Indian Journal of Entomology Online published Ref. No. e24884 DoI. No.: 10.55446/IJE.2024.1884

REPRODUCTIVE BEHAVIOUR OF MUGA SILK MOTH, *ANTHERAEA ASSAMENSIS* HELFER

Dipanjali Biswas[1] and Arup Kumar Hazarika[1]*

[1]Department of Zoology, Cotton University, Guwahati 781001, Assam, India
*Email: arup.hazarika@cottonuniversity.ac.in (corresponding author): ORCID ID 0000-0001-6206-3195

ABSTRACT

Reproductive behaviour including calling, courtship and mating behaviour of muga silk moth, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) has been reported here. Muga silk moths are nocturnal and moth emergence occurs from late evening till midnight. Both male and females are sexually mature by the time of eclosion from cocoon, displaying reproductive behaviour from the first day of emergence. Virgin females start releasing sex pheromone exhibiting characteristic calling behaviour after dusk, 1.5 to 2 hr after emergence. These display simple courtship behaviour of 4-12 min duration and virgin females tend to mate with the first male coming in contact. However, once mated females reject further mating attempts by approaching males by walking away or vigorous wing fluttering. Moths start mating (coupling) on the first night onwards, after 2 to 3 hr of emergence. Mating duration ranged from 10 - 16 hr. Male moths mated with multiple females within 4 days of emergence, but no moths mated more than once in a night. Female moths typically mate only once in their lifetime, but multiple mating with intermittent egg laying by female was observed during summer.

Key words: reproductive behaviour, silkmoth, *Antheraea assamensis*, nocturnal, calling behaviour, courtship, mating, natural coupling, mating duration, lifetime mating frequency, male, female moth

Lepidopteran insects (moths and butterflies) as a norm reproduce sexually and use mainly chemical signals termed as 'sex pheromones' for mate attraction. In most moths, pheromone released by the female insect attracts the male from long distance and acts as the cue which elicits typical courtship behaviour in males and mating depends only on acceptance of a conspecific male by the female (Castrovillo and Carde, 1980; Shimizu and Tamaki, 1980; Grant, 1987). Sexually mature virgin females display 'calling behaviour' which involves the extrusion, often rhythmically, of the pheromone gland at the tip of the abdomen, and a stereotyped calling posture during which pheromone is released into the environment (Stepien et al., 2020). Courtship behaviour is the short-range behavioural sequence before copulation (Alexander et al., 1997). Insects use courtship behaviour as mechanisms to ensure successful conspecific mating and select phenotypes among sexual partners (Cade 1985; Alexander et al.1997). Courtship sequence is stereotyped, often consists of at least certain components unique to a species and thus acts as a powerful mechanism for pre-mating reproductive isolation (Ehrman, 1964). Pheromone mediated mate attraction, calling, courtship and mating behaviour have been widely studied in economically important insects such as pest species and silkmoths. The knowledge of reproductive behaviour of pest insect comes handy in designing and employing integrated pest control methods through mating disruption. These behaviours have been studied in the model insect, the mulberry silk moth *Bombyx mori* (Obara, 1979; Biram Saheb et al., 2005) and other silk moths (Riddiford and Williams, 1967; Kato and Katsu, 1981; Mitamura, 2003; Morton, 2009) which helps in their better management. Detailed accounts of courtship and mating behaviour of various lepidopteran species have been reported (Joshi, 1985; Tomislav Curkovic et al., 2006; Paula Altesor et al., 2010).

Muga silk worm *Antheraea assamensis* Helfer produces highly durable silk possessing a rich naturally golden sheen which is highly valued in domestic as well as international market, but its production is limited (Anonymous, 2020; Anonymous, 2021). This economically important non-mulberry silk worm is cultivated exclusively in the North Eastern region of India, as a semi-domesticated species especially in the Brahmaputra valley of Assam, some parts of Nagaland and Meghalaya and Cooch Bihar area of West Bengal. This silkworm breeds throughout the year and completes five to six generations in a year. The wild populations of *A. assamensis* are distributed in restricted geographical area in the North East India, parts of Tibet and Burma and is represented by small, fragmented populations in the wild habitats facing various threats. This species is also speculated as the ancestor of all the species belonging to the genus *Antheraea* (Jolly et al., 1979). Mating behaviour of this species has been studied by several workers (Prasad and Sinha, 1981; Thangavelu et al., 1985). These mainly describe diurnal periodicity of mating in different seasons. Other aspects of reproductive behaviour are poorly understood. Present study describes the reproductive behaviours such as calling, courtship and mating behaviour of *A. assamensis*.

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 *Persea bombycina* (Som). The cocoons were spread thinly and stored in nylon cages for moth emergence. Grainage operation was carried out following the standard method. The study was conducted in four seasons viz. summer (June-July), autumn (Oct-Nov), winter (Dec-Feb) and spring (Mar-April) during 2021-22. Reproductive behaviour was studied in indoor conditions, under dim red light that was lit only during the observations. To study calling and courtship behaviour individual pairs of zero to one day old virgin female and male moths were enclosed within net cages (L30 x B30 x H60 cm) at dusk and was observed at regular intervals throughout the night. Female moth exhibiting characteristic posture protruding pheromone gland at abdominal tip was noted as "calling" behaviour. Virgin females were observed at every 30 min. interval for noting their calling behaviour throughout the night or until mating occurred. Male response towards calling female and male-female interaction was noted as “courtship behaviour”. When onset of courtship is noticed, the pair was continuously observed till the sequence of courtship behaviour is completed ending in mating. Unsuccessful mating attempts were also recorded separately. Thirty pairs of moths were studied in each season. Total duration of courtship was recorded with a digital stopwatch. Mating behaviour was observed in the same moth pairs noting the time of mating onset and total mating duration. To study lifetime mating frequency in both sexes, three to five pairs of moths (M: F = 1:1) were enclosed within net cages and observed every day for five consecutive days. Transfer of one spermatophore per mating is the norm in Lepidoptera, therefore female lifetime mating frequency was ascertained by dissecting and counting the number of spermatophores lodged in the bursa copulatrix. Total 30 moths of each sex were observed in each season for lifetime mating frequency.

RESULTS AND DISCUSSION

After emergence from cocoon, spreading and drying of wings took place within 50-60 min, then both male and female of *A. assamensis* moths rested with their wings parallel to the substratum and antennae along the costal margin of the forewings (Fig. 1A, B). Onset of sexual activity in female moths was marked by the characteristic calling posture adopted with wings slightly raised, extruding the tip of the abdomen, exposing the pheromone gland (Fig. 1C). Periodical

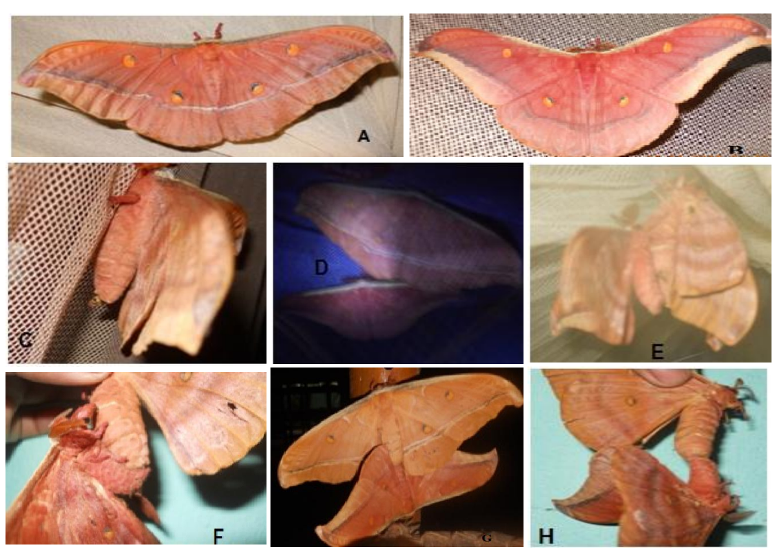

Fig. 1. Courtship sequence of muga silkmoth, *A. assamensis*. A- resting female, B- resting male, C-calling female, D-E – stages of courtship, F- mated pair (initial stage), G-H –mated pair during later stage

calling bouts were observed when silent vibration of the wings and rhythmic protrusion and retraction of the abdomen tip was seen, while they were also seen just extruding the pheromone gland without any wing vibration. Females started calling after dusk, 1.5 to 2 hr from the time of emergence. Calling females were observed throughout the night until mating occurred. Virgin females exhibited calling each night up to the third night from emergence, after that they started egg laying even if mating did not occur. Older females continuously extruded the pheromone gland without distinct retracting indicating senescence and after that they failed to attract males. On the other hand once mated females did not show calling behaviour during autumn, winter and spring seasons under study and started egg laying from the next evening. However, during summer season once mated females also displayed behaviour similar to calling, extruding the abdomen tip and a large proportion of once mated females re-mated on subsequent nights till the fifth day of emergence.

In presence of a calling female the male *A. assamensis* becomes highly active and responds with raised antennae and starts wing fluttering. Males took directional movement towards the calling females and at close proximity started courtship behaviour, the sequence of which has been represented here (Table 1). During mating, initially the mated pair remains attached venter-to-venter facing each other, the female clinging to the cage wall or any other upright object with the help of its legs and the male holding the female abdomen. Later they assume end-to-end position. The pair remains stationary for the rest part of the night and till the afternoon in the next day. The average mating duration was found to be 15.37, 15.5, 16.8 and 14.92 hr during summer, autumn, winter and spring seasons respectively. Natural couplings obtained during summer, autumn, winter and spring was 83, 87, 72 and 81% respectively on the 1st day of moth emergence. Natural coupling aptitude of muga silkmoth under indoor condition is very low (Dey et al., 2011); so in muga grainages (seed production) mechanical coupling

Table 1. Sequence of behaviour involved in courtship and mating in Muga silkmoth, *Antheraea assamensis* under captive condition

Stage	Behavioural sequence of courtship and mating	
	Males	Females
Resting	Wings spread horizontal with respect to the substratum. Antennae bent downwards.	Same as male moth
Start of activity	….. Antennae alert. Subsequently wing vibration at low amplitude and then vigorous wing vibration making fluttering sound.	Wings slightly raised, pheromone glands at abdomen tip extruded and retracted rhythmically at the beginning and then continuously extruded throughout the night or until mating occurs. Antennae at resting position. Periodical fanning of wings observed.
Flight	Males take short flights, land at some distance from the calling female and approaches her by walking while fluttering his wings. Flight may be absent in some cases.	Remains stationary.
Mate finding	Moves to and fro near the female finally locating her. Approaches the female from the rear end.	Remains stationary throughout the period.
Mating attempt	After first contact with the calling female the male curls his abdomen towards her abdomen tip attempting genital engagement.	A receptive female remains stationary. Female moving off by walking away or wing fluttering were unreceptive.
Mating/ Copulation	Initially male-female remain ventrally attached facing each other, the female clinging to the cage wall with the help of its legs and male holding the female abdomen. Later they assume end-to-end position. The pair remains stationary for the rest part of the mating period and till the afternoon in the next day.	
End of copulation	The copulating pair separates while the female walks upwards/forward, liberating the male, or the male release his grasp off the female genitalia and flies away after a while.	

by hand pairing is routinely employed for the female moths which fail to mate on the night of emergence. However, the success of such practice largely depend on the skill of the worker and also time of mechanical coupling (usually done at dawn). We observed that it is difficult to obtain natural coupling in muga silkmoth in poorly ventilated enclosed area. Repeated attempts to obtain coupling inside the BOD incubator for experimental purposes failed each time. This indicate that pheromone mediated courtship behaviour prior to mating plays important role in mating success of this semi-domesticated silkmoth species.

The present study observed some abnormal/ anomalous courtship and mating behaviour (Fig. 2) such as one female courted by multiple males and male- male pseudo-mating as reported in eri silkmoth (Joshi, 1985) and also in other Lepidoptera (Caballero-Mendieta and Cordero, 2012). Dey et.al., (2011) reported that at higher density of males, a female *A. assamensis* was simultaneously courted by two or more males and one or more males were found closely in association with a copulating pair. Similar findings were reported in case of eri silk moth (Joshi, 1985); though the fate of such interaction has not been reported. In case of muga silkmoth, the second male which approached a female after she mated with the first male reaching her, was seen rubbing its abdomen tip at the rear end of the mated female thus actively trying to dislodge the mating male. However, no such attempt to disrupt copulation was successful (once the first male established genital engagement to the female). The extra male remained clinging till the mated pair separated. In three instances (out of total 8 cases) copulated with the female within few seconds when the first mating ended.

In the present study during summer 56.67% of the females mated at least twice within 4 days of emergence. Re-mating either with the same male (repeated mating) or a different male (polyandry) were observed. Some of the females mated for the 3rd time as confirmed by counting the spermatophore lodged in the Bursa copulatrix. During autumn, winter and spring seasons, however, fewer females mated more than once (autumn 6.67%, n=30; winter 17.24%, n=29; spring 13.33%, n= 30). Lifetime mating frequency was 1.93, 1.06, 1.10 and 1.13, respectively during summer, autumn, winter and spring seasons, respectively. Similar observations were reported by Katsuki and Miyatake (2009) in the adzuki bean beetle *Callosobruchus chinensis* where female remating frequency at lower temperature (17 °C) was lower than at other temperatures (25°C and 33°C); and number of ejaculated sperm was significantly lower at 33°C than at 17°C. Multiple mating by male muga silk moth is common (Biswas et al., 2018) as reported in males of other species (Joshi, 1985, Morton, 2009). In the present study also male moths were observed to mate more than once in all seasons. Earlier studies reported that male fertility is adversely affected during the summer due to exposure of the larval and pupal stages to high temperature during developmental stages (Thangavelu et al. 1988; Sahu, 2005). Thus, higher multiple mating by female moth during summer may be attributed as a measure to acquire adequate amount of viable sperm.

Fig. 2. Anomalous mating behaviour in *A. assamensis*-I- Extra male clinging to already mated pair; J- male-male pseudo mating

Female calling and mating behaviour has been reported in a number of saturniid species such as *A. polyphemus* (Riddiford and Williams, 1967), *Callosamia angulifera*, *C. promethean* (Morton, 2009). Apart from the chemical nature of the sex pheromone, the timing of release of these chemicals is crucial to achieve sexual isolation of closely related species. The cues which trigger female calling behaviour is different for different species. Females of *Antheraea polyphemus* requires the presence of a volatile, trans-2- hexenal (Riddiford and Williams, 1967) emanating from oak leaves for displaying calling and successful mating in the laboratory. Virgin *Antheraea pernyi* females show no overt calling behaviour under laboratory conditions, however, the sex pheromone is continuously released to provoke mating at any time of day or night (Riddiford, 1970). In the present study it was observed that onset of calling behaviour in *A. assamensis* was temporally correlated with onset of dusk and female moths displayed calling behaviour throughout the night or until mating occurred. Both males and females increase their flight activity upon the onset of dusk, and they remain inactive throughout the day. In *Bombyx mori* elaborated courtship is lacking and these domesticated silk moths have a tendency to pair immediately after emergence. The pair come together immediately and copulates (Mathur and Sarkar, 2008). Contrary to this, muga silk moths display typical courtship behaviour prior to successful mating. The courtship sequence in *A. assamensis* best matches the 'simple' courtship behaviour model presented by Phelan and Baker (1990), i.e., a sequence where the male, after locating and contacting a calling female, simply attempts copulation, thus lacking specialized elements as found in the 'interactive' type of courtship behaviour (Phelan and Baker, 1990).

Environmental conditions play important role in reproductive behaviour of insects. In the present study the male muga silkmoths were found to mate several times within their adult lifespan of 5-7 days and female multiple mating was also common, at least during summer season. Grandela et al. (2023) found highly detrimental effects of heatwave on mating behaviour when males of *Drosophila subobscura* were subjected to prolonged heatwave, with longer courtship and copulation latencies and lower mating occurrence. Behaviour of females that mated with stressed males was also adversely affected, by often refusing male nuptial feeding and reduced fecundity and reproductive success. The effect of temperature and relative humidity on reproductive behaviour such as courtship, mating success, sperm transfer, mating pattern as well as effect of male and female multiple mating on fecundity and fertility in *A. assamensis*, a valuable silk moth species is worth to be investigated. In the present study our observations on calling, courtship and mating behaviour have been reported which will help to design further studies in this direction.

ACKNOWLEDGEMENTS

The authors express gratitude to Shri S A S Rahman, Scientist-D, RSRS, CSB, Boko for allowing us to carry out the study by providing muga silkworm sample and rearing facility. We are indebted to Dr A K Sahu, former Scientist- D of RSRS for sharing his valuable suggestions. Nripen Rabha, field worker of RSRS, Boko is acknowledged for sharing his experience on muga culture.

FINANCIAL SUPPORT

None.

AUTHOR CONTRIBUTION STATEMENT

DB and AKH conceived and designed the research. DB conducted experiments and wrote the manuscript. AKH contributed in writing and editing the manuscript. Both authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Alexander R D, Marshal D C, Cooley J R. 1997. Evolutionary perspective in insect mating, pp, 4-31. In Book "Mating system in insects and Arachnids" edited by Choe J C and Crespi B J. Cambridge University Press, Cambridge, United Kingdom.

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

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

Biram Saheb N M, Singh T, Kalappa H K, Saratchandra B. 2005. Mating behaviour of mulberry silkworm, Bombyx mori (L.). International Journal of Industrial Entomology 10(2): 87-94.

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.

Caballero-Mendieta N, Cordero C. 2012. Enigmatic liaisons in Lepidoptera: A review of same-sex courtship and copulation in butterflies and moths. Journal of Insect Science 12:138. Available online: http://www.insectscience.org/12.138

Cade W H. 1985. Insect mating and courtship behaviour. Comprehensive Insect Physiology Biochemistry and Pharmacology 9: 591-619.

Castrovillo P J, Carde´ R T. 1980. Male codling moth (*Laspeyresia pomonella*) orientation to visual cues in the presence of pheromones and sequences of courtship behaviors. Annals of Entomological Society of America 72: 173-188.

Dey S, Singh S, Dey S, Choudhury S, Chakraborty R, Hooroo R N K, Sharma D K. 2011. UV-Reflecting Wing Scales in the Silkmoth *Antheraea assamensis*: Its Biophysical Implications. Microscopy Research and Technique 74:28-35.

Ehrman L. 1964. Courtship and mating behaviour as a reproductive isolating mechanism in *Drosophila*. American Zoologist 4: 147-153.

Grandela A, Antunes M A, Santos M A, Matos M, Rodrigues L R, Simões P. 2023. Detrimental impact of a heatwave on male reproductive behaviour and fertility. Acta Ethologica (published online) https://doi.org/10.1007/s10211-023-00431-7

Grant C G. 1987. Copulatory behaviour of spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae): experimental analysis of the role of sex pheromone and associated stimuli. Annals of Entomological Society of America 80: 78-88.

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.

Joshi K L. 1985. Some observations on courtship and mating behaviour of eri silkmoth, *Smia ricini* (Lepidoptera: Saturniidae). Indian Journal of Sericulture XXIV (1): 18-21.

Kato Y, Katsu Y. 1981. Reproductive behaviour of the Japanese oak silkmoth' Antheraea yamamai (Lepidoptera: Saturniidae): Calling, copulation and oviposition. Japanese Journal of Applied Entomology and Zoology 25: 249- 252.

Katsuki M, Miyatake T. 2009. Effects of temperature on mating duration, sperm transfer and remating frequency in Callosobruchus chinensis. Journal of Insect Physiology 55(2): 113–116 http://dx.doi.org/10.1016/j.jinsphys. 2008.10.012

Mathur V B, Sarkar K. 2008. Impact of egg laying duration on the occurrence of fertilized and unfertilized eggs of the newly evolved race of *Bombyx mori*, L. International Journal of Industrial Entomology 16 (1):1-5.

Mitamura T. 2003. Mating behavior of wild silkmoth, Rhodinia fugax Butler (Lepidoptera: Saturniidae). International Journal of Wild Silkmoth and Silk 8: 73-80.

Morton E S. 2009. The function of multiple mating by female *Promethea* Moths, *Callosamia promethea* (Drury (Lepidoptera: Saturniidae). The American Midland Naturalist 162(1): 7-18. doi:10.1674/0003-0031-162.1.7

Paula Altesor, Venusa R Horas, Maria P Arcia, Carmen Rossini, Paulo H G Zarbin. 2010. Reproductive behaviour of *Crosidosema (=Epinotia) aporema* (Walsinghm) (Lepidoptera: Tortricidae): Temporal Pattern of female calling and mating. Neotropical Entomology 39(3): 324-329.

Phelan P L, Baker T C. 1990. Comparative study on courtship in 12 phycitine moths (Lepidoptera: Pyralidae). Journal of Insect Behavior 3: 303-329.

Prasad G K, Sinha S S. 1980-81. Annual report, Central Muga Eri Research Station, Titabar, Assam.

Riddiford L M, Williams C M. 1967. Volatile principle from oak leaves: role in sex life of the Polyphemous moth. Science 155(3762): 589-590. Doi: 10.1126/science.155.3762.589

Riddiford L M. 1970. Antennal proteins of saturniid moths: their possible role in olfaction. Journal of Insect Physiology 16: 653- 660.

Rutowski R L. 1982. Mate Choice and Lepidopteran Mating Behavior. The Florida Entomologist 65(1): 72-82.

Sahu A K, Das P K, Suryanarayana N. 2005. Male sterility and hatching failure in muga silkworm, *Antheraea assama* Ww (Lepidoptera: Saturniidae) during summer. In Advances in Tropical Sericulture, edited by S B. Dandain S B, Mishra R K, Gupta V P and Reddy Y S, National Academy of Sericulture Sciences of India, Bangalore, pp. 459-462.

Saikia D, Dutta L C, Saikia M, Singha T A, Gogoi I, Das D. 2023. Effect of season on reproductive parameters of muga silkworm (*Antheraea assamensis* Helfer). Ecology Environment and Conservation 29(1): 309-313.

Shimizu K, Tamaki Y. 1980. Releasers of male copulatory attempt in the smaller tea tortrix moth (Lepidoptera: Tortricidae). Applied Entomology and Zoology 15: 140-150.

Stepien T L, Zmurchok C, Hengenius J B, Rivera R M C, D'orsogna M R, Lindsay A E. 2020. Moth calling: Modelling female pheromone calling and male navigational strategies to optimize reproductive success. Applied Science 10: 6543. doi.10.3390/app10186543 (accessed on 1/9/2023)

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

Tomislav Curkovic, Brunner J F, Landolt P J. 2006. Courtship behaviour in *Coristoneura rasaaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae). Annals of the Entomological Society of America 99(3): 617-624.

(Manuscript Received: January, 2024; Revised: April, 2024;
Accepted: April, 2024; Online Published: May, 2024)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e24884