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<論 文>

Light and Scanning Electron Microscope Observatt-ons on Sexual Dimorphism in Pupa of Mulberry Silkworm, *Bombyx Mori* Linn (Lepidoptera : Bombycidae)

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ABSTRACT

Under Light and Scanning electron microscope, the pupal morphology of mulberry silkworm *Bombyx mori* Linn. revealed the reduced prothorax and metathorax, well developed mesothorax, less defined last pair of spiracle, well exposed prothoracic femora and wing pads approaching the anterior margin of Ab III. The important sex separating characters viz. pupal size, weight, antennal elevations, intersegmental lines and genital openings have been discussed. Further, two separate openings bursa copulatrix and ovipositional opening were observed, performing different functions in adult moth.

key words : sexual dimorphism, *Bombyx mori*, SEM

INTRODUCTION

The domestic silkworm *Bombyx mori* Linn. , an economically important sericigenous insect which shares a major part of silk procuction in India, has been extensively studied. A number of workers have studied, the general morphology of lepidopterous pupae (Poulton, 1891 ; and Packard, 1895) ; classification of lepidopteran pupae (Mosher, 1916) ; sexing of lepidopterous pupae(Butt and Cantu, 1962 ; Kumar and Verma, 1980) ; genital structure of *Nocoleia octasema* (Franzmann and Garrett, 1978) ; a key for the identification of the pupae of known lepidopterous sugarcane borers (Issac and Venkatraman, 1941) and setal map on a lymantriid pupa (Kumar and Goel, 1986). The scan of literature further reveals that among the lasiocampid pupa of superfamily Bombycoidea the sex identification in *Trabala vishnu* Lef. pupa and gravimetrics of weight loss is on record studied by Rao and Goel, (1990). In recent past the sexual dimorphism and gravimetrics on weight loss in *Dicrisia obliqua* pupa (Goel and Kumar, 1983). certain useful features in sexing and weight loss in pupa of mango defoliator *Lymantria marginata* (Singh and Goel, 1985). pupal morphology and sex differentiation on a sunflower pest *Plusia orichalcea* (Kumar and Goel, 1988) have been studied.

Most of the information on pupal morphology have been generated on the basis of the light microscopy and visual observations. So far, no attention has been paid on *B. mori* to develop an understanding at surface ultrastructure level pupal morphology especially on genital structures which are of great significance, since separation of the sex by visual observation is practised in large scale commercial grainages for silkworm egg (seed) production. Therefore, in the present paper an attempt has been made to study the sexual dimorphism in *B mori* pupa under light microscope and scanning electron microscope.

MATERIALS AND METHODS

The disease free layings of popular hybrid, PM X NB4D2 strain were reared in laboratory on mulberry leaves until they reansformed into adults under an average temperature 28.5±0.25°C and 75.08+1.37% relative humidity. The pre-pupal stage was characterized by cessation of feeding and body shrinkage.

Three days old male and female pupae were removed from cocoons and the genital and anal parts of both the sexes were dissected, washed thoroughly in distilled water, treated with 2% KOH to remove the muscles and fat bodies and studied under binocular microscope

(Leica). and photographed with attached camera.

For SEM studies the dissected body parts were fixed in 2.5% gluteraldehyde prepared in 0.2M Cacodylate buffer, (PH 7.2) for two hours, washed with Cacodylate buffer, fixed in 1% Osmium tetroxide for half an hour, washed in double distilled water, dehydrated in graded alcohol series, critically dried in critical point dryer (EMS 850). After drying, the samples were mounted over copper stubs by double side sticking tape and coated with gold (30 nm thickness) in Sputter Coater (EMS 550). The coated specimens were observed under electron microscope (JEOL 100 CX II-ASID-4D) at 20 kV.

RESULTS

The last instar larva spins an elaborate silken cocoon and transform into pre-pupa and finally it changes into a quiescent pupa. The change from the larva to the pupa, initially becomes evident by cessation of feeding. The ecdysis of the larva takes place inside the cocoon making the cuticle soft and yellowish in colour.

Table 1. *Sexual dimorphism in the pupa of mulberry silkworm bombyx mori linn.*

character	male	female
size	Small, average length 2.316=0.019 cm and width 0.780=0.013	Large, averag elength 2.493=0.029 cm and width 0.840=0.011 cm
weight	Light, average weight 1.263=0.016 gm	Heavey, average weight 1.520=0.0.03 gm
Antennal	More elevated	Less elevated
Position of the genital opening	Mid-ventral, situated on the caudal margin of Ab IX	Mid-ventral, extending on both Ab VIII-Ab IX with bursa copulatrix on the posterior margin of Ab VIII and ovipositional opening in the Ab IX
Intersegmental lines	Discontinuous and straight	Intersegmental lines of Ab VIII and Ab IX are stretched towards the female genital opening
Genital pads	Present	Absent

However, after 2-3 hrs the cuticle becomes hard and brown. The late age spindle shape pupa is dark brown in colour and wriggles when irritated. The silkworm pupal stage is a closed system, though the metabolic activity is high due to histolysis and histogensis leading to metamorphosis. The male pupa is smallar than female having 2.316±0.019 cm length and 0.780±0.013 cm width, while the female pupa is of 2.493±0.029 cm length and 0.840±0.011 cm width (Table 1).

The broad and pectinate antrnnae (An) is attached to the front of the vertex (V), extending lateroventrally half to the length of mesothoracic wings in male and 1/4 in the female pupa. Small vertex (V) infront of the

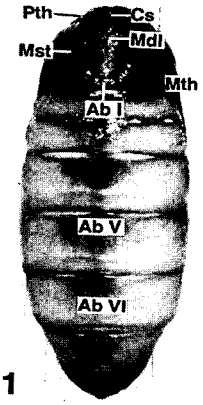

Fig. 1. *Dorsal view of B. moro pupa showing prothorax (Pth), mesothrax (Mst), metathorax (Mth), coronal suture, (Cs), middorsal line(Mdl), abdominal segement I (Ab I), abdominal segement V (Ab V) and abdominal segement VI (Ab VI).*

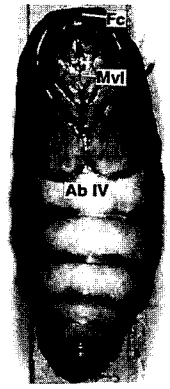

Fig. 2. *Ventral view showing frontoclypeus (Fc), midventral line (Mvl) and abdominal segement IV (Ab IV)*

prothorax (Pth) is bisected by the coronal stem (Cs) of the epicranial structure (Es) dorsally. The frontoclypeal sclerite (Fc) forms an anterior connate lobe extending caudlly into the labrum (Lb). After sixthday of pupation, the prominent eyes are clearly distinguished to a glazed eye (Ge) and sculptured eye piece (Se). The labial palps (Lp) are observed on the meson and caudad to labrum. The two labial palpi (Lp) aer caudally separated by the mid-ventral line (Mv1) of junction (Figs. 1-4). The distal end of macilla (Mx) separates the prothoracic femora (Pf), thoracic legs (TL1, TL2, TL3) and two wing pads. The small wing pads reaches upto the end of Ab III segment ventrally. The antena reaches upto the Ab I in the male while in female it reaches upto the metathorax (Mth). The prothoracic spiracles (Ps) are well defined, and six pairs of oval and conspicuous spiracles (S) which are clearly visible on both the lateral sides of the pupa from Ab II to Ab VII. However, the seventh pair of spiracle is less defined and located at the lateral side of the Ab VIII which unites with Ab IX and

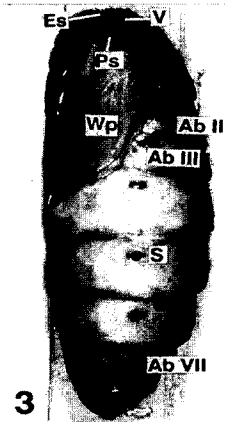

Fig. 3. *Lateral view showing epicranial suture (Es), vertex (V), prothoracic spiracle (Ps), wing pad (Wp), abdominal segment II (Ab II), abdominal segment III (Ab III), spiracle (S) and abdominal segment VII (Ab VIII).*

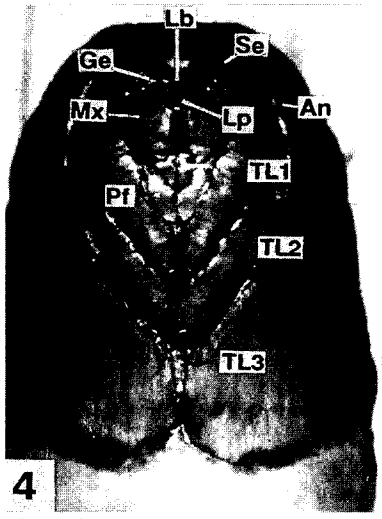

Fig. 4. *Anterolateral view showing labrum (Lb), glazed eye piece (Ge), sculptured eye piece (Se), maxilla (Mx), labial palpi (Lp), prothoracic femora (Pf), prothoracic leg (TL1), mesothoracic leg (TL2), and metathoracic leg (TL3).*

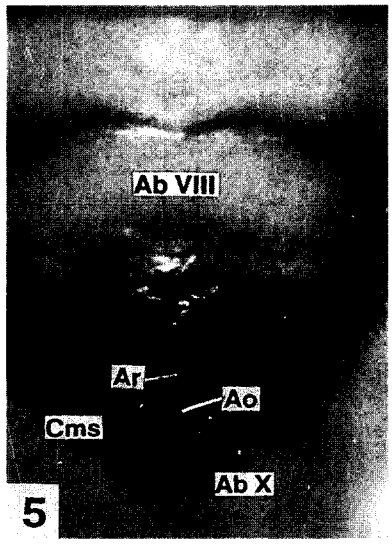

Fig. 5. *Posteroventral view of male pupa showing anal arise (Ar), anal opening (Ao), cremastral setae (Cms), abdominal segment VIII (Ab VIII) and abdominal segment X (Ab X).*

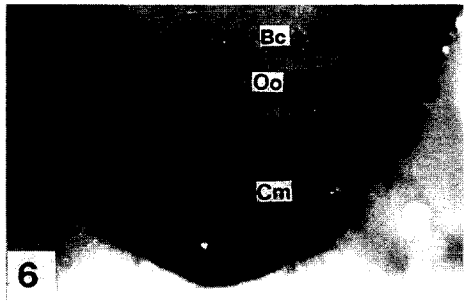

Fig. 6. *Posteroventral view of female pupa showing bursa copulatrix (Bc), ovipositional opening (Oo) and cremaster (Cm).*

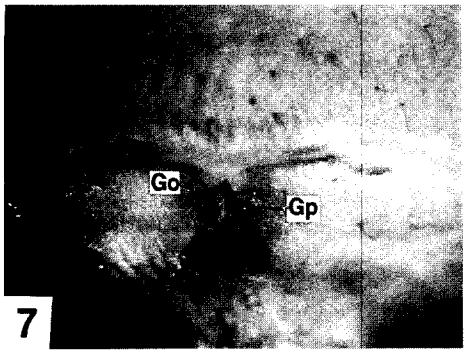

Fig. 7. *Male genital structures showing genital pads (Gp) and male genital opening (Go).*

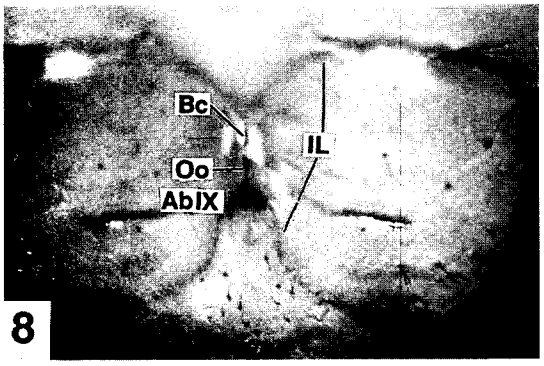

Fig. 8. *Female genital structures showing bursa copulatrix (Bc), oviposition al opening (Go), intersegmental lines (IL) and abdominal segment IX) (Scale bar=100 μm).*

Ab X to form a cap like structure, where genital openings and anal openimhs are situated ventrally in both the sexes (Figs. 1-8)

In the three segmented thorax, the prothorax is reduced and having a mid dorsal line (Md1), whereas mesothorax (Mst) is the largest of the thoracic part laterally extends into wing pads (Wp). A prominent line appears to be a contnuation of the coronal stem (Cs) of the vertex (V) extending through pro- and mesothorax. A narrow metathorax (Mth) which is laterally widen and visible in between mesothorax and Ab I. Wing pads (Wp) are wrapping the pupa upto second abdominal segment ventrally in both the sexes. Prothoracic femora (Pf) is clearly visible, prothoracic tarsals (TL1) are shorter than antenna and mesothoracic tarsals (TL2) are equal to antenna whereas the metathoracic tarsals (TL3) are bigger than antenna and the tips of TL3 are visible

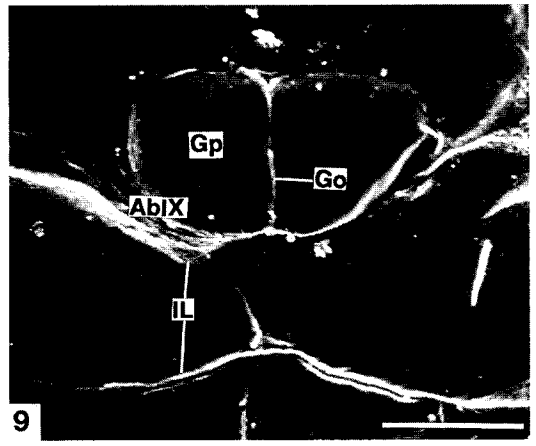

Fig. 9. *Male genital sturctures showing male genital pads (Gp), male genital opening (Go), intersegmetal lines (IL) and abdominal segment IX (Ab IX) (Sclale bar =100 μm).*

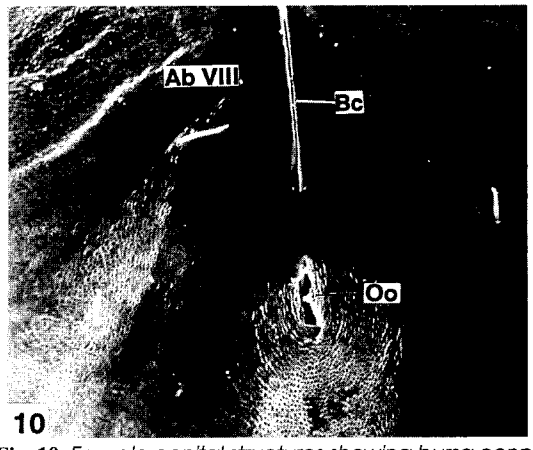

Fig. 10. *Female genital structures showing bursa conpulatrix (Bc), oviposition al opening (Oo) and abdominal segment VIII (Ab VIII) (Scale bar=100 μm).*

on the ventral side of Ab IV of the pupa (Figs. 1-4).

The abdominal segments fourth to seventh are the only segments with telescopic movement out of the ten abdominal segments. First three abdominal segments are covered by the wings ventrally in both the sexes. Abdominal segments Ab VIII to Ab X are fused immovable into a single unit though evidently demarcated by intersegmental lines (IL) in both the sexes in different fashion. The cremaster (Cm) is studded symmetrically with several pairs of cremastral setae (Cms) in both the sexes (Figs. 5,6).

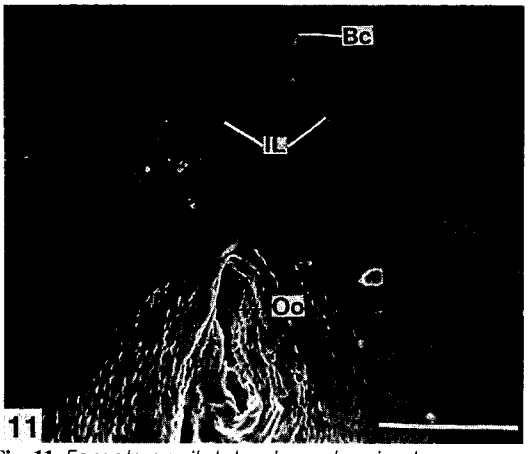

Fig. 11. *Female genital structures showing bursa copulatrix (Bc), ovipositional opening (Oo) and intersegmental lines (IL) (Scale bar=50 µm).*

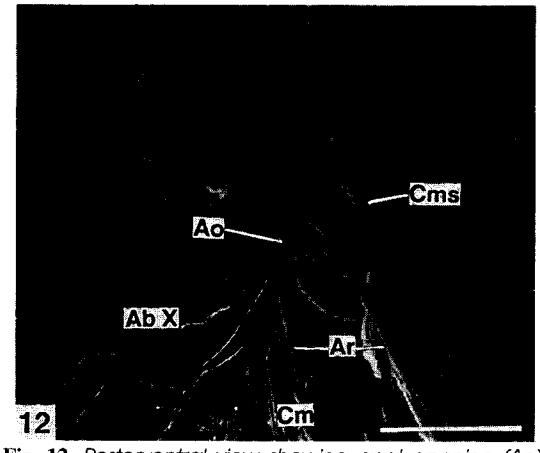

Fig. 12. *Posterventral view showing anal opening (Ao), analarise (Ar), cremastral setae (Cms), cremaster (Cm) and abdominal segment X (Ab X) (Scale bar=50 µm)*

The male genital opening (Go) is longitudinal and elongated between the two lateral rectangular elevated genital-pads (Gp) which are located at Ab IX on ventral side. In male straight and intersegmental lines are discon tinued. A single slit like medianal opening (Ao) is situated on anal arise (Ar) on Ab X, surrounded by intersegmental wrinkles in both the sexes (Figs. 5, 7, 9). A complete female genital structure comprises two separate openings an elongated slit like structure called bursa copulatrix situated at Ab VIII whereas ovipositional opening (Oo) is oval in shape and situated at Ab IX. However, both the openungs have different function in adult female moth. In female, intersegmental line (IL) of Ab VIII is directed towards Ab IX, and the intersegmental lines of Ab IX are stretched towards the Ab VIII midventrally, where the bursa copulatrix and ovipositional openung are situated. Various cremastral setae are observed adjacent to the posterior end of male and female anal opening and anal arise (Ar) (Fig. 12).

The pupal period lasts for about 10.00±0.11 days in male and 11.667±0.12 days in female. The female pupa is heavier with an initial pupal weight of 1.520±0.03 gms whereas in male it is 1.263±0.016 gms and the subsequent final pupal weight is 1.23±0.02 gms and 0.925±0.02 gms, respectively. The sexual dimorphism is distinct and summerized in the table.

DISCUSSION

The information are scanty on the morphology and sexing of the pupae of superfamily Bombycoidea. However, several authors have studied different lepidopterous pupae and differentiated the male and female pupae on the basis of important characteristic features (Goel and Kumar, 1983; Singh and Goel, 1985; Kumar and Goel, 1988; Rao and Goel, 1990). Mosher (1916) classified Bombycoidea into two families Bombycidea and Lasiocampidae based on the different characteristic features of each family. The sexual dimorphism in lepidopterous pupae have been studied on the basis of certain important features viz. the shape, size, weight, antennae and genital organs. The long and broad antenna in male than of female in *Lymantria marginate* have been reported by Singh and Goel (1985) similar to that of *B. mori* contrary to that a short antenna in male than female in a noctuid *Agrotis biconica* (Singh *et al.* 1991). Rao and Goel (1990) observed long, broad and pectinate antenna in male than of female in a lasiocampid pupa *Trabala vishnu* similar to *B. mori*, as both share the common features being the members of super family Bombycoidea. A small vertex infront of the prothorax is bisected by the coronal stem of epicranial suture and the front is rather a smooth *sclerite*, laterally supported by the large, broad antennae and the eye pieces, have been observed in *B. mori*. Similar observations have been made in I. *vishnu* (Rao and Goel,

1990). Further, labrum lies in between the eye pieces and not separated from the terminal sclerite of the frontoclypeus in *B. mori* identical to a lasiocampid pupa I. *vishnu* (Rao and Goel, 1990). Rao and Goel (1990) observed the labial palpi, triangular small sclerite caudad to labrum in between the maxillae similar to *B. mori*. Each eye is marked into a narrow glazed and sculptured eye piece in *B. mori* and I. *vishnu* (Rao and Goel, 1990), The glazed eye piece has been regarded as true pupal eye in lepidopterous pupa (Imms, 1973.).

The presence of exposef prothoracic femora in a noctuied pupa *Plusia orichalcea* (Kumar and Goel, 1988), confirms the characteristic of family Noctuidae of lepidoptera (Mosher, 1916). However, the exposed prothoracic femora caudad to maxilla has also been observed in *B. mori*. A small tarsal portion of the metathoracic leg is exposed on Ab IV on ventral side and the cremaster bears the prominent longer hooked seyae in both the sexes of *B. mori* similar to I. *vishnu* (Rao and Goel, 1990).

Rao and Goel (1990) reported two separate opening i.e. bursa copulatrix at Ab VIII and ovipositional opening at Ab IX comprising female genital system in a lasiocampid pupa T. *vishnu* similar to *B. mori*, showing the characteristic of superfamily Bombycoidae. The similar observations have also been made on a arctiid Diacrisia obliqua by Goel and Kumar (1983) and on Pieris brassicae (Pieridae) by Lal and Chandra (1975). However, the ovipositional opening was named as "Aperture of oviduct". Two confluent openings i.e. bursa copulatrix and ovipositional opening forming a narrow slit like sturcture has been observed in *Lymantria marginata* (Singh and Goel, 1985): *Plusia orichalcea* (Kumar and Goel, 1988) and *Agrotis biconica* (Singh *et al.*, 1991). Tazima (1978) studied *B. mori* pupa for sex separation and reported a vertical line across the ventral side of Ab VIII and a genital opening at Ab IX in female pupa whereas we have observed two separte openings i.e. bursa copulatrix at posterior margin of Ab VIII and ovipositional opening at Ab IX by scanning electron microscope. Further we have observed the male genital aperture at Ab IX which is similar to the observation made by Tazima (1978).

B. mori pupa shows slightly shorter pupal duration than female similar to lepidopterous pupa *Diacrisia obliqua* (Goel and Kumar, 1983) and identical pupal duration in both the sexes in lymantriid *Lymantria marginata* (Singh and Goel, 1985), Rao and Goel (1990), however, observed female pupal period longer than of male in a lasiocampid pupae *Trabala vishnu*.

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