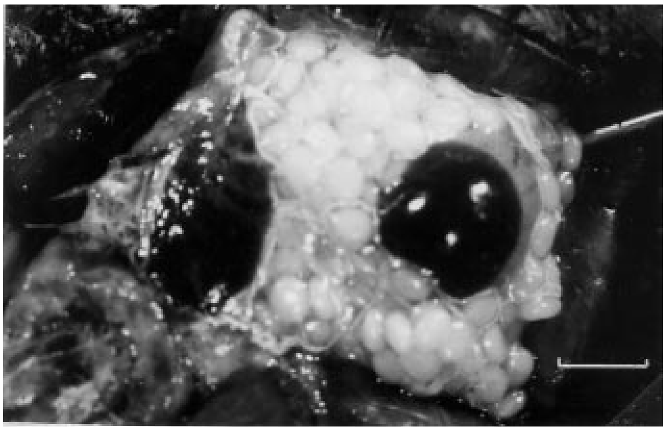

Figure 1. General view of the abdominal cavity of the female silkworm at the late pupal stage. Rectal gland is very conspicuous among the egg filled ovarioles. Scale bar=3mm

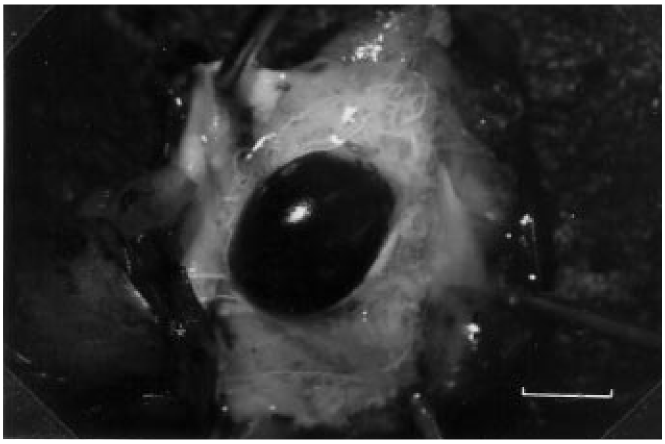

Figure 2. General view of the abdomen of male silkworm at the late pupal stage. Rectal gland appears as a distinct structure. Scale bar = 3mm

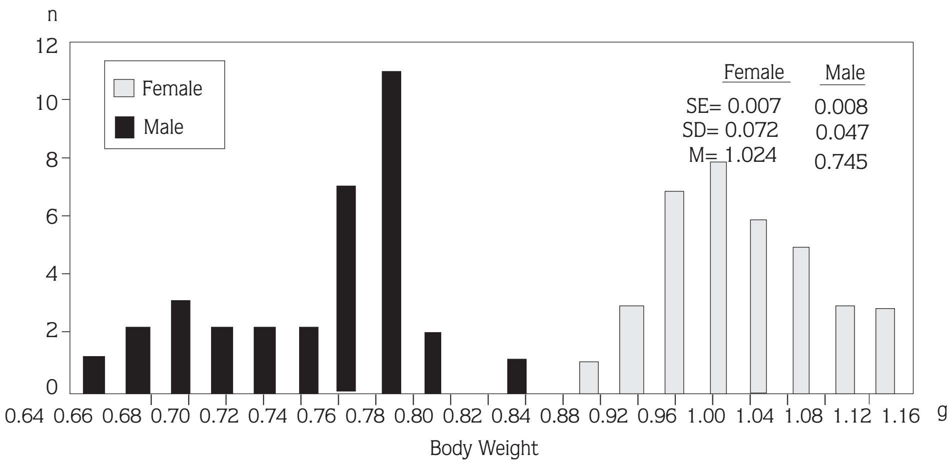

Figure 3. Comparison of the body weight of the late pupae of female and male silkworms.

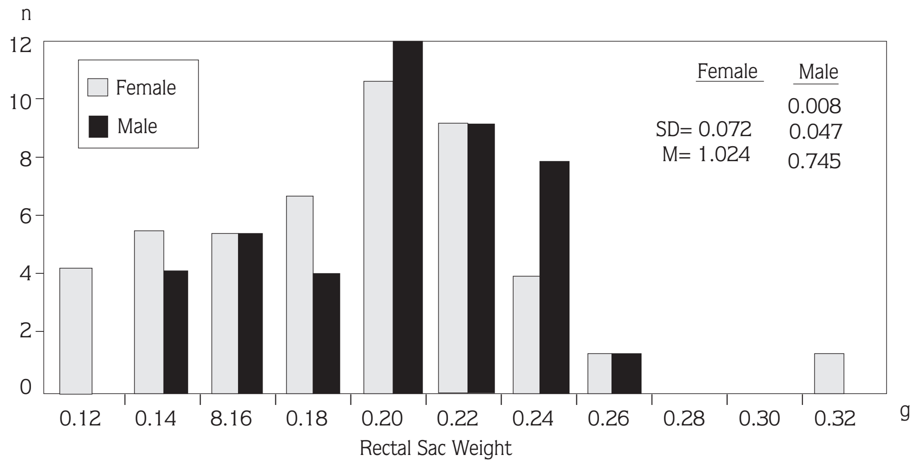

Figure 4. Comparison of the rectal sac weights of female and male pupae of silkworms.

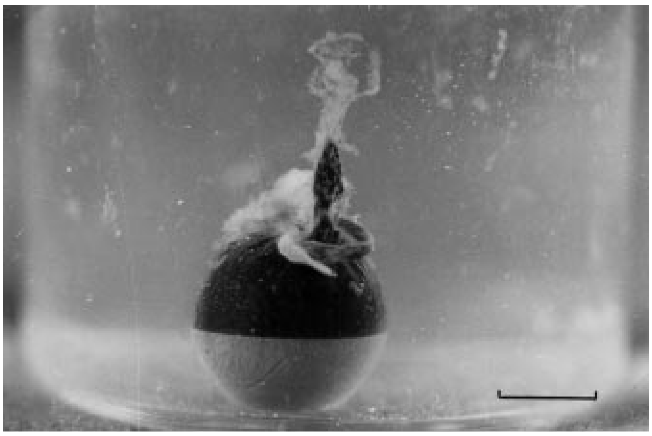

Figure 5. Rectal gland, as a globular sac, contains two separate phases of fluid. The dense material is in the lower half while the apex is filled with a material lighter in density but darker in colour. Scale bar = 3 mm

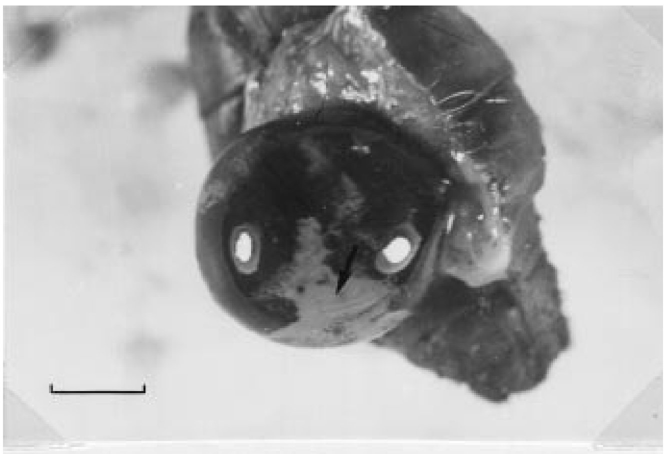

Figure 6. Rectal sac protrudes easily from the abdomen and the dense material flows downward when the organ is tilted. The arrow points to this shifting process. Scale bar = 2 mm

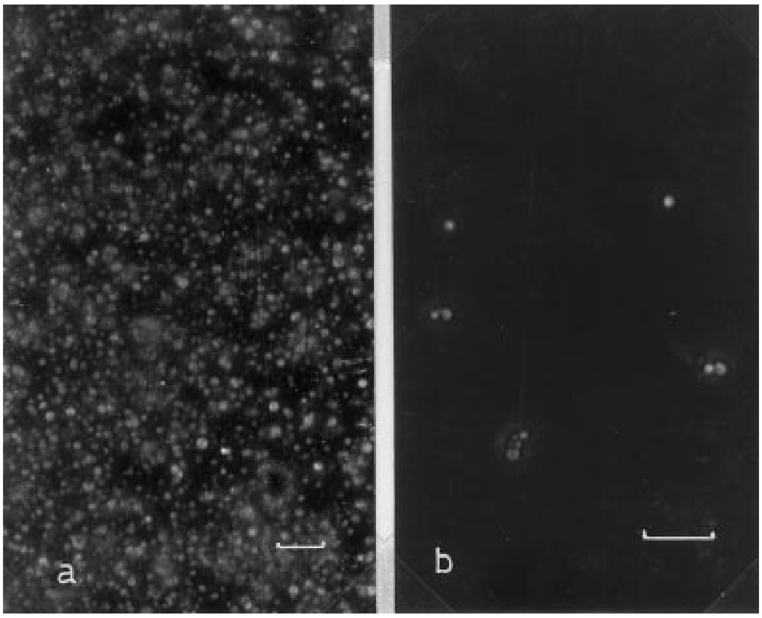

Figure 7. The density difference in two phases of the content of the rectal gland. The lighter phase contains numerous small spheres even when diluted tenfold while the darker phase was clear liquid. Scale bar = 10 μm

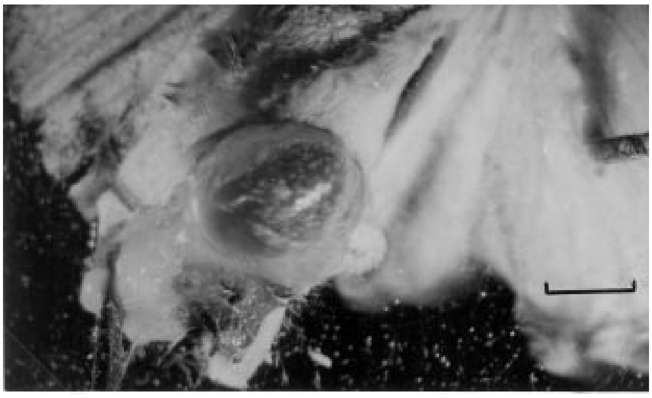

Figure 8. Discharged membranous sac remains within the abdomen of the moth. Scale bar = 2 mm.

of numerous spherules, the darker phase was only liquid, and this difference is very clear in Figs. 7a and b, even in tenfold diluted lighter phase.

During the process of pupal development, the amount of dark fluid was greater but as pupae develop became less. Soon after the moths freed themselves, the sac diminished almost totally or appeared to be an air-filled membranous structure (Fig. 8). At the time of emergence, adult silkworms spewed some fluid which was the same colour as the dark fluid in the sac. When compared, it was found to be similar to the colour of the stain formed on the cocoons by the moths (Fig. 9) in order to be able to emerge. When the gland was placed into a fixative the darker fluid diffused out quickly whereas the lighter one remained much longer. The empty gland appeared to be a membranous sac (Fig. 10).

Discussion

Wild and domesticated silkworms are protected in silken cocoons in the course of their pupal development. This heritable character of this group of animals which is a result of the direct relationship between the physical and behavioural abilities of their larvae, has been focus of attention for a large number of investigators (5). The structure of the silk gland and silk fibre, as well the physiology of the silk gland and the biochemical aspects of silk proteins have, therefore, been thoroughly investigated (6-8).

The protective mechanism of pupae generates another problem: the escape of the enclosed adults from the cocoons. In *Bombyx mori* and in wild silkworms the cocoon wall is softened and dissolved with liquid containing a proteolytic enzyme secreted from the mouth of the pharate adults (3, 9, 10).

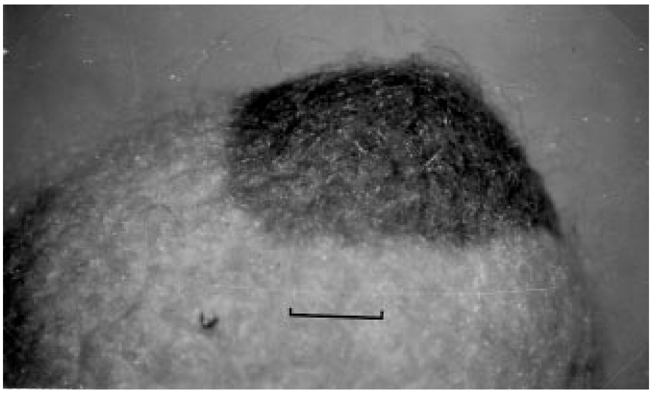

Figure 9. A stain is formed by the rectal fluid discharged by the pharate adult at the time of emergence. Scale bar = 2 mm

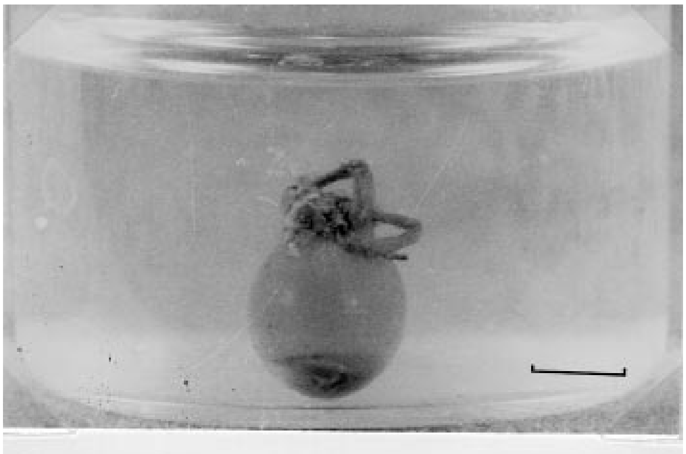

Figure 10. During fixation rectal gland becomes an empty-memsranous sac, with only a small amount of fine grains in the organ base. Scale bar = 2 mm.

It has been suggested that the midgut was the source of proteases in pharate adult of *B. mori*. According to Eguichi and Ivamoto (4), some reservoir organs that are concerned with the production and utilization of cocoon-digesting enzymes, work in a combined system with the midgut. The same researches have shown that the proteolytic activity in the midgut of pharate adults reaches a peak just before emergence and falls steeply afterwards and only a traceable amount of enzyme exists in the midgut. Although the existing literature has mainly been focused on the crop, in the present study the appearance of the rectal sac in the late pupae and pharate adult was found to coincide with the storage function related not only with the excretion but also with some other activities. The light phase with spherules probably contains uric acid in relation to the main role of the gland because it was previously demonstrated that uric acid is essentially an end-product of the nitrogen metabolism in silkworms (11, 12). In reference to the dark ingredient, however, the rectal sac was regarded in relation with the crop, in the cocoon-digesting function. Because the rectal sac is another reservoir organ closely related with the midgut, it was considered that the discharge of the midgut content, in relation to cocoon-digestion, was achieved as anal emission through the rectal sac while the crop caused an oral emission. Some insects were also found to eject some chemical compounds with the rectal fluid for protective purposes (13). It is very probable that other compounds could be discharged to free the insect from the cocoon shell.

Despite the wealth of information about the digestive enzymes in silkworms (14-16) and about their complex excretory systems (1, 17), little is known about the morphology and the complete role of the rectal sac especially in their pupal stage. As far as we know, the work reported in this paper is the first example on this subject. We have as yet very little information about the significance of the differences in the rectal gland content as discernable in phases, in relation to he behaviour of the moth to manage the flight from the enclosed chamber. To solve this problem, further biochemical studies on the physiological and behavioral aspects are being carried out.

References

1. Hickman C. P. Jr., Roberts L. S. and Larson A.: Integrated Principles of Zoology (9 th Ed.), Mosby-Year Book, Inc., St. Louis, 1993.
2. Wigglesworth V. B.: The Principles of Insect Physiology (8 th Ed.), Chapman & Hall, London, 1984.
3. Eguchi M. and Iwamoto A.: Role of the Midgut, Crop and Maxillae of *Bombyx mori* in the Production of Cocoon-Digesting Enzyme., J. Insect Physiol. 20, 1366-1372, 1975 a
4. Eguchi M. and Iwamoto A.: Hydrolysis of Solubilized Fibroin and Silk Proteins in the Midgut of the Pharate Adult of *Bombyx mori*., J. Insect Physiol., 21, 577-588, 1975 b.
5. Tazima Y. The Silkworm: an important laboratory tool. Kodansha, Tokyo, 1978.
6. Akai H., Imai I. and Tsuboichi K.: Fine Structural Changes of Liquid Silk in the Silk Gland During the Spinning Stage of Bombyx larvae., J. Seric. Sci. Jpn. 56, 131-137, 1987.
7. Akai H., Kiuchi H. and Tamura T.: Ultrastructures of Silk Glands and Cocoon Filaments of Wild Silkmoths, *Antheraea yamamai* and *Antheraea pernyi*., Wild Silkmoths'88 (Ed. Akai H. & Wuz. S.), pp. 9-23, Ins. Soc. Wild Silkmonths, Tsukuba, 1991.
8. Narumi T., Kobayashi M. and Mori T.: Electron Microscopic Observations of Voids in Cocoon Filaments of Silk-Spinning Moths. J. Seric. Sci. Jpn., 62, 489-495. (Japanese with Eng. Sum.)., 1993.
9. Kafatos F. C. and Williams C. M.: Enzymatic Mechanism for the Escape of Certain Moths from Their Cocoons., Science (Wash.), 146, 538-540, 1964.
10. Hruska J. F., Felsted R. L. and Law J. H.: Cocoonase of Silkworm Moths: Catalytic Properties and Biological Function., Insect Biochem., 3, 31-43, 1973.
11. Tojo S.: Uric Acid Production in relation to Protein Metabolism in the Silkworm, *Bombyx mori*, during Pupal-adult Development. Insect Biochem., 1, 249-263, 1971.
12. Cochran D. G.: Nitrogenous excretion. In "Comprehensive Insect Physiology, Biochemistry and Pharmacology", Ed. G. A Kerkut and L. I. Gilbert, Vol. 4, pp. 467-506, Pergamo~Press, Oxford, 1985.
13. Eisner T., Ziegler R., McCormick J. L., Eisner M., Hoebeke E. R. and Meinwald J.: Defensive Use of an Acquired Substance (carminic acid) by Predaceous Insect Larvae., Experientia 50, 610-615, 1994.
14. Watanabe T.: On the Cocoon-Digesting Enzyme in the Crop Fluid from Moths of *Bombyx mori*., Sakurakaishi, 19, 21-31, 1926.
15. Akabori S. and Uehara K.: On the Enzyme in the Crop Fluid from Moths of *Bombyx mori*., Bull. Inst. Sreicult. Sci. 1, 9-14, 1942.
16. Kafatos F. C, Tartakoff A. M. and Law J. H.: Cocoonase-I. Preliminary Characterization of a Proteolytic Enzyme from Silk Moths., J. Biol. Chem., 242, 1477-1488, 1967.
17. Wall B. J. and Oschman J. L.: Structure and Functions of the Rectum in Insects. Fortschr. Zool., 23, 193-222, 1975.