Photoperiodic modulation of circadian rhythms in the silk gland protein profiles of *Bombyx mori* and its influence on the silk productivity and quality

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Abstract: Circadian rhythms in the silk gland protein profiles of *Bombyx mori* were analyzed under 12 h light and 12 h dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions. The phase response curves of protein rhythms indicate the prevalence of a series of silk cycles, each comprising three phases; transcription, translation and consolidation of silk proteins. In the 24h- protein rhythm, the silk cycle repeats every 3h, 42 m under LD, 2h, 36m under LL and 3h under DD. The light and dark conditions advanced the rhythm of each silk cycle by 48m and 24m respectively. As a result the silk gland completes 7 rounds of protein synthesis under LD, 9 rounds under LL and 8 rounds under DD during the 24h-free running time of the rhythm. The light-induced clock-shift in the protein rhythm caused significant gains in economic parameters of sericulture with positive signals for enhancing silk productivity and quality*.*

Keywords: *Bombyx mori***,** Circadian rhythms, Photoperiod, Proteins, Silk gland

INTRODUCTION

The silkgland of *Bombyx mori* is the principal organ that converts nutrients of mulberry leaves into silk proteins. The productivity and quality of the silk, the exquisite fabric of the sericulture industry, largely depends on the growth of the silk gland and the timing of the silk proteins synthesized in it. In view of its economic importance, the silk gland of *Bombyx mori* and other silk moths has been extensively probed. (eg. Obara and Suzuki, 1988; Michaille *et al*., 1989; Gizelak, 1995; Inoue *et al*., 2000; Yong Hou *et al*., 2007; Durand *et al*., 1992 and Fukuta *et al*., 1993). One of the important lines of investigations concentrated on the identification, isolation and cloning of circadian clock genes and their products and unraveling the molecular and genetic mechanism of circadian rhythms. A large number of circadian clock genes such as period (per), timeless (tim), double time (dbt) and the clock proteins such as the PER (PERIOD), TIM (TIMELESS), CRY (CRYPTOCHROME), CYC (CYCLE), DBT (DOUBLE TIME), CLK (CLOCK), were identified in silk moths and specific functions were assigned to them (Sharma, 2003; Sehadova *et al*, 2004 and Iwai *et al*., 2006). Concerted efforts were also made to localize the endogenous pace makers or circadian clocks or oscillators in a variety of insects (Glossop and Hardin, 2002; Sehadova *et al*., 2004 and Reppert, 2006).

The studies on circadian rhythms largely highlighted the transcriptional and translational profiles of clock genes in *Bombyx mor*i. The molecular and genetic mechanism of circadian clock genes needs to be substantiated in terms of biochemical and physiological manifestations. No effort has since been made to elucidate the biochemical basis of circadian clock mechanism and its implications for silk production. Studies on the silkgland circadian behaviour could provide vital clues on the mysteries of silk genes and the timing of their expression that could have strong influence on the quality and productivity of the silk. The present investigation aims at making a preliminary attempt in this direction.

MATERIALS AND METHODS

The present investigation was carried out on the Pure Mysore x CSR_2 hybrid variety of the silkworm *Bombyx mori,* reared under standard environmental conditions of 28^0 C, 85 % RH, as described by Krishnaswami (1986). After hatching the worms were fed with M_5 variety of mulberry leaves, 5 times a day at 6AM, 10 AM, 2 PM, 6PM and 10 PM under 12h light and 12h dark cycle. After the third moult, the larvae were divided into three batches and reared separately under three different photoperiodic conditions, viz., 12h light and 12h dark cycle (LD), continuous light (LL) and continuous dark (DD) with five feeds a day as earlier. The investigation was divided into two parts; the assay of circadian rhythms in the silk gland protein profiles and the analysis of economic parameters of sericultural importance.

i) Assay of circadian protein rhythms: Circadian rhythmicity in the protein profiles of the silk gland was analyzed on the fifth day of fifth instar throughout the day. The silk glands, isolated every hour by dissecting the silkworm larvae in ice-cold Silkworm Ringer (Yamaoka

et al., 1971), starting from 8 AM on day-5 till 8 AM on day-6 (i.e. for 25 hours), were used for the assay of proteins. Hour-to-hour changes in protein profiles of the silk gland were analyzed and the levels of total and soluble proteins of the silk glands were estimated in 1% homogenates of the tissue in ice cold distilled water, by the method of Lowry *et al.* (1951). The levels of structural proteins were deduced from the levels of total and soluble proteins by subtracting the later from the former.

The experiment lasted for two consecutive days encompassing 12: 12 hours of light and dark cycle (LD) for the first batch, continuous light (LL) for the second batch and continuous dark (DD) for the third batch. In the LD condition, the study duration was divided into two photo (light) phases and one scoto (dark) phase. The first light phase extended from 8 h to 18 h on day-5 lasting for a duration of 10 hours, and the second light phase extended for two hours on day-6, i. e from 6 h to 8 h. The duration of dark phase lasted for 12 h, i.e. from 18 h on day-5 to 6 h on day-6 at a single stretch. The batch of the silkworm larvae reared under LD was treated as the control and those reared under LL and DD as the experimental samples.

ii) Analysis of economic parameters: Some important economic parameters of the sericulture industry were analyzed as per the methods given by Bohidar *et al.* (2007) and Rahmathulla *et al.* (2007). The ripened worms were mounted separately and the economic traits such as the larval weight, silk gland weight, gland–body ratio, cocoon weight, cocoon-shell weight, cocoon - shell ratio, renditta, raw silk – larval body ratio, raw silk percentage, filament weight, denier (filament size), floss-shell ratio were analyzed separately in each batch. Additionally, the protein content of the shell and floss of the cocoon was also ascertained as per the method given by Lowry *et al.* (1951).

RESULTS

The results of the current study encompasses two aspects; photoperiodic modulation of circadian rhythms in the silk gland protein profiles and its impact on economic parameters of sericulture.

Photoperiodic modulation of circadian changes in protein profiles: Hour-to-hour changes in the levels of total, soluble and structural proteins of the silk gland, assayed under LD, LL and DD conditions for a period of 24h is designated as the free running period or the *tau*. The data pertaining to protein profiles are presented graphically in the form of phase response curves (PRCs) in figure 1. The elevated points (both major and minor) in the protein levels were treated as peaks and the low points or depressions (both major and minor) as troughs, which represent two phases of the protein rhythm.

i) Total proteins: Under 12:12 light / dark cycle, the total protein content of the silk gland showed 7 peaks,

(8h,13h,16h,20h,23h,2h and the last one next day at 6h) and 7 troughs (12h, 15h, 18h, 22h, 00h, 3h and the last one next day at 8h)) during the 24 h free running period of the circadian rhythm (Fig. 1 A). Of the 7 peaks and 7 troughs, four each were recorded during the photo phase and three each during the scoto phase of the light-dark cycle. Under LL, the protein rhythm recorded 9 peaks (8h, 10h, 12h, 15h, 17h, 19h, 21h, 3h and the last one next day in between 6 - 7h) and 9 troughs (9h, 11h, 13h, 16h, 18h, 20h, 23h, 4h and the last one next day at 8h). Similarly, 7 peaks (10h, 13h, 15h, 19h, 00h, 4h, and 7h) and 7 troughs (11h, 14h, 16h, 21h, 2h, and the last two next day at 6h and 8h) were recorded under DD. During the remaining free running period of rhythm, all the protein levels maintained constant fluctuations that are not statistically significant to be counted as peaks and troughs. The interval between different peaks and troughs ranged from 2 to 7 hours under three photoperiodic conditions (Tables 1 and 2). However, the computed mean interval between two peaks and two troughs was ~3h under LD, ~2.5h under LL and ~3h under DD.

ii) Soluble proteins: The soluble protein levels of the silkgland showed 7 peaks (8h, 16h, 19h,23h,2h,5h and the last one next day at 8h) and 7 troughs (9h, 14h, 18h, 21h, 00 -01h, 4h and the last one next day at 6h) under LD, of which three appeared during the photo phase and four during the scoto phase of the rhythm, Likewise, the soluble protein profiles of silk gland showed 9 peaks (8h, 11h, 13h, 15h, 18h, 20h, 22h, 2h and the last one next day at 8h) and 9 troughs (9h, 12h, 14h, 16h, 19h, 21h, 23h, 3-4h and the last one next day at 6h) under LL, but 9 peaks (10h, 13h,15-16h, 19h, 21h, 00h, 3h, 5h and the last one next day at 7h) and 10 troughs (8h,12h, 14h,17h, 20h, 22h, 2h, 4h and the last two next day at 6h and 8h) under DD (Fig. 1-B). During the remaining period of the rhythm, soluble protein levels maintained minor ups and downs, which are of least significance to be considered as peaks and troughs (Fig. 1-B). Though, the individual intervals between any two peaks or troughs ranged from 2 to 6h, the computed mean value stood at ~3h under LD, 2.5h under LL and ~2h under DD (Tables 1 and 2).

iii) Structural Proteins: Under 12:12 light / dark cycle (LD), the levels of structural proteins showed 8 peaks (8h, 13h, 17h, 20h, 23h, 2h, 4h and the last one next day at 6h)) and 8 troughs (12h, 15h, 19h, 22h, 00h, 3h, 5h and the last one next day at 8h) during the 24h-free running period of the circadian rhythm (Fig.1. C). Of all, 4 peaks and 3 troughs appeared during the photo phase and the remaining ones appeared during scoto phase. Under continuous light (LL), 8 peaks (8h, 12h, 14-15h, 17h, 19h, 21h, 3h and the last one next day in between 6-7h) and 8 troughs (11h, 13h, 16h, 18h, 20h, 23h, 4h and the last one next day at 8h) were recorded in the levels of structural proteins of the silk gland.Under continuous dark (DD)
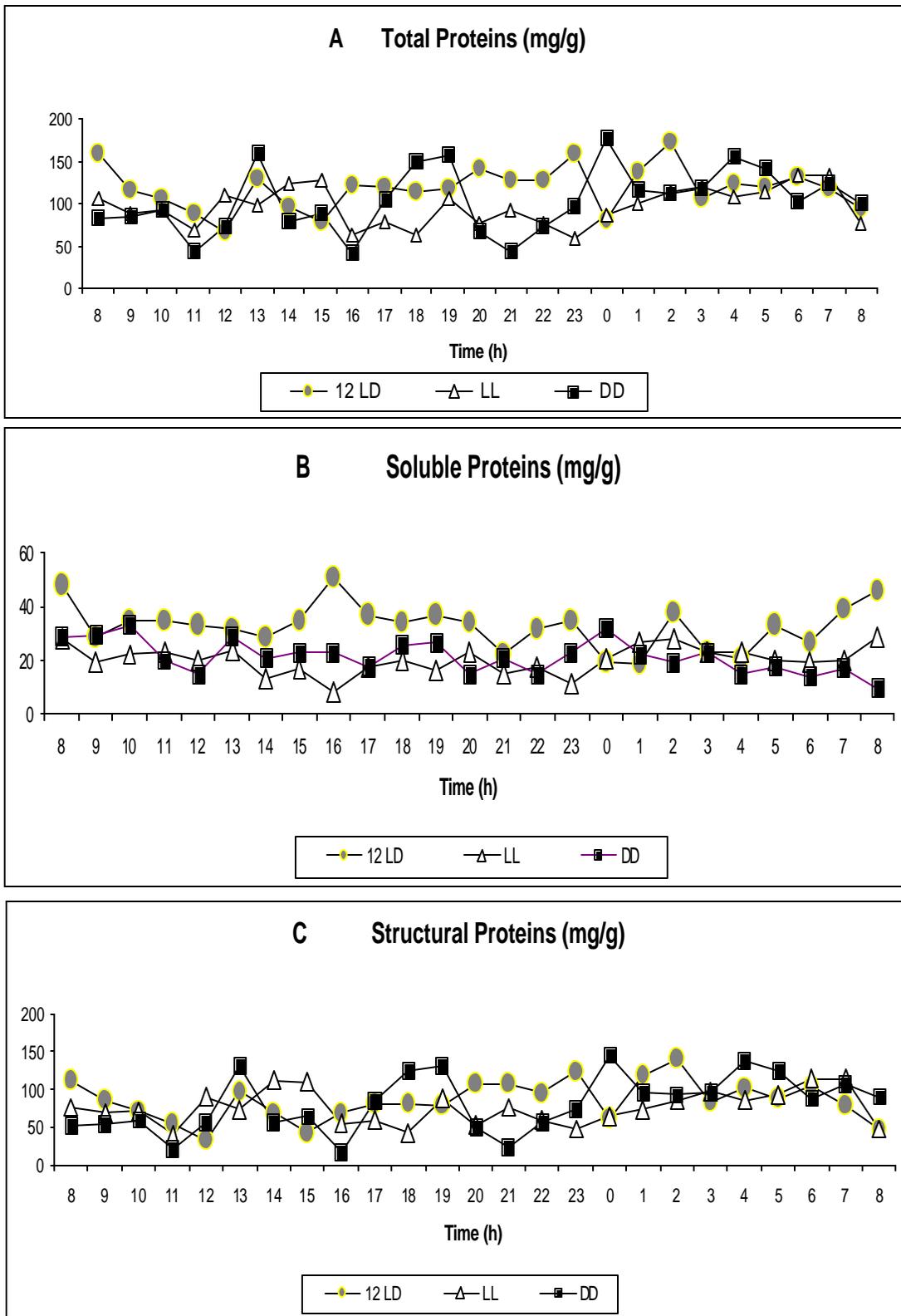
Fig. 1. *Phase response curves (PRC) of the circadian protein rhythms in respect of total (A), soluble (B) and structural (C) proteins in the silk gland of Bombyx mori, under 12h light: 12h dark cycle (LD); continuous light (LL) and continuous dark (DD) conditions. Each phase represents the hourly assay of protein levels, starting from 8 h on day-5 to 8 h on day-6 during fifth instar development. Each value, expressed as mg protein / g wet weight of tissue represents the mean ±S.D of four separate observations; (P values: <0.001).*
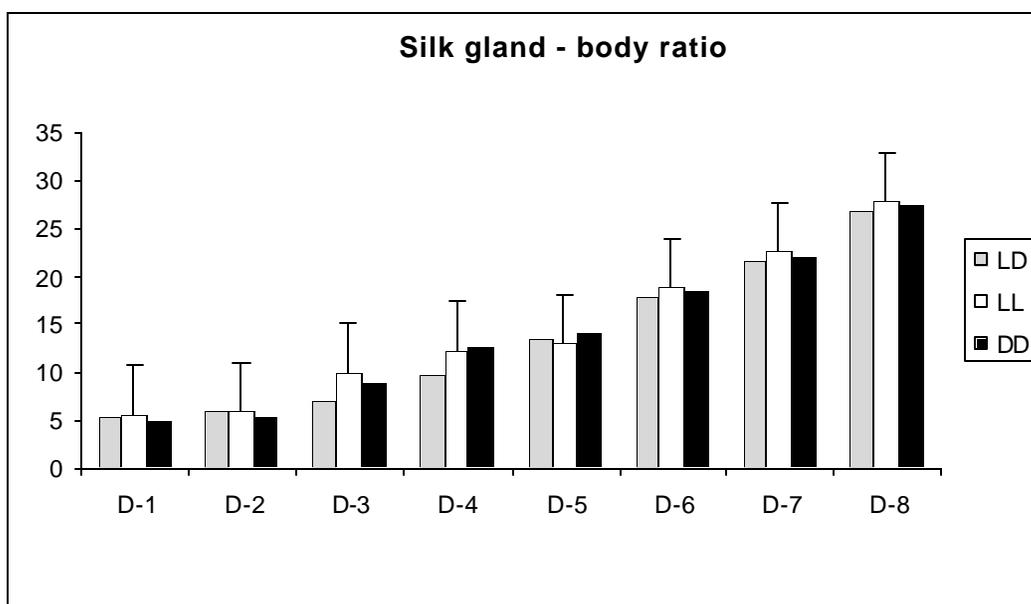
Fig.2. *Growth of the silk gland – body ratio during fifth instar development of Bombyx mori, under 12h light and 12h dark cycle (LD), continuous light (LL) and continuous dark (DD). Each value is a mean, ± standard deviation of four individual observations. (P value < 0.001). D -1 to D- 8: Day-1 to day–8 of fifth instar.*

the structural protein content of the silk gland recorded 7 peaks (10h, 13h, 15h, 19h, 00h, 4h and the last one next day at 7h) and 7 troughs (11h, 14h, 16h, 21h,1-2-3h and the last two next day at 6h and 8h) during the 24 h free running period of the rhythm (Fig.1. C). During the remaining period of the free running time, the structural protein levels maintained minor ups and downs, which are of least significance. Though, the individual intervals between any two peaks or troughs ranged from 2 to 6h, the mean interval between any two peaks or troughs stood at 2.8h under LD, 2.6h under LL and ~3h under DD (Tables 1 and 2).

Photoperiodic modulation of economic parameters: The data pertaining to the economic parameters are presented in Table 3 and Fig. 2.

The growth of larval body and the silk gland under three photoperiodic conditions, measured in terms of daily changes in their weights during the fifth instar development is presented in Fig. 2. Under 12 h light / 12 h dark cycle (LD), the bodyweight recorded significant increases of 342% from day-1 through day-8 of the fifth instar. At the same time, the silk gland exibits a growth rate of about 2014%. Under continuous light condition, the larval body weight showed an overall increase of about 359% and the silk gland had an increase of 2139% during fifth instar. Under continuous dark condition, the larval body weight increased to about 364%, while the silkgland grew about 2458%. The silk gland body ratio

Table 1. Interval between peaks (elevated points) of protein levels in the silk gland of *Bombyx mori* during the free running period of the protein rhythm under 12h light / 12h dark cycle (LD); continuous light (LL) and continuous dark (DD).

Protein type	Photo-period	No. of peaks	Interval between peaks in hours									Mean interval in hours
			1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	
Total Proteins	LD	**7**	5	3	4	3	3	4	-	-	-	3.1
	LL	**9**	2	2	3	2	2	2	7	3	-	2.3
	DD	**7**	3	2	4	5	4	3	-	-	-	3.1
Soluble proteins	LD	**7**	7	3	4	3	3	3	-	-	-	3.3
	LL	**9**	3	2	2	3	2	2	4	6	-	2.7
	DD	**9**	3	2	3	2	3	3	2	2	-	2.2
Structural Proteins	LD	**8**	5	4	3	3	3	2	2	-	-	2.8
	LL	**8**	4	2	2	2	2	6	3	-	-	2.6
	DD	**7**	3	2	4	5	4	3	-	-	-	3.0

Source: Fig. 1

Table 2. Interval between troughs (low points) of protein levels in the silk gland of *Bombyx mori* during the free running period of the protein rhythm under 12h light / 12h dark cycle (LD); continuous light (LL) and continuous dark (DD).

Protein type	Photo-period	No. of troughs	Interval between troughs in hours									Mean interval in hours
			1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	
Total Proteins	LD	7	3	3	4	2	3	5	-	-	-	2.9
	LL	9	2	2	3	2	2	3	5	4	-	2.6
	DD	7	3	2	5	5	4	2	-	-	-	3.0
Soluble proteins	LD	7	5	4	3	3	4	2	-	-	-	3.0
	LL	9	3	2	2	3	2	2	4	3	-	2.3
	DD	10	4	2	3	3	2	4	2	2	-	2.2
Structural Proteins	LD	8	3	4	3	2	3	2	5	-	-	2.8
	LL	8	2	3	2	2	3	5	4	-	-	2.6
	DD	7	3	2	5	4	3	2	-	-	-	2.7

Source: Fig. 1.

showed an increase of 379% under LD, of 390% under LL and under DD conditions it rose to 450% (Fig.2).

The remaining 12 other economic parameters, viz., weight of single cocoon, shell with floss, shell without floss, floss of single shell, floss of shell ratio, shell proteins of single cocoon, floss proteins of single cocoon, silk filament weight per shell, raw silk-larval body ratio, raw silk percentage, renditta and denier (Table 3) have shown positive growth trends in the silkworms reared under continuous light (LL) and continuous dark (DD) conditions, compared to those reared under 12 h light 12 h dark cycle (LD).

DISCUSSION

The silk gland is a repository of over 93 proteins that includes both soluble and structural components (Jin *et al*., 2004; Zhang *et al*. 2006 and Yong Hou *et al*., 2007). Prominent soluble proteins of the silk gland in *Bombyx mori* include chaperones, metabolic enzymes like triose phosphate isomerase, enolase and inorganic pyrophosphate, heat shock proteins, immuno proteins, serpins, transport proteins and those involved in replication, transcription and translation of silk proteins, (Yang Hou, *et al*., 2007; Kyung*et al*., 2006). The structural proteins comprise several cocoon proteins (Nirmala *et al*., 2001) and silk proteins (Inoue *et al*., 2000; Takasu *et al*., 2005), and cytoskeleton proteins like actins, tubulins, transmembrane proteins and many more (Yong Hou *et al*., 2007).

The current study demonstrates the prevalence of circadian protein rhythm in the silk gland of *Bombyx mori* that manifests in the profiles of total, soluble and structural proteins (Fig.1). Our findings are in consistence with reports that highlighted the existence of tissue specific circadian rhythms in *Bombyx mori* (Sehadova *et al*., 2004; Iwai *et al*., 2006; Kyung *et al*., 2006; Yong Hou *et al*., 2007). Circadian rhythms enable organisms to live in

harmony with the rhythms of the nature by re-adjusting their physiological events to occur at an appropriate time of the day (Saunders, 2002). Light being the most dominant zeitgeber (time giver), modulates the circadian clock mechanism by entrainment or clock-shifting phenomenon during the larval development (Peschel *et al*.,2009) which involves the continuation of the circadian rhythm, but setting it ahead or behind the normal free running time (Wallace *et al*., 1991).

The phase response curves (PRCs) of the protein rhythm, with its characteristic ups (peaks) and downs (troughs) show the profound effect of altered photoperiodic conditions on the circadian protein rhythm in the silk gland of *Bombyx mori*. We presume that the peaks represent intense phases of protein biosynthesis and the troughs the declining trend in protein synthesis during the free running period of the rhythm. The troughs may also indicate the mobility of some clock related proteins from the cytosol to the nucleus and the time-bound homeostatic mechanism that facilitate the degradation of selected proteins by intracellular proteolysis (by proteases) that characterize the silkworm metamorphosis (Chen and You, 2004 and Ciechanover, 2005). The analysis of PRCs, in terms of the mean number of peaks and troughs and mean interval between them gives meaningful results. Notwithstanding the individual fluctuations in the number of peaks and troughs, their combined mean value is of ~7 under LD, ~9 under LL and ~8 under DD, while their combined mean interval is ~3.4 h under LD, 2.6 h under LL and exactly 3 h under DD.

On the basis of rhythmic changes in the protein profiles observed in the current study, we propose a 24h-circadian silk rhythm that could possibly operate in the silk gland of *Bombyx mori* during the fifth instar development. This rhythm includes a series of silk cycles that are repeated ~7 times under LD, ~9 times under LL and ~8 times under

Table 3. Photoperiodic modulation of economic parameters of the silkworm, *Bombyx mori*, under 12h light and 12h dark cycle (LD), continuous light (LL) and continuous dark (DD). The weight of the cocoon, shell and floss represents the mean of 25 individual cocoons. Each value is a mean ± standard deviation of four individual observations. The values in parentheses represent the percent changes computed by taking the LD as the control.

Parameter		12 h light / 12 h dark cycle (L D)	Continuous Light (L L)	Continuous Dark (D D)
1. Weight of single green cocoon (g)	Mean	0. 976	1. 000	0. 984
	P.C	-	(+2.5)	(+0.8)
	S.D	±0.001	±0.001*	±0. 001*
2. Weight of the single shell with floss (g)	Mean	0. 189	0. 189	0. 188
	P.C	-	(+0.0)	(-0.5)
	S.D	±0.001	±0.001**	±0. 001**
3. Weight of single shell without floss (g)	Mean	0. 166	0. 172	0. 170
	P.C	-	(+3.6)	(+2.4)
	S.D	±0.001	±0.001*	±0. 001*
4. Weight of the floss of single shell (g)	Mean	0. 023	0. 017	0. 018
	P.C	-	(-26.1)	(-21.7)
	S.D	0.001	±0.001*	±0. 001*
5. Floss – shell ratio	Mean	13. 9	9. 9	10. 6
	P.C	-	(-28.8)	(-23.7)
	S.D	0.01	±0.01*	0. 01*
6. Total protein content of single shell without floss (mg)	Mean	43.4	46.2	39.6
	P.C	-	(+6.5)	(-8.8)
	S.D	±0.10	±0.10*	±0. 01*
7. Total protein content in the floss of a single shell (mg)	Mean	6.1	4.1	4.9
	P.C	-	(-32.8)	(-19.7)
	S.D	±0.01	±0.01*	±0. 01*
8. Silk filament weight per shell (g)	Mean	0. 126	0. 143	0. 132
	P.C	-	(+13.5)	(+4.8)
	S.D	±0.001	±0.001*	±0. 001*
9. Raw silk - larval body ratio	Mean	5. 70	6. 12	5. 84
	P.C	-	(+7. 4)	(+2. 5)
	S.D	±0.01	±0.01*	±0. 01*
10. Raw silk percentage	Mean	12. 91	14. 30	13. 41
	P.C	-	(+10.8)	(+3.9)
	S.D	±0.01	±0.01*	±0. 01*
11. Renditta	Mean	7. 93	7. 01	7. 56
	P.C	-	(-11.6)	(-4.7)
	S.D	±0.01	±0. 01*	±0. 01*
12. Denier	Mean	17. 1	14. 4	13. 5
	P.C	-	(-15.8)	(-21.1)
	S.D	±0.01	±0. 01*	±0. 01*

* Statistically significant. ** Statistically not significant.

DD. Each round of the silk cycle includes two vital phases of silk gene expression, i.e., transcription, translation apart from the phase of consolidation. The timing of troughs probably indicate the timing of transcriptional phases, while those of peaks reflect the timing of translation phases. The silk proteins synthesized during the translation phase may assemble and assume secondary and tertiary structures and achieve their functional status during the consolidation phase, which probably occurs during the scotopic phase of the rhythm. Obviously, the ups (peaks) and downs (troughs) in protein levels of the silk gland provide vital clues regarding the expression of silk genes. Presumably, the ups and downs in the levels of soluble and structural proteins indicate the synthesis and consolidation of silk proteins during peak phases that are subjected to modulation by the dark and light cues. The synthetic and consolidation phases are apparently stimulated by the dark cues as evidenced by the appearance of higher peaks in the levels of soluble and structural proteins under LD and DD, compared to those under LL. On the other hand the light seems to have stimulatory effect on the number of protein synthetic cycles as reflected in the increase in the number of peaks and troughs during the free running time of the rhythm (Fig. 1. A, B, C). Thus, the intensity (height of peaks) and frequency (number of peaks) of silk gene expression in *Bombyx mori* are independently modulated by the dark and light cues respectively. We do not have supportive

data to establish this fact conclusively. Nonetheless, the available data on circadian clock genes and their timing of expression indirectly lends some support (Grima *et al*., 2004; Hardin 2004; Shafer *et al*., 2004; Stoleru *et al*., 2004). Further, it is evident that the photoperiod is known to cause behavioural adjustments by altering clock gene expressions and the clock time could be advanced by the degradation of TIM during the later half of the night. Further, our assumption is based on the premise that the mRNA synthesis of clock genes was found to be higher on short days than under longer days and that it peaks during the scoto phase (Goto and Denlinger, 2002; Syrova *et al.*, 2003).

The genetical and molecular mechanism of the circadian clock is not corroborated by biochemical studies; as direct evidence for protein rhythms remains unavailable. However, our findings are in agreement with the earlier observations that more proteins are synthesized under altered conditions of photoperiod (Kenny and Saunders, 1991; Koga *et al*., 2005;Peschel *et al*., 2009). The control mechanism of circadian behaviour of tissue protein is not known; but it is likely that the juvenile hormone secreted by the corpora allata (Dolezel *et al*., 2008) does so by modulating the peripheral oscillators of the silk gland. Further, the silk gland maintained the circadian rhythm more or less at a constant rate even under continuous dark condition (DD), probably by taking cues from the diet, in the absence of light. This fact is substantiated by earlier reports (Damiola *et al*., 2000; Kita *et al*., 2002; Stokkan *et al*., 2001) which state that the food can act as prime zeitgeber and that the restricted feeding quickly resets clocks in peripheral organ. Since, the silkworms, reared under continuous dark were also fed 5 times a day, they carried through the rhythm more or less on the same lines as done by the worms grown under LD. It is likely that silkworm might have a dual mode operating system with at least two-oscillators in the silk gland, in which one responds to the light and the other responds to the dark, as observed by Forster (2000) with respect to locomotory rhythm in Drosophila flies.

Despite the prevalence of minor variations in the levels of proteins under different photoperiods, our study revealed a clock-shift in all the three types of proteins in the silk gland of *Bombyx mori* under constant light (LL) and constant dark (DD) conditions. Accordingly, in the silkworms that are deprived of natural light cues, the circadian silk rhythm shifts from the standard 24h pattern. In the present case the shift resulted in the advancement of each silk cycle by 48m under LL and by 24m under DD conditions, thus disturbing the free running time of the circadian silk rhythm both in photo and scoto phases. The 24h-circadian protein rhythm of LD condition, with 7 rounds of protein synthesis, has been clock-shifted to about 18.2h under LL and 21h under DD conditions. With

the result, the silk gland is able to accomplish 2 additional rounds of protein synthesis under LL and one round under DD during the 24h-free running time of the silk rhythm.

Influence of protein rhythms on economic parameters: The productivity and profitability of the sericulture industry largely depend on the quantity and quality of silk proteins. Both these parameters are influenced by the rhythm it maintains with regard to protein synthesis. Any progressive change in the style of functioning of silk gland will have long-lasting impact on the economic parameters of sericulture. The silk gland of the silkworms reared under LL and DD conditions showed promising growth trends compared to those reared under LD.

The positive impact of LL and DD on economic parameters, observed in the present study, is attributable to the increase in the frequency (number of peaks) and intensity (height of peaks) of protein synthetic cycles during the free running time of the circadian protein rhythm. Two additional rounds of protein synthesis in the silk gland under LL (total 9 rounds) and one additional round under DD (total 8 rounds), and a decrease in the interval between protein synthetic cycles (from 3.4h under LD to 2.6h under LL and 3h under DD) have advanced the silk cycle by 48 m under LL and 24m under DD, resulting in significant rise in all the economic parameters analyzed. For instance, in the silkworms grown under continuous light during the fourth and fifth instars, the silkgland-body ratio significantly increased from 26.8 under LD to 27.9, thus indicating the fact that the silk gland grows at a faster rate under LL compared to LD. Likewise, one additional round of protein synthesis and 24 m advancement of the free running time of the protein rhythm in the silk gland resulted in raising the gland-body ratio from 26.8 to 27.5 under DD (Fig.2). The other 12 economic parameters were also altered under LL and LD conditions (Table 3).

Thus, the circadian protein rhythm modulates the productivity and quality of silk produced by the silk gland. The photoperiod -modulated rhythm under LL has resulted in improving the productivity and quality of silk in two ways; i.e., increasing the silk output and at the same time decreasing the wastage. This is achieved by increasing the synthesis of fibroin, the core silk protein (Inoue *et al*., 2000; Yong Hou *et al*., 2007) and by decreasing the synthesis of sericin proteins which constitute the bulk of floss that is chiefly distributed in the outer most layer of the cocoon and is removed as wastage during silk reeling (Takasu *et al*., 2005). The increasing effect of photoperiod on the fibroin synthesis vis-à-vis its modulatory role on circadian rhythms, is evidenced by improvements in several economic parameters such as the shell weight, silk-body ratio, gland-body ratio, protein content of the shell, raw silk

percentage and weight of silk thread obtained from a single cocoon. Similarly, the decrease in wastage is substantiated by similar changes in floss-shell ratio, floss protein content and floss weight. Further, the productivity is enhanced significantly by reducing the renditta, from ~8 under LD, to ~7 under LL and ~7.6 under DD. The decline in the denier, which is the measure of thickness of the silk thread, from 17.1 in LD to 14.4 (-16%) in LL and 13. 5 (-21%) in DD indicates that the silk produced under LL becomes thinner and its texture becomes smoother than those produced under normal day-night cycle. This becomes still thinner in case of the silk thread produced under DD.

The analysis of PRCs (Fig.1), in terms of height (higher protein levels) and number of peaks (silk cycles) provides further insights into the modulatory role of photoperiod on the silk production and productivity. The higher peaks in the protein levels observed under LD and DD, coupled with the trends in floss-related economic parameters, indicate that the dark-light condition (both in LD and DD) stimulates the synthesis of both fibroin and sericin with more emphasis on the later, resulting in the production of more sericin that constitutes the floss of the cocoon. Similarly, more number of silk cycles (i.e. 2 more than LD and 1 more than DD), but with lower protein levels, observed under LL condition, coupled with positive trends in fibroin-related economic parameters reflect that light condition stimulates the synthesis of more fibroin, the core silk protein. If, the observed trends in the economic parameters are an indication, it is presumed that the light condition enhances silk productivity by stimulating fibroin gene expression, while the light-dark condition contributes to higher wastage in silk output by enhancing sericin gene expression, Though, several reports are available on economic parameters of sericulture (Bohidar, *et al.*, 2007; Rahmathulla *et al.,* 2007), our investigations have no parallel supportive documents. Moreover, the study is not exhaustive; nevertheless it provides insights into the utility of photoperiod for profitable sericulture. Extensive field trials involving post-treatment studies with continuous light may go a long way in validating the positive impact of light on the economic parameters of silkworm. The possibility of coupling economic parameters with circadian biochemical rhythm and silk gene expression is thus open for future investigations.

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