Moulting patterns in the silkworm, *Bombyx mori* L. (PM × NB4D2) under different photoperiodic conditions

N SIVARAMI REDDY†, T PAVAN KUMAR** and K SASIRA BABU

Department of Zoology, S V University, Tirupati 517 502, India
Present address: *Central Sericultural Research and Training Institute, Srirampuram, Manandavadi Road, Mysore 570 008, India
**Central Silk Board, United Mansions, 39, M G Road, Bangalore 560 001, India

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Abstract. Moulting patterns in the silkworm, *Bombyx mori* L. (PM × NB4D2) under different photoperiodic conditions were studied. Moulting phenomenon, in *Bombyx mori* seems to be under the control of the circadian phenomenon. The peaks for the second moult were observed during the dark phase of the photoperiodic schedule expressing nocturnal predominancy. Consequently these peaks for consecutive moults moved into the light part of the 24 h cycle. This clearly demonstrates the instar dependency of the moulting expression. Under LL condition, a damp-out situation in moulting was observed. Moulting durations were greatly affected by photoperiods. Shortest moulting durations were recorded for LD 11:13.

Keywords. Moulting; photoperiods; *Bombyx mori*.

1. Introduction

Insect growth is characterised as discontinuous and is manifested by a series of moults. It is well established that the major features of the moulting cycles are regulated by a sequential action of 3 hormones; prothoracicotropic hormone (PTTH), ecdysone and juvenile hormone (JH) (Reynolds 1980; Happ 1984), the release and action of the PTTH is controlled by neurosecretory cells in the brain (Truman 1972). It is amply demonstrated that the optic lobes [the pupative biological clock(s) in insects] have a direct influence on the neurosecretory system (Page et al. 1977). Photoperiodic effects on the timing of the moulting cycles can be accounted for on the basis of the neurosecretory system (Truman 1972; Beck 1980) being responsible for the secretion of PTTH, the first hormone in the moulting cycle sequence.

In our earlier communications (Sivarami Reddy et al. 1984; Sivarami Reddy and Sasira Babu 1990), we have reported how the hatching, in *Bombyx mori* (L.) is influenced under different photoperiodic schedules. In the present paper, studies on the moulting in the commercial silkworm, *B. mori* (PM × NB4D2), under different photoperiodic conditions are reported.

2. Material and methods

Disease free layings (DFLs, each DFL consists of 350–450 eggs laid by a single silkmoth on a single day) of the silkworm, *B. mori* (PM × NB4D2, a hybrid of Pure

†To whom all the correspondence should be addressed.
Mysore, a multivoltine and NB4D2, a bivoltine) were procured, third day after oviposition, for the study from the Government Silk Farm, Palamaner, Andhra Pradesh. The DFLs were transported to the laboratory during evening hours and immediately spread in the rearing trays. The same day, the DFLs were introduced, till completion of larval period, to normal day (LD 12:12), DD (continuous dark), LL (continuous light) and other different photoperiodic regimens, starting from LD 4:20 (short-day) to LD 20:4 (long-day). For all the photoperiodic combinations, the light phase (photophase) (around 50 lux) of the LD cycle commenced from 0600 h (for example for LD 12:12 condition the photophase was from 0600–1800 h and the scutophase spanned from 1800–0600 h). Optimum temperature (25 ± 1°C) and Rh (80 ± 5%) (Krishnaswami 1986) were maintained in the laboratory all through the experimentation. The experiment was conducted 5 times during different months in a year.

Two parameters in the moulting process of *B. mori*; one—the phase of settling for moulting (SM), when the larva stop feeding, with its thoracic segments bulging and fixing legs to the substratum adopting an ‘S’ shaped posture for moulting from which the larvae are not supposed to be disturbed (Krishnaswami 1986), and the other parameter, the completion of moult (CM), the time at which the larva casts out its old cuticle and enters the next larval instar, were studied. Precise time (1 h interval) of SM and CM were recorded from all the (5) replications. Since the first instar larvae are very small in size for taking accurate observations, the observations on the first moult, both SM and CM, however, were not recorded. Further, the process for SM was already reported by us (Sivarami Reddy et al 1984) for two photoperiodic conditions; LD 12:12 and LD 11:13. The moulting duration, from SM to CM (between peak hours) for all the photoperiodic conditions was also computed based on the observations recorded for SM and CM.

3. Results

3.1 Moulting under LD 12:12 conditions

Second moult (both SM and CM), under LD 12:12 photoperiodic conditions recorded nearly 180° out of phase over hatching (Sivarami Reddy et al 1984; Sivarami Reddy and Sasira Babu 1990), with crests for SM and CM occurring at 20 h during the dark phase of the LD cycle (figures 1 and 5), thus expressing a nocturnal predominancy for the second moult. The peaks of SM and CM for third moult crested at 17 and 16 h respectively (figure 1). For fourth moult, the peaks for SM and CM crested at 13 and 9 h respectively. Thus successive moults (from II moult) crested gradually through the light phase of the LD cycle.

3.2 Moulting under continuous dark (DD) and light (LL) conditions

A definite rhythmic pattern in SM and CM has been observed under DD conditions for all 3 (II, III and IV) moults (figure 1). The trend in expression of the crests for consecutive moults from the second moult onwards was as that observed for LD 12:12 (figure 1). Thus the moulting peak of SM and CM for second moult was observed during late hours of the 24 h subjective cycle, showing a creeping
Moultinq in Bombyx mori

Figure 1. Moultinq patterns in B. mori (PM x NB4D2) under natural solar day (LD 12:12), continuous dark (DD) and light (LL) conditions for the second, third and fourth moults. Note the nocturnal expression for the second moult shifting to a diurnal expression for the fourth moult through the third moult. Also, note the 'damp-out' expression in the moulting under LL conditions. Cross-hatched area represents the scutophase imposed.

phenomenon of crests for other successive moults into the first half cycle of the 24 h subjective cycle. However, the peak for the SM of fourth moult has been observed at 9 h while the same for CM continued expressing in the initial hours of the second half cycle.

Under continuous light (LL) conditions, the second moult was undoubtedly rhythmic (figure 1) with a distinct peak appearance. The expression of peaks for the successive moults too was rhythmic, however, with less defined peak appearance reminding a state leading to a damp-out expression. Except for the SM of the second moult, the quantum of larvae moulted at peak hour (observed under DD and LD) for all the other moults was not more than 20% (figure 1). For all the 3 moults studied, the moulting peak was observed during the second half of the 24 h day, predominantly after 16 h. Further, the moulting process was continuous spreading all through the 24 h day, the expression being more predominant for SM of fourth moult and CM of third and fourth moult.

3.3 Moultinq under different photoperiodic conditions

Moultinq under all the photoperiodic conditions was rhythmic, their peaks for the
second moult observed during the dark phase of the photoperiodic schedule and consequently these peaks for consecutive moults moved into the light part of the 24 h cycle (figures 2–4), thus indicating nocturnal expression for the initial moults, shifting to the diurnal expression for the later moults. This also clearly demonstrates the instar dependency of the moulting expression.

Under all the short-day conditions (LD 4:20 to LD 11:13), expression of the peak for SM (figure 2) has been observed between 18–22 h, thus during the early hours of imposed dark schedules, except for LD 11:13 condition where it was during late hours of the subjective day. The peak for CM also expressed a clear cut nocturnal predominancy for the second moult. Under long-day schedules (LD 13:11 to LD 20:4), the peaks for both the SM and CM were observed during the late hours of the imposed light schedules (figure 2), except for LD 13:11 and LD 14:10, for which these have been observed during early hours of the imposed night.

The crests for third moult (SM and CM) have been observed creeping towards light part of the 24 h subjective cycle (figure 3). Thus, under the short-day conditions, the moulting crests were observed during the early hours of the subjective night up to LD 9:15. For LD 10:14 and LD 11:13, these were observed

![Figure 2](image-url)

**Figure 2.** Moulting patterns of second moult in *B. mori* (PM x NB4D2) under different photoperiodic combinations. Note the nocturnal expression of moulting. Cross-hatched area represents the scutophase imposed.
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Figure 3. Moulting patterns of third moult in *B. mori* (PM x NB4D2) under different photoperiodic combinations. Note shifting of nocturnal expression in the second moulting towards light phase in third moult. Cross-hatched area represents the scutophase imposed.  

during late hours of subjective day (figure 3). Also, under long-day conditions peaks of both SM and CM were observed during late hours of the imposed light schedules. Interestingly, the distribution time for SM under long-day conditions (figure 3), (LD; 18:6 to 20:4), was more over that of the other LD schedules showing a broadening tendency of the ecdysial distribution under (extreme) long-day conditions. However, the same for CM was comparable to that of the other photoperiodic regimes. Also under extreme long-day conditions, the moulting was confined to the late hours (20–24 h) of the 24 h cycle.

A distinct expression of the trend in shifting of the crests in moulting during subjective night hours for the second moult to a diurnal expression for the peak of fourth moult is clearly demonstrated, especially with the LD schedules very close to LD 12:12 (figures 4 and 5). In general, the peak moulting was observed during early hours (6–12 h) of the imposed light schedules under LD cycles close to LD 12:12 (figures 4 and 5). However, moulting was still nocturnal under short-day conditions from LD 5:19 to LD 8:16. Interestingly, for LD 4:20 the SM was observed during early hours of the imposed light part (figures 4 and 5), while the same for CM was during late hours of the subjective dark period indicating long
Figure 4. Moulting patterns of fourth moult in B. mori (PM x NB4D2) under different photoperiodic combinations. Note the nocturnal expression of moulting in the second moult shifted to diurnal in the fourth moult, especially with the photoperiodic conditions close to LD 12:12. Cross-hatched area represents the scutophase imposed.

Figure 5. Expression of peak hours in moulting, in B. mori (PM x NB4D2) under different photoperiodic combinations including LD 12:12, DD and LL. Note the nocturnal expression, in moulting for the second moult shifting to a diurnal component for the fourth moult through the third moult, especially with the photoperiodic combinations close to LD 12:12 (including LD 12:12). Each horizontal bar represents a photoperiodic combination in which peak hour of SM (○) and CM (●) are depicted. Cross-hatched area represents the scutophase imposed.
moulting duration (between peaks of SM and CM). Under long-day schedules, on the contrary, the moulting continued cresting during the late hours of the imposed light cycles. As observed for the third moulting, the distribution time for SM, under extreme long-day conditions, was more compared to that under the other LD cycles.

3.4 Moulting durations

It is very interesting to note the effect of the photoperiod on the moulting duration, the time interval between the crests of SM and CM. Under extreme long and short-day conditions, the duration was observed to be more than 24 h while for LD conditions close to L 12:12, the same was around or less than 24 h (figure 6), thus indicating differential implications of photoperiodism. Significantly, shortest moulting durations for all the 3 moults studied (\( P < 0.05 \)), was recorded for LD 11:13 condition.

4. Discussion

Truman’s (1972) extensive studies revealed that the larval ecdysis in *Antheraea pernyi* and *Manduca sexta* occur at a particular time of the day depending on species, the instar and the photoperiod. He reported that the larval moulting distribution time tend to broaden, in *A. pernyi* from initial instars to later ones. The first and second moults occurred during the light phase of both LD 12:12 and 17:7, but that of the third moulting occurred in the following night and that of the fourth moulting was delayed, occurring in the light phase again. A series of ligation experiments between the head and thorax at various times by Truman (1972) during the light cycles demonstrated that the release of the brain hormone (PTTH) was a gated phenomenon.

Beck (1980) pointed out that gated rhythms of larval (or nymphal) ecdysis have not been demonstrated and further this phenomenon has been considered by Beck (1980) as ‘fortuitous synchrony’ being the result of a gated rhythmic hormonal

![Figure 6](image-url)
release (Truman 1972). Thus, the larval ecdysis themselves were not gated, however, but occurred after a fixed number of hours of hormonal (PTTH) release (Saunders 1982).

The observed patterns of rhythmicity in larval ecdysis, in the present study, the SM and CM (figure 1) under LD 12:12 conditions and the persistence of the LD 12:12 pattern under all the other photoperiodic conditions including the continuous conditions (figures 1–5) suggests the endogenously free-running nature. Moreover, a damp-out (as less defined peak expression observed) stage of moulting expression has been observed under LL condition (figure 1), especially for third and fourth moults, supporting the above observation. It has amply been demonstrated that the eclosion rhythm in *Drosophila pseudoobscura* (Pittendrigh and Bruce 1957; Chandrashekaran and Loher 1969) showed a fairly rapid damping out to eventually arrhythmicity under LL conditions. Saunders (1982) stated that although the eclosion may be aperiodic in LL, a rhythm becomes apparent after transfer of the culture from LL to darkness (Winfree 1970, 1972). Thus the present results on moulting seems to be gated in nature (after fixed hours of PTTH release, a subject for further probe).

Beck (1980) viewed that, though the experimental data of Truman (1972) strongly suggests that the gating rhythm was circadian, its endogenous nature was not actually demonstrated. Most significantly, Truman (1972) found that though the release of PTTH is a gated event, the secretion of growth hormones (ecdysone and JH) coming into act, subsequent release of PTTH in the moulting cycle were not gated. However, the synchrony of larval ecdysis appeared to be dependent solely on the gated secretion of PTTH. In the present study, the synchrony of the CM appears to be dependent on the SM. However, the question whether the SM in *B. mori* is the immediate response of PTTH hormonal release or not would be an interesting subject for further research.

Anantha Narayana et al (1978) demonstrated that in multivoltine (Pure Mysore) *B. mori* the light-on signal is taken as reference point in rhythms of eclosion and egg hatching while light-off for oviposition rhythm. For bivoltines, they reported that light-off is taken as reference point for eclosion rhythm and neither on or off for oviposition rhythm. Sivarami Reddy and Sasira Babu (1990) also reported light-on as a possible reference point for egg hatching in *B. mori*. For larval moulting, however, neither light-on nor off seems to be taken as a reference point. However, the peak moulting expression, in the present study seems to be instar dependent as reported for *A. pernyi* and *M. sexta* (Truman 1972) which was attributed to the differences in duration of successive stadia or instars. Krishnaswami (1986) clearly reported significant variations in the durations of different larval stadia in *B. mori*.

The time intervals between the release of hormone (PTTH) and the completion of larval ecdysis is again reported as instar specific and temperature dependent (Truman 1972; Beck 1980; Saunders 1982) as Truman (1972) demonstrated a 3°C raise in the temperature from 25–28°C resulted in 2 h advancement of the moult. Since the larvae of *B. mori*, in the present study, were maintained at a constant temperature (25 ± 1°C), the duration seems to be influenced by photoperiod as also the instar, as suggested by Truman (1972).

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