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Effect of photoperiod on the DNA, RNA and protein concentration in the silkgland of tasar silkworm, *Antheraea mylitta* (D) (Lepidoptera, Saturniidae)

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ABSTRACT: In *Antheraea mylitta* (D) (Lepidoptera, Saturniidae), the effect of photoperiod on total DNA, RNA, and protein concentration in the middle silk gland (MSG) and posterior silk gland (PSG) was studied. The photoperiod caused enhancement in the secretory activity and stimulates the sericogenesis process in MSG and PSG. The total nucleic acids and protein concentration of MSG and PSG under 24L:00D condition reached maximum level and then it decreased. Maximum nucleic acids and protein concentration were observed during long-day period and the minimum during short-day period. The photoperiod activated or inhibited the biological clock which acted upon the respective endocrine glands accordingly and control the process of sericogenesis. The larvae underwent significant changes with high DNA, RNA and protein concentration under 24L:00D, which is highly suitable for the silk protein synthesis. © 2024 Association for Advancement of Entomology

KEY WORDS: Sericogenesis, middle silk gland, posterior silk gland, biological clock

INTRODUCTION

Commercially there are four types of silks *viz.*, mulberry, tasar, eri and muga. India is unique in producing all these four varieties of silks. The mulberry silk produced in India, is recognized as one of the major one. Silk is produced from the silkglands which are nothing but the modified labial glands. The silkgland pass through four consecutive phases- the growth phase, the secretory phase, the regression phase, and the degeneration phase. The silkgland complex is well-developed in the last instar larva and differentiated into three regions: anterior, middle, and posterior. The lumen of anterior silkgland (ASG) is empty during the growth phase and filled with protein secretion in middle silk gland (MSG) and posterior silk gland (PSG) during the spinning period. Lumen transports silk secretion from MSG and PSG to the spinneret. As the MSG secretes sericin and the PSG secretes fibroin which later on modifies into true silk fibre oozing out from the spinneret at the time of cocoon spinning (Akai, 1965; Suzuki and Suzuki, 1974; Akai and Kataoka, 1978; Matsumura, 1980; Minagawa, 1980; Akai *et al.,* 1987; Sehnal and Akai, 1990; Motoyuki *et al*., 1993; Ishimuras and Numata, 1994; Minoura *et al*., 1995), these MSG and PSG were used for the estimation of DNA, RNA and protein concentration.

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Tasar silkworm *Antheraea mylitta* (D) (Lepidoptera, Saturniidae), contributes economically to the tasar culture in India (Jolly *et al.,* 1979). The development, cytological structure, and cyclical activity of the silkgland were previously studied (Barsagade and Tembhare, 2000). Further, research on the hormonal activity in relation to silk production, particularly secretion of sericotropic hormones had contributed to understanding medial neurosecretory cells of the insect brain (Tembhare and Barsagade, 2000). Endocrine and nervous factors are involved in insect circadian rhythm (Harker, 1960). The photoperiod acts directly on the brain and regulates the biological clock which determines various physiological and behavioural activities in most of the saturniid silkworms (Williams and Adkisson, 1964). The photoperiodic clock in various saturniid silkworms is known to be confined in the brain,which controls the release or inhibition of some brain neurohormones during diapauses, induction, and termination respectively (Williams, 1969). Jolly *et al.* (1970) studied the influence of temperature and photoperiod on termination of pupal diapauses in *A. mylitta*. The Medial Neurosecretory Cells in the brain also secrete the allatotropic hormone and prothoracicotropic hormone which stimulates the secretion of the juvenile hormone and ecdysone respectively. Prudhomme (1976) discussed the role of these hormones in the sericogenesis and silkgland degeneration in various species of silkworms. Unni and Pant (1985) studied the photoperiodic response on the silkgland of *P. ricini* (eri silkworm) during the fifth instar development and spinning period. The silk protein secretion is also known to be controlled by the brain neurohormone particularly the sericotropic hormone (Kodrik and Sehnal,1991: Tembhare and Barsagade, 2000). There is, however, no report on the effect of photoperiod on silk protein biosynthesis in *A. mylitta*. In India, the species, *A. mylitta* (Drury) occurs in 25 ecotypes or races (Jolly *et al*., 1979). Dabha race has been regularly cropped over the last 20 years at the Central Tasar Research and Training Institute (CTRTI), Basic Seed Multiplication and Training Centre (BSMTC) Dawadipar, Bhandara (Maharashtra State), India. The present research work on the effect of photoperiod on silkgland secretory activity in *A. mylitta*, is reported on the local 'Dabha Race' which is found in the forest zone of Madhya Pradesh, Bihar and Vidarbha Region of Maharashtra.

MATERIALS AND METHODS

The late fourth instar larvae were reared in a laboratory and were kept in specially prepared wire-grid cages (0.75x 0.5 x 0.5m) with top sliding glass covers. Their diet consisted of leaves of *Terminalia tomentosa*. The newly emerged fifth instar larvae were also reared similarly. To study the photoperiodic control of the secretory activity of the silkgland, the 10-15 day-old fifth instar larvae of *A. mylitta* were kept at various photoperiodic regimes such as-

1. 24L:00D (24h Light:00h Dark)
2. 15L:09D (15h Light:09h Dark)
3. 12L:12D (12h Light:12h Dark)
4. 09L:15D (09h Light:15h Dark) and
5. 00L:24D (00h Light:24h Dark)

During the experimental period, larvae were fed fresh leaves of *Terminalia tomentosa*. The cages were cleaned after every 6-hour. The larvae were sacrificed after 24-hours, and MSG and PSG were dissected out and the extracts were subjected to estimate the total concentration of DNA, RNA and protein by Burton's Diphenylamine technique, Dische-Orcinol technique (Lowry *et al.,*1951; Endo, 1970).

Silkgland DNA estimation: Live larvae that were dissected out in ice cold insect Ringer's solution and the silkglands were pulled out from the body. The tracheae and the adhering tissues were removed and the middle and posterior regions of the silkglands were separated. The silkglands were homogenized for 5 minutes at 0°C in different volumes of ice-cold distilled water, Ringer's solution using a pestle and mortar for nucleic acid and proteins respectively. DNA was estimated by Burton's Diphenylamine technique (Searcy and Maclnnis, 1970, 1970a). The standard DNA solution was prepared by dissolving 5mg of Standard DNA calf thymus in 5ml of distilled water(1mg/ml). The blank and unknown tube contains 2ml of water and 2ml of tissue extract respectively. Now 4ml of

Dipheylamine reagent was added to all the tubes. All the tubes were kept in boiling water bath for 10 minutes. The color intensity was observed at 500 nm on the spectrophotometer. The standard calibration curve was prepared with known tubes of standard DNA calf thymus and from this, the actual amount of DNA was determined from the extracted sample.

DNA concentration = 50µg/ml x OD_{500} x Dilution Factor (50)

Where, OD = Optical Density of sample

Silkgland RNA estimation: The silkgland RNA was estimated by Dische-Orcinol technique (Endo, 1970). The standard RNA solution was prepared by dissolving 1mg of commercial yeast RNA in 6ml of distilled water. The solution was assisted by adding 0.1N NaoH (0.166 mg/ml). The blank and unknown tube contains 3ml of distilled water and 3ml of tissue extract respectively. Now 6 ml of acid-Orcinol reagent and 0.4 ml of Orcinol-alcohol reagent was added to all the tubes. All the tubes were kept in boiling water bath for 20 minutes. The colour intensity was observed at 660nm on the spectrophotometer (Milton Roy). The standard calibration curve was prepared with four known tubes of yeast RNA and from this, the actual amount of RNA was determined from the extracted sample.

RNA concentration = 50µg/ml x OD_{660} x Dilution Factor (50)

Where, OD = Optical Density of sample

Silkgland protein estimation: The protein was estimated by Lowry *et al.* method (1951). The standard protein solution was prepared by dissolving 5mg of standard protein bovine serum albumin in 1ml of distilled water. The blank and unknown tube contains 4 ml of distilled water and 4ml of tissue extract respectively. To each test tube, 5.5 ml of reagent C (50ml of 2% sodium carbonate in 0.1N NaoH + 1ml of 0.5% copper sulfate solution in 1% sodium potassium tartarate solution) was added which was kept undisturbed for 10-15min. Then to each test tube, 0.5 ml of Folin-Ciocaltteau reagent with an equal amount of water was added with vigorous shaking and was kept undisturbed for 30 minutes. The colour intensity with blue colour was observed at 650 nm on the spectrophotometer, (Milton Roy). The standard graph was drawn with four known tubes of bovine serum albumin, and from this, the actual amount of protein was determined from the extracted sample.

$\frac{\text{Unknown}}{\text{Standard}}_{x}$ 0.2 x100= mg/ml

Where, Unknown = Optical Density of sample

Standard = Optical Density of standard protein

0.2 = Protein in standard

100 = Dilution Factor

Data were statistically analyzed according to Daniel (2000) and standard deviation, standard error, student-t test, and P-values of the data were derived.

RESULTS AND DISCUSSION

The present studies undertaken to determine the effect of photoperiod on the secretory activity of the silkglands in *A.mylitta,* showed maximum concentration of nucleic acids and proteins, when exposed to maximum day light as compared to night, indicating that the sericin and fibroin in MSG and PSG respectively increased in daylight with maximum photoperiod.

Total DNA concentration: There was a significant gradual decrease in the level of total DNA concentration in MSG. In 24h light condition it measured at 8.00±0.008µg/mg, but it declined gradually to 6.00±0.084, 5.00±0.02 and 1.5±0.14µg/mg respectively in the photoperiods of 15, 12 and 9h light. The concentration decreased to 0.5± 0.1 µg/mg in total dark period. In PSG the total DNA concentration measured at 10.9± 0.008µg/mg in 24h light period. It gradually decreased in the photoperiods of 15, 12 and 9h light to (10.4± 0.084, 10.4±0.02 and 3.00 ±0.14µg/mg respectively). The total DNA concentration was low (2.5 ±0.1 µg/mg in the total dark period (Table 1).

Total RNA concentration: In MSG total RNA concentration was 1.8 ±0.16µg/mg in 24h light condition. It declined in the photoperiods of 15h, 12

Table 1. DNA concentration(µg/mg) at various photoperiods in silk gland

Photoperiod	Hours	MSG	PSG
24L:00D	2.00 pm	8.00±0.008*	10.9± 0.008*
15L:09D	8.00 am	6.00± 0.084*	10.4± 0.084*
12L:12D	12.00 pm	5.00± 0.02**	10.4± 0.02**
09L:15D	8.00 am	1.5 ±0.14	3.00 ±0.14
00L:24D	2.00 pm	0.5± 0.1*	2.5 ±0.1*

Each value represents total of one larva, four replicates ± standard error of means (SEM), Significance *P<0.05, **P<0.01.

Table 2. RNA concentration(µg/mg) at various photoperiods in silkgland

Photoperiod	Hours	MSG	PSG
24L:00D	2.00 pm	1.8 ±0.16	2.1± 0.16
15L:09D	8.00 am	1.7 ±0.11	2.0± 0.11
12L:12D	12.00 pm	1.6 ±0.141	1.8 ±0.141
09L:15D	8.00 am	0.9 ±0.081*	1.5 ±0.081*
00L:24D	2.00 pm	0.4± 0.2	0.52± 0.2

Each value represents total of one larva, four replicates ± standard error of means (SEM), Significance *P<0.05.

and 9h light (1.7 ±0.11, 1.6 ±0.141 and 0.9 ±0.081µg/mg respectively) and it was low in total dark period (0.4± 0.2µg/mg). RNA concentration in PSG in 24h light period was 2.1± 0.16µg/mg. It was 2.0± 0.11, 1.8 ±0.141 and 1.5 ±0.081µg/mg respectively at 15, 12 and 9h light. It was very low (0.52± 0.2µg/mg) in total dark period (Table 2).

Total protein concentration: In MSG the total protein concentration measured 6.1 ±0.002µg/mg in 24-hour light condition. It declined gradually in the photoperiods of 15, 12 and 9h light and measured 5.5±0.0031, 4.2±0.0025 and 3.0±0.005µg/mg respectively. The concentration decreased to 3.0±0.0058µg/mg in the total dark period. Total protein concentration in PSG in 24h light measured 27.0± 0.002µg/mg. But it low in of 15, 12 and 9h light to 25.0±0.0031, 23.0±0.0025 and 20.0±0.005µg/mg respectively. The concentration decreased to16.9 ±0.0058 µg/mg in total dark period (Table3).

In *A. mylitta* the total protein concentration in MSG and PSG under 24L: 00D (total light photoperiod) condition reached to maximum level along with the total DNA and RNA concentration suggesting that the total light condition is highly suitable for the silk protein synthesis. DNA, RNA and protein concentration in MSG and PSG gradually decreased in 15L:09D (long day photoperiod) and 12L:12D (normal-day photoperiod) conditions and reached the minimum in 09L:15D (short-day photoperiod) and 00L:24D (total dark photoperiod) conditions suggesting that upto 12L:12D photoperiod regime, the MSG and PSG are quite efficient in protein secretion but their activity is declined greatly during the low photoperiod *i.e,* 09L:15D and 00L:24D.

In *A. mylitta* it is well-established that the photoperiod induced and terminated the diapause at the early pupal stage of the third generation (during December - January and June - July respectively). Besides the diapauses, the photoperiod is also known to determine a time regime during the day cycle for the moth-emergence, male-female coupling, egg-laying, and egg-hatching (Jolly *et al.,* 1971, 1979). Unni and Pant (1985) studied the photoperiodic response on the silkgland of *P. ricini* at the time of development of the 5th instar and period of spinning. The photoperiodic effect on larval-pupal characters, fat body, nucleic acids and protein of silkworm *Bombyx mori* L. showed that the nucleic acids and protein contents increased at 24L over other photic regimes (Janarthanan *et al.,* 1994). MSG and PSG are quite efficient in nucleic acid and protein secretion in high photoperiod but their activity declines greatly during the low photoperiod. Studies also shows that long photoperiod promotes and short photoperiod delays development of the silkgland. The study also shows that long day photoperiod promotes the better silk production (Zothanmawii *et al*., 2017). Shewale *et al*. (2019) showed that larval weight, silkgland weight increases in both M5 and V1 mulberry varierty fed larvae when exposed to 18hrs light and decreases when exposed to 18hrs dark. The larvae were exposed to the electric bulb and the cages were covered with black cloth to study the nucleic acids and protein to the light and dark conditions respectively.

Table 3.Protein concentration (µg/mg) at various photoperiods in silkgland

Photoperiod	Hours	MSG	PSG
24L:00D	2.00 pm	6.1 ±0.002*	27.0± 0.002*
15L:09D	8.00 am	5.5± 0.0031*	25.0 ±0.0031*
12L:12D	12.00 pm	4.2± 0.0025*	23.0± 0.0025*
09L:15D	8.00 am	3.0± 0.005**	20.0 ±0.005**
00L:24D	2.00 pm	3.0± 0.0058**	16.9 ±0.0058**

Each value represents the total of one larva, four replicates ± standard error of means (SEM), *P<0.001, **P<0.002.

The effect of photoperiod impact on diapause in saturniid silkworm pupae was however extensively studied (Tanaka, 1951; Williams, 1969; Takada *et al*., 1997). In saturniid silkworms it is now well-established that the photoperiod acts directly on the brain and regulates the biological clock which determines various physiological and behavioral activities (Williams and Adkission, 1964). The present study showed that the photoperiod activated or inhibited the biological clock which acts upon the respective endocrine glands accordingly and controls the process of sericogenesis in *A. mylitta,* causing enhancement in the secretory activity. According to Saunders (1976), the rhythms are used by the organisms to measure the passage of time and establish a biological clock to perform various physiological activities. The 24h light-dark cycle initiates daily rhythms, influencing greatly physiological activities, behavior, body temperature, and hormone production in many vertebrate and invertebrate species (Binkley, 1993; McEachron and Schull, 1993).

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