

**EMBRYONIC DEVELOPMENT IN MUGA SILKWORM,
ANTHRAEA ASSAMENSIS HELFER
(LEPIDOPTERA: SATURNIIDAE)**

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ABSTRACT: Chronological variations during embryonic development of normal eggs of *Antheraea assamensis* Helfer were recorded from 24 h to till hatching. During 10-16 h of the embryonic development, the ventral plate is formed and by 24 h trough shaped embryonic premodium floats in yolk and the protocephalon and protocorn are separated by transverse furrows. After 96 h the embryo became C-shaped. Although, mouth parts are yet not developed completely but they were at the advance stage of development. The thoracic region is clearly divisible into three thoracic segments. After 120 h, organogenesis in embryo takes place. The antennae bear segments and head region was detachable into three segments. The head capsule formation completed in after 144 h and mouth parts got matured. Three segmented antennae with antennal setae, the mandibles and labrum are well developed. After 168 h old, mandible become sclerotised and pigmented at distal ends. Larval eyes appear as six brown spot on either side of head. The spiracles are clearly visible on the sides of the body. Fully developed muga silkworm larva comes out from the egg cell after 8th day of oviposition rupturing the anterior part of egg shell by the mandibles.

KEY WORDS: *Antheraea, assamensis*, embryonic development, different stages, organogenesis.

Muga silkworm, *Antheraea assamensis* Helfer is the lustrous golden yellow silk producing lepidopteran insect endemic to North-East India, which serves as an exclusive ecological niche for this species. The muga silkworm is semi-domesticated, polyphagous and multivoltine in nature having five to six generations in a year. 'Som', *Persea bombycina* Kost and 'Soalu', *Litsaea polyantha* Juss are the two primary food plants of muga silkworm.

Egg is considered as the key factor of sericulture industry. Only quality eggs can ensure a good harvest and healthy crop. The oviposition and hatching of eggs of muga silkworm are severely affected during the adverse summer months of the year i.e. July to September owing to the prevalence of high temperature coupled with very high humidity. The non-synchronized moth emergence results in wastage of valuable biological material and absence of technology for silkworm egg preservation etc. are among the main constrains of muga silk industry. Postponement of hatching sometimes becomes inevitable for solving problems associated with synchronized brushing, as per suitability of season and availability of good foliage (Pandey et al., 1992). In traditional practice, muga silkworm eggs are preserved at room condition/BOD incubator at 26 ± 2 °C for incubation just after the completion of the daily grainages in a piece meal system during all the seasons of rearing. The eggs are collected after 72 hours of laying. This practice creates problems like, synchronizing hatching with leaf sprouting, non uniform hatching due to mixing of eggs of different ages leading to unequal development of worms during rearing, timely distribution of eggs in bulk with

uniform hatching for commercial rearing, etc. which resulted in low productivity. Therefore, in commercial grainages, it becomes necessary to develop appropriate technologies of short and long term preservation of eggs in muga culture to postpone hatching for synchronizing hatching with leaf sprouting, to skip unfavourable seasons and timely supply of eggs in bulk with uniform hatching. This will protect the wastage of valuable biological material. Before proceeding to long term cold preservation of embryo, it is essential to know in details about the different embryonic developmental stages and confirm the suitable embryonic stage for preservation at low temperature. Technologies for preservation of multivoltine muga silkworm eggs have not been established thereby facing a big hurdle in the management of seed sector of the industry. The non-diapause eggs of mulberry silkworm, *B. mori* can be preserved at low temperature for more than 70 days without affecting the hatching (Rajanna et al., 2009). In mulberry silkworm, the longest embryonic stage i.e. stage 15 has been identified as the suitable stage of effective preservation for longer duration (Vemananda Reddy et al., 2003). In muga silkworm, the different embryonic developmental stages have not been studied. Therefore, in the present study, we present the different embryonic developmental stages of muga silkworm eggs which will help to find out the suitable stage for longer duration of preservation of eggs.

MATERIAL AND METHOD

Muga silkworm eggs were collected from the Muga Silkworm Breeding Section, Central Muga Eri Research and Training Institute, Central Silk Board, Jorhat, India. To isolate the different embryonic developmental stages of muga silkworm in different ages, the standard technique developed by Vemananda Reddy et al. (2003) for mulberry silkworm was followed with slight modification as the eggs of muga silkworm has thick chorionic layer covered with thick gummy substances.

The zero age eggs of muga silkworm, *A. assamensis* were collected and incubated at 26 ± 2 °C and relative humidity of 75-85 % for different durations from 24 h to till hatching and the stages of embryonic development at the different ages were studied at an interval of 12 h. The egg samples of different age groups were boiled in 3 – 4 % KOH solution for 2-3 minutes and then washed in 60 °C water. Care is taken so that KOH does not dissolve the embryo. The embryo was then kept in distilled water maintained at room temperature in a transparent glass petridish and kept under the dissecting stereo zoom microscope. The water was squirted by using a Pasteur pipette over the eggs to release embryos. The egg shell was removed from the micropylar end using a sharp surgery blade. With the help of a pointed needle and soft brush, the embryo was freed from the yolk material. The embryos were arranged for taking photograph of that particular age to use as reference for future. After isolation of different stages, embryos were preserved in 70 % alcohol in the small glass vial for preparation of permanent slide.

RESULT AND DISCUSSION

Like in most other insects, life of muga silkworm begins as an independent egg. Each egg is manufactured within the female's genital system and is eventually released from her body through an ovipositor, a component of her external genitalia (Fig. 1). The cell's cytoplasm is usually distributed in a thin band just inside the vitelline membrane and in diffuse strands that run throughout the

yolk. The egg cell's haploid nucleus lies within the yolk, usually close to one end of the egg. The egg's anterior/posterior polarity is determined by the relative positions of the nucleus and the oosome. The egg is covered by a protective "shell" called the chorion made of protein secreted before oviposition by accessory glands in the female's reproductive system. The chorion is perforated by microscopic pores called aeropyles that allow respiratory exchange of oxygen and carbon dioxide with relatively little loss of water. The micropyle, a special opening near the anterior end of the chorion, serves as a gateway for entry of sperm during fertilization.

Embryogenesis is a developmental process that usually begins once the egg has been fertilized. After two hours of oviposition, male and female pronuclei unite at a definite position near the anterior pole to form zygote (Takami, 1969). The zygote yields about 5000 daughter nuclei through 12-13 cycles of mitosis without cytokinesis. The cleavage nuclei migrate through the yolk toward the perimeter of the egg. They settle in the band of periplasm where they engineer the construction of membranes to form individual cells and a one-cell-thick layer, the blastoderm is formed. Blastoderm cells on one side of the egg begin to enlarge and multiply. This region, known as the germ band is where the embryo's body will develop. The rest of the cells in the blastoderm become part of a membrane that forms the yolk sac. Cells from the serosa grow around the germ band, enclosing the embryo in an amniotic membrane.

At the cellular blastoderm stage, when secondary membrane is formed between blastoderm cells and the yolk system, some cleavage nuclei migrating towards egg periphery are prevented from entering into the periplasm and they remain attached to the secondary yolk membrane. These become vitellophages. Cleavage nuclei remaining in yolk becomes the centers of yolk granules to supply nutrition to the developing embryo. Yolk membrane folds during late germ band stage after which the yolk system divided into masses each enclosing one or several nuclei and yolk organelles. This process is known as yolk segmentation (Miya, 1984).

The embryonic developmental stages in *Bombyx mori* were serially numbered from 1-30 (Takami & Kitazawa, 1960). The embryonic developmental stages in eri silkworm, *Samia ricini* has been studied and identified the suitable stage for cold preservation (Sarkar et al., 2012). The fertilization is considered as stage-1, Cleavage is stage -2 and Blastoderm is stage -3. The earliest embryonic stage that can be isolated and removed is from stage 4 i.e. germ band onwards.

The chronological variations during embryonic development of normal eggs were recorded from 24 h to till hatching (Fig. 2A-N). Thirteen different embryonic stages were detected and among these stages the longest stage viz. Hei - B stage was observed at 68 h to 72 h old embryo.

Stage-4: This stage is called germ band, which develops to an embryo. A group of cells attaches to the inside at a specific region of the germ band. The cytoplasm of these cells appears dense than that of germ band cells. These are primordial germ cells. The germ band is concave on the inner side and the shape is of an oval plate. In muga silkworm, it forms within 24 h of oviposition (Fig. 2A). Throughout 22-28 h, the germ band becomes slender and elongated and by 28-34 h, a long narrow depression called the primitive groove or streak or median plate is formed along the mid portion of the germ band on upper side. By 24-36 h, the development of embryo was well differentiated into head and trunk region (Fig. 2B).

Stage-5, 6 & 7: The embryo gradually contracts to the shape of Dharuma (Japanese doll). Gradually the head and tail region can be identified. After 48 h,

segmentation of the body is clearly visible. The head end is called the protocephalon and the tail end is called caudal lobe. This stage is continued from 36 h to 60 h in muga silkworm egg (Fig. 2B-2D).

Stage-15: In 68 h to 72 h of age, the embryo reaches this stage wherein the metamerism of mesoderm is completed and mesoderm is arranged segmentally (Fig. 2E). In 72 h, embryo showed three well differentiated distinct regions of the body i.e. head, thorax and abdomen. The segmentations with enough length and the amniotic fold covering the embryo were clear and serosa was completely covered with the yolk. The embryo was slender with a well-defined head and caudal region. The head has a clear cut depression in the middle. Mesoderm segments are clearly visible.

Stage-16-20: In 84-96 h, rudiments of appendages appear in thoracic region and cephalic region formed by the beginning of stomodaeum.

Stage-21: The process of blastokinesis begins in 108-120 h after oviposition. Embryo starts to move around. Blastokinesis first start in the abdominal region and extend toward heads. Posterior abdominal segments are first turned vertically so that the abdominal region as a whole forms a straight line. The abdominal region then turns towards anterior side and reaches the level of prothorax. The anterior and posterior ectodermal invaginations extends to form the fore gut and hind gut respectively.

Stage-22: The head capsule formation completed in after 132 h and mouthparts got matured (Fig. 2J). Three segmented antennae with antennal setae, the mandibles and labrum are well developed. Yolk mass inside the eggs serves as a source of nutrients for the developing embryo and also help in holding the embryo on its surface as a necessary foundation.

Stage-23: After 144 h lateral walls complete and tips of labrum and labium become segmented (Fig. 2K). Thoracic legs become segmented with claws at distal end. Rudiments of the setae develop on the body surface.

Stage-24: At about 156 h, entire body of embryo is covered by strong setae and embryonic moult occurs in this stage. The caudal horns also occur in this stage.

Stage-25-29: After about 168 h, mandible become sclerotised and pigmented at distal ends (Fig. 2M). Larval eyes (i.e. ocelli) appear as six brown spot on either side of head. The spiracles are clearly visible on the sides of the body. Head capsule and mouth appendages are sclerotised and well pigmented. The amnion and serosa disappear by fragmentation. Embryo ingests the embryonic membranes and sensitive for adverse environmental condition. Entire body of embryo becomes sclerotised.

Stage-30 (Newly born muga larvae): Fully developed muga silkworm larva comes out from the egg cell on the 8th day of oviposition rupturing the anterior part of egg shell by the mandibles and swallowing the portion of the chorion in the early morning of exposure of light (Fig. 2N). Newly born larvae are generally blackish or brownish in colour. Generally healthy larvae are blackish brown in colour with distinct yellow lines at the intersegment region. Head portion is shining black with elongated spot and larval body is yellowish with blackish tubercle.

Present finding indicates that, the embryonic development starts within a few hours of egg laying and it requires proper incubation for healthy development of embryos. Any change in temperature can hamper the development, hatching and rearing performance. Embryonic development and hatching were hampered at the stressed temperature and humidity condition because high temperature and low humidity were unfavorable condition for embryonic development (Dinesh et al., 2012). Due to global warming, this type of condition prevails during seed crop

rainages of summer seasons of *A. assamensis*. Higher temperature and low humidity during embryonic development leads to death of embryo during early age. Temperature stress caused poor egg laying, delay and poor hatching, depression of eggs and death of fully formed larvae inside egg. The result of the present study will help to find out the particular embryonic stage suitable for long term egg preservation which will help to skip the unfavourable season and can synchronize rearing with availability of leave.

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Figure 1. A. Mother moth laying eggs, B. Eggs (enlarged).

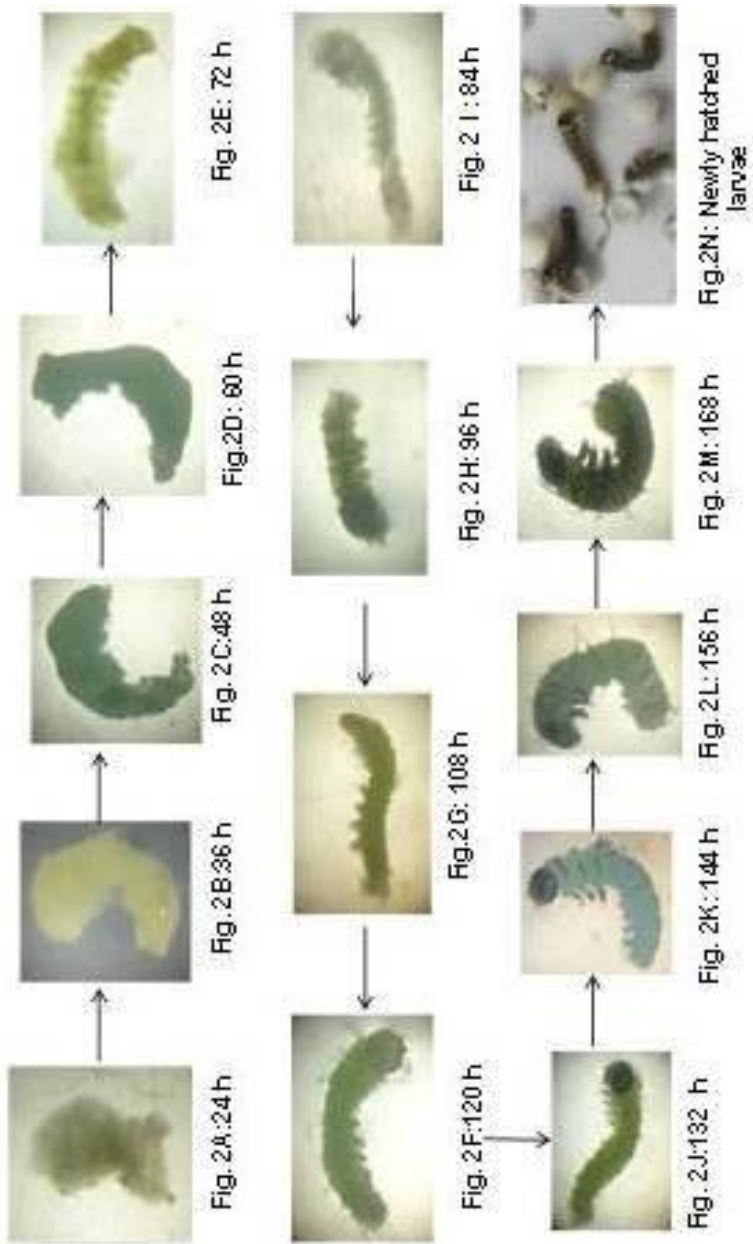


Figure 2. Different developmental stages of embryos of muga silkworm (24 h to till hatching).