

Hatching patterns in the silkworm *Bombyx mori* L. (PM × NB4D2) under different photoperiodic combinations

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Abstract. Hatching pattern in the silkworm, *Bombyx mori* L. (PM × NB4D2) under different photoperiodic conditions was studied. Hatching was predominantly diurnal under natural solar day conditions (LD 12:12). The observed LD pattern prevailed under all the photoperiodic conditions. Hatching confined to a single day under all the short-day conditions as also LD 12:12 and DD conditions. All the long-day photoperiodic conditions, as also continuous light (LL), resulted in broadening of the hatching peaks with reduced amplitudes, with extended hatching for two days apart from long hatching durations with more hatching on the second day. The hatching rhythmicity seems to be under circadian control. Maximum hatching was observed under LD 11:13 photoperiodic combination. Shortest hatching duration was observed under LD 11:13 condition.

Keywords. Photoperiod; hatching; circadian control; *Bombyx mori*.

1. Introduction

Under light-dark schedules of natural day (\leq or \geq 24 h; Saunders 1978, 1982) animals exhibit rhythmic fluctuations (Solberger 1965). Embryonic diapause in response to photoperiods characterizing short-day nature (Kogure 1933), growth rates (Hirasaka and Koyama 1970, 1972; Sivarami Reddy *et al* 1984) and various other phenomena showing cyclical fluctuations in *Bombyx mori* L. were reported. *B. mori* was reported as a short day insect (Kogure 1933; De Wilde 1962; Danilevskii 1965; Lees 1968; Beck 1980; Saunders 1982), while Shimizu (1982) showed that an artificial diet produced long day characteristics for individuals that passed through embryonic diapause in the next generation. The present investigation describes rhythmic pattern in hatching under different photoperiodic combinations in *B. mori* (PM × NB4D2 a hybrid of pure Mysae, a multivoltine and NB4D₂, a bivoltine).

2. Materials and methods

Disease free layings (DFLs, each DFL consists of 350-450 eggs laid by a single silkworm on a single day) of the silkworm, *B. mori* (PM × NB4D2) 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, DFLs were introduced till hatching, to normal day (LD 12:12), DD (continuous dark

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condition), 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 ($25 \pm 1^\circ\text{C}$) and Rh ($80 \pm 5\%$) (Krishnaswami 1986) was maintained in the laboratory all through the experimentation. Five DFLs were kept under each photoperiodic conditions. Rhythmic pattern in the hatching was studied under these photoperiodic schedules. Precise timings, in hours, for hatching was determined and recorded.

3. Results

Hatching was predominantly diurnal with about 95% of hatching observed at early hours of the light part of the natural LD cycle (LD 12:12) (figure 1J). The observed LD 12:12 pattern prevailed for hatching under all the other photoperiodic conditions also. For all the short-day photoperiodic combinations, the hatching was confined to a single day. However, under all the long-day photoperiodic combinations the hatching was observed on the next (second) day also. The occurrence of peaks, in hatching was observed moving towards the early hours of the photophase under short-day conditions and the same under long-day conditions towards late hours (figure 1) with the hatching spreading over long duration of time. Further, the hatching peaks under photoperiodic combinations longer than 12 h of light has been observed broadening with less amplitudes. Except under LD 13:11 and 18:6, where hatching is more on the first day itself, hatching was more on the second day under all other long-day combinations (figure 3). Under continuous conditions (LL and DD) negligible and absolutely no hatching respectively was observed on the first day (figures 1A, S and 3). Under all the photoperiodic combinations, the hatching was predominantly diurnal. Interestingly, the magnitude of first day hatching was more at the peak hours under short-day conditions as also DD when compared to that under long-day and continuous light conditions (figures 1, 2) indicating broadening of peaks.

Total hatching was highest under LD 11:13 condition (99%) (figure 4). Hatching under LD 10:14 (98%) was second to LD 11:13 condition. Total hatching, under all the conditions was equal to or more than 90%. However, it was less than 95%, observed for LD 12:12 condition, under extreme long or short photoperiodic conditions (figure 4).

Hatching duration of the silkworm eggs (from initiation to the completion of hatching) (figure 5) had been very minimum, ranging from 1–3 h for the eggs under short-day conditions as also LD 12:12 condition. Lowest hatching duration (≈ 1 h) was observed under LD 11:13 photoperiodic condition. Interestingly, the hatching under all the long-day photoperiodic conditions was prolonged, the duration being more than 24 h. Under DD condition, however, eventhough the hatching was observed only on the second day the duration was about 5 h. On the other hand, the same under LL condition was around 24 h apart from occurring for two consecutive days.

4. Discussion

The rhythmic pattern in hatching of the eggs has been studied only in limited

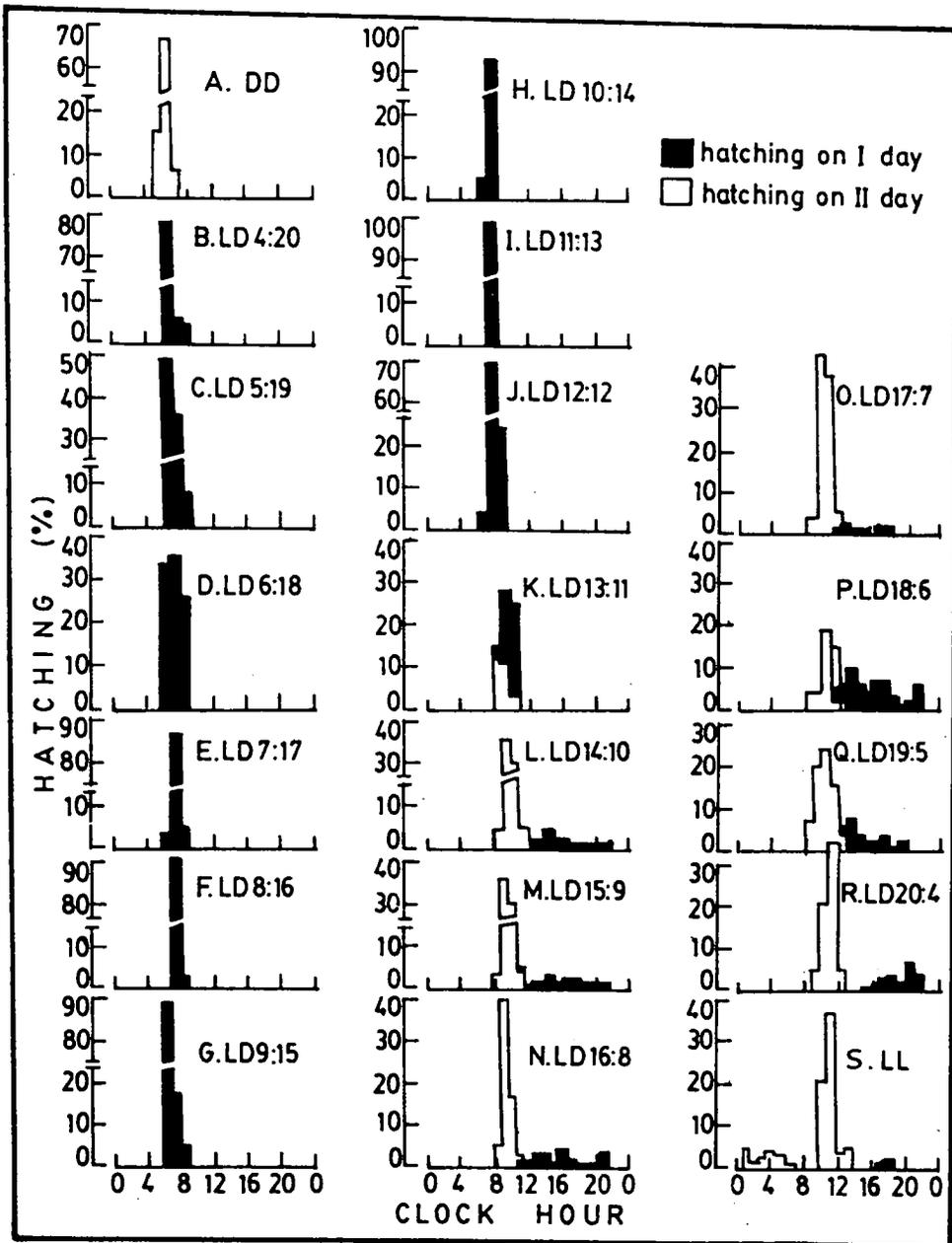


Figure 1. Distribution of hatching (%) in the silkworm, *B. mori* (PM × NB4D2) under different photoperiodic conditions; continuous dark (DD) (A), short-day (LD 4:20 to 11:13) (B-I), natural day (LD 12:12) (J), long day (LD 13:11 to 20:4) (K-R) and continuous light (LL) (S). Note hatching on single day under DD to LD 12:12 with shorter hatching duration and phase moving towards early hours of photophase (A-J) and two days under LD 13:11 to LL with broadening of hatching peaks, prolonged hatching durations and phase moving towards late hours of the photophase (K-S)

insects. In *Pectinophora gossypiella* (Minis and Pittendrigh 1968) eggs under continuous photoperiodic (DD/LL) conditions were arrhythmic in hatching but

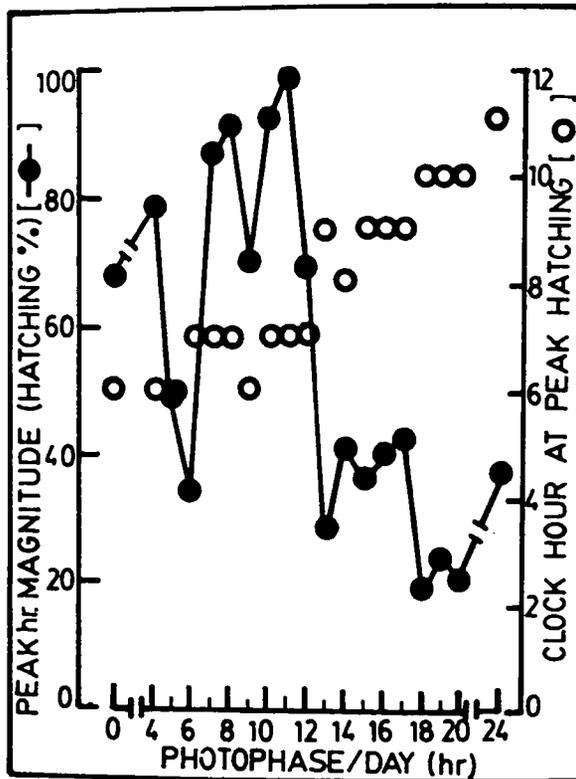


Figure 2. Hatching (%) in *B. mori* (PM × NB4D2) at peak hour and clock hour at peak hatching under different photoperiodic conditions. Note maximum hatching for LD 11:13 and 10:14 and less hatching for extreme long/short-day photoperiodic conditions including continuous conditions (DD and LL). Also note phase delay for long-day and phase advance for short-day photoperiodic conditions.

under LD 12:12 condition there occurred a distinct rhythm, with hatching peaks just before dawn. For *Aedes* sp., hatching was observed as a direct response to environmental factor (Gillett 1955). Nayar (1967), however, reported that the preconditioned eggs of *Aedes taeniorrhynchus* hatch at any time of the day with 15 min of immersion in deoxygenated water. Rhythmic patterns in eclosion, oviposition, and hatching, as effected by photoperiods in silkworm have been reported (Yamaoka and Hirao 1975; Yamaoka *et al* 1976; Anantha Narayana 1980). Anantha Narayana *et al* (1978) explained the hatching peak in Mysore race (PM) of *B. mori* to be very close to dawn under alternating cycles of light and dark, the system links the 'light-on' as the synchronizing signal. In the present study also, hatching in *B. mori* under short-day photoperiodic regimes has been observed very close to dawn (light-on). It could be inferred that the occurrence of hatching during early dawn hours might be due to the relatively high humidity in the micro/macro environment which minimizes 'the risk' of desiccation as Pittendrigh (1966) demonstrated for eclosion in *Drosophila*.

Pupal eclosion rhythm in *D. pseudoobscura* (Pittendrigh 1966) suggests the existence of a self-sustained oscillator which partitions a mixed-age population into

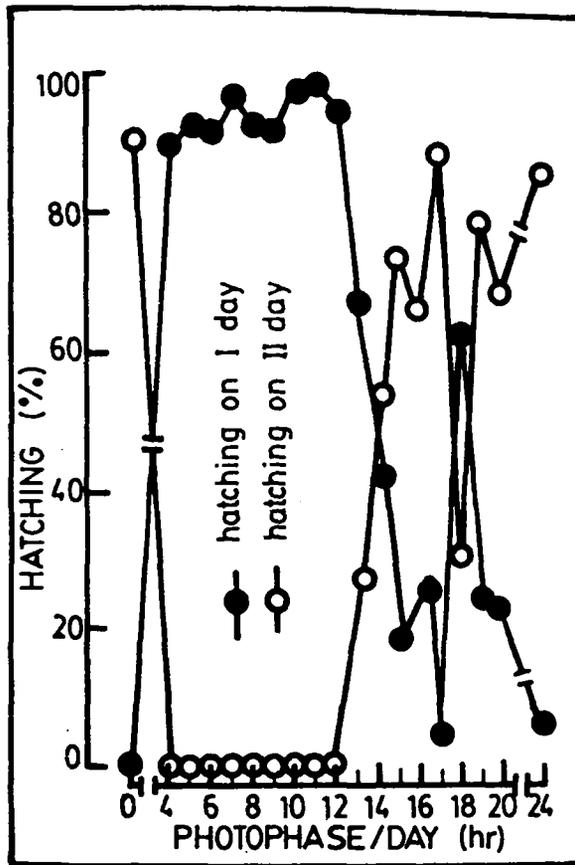


Figure 3. Hatching (%) in *B. mori* (PM x NB4D2) under different photoperiodic conditions. Note single day hatching under continuous dark (DD) and short-day combinations (LD 4:20 to 11:13) as also normal day (LD 12:12) conditions. On the other hand, hatching is less on the first day and more on the second day for all the long-day (LD 13:11 to 20:4) as also continuous light (LL) conditions, apart from prolonged hatching for two days.

daily active peaks; certain hours of the day constitute 'forbidden zones' and certain other hours of the day are 'allowed zones or gates', and are directed by the clocks. Works by Skopik and Pittendrigh (1967) and Pittendrigh and Skopik (1970), indicate that if the animals are not at the 'correct' morphogenic state to utilise one particular allowed zone or gate, they are required to remain waiting till the onset of next gate for utilisation, the intervening hours consisting a 'forbidden zone'. The gate or forbidden zones recur with circadian frequency after the LL/DD transfer. The gating of certain developmental stages by such a mechanism is probably ubiquitous in insect population rhythms (Saunders 1982). It is evident, for example, in pupal eclosion of *Antheraea pernyi* (Truman 1971) egg hatching (Minis and Pittendrigh 1968) and oviposition in *Pectinophora gossypiella* (Pittendrigh and Minis 1964) and in the oviposition rhythm of *Oncopeltus fasciatus* (Rankin *et al* 1972).

Early investigations on the effect of LL on the eclosion rhythm in *D. pseudoobscura* (Pittendrigh and Bruce 1957; Chandrashekar and Loher 1969)

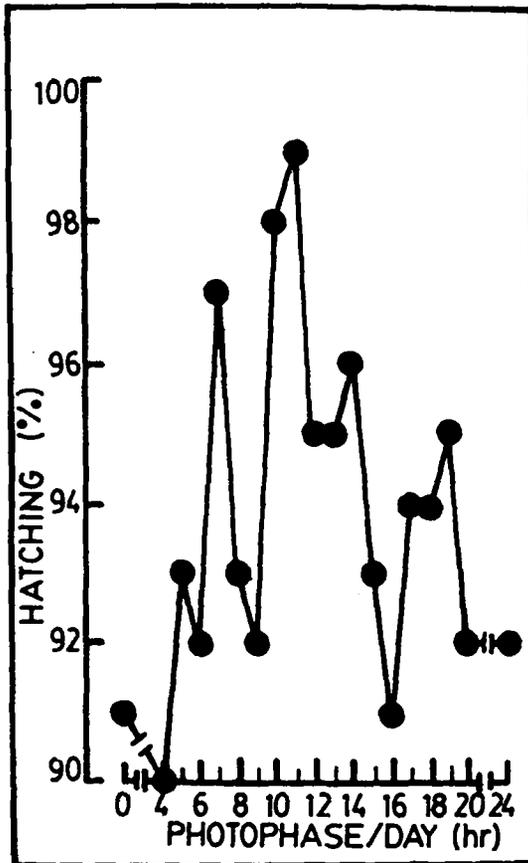


Figure 4. Total hatching (%) in *B. mori* (PM x NB4D2) under different photoperiodic combinations. Note maximum hatching under LD 11:13. Hatching under all the other photoperiodic conditions was <95% but \geq 90%.

showed fairly rapid damp-out to eventual arrhythmicity under LL condition. The results given in Pittendrigh (1960, 1966) implies that, photoperiods longer than 12 h not only damp-out the oscillation but hold the oscillations (in each individual in the population) in the same fixed stage which corresponds to that at circadian time 12, so that, following entry or re-entry into the dark, all the oscillations in the population resume their motion at the same phase. Saunders (1982) is of the view that although the overt rhythm may be damped out in LL, constant light of quite high intensity does not suppress the motion of the underlying pace maker (although it may reduce its amplitude) as he (Saunders 1979) observed broadening of eclosion peaks in *Sarcophaga argyrostama* with higher intensities of continuous light.

Though experimentations were not carried with mixed-age populations in the present investigation, it is clear that the underlying driving oscillation in the hatching of *B. mori* is under the control of the circadian system as hatching follow the gating pattern. Further, hatching peak is broadening with photoperiods more than 12 h as also under LL conditions though not expressing the damp-out situation of the rhythmicity since, the number of photoperiodic cycles experienced

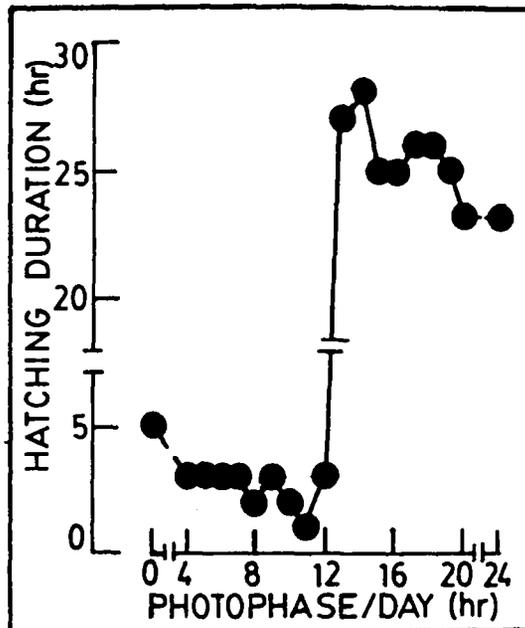


Figure 5. Hatching in *B. mori* (PM × NB4D2) under different photoperiodic conditions. Note less hatching duration under DD and short-day photoperiodic conditions (LD 4:20 to 11:13) as also LD 12:12. On the other hand, hatching duration is more for all long-day combinations as also LL.

by the experimental population is limited (7–8 cycles). Such instances have been widely reviewed by Saunders (1982). A state of arrhythmicity in hatching patterns has been demonstrated in *B. mori* by Anantha Narayana *et al* (1978) after exposure to LL condition from its previous generation.

Eversince the report of Kogure (1933), this species of silkworm was considered as a short-day insect (De Wilde 1962; Danilevskii 1965; Lees 1968; Beck 1980; Saunders 1982). However, Shimizu (1982) demonstrated that with artificial diet, the embryonic diapause in the next generation characterized long-day photoperiodic regime. On a overview, the short-day schedule, in the present investigation, resolved maximum hatching with the shortest hatching duration in *B. mori* offering a chronobiologic approach for a greater return of commercial product (Tucker and Ringer 1982; Sivarami Reddy *et al* 1984).

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