Indian Journal of Entomology Online published Ref. No. e23026 DoI. No.: 10.55446/IJE.2023.1510

REPRODUCTIVE BIOLOGY OF MUGA SILK MOTH *ANTHERAEA ASSAMENSIS* HELFER

Dipanjali Biswas[1], Arup Kumar Hazarika[1], Anup Kumar Sahu[2] and Dulumoni Das[3]

[1]Department of Zoology, Cotton University, Guwahati 781001, Assam, India
[2]Regional Muga Research Station, Central Silk Board, Boko 781123, Kamrup, Assam, India
[3]Department of Statistics, Cotton University, Guwahati 781001, Assam, India
*Email: arup.hazarika@cottonuniversity.ac.in (corresponding author): ORCID ID 0000-0001-6206-3195

ABSTRACT

Results of morphological and morphometric study of male and female reproductive systems of *Antheraea assamensis* Helfer (Saturniidae: Lepidoptera), a silk moth cultivated exclusively in the North Eastern Region of India are reported herein along with some observations on its reproductive performance in four seasons. The male reproductive system consists of a pair of testes, paired vasa deferentia, seminal vesicle, ductus ejaculatorius duplex, ductus ejaculatorius simplex, an aedeagus and a pair of male accessory glands. The female reproductive system consists of a pair of ovaries, each with four ovarioles, paired lateral oviducts, a common oviduct which has a dorsal protrusion, the vestibulum, a bursa copulatrix, a spermatheca and a pair of female accessory glands with their reservoirs. Fecundity has been found to be positively correlated with female pupal weight in each season. Hatching % of eggs has been found lowest and significantly different ($p<0.001$) in summer as compared to autumn, winter and spring seasons. Morphology of eupyrene and apyrene sperm bundles and sperm has also been reported.

Key words: Reproductive system, *Antheraea assamensis,* morphometrics, male, female, fecundity, hatching, season, apyrene, eupyrene, sperm bundle, reproductive system, Morphometrics, seas and variations

Muga silk moth *Antheraea assamensis* Helfer is a non-mulberry silk moth found exclusively in the North Eastern Region of India. It produces naturally golden lustrous silk which is a Geographical Indication (GI) registered product of the state of Assam. Though high in demand production muga its production is limited muga silk accounts for only 0.71% (239 mt) of total 33,739 mt of raw silk produced in India (Anonymous, 2020; 2021). *Antheraea assamensis* is a multivolotine species completing five to six generations in a year. Frequent crop failures during its outdoor rearing and shortage of timely supply of good quality seed has been a perennial problem in muga silk industry (Kakati, 2002; Bindroo et al., 2008; Saikia, 2011; Rabha, 2022). Though, general biology and some aspects of reproductive biology of this species have been studied (Jolly, 1979; Prasad and Sinha, 1980-81; Choudhury, 1981; Thangavelu et al., 1988; Sahu et al., 1997; 1998; Dutta et al., 2013), its reproductive biology remains poorly understood. Apart from the preliminary study by Sahu et al. (1999-2000) on development of reproductive apparatus in *A. assamensis* during larval, pupal and adult stage, no detailed study on structure of reproductive system is available.

Studies on reproductive system of different species of lepidopteran insects such as *Ephestia kuehniella* (Norris, 1932), *Heliothis zea* (Challahan, 1958), *Spodoptera litura* (Etman and Hooper, 1979), *Bombyx mori* (Omura, 1936, 1938a, 1938b), *Antheraea mylitta* (Jolly et al., 1979; Dubey et al., 1992) reveal that though the general structure of male and female reproductive systems is similar there exist considerable differences in the finer details. It is also reported that lepidopteran insects produce two types of sperms, nucleated eupyrene sperms and apyrene sperms without nucleus. Eupyrene sperms fertilize the eggs and the apyrene sperms play indispensable role assisting in fertilization (Silberglied et al., 1984). The production and role of these two types of sperms in reproduction of many species of Lepidoptera have been reported (Omura, 1936; Silberglied et al., 1984; Friedlander, 1997; Kawamura and Sahara, 2002). No such study has been carried out in *A. assamensis*. Therefore, the present study on morphology and morphometrics of male and female reproductive systems of *A. assamensis* along with some observations on its reproductive performance in four seasons viz. summer, autumn, winter and spring.

MATERIALS AND METHODS

Antheraea assamensis seed cocoons were collected from Regional Sericultural Research Station (RSRS), Boko (formerly Regional Muga Research Station,

RMRS) from the cultivated stock reared outdoors on its primary host plant som *Persea bombycina*. Male and female cocoons and pupae were weighed separately using digital balance. The cocoons were spread thinly and stored for moth emergence. Grainage operation was carried out following the standard method. Fecundity was assessed by counting the total number of eggs produced by individual mated females in its lifetime of 5 to 7 days. Hatching % was calculated as the ratio of number of eggs hatched to the number of eggs laid by individual female moths. Laboratory studies were conducted in the Advanced Biotech Hub in Jawaharlal Nehru College, Boko. The study was conducted in four seasons viz. summer (June-July), autumn (Oct-Nov), winter (Dec-Feb) and spring (Mar-April) during 2021-22. The minimum-maximum temperature during the four seasons were 24°C- 34.5°C, 18°C- 31°C, 12°C- 26°C and 19.5°C-32.5°C and relative humidity (RH %) were 69-92, 78-88, 74-86 and 47-82 during summer, autumn, winter and springs, respectively.

Morphological studies of the reproductive systems were done following the method of Etman and Hooper (1979). The morphology was studied by dissecting newly emerged moths in Belar's saline. Measurements of the parts were done in three newly emerged individuals of each sex in every season. Sperm bundles were studied following the method of Etman and Hooper (1979) with some modification such as instead of lacto-orcein stain used by them; sperm bundles were stained with acetocarmine for 10 min. Then the preparation was examined under the microscope at 400x magnification and photographed with an Olympus digital camera. Individual sperm was studied in unstained preparation from diluted content by dissecting the spermatheca of mated female moths. Statistical analysis was done using SPSS software. t test was employed as a test of significance. ANOVA was performed to study the effect of season on cocoon weight and fecundity. Correlation studies were done between cocoon and pupal weight and also female pupal weight and fecundity.

RESULTS AND DISCUSSION

Studies on morphology of male and female reproductive systems in different lepidopterans show that there is considerable variation in the nomenclature of parts. In this paper the terminology of Callahan (1958) followed by Etman and Hooper (1979) has been adopted.

Morphology

The male reproductive system consists of a pair of testes, paired vasa deferentia, ductus ejaculatorius duplex, a ductus ejaculatorius simplex, an aedaegus and a pair of male accessory glands (Fig. 1). In newly emerged moth the testes appear yellow coloured due to adhering fat body and thus can easily be located on the dorsal side of the fifth abdominal cavity on either side of the dorsal vessel. The testis consists of four lobes each containing the germ cells enclosed within distinct cysts arranged centripetally, eupyrene and apyrene sperm

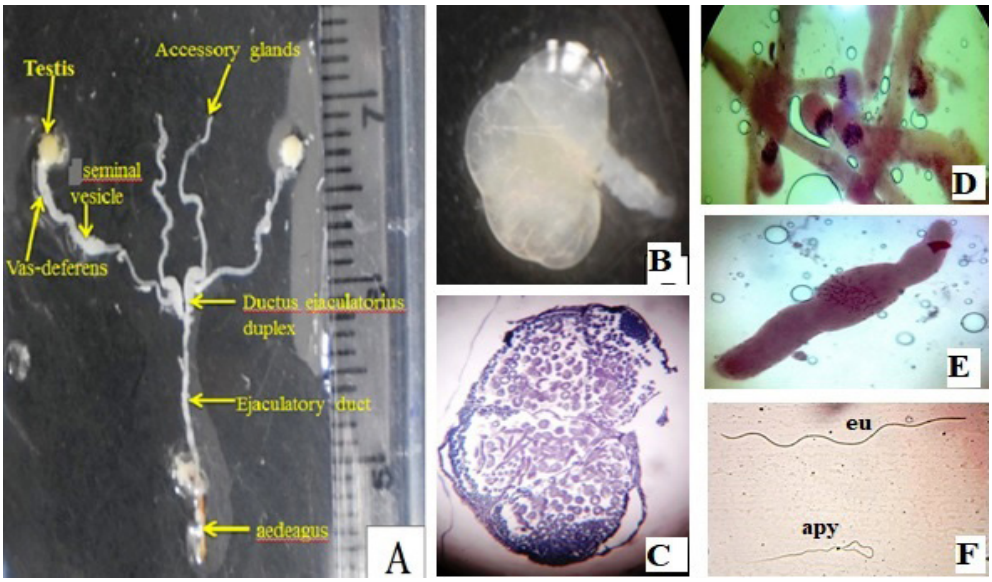

Fig. 1. A- Parts of the male reproductive system of *A. assamensis*, B- pupal testis, C- internal structure of pupal testis, D-epyrene sperm bundles inside testes, E-aupyrene sperm bundle, F- single eupyrene and apyrene sperm (from spermatheca of mated female)

bundles being developed in separate cysts similar to that reported in *B. mori* (Omura, 1936). The vas deferens arising from each testis runs towards the ventral side of the body and all other attached organs are located ventrally in the hind body. The paired male accessory gland are slender tubular organs with uniform diameter throughout the entire length and consist of two distinct morphological regions, 1/6th of its length towards the free end being translucent and the rest part opaque milky white in colour. Both the accessory glands lie coiled together over the ductus ejaculatorius duplex on the ventral side of the hind body.

Each vas deferens is a long tubular organ with variable diameter throughout its length, being wider proximal to the testis, considerably dilated in the middle forming the seminal vesicle and narrowest distally. In the newly emerged moth the seminal vesicle is filled with spermatozoa and appears milky white. The ductus ejaculatorius duplex is a paired glandular organ with two arms, each with a sharp bend at about the midpoint where the vas deferens joins. The duplex serves as a reservoir for sperm and secretions of accessory reproductive glands. The two arms of the duplex joins together and leads to the ductus ejaculatorius simplex or the common ejaculatory duct, a tubular organ that carries semen to the male copulatory organ aedaegus which is a dark brown sclerotized organ that transfers semen into the female bursa copulatrix during mating. The dimensions of each organ of the system vary with age and size of the individual as well as variations was found in different seasons (Table 1). Statistical analysis showed that testis dimensions did not vary significantly ($p<0.05$) in different seasons. Length of vas-deferens significantly varies in summer and winter, in summer and spring, in autumn and winter. Length of duplex significantly varies only in summer and winter. Length of simplex significantly varies in summer and winter, in summer and spring. Length of Male accessory gland varies significantly in summer and autumn, in summer and winter, in summer and spring and in autumn and winter.

Testis size was ranging from 2.23 mm to 2.00 mm in length and 2.03 mm to 1.80 mm in breadth. The maximum size of testis in of *B. mori* measured in an average 3.94 mm in length 2.05 mm in breadth in pupa 4 to 5 days before emergence and it reduced to 2.5 mm in length, 1.3 mm in breadth in 12 hours old moth (Omura, 1936) while in *Philosamia ricini* average size of testis was found 4.5 mm in length and 2.0 mm in breadth (Hurkadli et al., 1985). In lepidopteran insects, sperms are not mature in the testis and change morphologically when passing through the vas efferens from the testis to the vas deferens. Hiroyoshi and Reddy (2021) discussed positional relationships of male reproductive organs and how this relates to their morphology and function with a focus on sperm maturation and transfer. However, Omura (1938b) reported that though spermatozoa attain full maturity in the testis in *B. mori* they remain inactive throughout the post testicular organs and become vigorously active only when get mixed with

Table 1. Measurements of various parts of the male reproductive system of *A. assamensis* Helfer

Parts	Measurements (mm)	Season			
		Summer	Autumn	Winter	Spring
Testis	Length	2.23± 0.05	2.20± 0.16	2.00± 0.16	2.20± 0.08
	Breadth	2.00± 0.08	2.03± 0.12	1.80± 0.22	1.93± 0.09
Vas-deferens (VD)	Length	15.17± 0.62	14.17± 0.24	12.33± 0.47	13.00± 1.00
	Breadth	0.63± 0.12	0.57± 0.05	0.53± 0. 05	0.53± 0.05
Seminal vesicle (SV)	Length	3.50± 0.41	3.17± 0.24	2.93± 0.33	3.33± 0.24
	Breadth*	3.33± 0.62	3.00± 0.41	2.50± 0.41	2.97± 0.05
Ductus ejaculatorius duplex (DD)	Length	4.77± 0.21	4.16± 0.24	3.67± 0.24	4.17± 0.24
	Breadth*	1.43± 0.17	1.43± 0.33	1.33± 0.24	1.40± 0.14
Ductus ejaculatorius simplex (DS)	Length	12.67± 0.94	11.17± 0.62	10.50± 0.41	11.00± 0.41
	Breadth	0.60± 0.14	0.53± 0.05	0.50± 0.00	0.54± 0.05
Aedeagus/ penis	Length	5.03± 0.12	5.07± 0.09	4.87± 0.19	5.00± 0.16
	Breadth	1.20± 0.16	1.13± 0.09	1.13± 0.09	1.13± 0.09
Male accessory gland	Length	17.33 ±1.69	13.67± 1.25	11.50± 0.41	13.17± 1.03
	Breadth	1.33± 0.51	1.06± 0.09	1.06± 0.09	1.16± 0.12

All data mean ±SD; *indicate breadth at the widest part.

secretions from glandula prostatica. In *A. assamensis* also inactive eupyrene sperm bundles mixed with loose apyrene sperms were observed in the seminal vesicle and duplex whereas ejaculated sperms obtained from mated female were actively motile.

The female reproductive system consists of a pair of ovary, each with four ovarioles, paired lateral oviducts which joins to form a common oviduct which has a dorsal protrusion, the vestibulum, a bursa copulatrix, a spermatheca and a pair of female accessory glands or dorsal colleterial glands with their reservoirs (Fig. 2). The dimension of these various parts has been given (Table 2). Each ovariole is polytrophic, meroistic type as described in model lepidopteran insect by Wigglesworth (1965). Four ovarioles in each side remain coiled together, tightly bound by trachea to accommodate inside the abdominal cavity of the female. When stretched each ovariole is long tubular organs with varying diameter throughout the length. The tip of the four ovarioles in each side containing immature previtellogenic ova still remained enclosed within a thin capsule. The paired lateral oviducts are short muscular tubes which join together forming a common oviduct. The common oviduct has a thicker musculature and its posterior end has a dorsal evagination/ protrusion forming the vestibulum which is followed by the vagina. The Bursa copulatrix is a thin walled dilated chamber 3-4 mm in length with a narrow sclerotized tubular neck, the 'ductus bursae' which opens externally at the 'ostium bursae'on the ventral side of the abdomen which receives male ejaculate forming a spermatophore during mating. From the dorsal side of the neck of the bursa starts the seminal duct which opens into the vestibulum and the 'S' shaped spermathecal duct from the sperm storage organ (spermatheca) also joins the vestibulum. The spermatheca in *A. assamensis* is double chambered as reported in *B. mori* (Omura, 1938a) but the spermathecal gland is Y shaped with little variation in shape among different individuals unlike those widely variable structures as observed in *B. mori* (Omura, 1938a). The paired long coiled colleterial glands secrete a dark adhesive that glues the eggs to the substratum when eggs are laid.

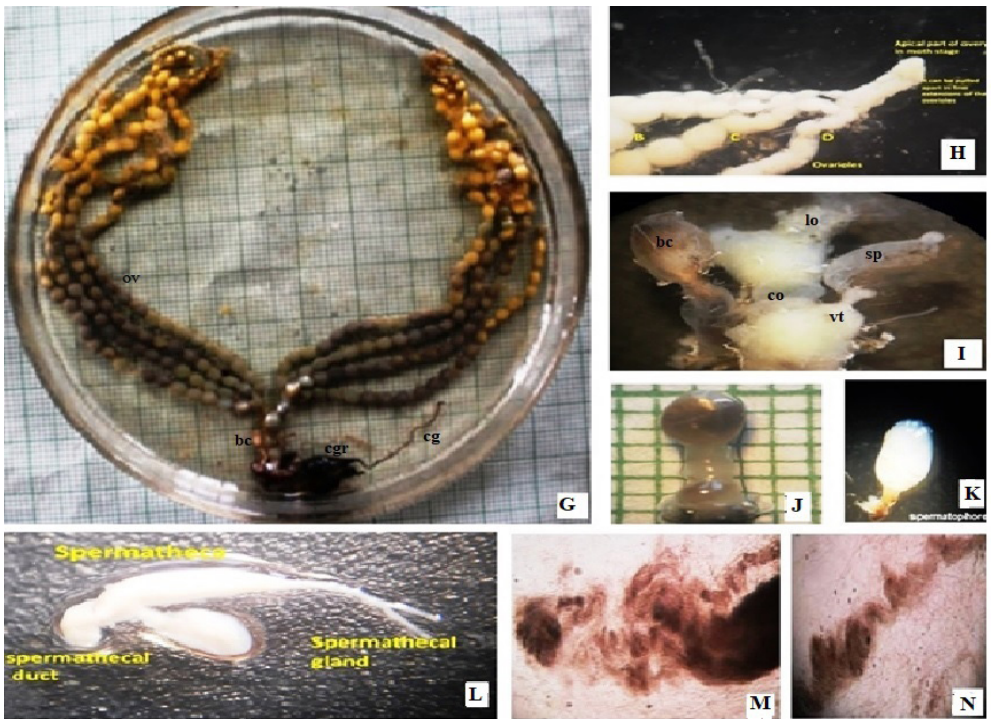

Fig. 2. G- Parts of the female reproductive system of Muga Silkmoth *A. assamensis* H- apical part of ovary of muga moth, I- enlarged view of common oviduct (co), lateral oviduct (lo), bursa copulatrix (bc) with a spermatophore within it, spermatheca (sp) with its duct and vestibulum (vt) of female moth just after end of mating before sperms reach the spermatheca, J- bursa copulatrix with a spermatophore within, K- spermatophore (dissected out of Bursa copulatrix of mated female), L- bilobed spermatheca with spermathecal gland, M and N- sperms (both eupyrene and apyrene mixed together) from dissected spermatheca.

Table 2. Measurements of female reproductive system of *A. assamensis*

Parts	Measurements (mm)	Season			
		Summer	Autumn	Winter	Spring
Ovarioles	Length	102.33± 5.56	95.33± 1.70	82.00± 3.27	90.33± 2.05
	Breadth	Variable throughout the length	Variable throughout the length	Variable throughout the length	Variable throughout the length
Common oviduct	Length	5.60± 0.53	5.00± 0.41	4.67± 0.24	5.00± 0.41
	Breadth	2.17± 0.24	2.17± 0.24	2.00± 0.41	2.17± 0.24
Bursa copulatrix: Corpus bursae	Length	3.07± 0.12	2.77± 0.21	2.33± 0.24	2.83± 0.12
	Breadth	2.27± 0.17	2.23± 0.21	2.16± 0.24	2.23± 0.21
Ductus bursae	Length	2.97± 0.05	2.83± 0.24	2.67± 0.24	2.90± 0.29
	Breadth	1.80± 0.29	1.60± 0.29	1.56± 0.33	1.70± 0.22
Seminal duct	Length	1.63± 0.12	1.60± 0.14	1.53± 0.47	1.63± 0.12
	Breadth	1.50± 0.08	1.47± 0.02	1.33± 0.24	1.47± 0.05
Spermathecal duct	Length	3.17± 0.47	2.90± 0.54	2.67± 0.24	2.43± 0.17
	Breadth	1.67± 0.94	1.43± 0.09	1.33± 0.12	1.50± 0.08
Spermatheca: Main chamber	Length	4.63± 0.26	4.17± 0.24	3.83± 0.12	3.90± 0.07
	Breadth*	3.23± 0.19	3.17± 0.24	2.77± 0.21	3.07± 0.09
Lateral chamber	Length	3.40± 0.14	3.10± 0.08	2.83± 0.24	3.17± 0.12
	Breadth*	3.03± 0.41	3.07± 0.09	2.60± 0.14	2.93± 0.25
Spermathecal gland	Length	7.10± 0.65	5.50± 0.41	4.67± 0.24	5.23± 0.21
	Breadth	1.53± 0.21	1.40± 0.14	1.10± 0.14	1.30± 0.24
Colleterial gland/ female accessory gland	Length	62.33± 4.92	57.33± 2.05	54.00± 1.41	56.77± 1.70
	Breadth	1.60± 0.14	1.53± 0.05	1.43± 0.09	1.53± 0.05

All data mean ±SD; *indicate breadth at the widest part.

Statistical analysis (t test) revealed that length of corpus-bursae significantly varies only in summer and winter. Length of spermathecal gland significantly varies in summer and autumn, summer and winter, summer and spring, autumn and winter and winter and spring. Length of colleterial gland significantly varies only in summer and winter at 5% level of Significance ($p<0.05$). Though ovariole length and dimensions of other parts of the female reproductive system varies considerably in different seasons, the difference was not found statistically significant.

Reproductive performance

Fecundity was 189± 5.75, 176± 7.59, 125± 5.98 and 167± 9.44 in summer, autumn, winter and spring seasons respectively. t-test shows that fecundity is significantly different under different seasons ($p<0.05$). Female pupal weight was 6.32± 0.16, 5.87± 0.02, 4.26± 0.16 and 5.28± 0.17 respectively during summer, autumn, winter and spring respectively. There is statistically significant positive correlation between fecundity and female pupal weight in every season as reported by previous studies (Barah and Sengupta, 1991). Unlike female *Bombyx mori* in which all the eggs (about 400 nos.) are mature by the time of moth emergence, in female muga silkmoth 60- 65% of total eggs were mature in newly emerged moth and rest of the eggs continued to mature till 5th day after emergence (Biswas et al., 2018). Prasad and Sinha (1980-81) found 42.5-50% oviposition on first day followed by 28-34% on second day, 10-17% on third day and 4-8% on the fourth day by the mated female *A. assamensis*. Hatching % of eggs was observed to be the lowest in summer season (21± 6.08) as compared to that in autumn (87± 3.90), winter (92± 2.94) and spring season (88± 2.69). Two tailed t-tests revealed that the difference in hatching % between summer and autumn, summer and winter and summer and spring season was significantly different ($p<0.0001$) whereas, the difference in hatching in autumn, winter and spring was not significant. Low hatching in summer (Aherua and Bhodia) crops of muga silkworm have been observed by previous authors as well (Sahu et al., 2005; Subharani and Jayprakash, 2015; Biswas et al., 2018). Studies by Sahu et al., (2005) inferred that extremely low hatching in summer was caused by partial or complete male sterility when developing larvae and pupae were

exposed to high temperature (34-36°C) during summer season in muga growing areas of Assam.

Sperm dimorphism

Production of dimorphic sperms had been reported in *B. mori* (Omura, 1936; 1938b; Katsuno, 1978) and many other lepidopteran insects (Etman and Hooper, 1979; Silberglied et al., 1984). Similarly, two types of sperms, nucleated eupyrene sperms (mean length= 608 µm) and anucleated apyrene sperms (mean length=401 µm) were observed in male *A. assamensis* (Fig. 1F). Inside the testis of *A. assamensis* both eupyrene and apyrene sperms were observed to occur in separate bundles whereas in seminal vesicle and duplex of male moth eupyrene sperms were still in bundles but apyrene ones were found as loose sperms. Similar observation has been reported in *B. mori* (Omura, 1938b). Biswas et al., (2018) in their preliminary study on *A. assamensis* observed that number of both eupyrene and apyrene sperm produced and transferred by males to females during mating was lower in summer season as compared to autumn and winter which might result in lower hatching percentage.

From the present study it has been observed that in *A. assamensis* morphology of the male reproductive system differs considerably from that in *B. mori* as described by Omura (1936; 1938b) and found similar with that in *A. mylitta* as depicted by Jolly et al., (1979). The structure of female reproductive system in *A. assamensis* was comparable to those reported by Jolly et al. (1979) in *A. mylitta* and *B. mori* though Omura (1936; 1938b) used somewhat different nomenclature. In lepidopteran insect male ejaculate is transferred into the female bursa copulatrix forming a discrete structure called the spermatophore (Norris, 1933; Omura, 1938a; Etman and Hooper, 1979) whose structure corresponds to that of the bursa copulatrix. In muga silk moth also a spermatophore is formed inside the bursa copulatrix (Fig. 2J, 2K) containing loose apyrene sperms, eupyrene sperm bundles and glandular secretions from the male reproductive tract. The eupyrene sperm bundles dissociate within the spermatophore soon after the end of mating and loose apyrene and eupyrene sperms has been observed in the spermatheca after one hour from the end of mating. In *B. mori* the whole path of migration of sperms within the female reproductive tract and detailed study on mechanism of fertilization has been worked out by Omura (1938a). Sperms are stored in the spermatheca and then migrate down into the vestibulum, the site of fertilization of the eggs (Omura, 1938; Karube and Kobayashi, 1999). Absence or decrease in the number of eupyrene sperm in spermatheca leads to occurrence of unfertilized eggs in *B. mori* grainages (Saheb et al., 2009; Chen et al., 2020). Though preliminary observations by Biswas et al. (2018) revealed that at elevated ambient temperature during summer season spermathecal sperm content in *A. assamensis* reduced considerably leading to lower hatching percentage of the eggs laid my female moths, detailed study in this aspect remains to be done.

The present study will help to better understand reproductive biology of *A. assamensis* and serve as a reference for detailed study. Further studies on development of eggs and spermatogenesis in larval, pupal and moth stage are necessary to pin point the stages most vulnerable to nutritional as well as abiotic stress and to find ways to mitigate problems such as low fecundity, low rate of egg laying, long oviposition span (5 to 7 days) and low hatching during seed production. Knowledge of detailed morphology of male and female reproductive system will also help in developing improved breeding technique such as artificial insemination for producing hybrids and to save valuable breeding stocks.

ACKNOWLEDGEMENTS

The authors express gratitude to Dr. Tapan Dutta, Principal, Jawaharlal Nehru College, Boko for providing laboratory facility in the Advanced Institutional Biotech Hub in the College and acknowledge the help extended by Shri S A S Rahman, Scientist-D, RSRS, CSB, Boko for allowing us to carry out the study by providing muga silkworm sample, rearing and grainage facility.

FINANCIAL SUPPORT

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

AKS and DB conceived and designed the research. DB conducted experiments and wrote the manuscript. DD analysed data. AKH and AKS contributed in writing and editing the manuscript. All authors read and approved the manuscript.

REFERENCES

Anonymous. 2020. Statistical handbook of Assam, Directorate of Economics and Statistics, Govt. of Assam. pp. 161-165.

Anonymous. 2021. Functioning of central silk board & performance of Indian silk industry as on 1st July, 2021. Central Silk Board, Bangalore. pp. 20-21.

Barah A, Sengupta A K. 1991. Correlation and regression studies between pupal weight and fecundity of muga silkworm, *Antheraea assama* Ww (Lepidoptera: Saturniidae), on different food plants. Acta Physiologica Hungarica 78(3): 261-264.

Biswas D, Sahu A K, Kalita J C. 2018. A preliminary study on reproductive performance of muga silkworm *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae), with reference to elevated ambient temperature during Summer Season. North Bengal University Journal of Animal Science 12: 19-26.

Callahan P S. 1958. Serial morphology as a technique for determination of reproductive patterns in the Corn Earworm, *Heliothis zea* (Boddie). Annals of Entomological Society of America 51: 413-428.

Chen S, Liu Y, Yang X, Liu Z, Luo X, Xu J, Huang Y. 2020. Dysfunction of dimorphic sperm impairs male fertility in the silkworm. Cell Discovery 6: 60 (2020). https://doi.org/10.1038/s41421-020-00194-6

Dubey O P, Bajpeyi C M, Kumar N. Thangavalu K. 1992. Morphology of female reproductive system in tasar silkworm *Antheraea mylitta* D. (Lepidoptera: Saturniidae). Indian Journal of Sericulture 31(2): 101-106.

Etman A A M, Hooper G H S. 1979. Developmental and reproductive biology of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Journal of Australian Entomological Society 18: 363-372.

Friedlander M. 1997. Control of eupyrene and apyrene sperm dimorphism in Lepidoptera. Journal of Insect Physiology 43: 1085-1092.

Hiroyoshi S, Reddy G V P (2021) Positional relationship among male reproductive organs in insects. In Book "Male reproductive anatomy" edited by Wi Wu. Doi: http://dx.doi.org/10.5772/intechopen.98798 (downloaded on 23/05/23)

Hurkadli H K, Hooli M A and Nadkarni V B. 1988. Testicular development and spermatogenesis in the larva, pupa and adult eri silkworm, *Philosamia ricini*- A histological study. Sericologia 28(2): 173-180.

Jolly M S, Sen S K, Sonwalkar T S, Prasad G K. 1979. Non-mulberry silks. FAO Agricultural Service Bulletin, Reprinted, Central Silk Board, Bangalore 4: 1-178.

Karube F, Kobayashi M. 1999. Presence of eupyrene spermatozoa in vestibulum accelerates oviposition in the silkworm moth, *Bombyx mori.* Journal of Insect Physiology 45: 947-957.

Katsuno S. 1978. Studies on eupyrene and apyrene spermatozoa in the silkworm *Bombyx mori* (Lepidoptera: Bombycidae) VII. The motility of sperm bundles and spermatozoa in the internal reproductive organs of males and females. Applied Entomology and Zoology 13: 91-96.

Kawamura N, Sahara K. 2002. In-vitro cultivation of spermatocytes to mature sperm in the silkworm *Bombyx mori*. Development, Growth and Differentiation 44: 273-280.

Norris M J. 1933. Contributions towards the study of insect fertility-II. Experiments on the factors influencing fertility in *Ephestia kuhniella* Z. (Lepidoptera, Phycitidae). Proceedings of the Zoologial Society of London 103(4): 903-933.

Omura S. 1936. Studies on the reproductive system of the male of *Bombyx mori*: I. Structure of the testis and the intra testicular behaviour of the spermatozoa. Journal of Faculty of Agriculture Hokkaido Imperial University, Sapporo XXXVIII (2): 151-181.

Omura S. 1938a. Structure and function of the female genital system of B*ombyx mori* with special reference to the mechanism of fertilization. Journal of Faculty of Agriculture Hokkaido Imperial University, Sapporo 40(3): 111-128.

Omura S. 1938b. Studies on the reproductive system of the male of *Bombyx mori* II. Post-testicular organs and post-testicular behaviour of the spermatozoa. Journal of Faculty of Agriculture Hokkaido Imperial University, Sapporo 40(3): 129-170.

Rabha P. 2022. Requirements and Benefits of Muga Silkworm Egg Production Unit (Grainage) in Private Sector. Just Agriculture, Multidisciplinary e-Newsletter 2(5): 1-5.

Saheb N M B, Singh T, Saratchandra B. 2009. Occurrence of unfertilized eggs in the mulberry silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) Review article. International Journal of Industrial Entomology 18(1): 1-7

Sahu A K, Sinha B B, Kumar N. 1999-2000. Studies on structure of reproductive apparatus of *Antheraea assama*. Annual Report, Regional Muga Research Station, Boko. pp. 25-26.

Silberglied R E, Shepherd J G, Dickinson J L. 1984. Eunuchs: The role of apyrene sperm in Lepidoptera? The American Naturalist 123(2): 255-265.

Subharani S, Jayaprakash P. 2015. High altitude seed zones: a ray of hope for muga silk industry. International Journal of Current Microbiology and Applied Sciences 4(6): 436-442.

Thangavelu K, Chakroborty A K, Bhagowati A K, Isa M D. 1988. Handbook of Muga culture. Central Silk Board, Bangalore. pp. 75-88.

Wigglesworth V B. 1965. The principles of insect physiology. London, Methuen.

(Manuscript Received: January, 2023; Revised: June, 2023;
Accepted: June, 2023; Online Published: July, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23026