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Implications of Temperature and Humidity on Pupation Patterns in the Silkworm, *Bombyx mori* L.

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The implications of temperature (25, 30 and 35°C) and relative humidity (60, 70 and 80%) on the pupation patterns were studied in the silkworm, *Bombyx mori* L. Larvae of two pure silkworm breeds, Pure Mysore (PM) and NB4D2 and their hybrid, PM × NB4D2 were reared under experimental conditions under natural day photoperiodic (LD 12: 12) condition. The three developmental marker events *viz.*, larval ripening, pharate pupal formation and pupal formation occurred at or around the beginning of the photophase. The computed of mean vector ($\bar{\phi}$), based on the circular statistics, also confirmed the above. However, the length of mean vector, r and the mean vector angular variance, s varied according to temperature and humidity conditions imposed; the variations being non-significant. Extreme temperature and humidity conditions, however, resulted in reduction in pupation rate (%) for PM and PM × NB4D2. On the other hand, in NB4D2 pupation percentage reduced below the economic level. The temperature and humidity together seems to exert synergic impact on the pupation rate at least in the silkworm *Bombyx mori L.*

Key words: Silkworm, *Bombyx mori*, Temperature, Humidity, Pupation

Introduction

Larvae of the commercial mulberry silkworm, *Bombyx mori* enters a period of rapid growth after its fourth and final larval to larval ecdysis. Late in the final instar, feeding ceases followed by ripening, a stage where the larval colour (light ash) turns into yellow. Following this phase, the caterpillars show complete lack of interest in available food, compared with the same situation reported for saturniid silkworms, *Hyalopora cercopia* and *Antheria pyrni* (Lounibos, 1976). At this stage, the larvae of *Bombyx* are picked and mounted on mountages (a device for the silkworm to construct their cocoon) for cocoon spinning (Krishnaswami *et al.*, 1973; Krishnaswami, 1986). The larvae stop feeding about 120 hrs, purge the gut content about 130 h and pupate about 190 h after the time of the first feeding following the fourth moult (Shimada, 1989). Three developmental marker events *viz.*, larval ripening, pharate pupal formation and pupal formation were identified in the pupation patterns of the silkworm, *Bombyx mori* (Sivarami Reddy *et al.*, 1993). The present investigation describes how the pupal ecdysis and its related developmental marker events, such as ripening, pharate pupal formation and pupation, in *Bombyx mori* are influenced under LD 12:12 photoperiodic conditions exposed to different temperatures and humidity conditions.

Materials and Methods

The silkworm, *Bombyx mori* L. was used for the present study. Three silkworm breeds/hybrids, *viz.*, PM, a multivoltine breed, NB4D2, a bivoltine breed and their hybrid, PM × NB4D2 were selected. The DFLs (disease free layings) of the silkworm were procured from the Government Grainages, Hindupur and Madakasira (Andhra Pradesh, India). Five DFLs of each silkworm breed/hybrid were introduced into experimental conditions (Sivarami Reddy and Sasira Babu, 1990). The silkworm rearing was conducted as per Krishnaswami (1986) under LD 12:12 (photophase from 6 hrs to 18 hrs of the day, around 50 lux and scotophase from 18 hrs to 6 hrs) till the completion of

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pupation. Three temperature conditions, *viz.*, 25, 30 and 35°C were imposed in the laboratory throughout experimentation, using an environmental chamber (Kolarstat). Further, three levels of relative humidity (RH) conditions, *viz.*, 60, 70 and 80% were induced. At the end of the 5^{th} instar feeding period, the larvae ripening stage, observations, at one-hour intervals, on the number of larvae ripened were recorded. The ripened larvae were separated from the experimental batches and mounted on a mountage kept in conditions that were identical to the experimental conditions of respective silkworm batch. The cocoons were harvested from the mountages before the expected hours of completion of spinning. Due care was taken that the spinning larvae inside the cocoon are not disturbed. The cocoons were carefully cut open vertically on one side. Half (vertical) portion of the cocoon shell was carefully separated to expose the animals for easy observation.

The animals in the cut-opened cocoons were kept in a tray, in a single layer for further observations on pharate pupal formation, a stage where the silkworm larvae loose their grip with the inner layers of the cocoon and starts shrinking. The observations on the number of pharate pupae formed were noted at one-hour intervals till the completion of pharate pupal formation in the experimental batch. Nearly 24 hrs after the formation of pharate pupa, the animals shed their larval cuticle (pupal ecdysis) to become pupae. Observations on the number of pupae formed were made at one-hour intervals. Data on ripening, pharate pupal formation and pupation were converted in to histograms (distribution diagrams) for each replication and the peak hour has been identified. Using the values on peak hour, the unit (individual) vector of each replication was calculated (resolving a day or 24 hrs into a complete cycle, ω=360°). From these values, the circular variables like mean vector ($\bar{\phi}$), length of mean vector (r) and angular standard deviation (s) corresponding to each developmental phase was computed through circular statistics (Chassé and Théron, 1988). The data on these values ($\bar{\phi}$, r and s) were then analyzed statistically using the test of Mardia-Watson-Wheeler (Mardia, 1972) to test the significance of differences, if any. Finally, the data on pupation rate (%) were also recorded and analysed statistically (ANOVA, 2 - way classification with 5 observations per cell).

Results

Three developmental marker events in silkworm pupation *viz.*, larval ripening, pharate pupal formation and pupal formation were studied. These phenomena for PM × NB4D2 under 25°C and 60, 70 and 80% RH is depicted in Figure 1 as distribution diagram. All the three developmental marker events occurred at or around the beginning

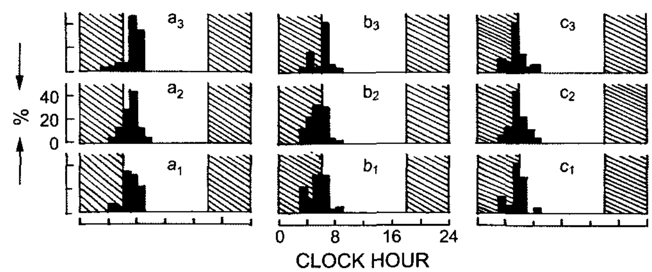

Fig. 1. Distribution of larval ripening (a), pharate pupal formation (b) and pupal formation (c) in the pupation patters of the silkworm, *B. mori* (PM × NB4D2) under LD 12:12 condition and 25°C and relative humidity of 60 (a1, b1 and c1), 70 (a2, b2 and c2) and 80% (a3, b3 and c3). Cross-hatched area represents the scotophase imposed. Note the occurrence of peak hour at or around initiation of photophase.

Table 1. Rhythmic characteristics (circular variables, $\bar{\phi}$, r and s) of developmental marker event, ripening in pupation in PM, NB4D2 and PM × NB4D2 of silkworm, *Bombyx mori* L. under LD 12:12 (n=5)

Breed	Circular variables	RH at 25°C			RH at 30°C			RH at 35°C		
		60	70	80	60	70	80	60	70	80
PM	$\bar{\phi}$	117°	108°	132°	99°	102°	111°	87°	96°	84°
	r	0.99	0.99	0.99	0.99	0.98	0.98	0.98	0.96	0.96
	s	5.99°	5.99°	5.99°	7.34°	11.19°	11.95°	11.23°	16.56°	15.22°
NB4D2	$\bar{\phi}$	129°	143°	145°	137°	129	144°	141°	138°	120°
	r	0.96	0.87	0.81	0.86	0.96	0.96	0.90	0.94	0.95
	s	15.21°	29.34°	34.87°	30.33°	15.21°	15.21°	25.37°	19.68°	17.38°
PM × NB4D2	$\bar{\phi}$	114°	117°	117°	105°	111°	108°	102°	87°	93°
	r	0.98	0.98	0.98	0.96	0.99	0.99	0.98	0.97	0.95
	s	11.95°	11.19°	11.19°	16.29°	7.34°	5.99°	11.19°	14.59°	17.31°

Table 2. Rhythmic characteristics (circular variables, $\bar{\phi}$, r and s) of developmental marker event, pharate pupal formation in pupation in PM, NB4D2 and PM × NB4D2 of silkworm, *Bombyx mori* L. under LD 12:12 (n = 5)

Breed	Circular variables	RH at 25°C			RH at 30°C			RH at 35°C		
		60	70	80	60	70	80	60	70	80
PM	$\bar{\phi}$	105°	102°	114°	96°	96°	99°	84°	84°	87°
	r	1.00	0.99	0.94	0.99	0.99	0.98	0.99	0.98	0.99
	s	0.01°	5.99°	20.22°	7.34°	7.34°	11.95°	7.38°	11.97°	5.99°
NB4D2	$\bar{\phi}$	123°	123°	114°	117°	120°	123°	108°	98°	102°
	r	0.98	0.99	0.98	0.98	0.97	0.97	0.86	0.89	0.88
	s	11.19°	5.99°	11.95°	11.19°	13.38°	14.65°	30.22°	27.13°	28.35°
PM × NB4D2	$\bar{\phi}$	99°	111°	111°	102°	99x	102°	84°	95°	96°
	r	0.99	0.99	0.99	0.97	0.99	0.98	0.98	0.99	0.99
	s	7.34°	7.34°	7.34°	14.59°	7.34°	11.19°	11.95°	9.64°	7.34°

Table 3. Rhythmic characteristics (circular variables, $\bar{\phi}$, r and s) of developmental marker event, pupal formation in pupation in PM, NB4D2 and PM × NB4D2 of silkworm, *Bombyx mori* L. under LD 12:12 (n=5)

Breed	Circular variables	RH at 25°C			RH at 30°C			RH at 35°C		
		60	70	80	60	70	80	60	70	80
PM	$\bar{\phi}$	96°	78°	102°	93°	96°	93°	78°	84°	87°
	r	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.99
	s	7.34°	5.99°	11.19°	5.99°	7.34°	5.99°	6.09°	7.34°	5.99°
NB4D2	$\bar{\phi}$	108°	108°	114°	84°	96°	105°	91°	84°	87°
	r	0.95	0.89	0.95	0.95	0.90	0.89	0.84	0.89	0.90
	s	17.38°	27.18°	17.90°	17.76°	25.84°	26.53°	32.78°	27.13°	25.38°
PM × NB4D2	$\bar{\phi}$	93°	102°	111°	102°	99°	102°	84°	85°	96°
	r	0.99	0.97	0.99	0.97	0.99	0.98	0.98	0.99	0.99
	s	5.99°	14.65°	7.34°	14.59°	7.34°	11.19°	11.95°	9.46°	7.34°

Table 4. Pupation rate (%) in PM, NB4D2 and PM × NB4D2 silkworm of *Bombyx mori* (L) at three temperature (25, 30 and 35 × C) and three relative humidity (60, 70 and 80%) conditions

Breed/ Hybrid	Pupation rate (% ± SD) at temperature (°C)/relative humidity (%) conditions								
	25/60	25/70	25/80	30/60	30/70	30/80	35/60	35/70	35/80
PM	94.00 ±2.191	94.80 ±2.135	96.80 ± 3.54	91.60 ± 3.77	92.00 ± 5.33	94.80 ± 2.64	91.20 ± 6.40	93.80 ± 3.31	94.40 ±2.94
NB4D2*	86.00 ±5.02	84.00 ± 3.74	88.40 ± 3.01	83.40 ± 2.94	82.40 ± 1.36	89.20 ± 2.56	72.00 ± 2.10	75.00 ± 2.83	77.00 ± 5.55
PM × NB4D2	95.40 ±2.65	95.80 ± 3.12	97.20 ± 3.76	96.60 ± 1.85	97.80 ± 1.72	95.40 ± 3.72	97.20 ± 0.75	97.00 ± 1.41	96.00 ± 1.27

*In PM and PM × NB4D2, the effects of temperature and humidity and their interactions are not significant. However, in NB4D2, the effects of temperature, humidity and their interactions were significant at 5% (with f values of 6.428, 39.748 and 2.881 respectively, with error of 15.067) as per ANOVA 2-way classification test.

of the photophase (Fig. 1). The rhythmic characteristics of these developmental marker events, the circular variables computed through circular statistics like mean vector ($\bar{\phi}$), length of mean vector (r) and angular standard deviation (s) of each event for larval ripening, pharate pupal formation and pupal formation are given in Table 1, 2 and 3, respectively. The data presented in Table 1, 2 and 3 clearly indicate that the marker events occurred at or around 6 hrs, onset of photophase, as the mean vector concentrated at $\bar{\phi}$ of 90°. However, the length of the mean vector (r) and the their angular variance (s) differed greatly. As the imposed temperature varied, these values also varied. The least value of the length of mean vector (r) were observed for NB4D2 pupal formation (0.84) under imposed temperature and RH of 35°C and 60% (Table 3). Similarly, highest value for s was observed for NB4D2 pupal formation (32.78°) under the same temperature and humidity conditions (Table 3). The highest value of r and least values of s were recorded for all silkworm breed/hybrids under imposed temperature and humidity of 25°C and 60%. It is generally observed that the length of mean vector and the angular variance are inversely related. However, these variations are not significant.

The results on silkworm pupation rate are furnished in Table 4. The pupation rate was more in hybrid, PM × NB4D2 followed by PM. Least pupation was recorded in NB4D2. Under low temperature (25°C) conditions, the pupation was high compared to other temperature (30 and 35°C) conditions. Further, pupation rate was more under high relative humidity (80%) conditions compared to other two humidity (60 and 70%) conditions. Effects of temperature and humidity and their interaction on pupation rate are more profound in NB4D2 only and the observations were significant at 5% level. On the other hand, the influence of temperature and humidity and their interaction on pupation rate are not significant in PM and PM × NB4D2.

Discussion

With the cessation of feeding and the empty stomach at the close of fifth instar, the fully matured *Bombyx* larvae become translucent and yellowish in appearance which is called the ripening or mounting stage (Krishnaswami *et al.*, 1973). At this juncture, the larvae are mounted on the mountages or cocooning frame for construction of the cocoon. Such ripening larvae normally crawl towards the periphery of the rearing trays in search of a suitable supporting place for cocooning. It is generalized that the transitional phase between larval and pre-pupal behavior can be determined by the cessation of feeding, change in larval colour and wandering in search of a suitable site for cocooning. This stage has been denoted as wandering stage by many workers (Lounibos, 1976; Piepho *et al.*, 1960; Riddiford, 1980; Truman and Taghert, 1981; de Wilde *et al.*, 1980). In *Bombyx* silkworm, this stage has been regarded as ripening stage, since this term is exclusively used in contemporary sericulture (Krishnaswami *et al.*, 1973; Krishnaswami, 1986; Sivarami Reddy *et al.*, 1993). Apart from larval ripening, two more distinctive developmental marker events, pharate pupal formation and pupal formation were reported (Sivarami Reddy *et al.*, 1993).

The observed rhythmic patterns in larval ripening, pharate pupal formation and pupation, in the present study, under all temperature and humidity conditions suggests the circadian and endogenous nature of the rhythmicity. The endogenously free-running nature and circadian control over the phenomenon has been demonstrated in *Bombyx mori* (Sivarami Reddy *et al.*, 1993). All the three developmental marker events exhibited diunal predominance as the events occurred at or around the beginning of photophase. Hatching in *B. mori* was reported as diurnal (Sivarami Reddy and Sasira Babu, 1990). Larval to larval ecdysis was reported to be instar dependent (Krishnaswami *et al.*, 1973; Krishnaswami, 1986; Sivarami Reddy *et al.*, 90). In the present study, the pupal ecdysis is diurnal, may be as a resultant of a long fifth larval stadium (around 8 days) (Truman, 1971; Sivarami Reddy *et al.*, 1993). A comparative analysis of marker events in the pupation patterns in the silkworm *Bombyx mori* under different temperature and humidity conditions through circular statistics (Chassé and Théron, 1988) served as good example of chronobiological markers in population rhythm. The non-significant variations in the $\bar{\phi}$, r and s values indicate the homogeneity nature of the rhythm under temperature and humidity conditions studied.

Insects generally require an adequate level of environmental conditions in order to keep their physiological system working normal (Mellors *et al.*, 1984; Bauer, 1976). Among the environmental conditions, temperature, relative humidity and photoperiod are the most important ones. Datta (1992) emphasized optimum environmental conditions of 30°C and 70% humidity during silkworm pupation stage for economical pupation (%). In PM, higher pupation rate (%) (Table.4) compared to bivoltine breed (NB4D2) was observed. The productive bivoltine breeds are reported to be susceptible to high temperature and low humidity (Kato *et al.*, 1979). As supported by the observation in the present study, pupation rate in NB4D2 is significantly (at 5% level) influenced by both temperature and humidity. The pupation rate (%) in the hybrid, PM × NB4D2 was higher over the two pure breeds, PM and NB4D2 (Table 4). Suresh Kumar and Yamamoto (1995) reported that the hybrids are more tolerant than the pure races. Bursell (1970) also viewed that tropical species are more resistant to temperature than temperate species. Through direct influence on various metabolic, neuronal and hormonal processes, temperature has been shown to exert a profound effect on the growth rate of the insects. On humidity, Bursell (1970) viewed that this environmental factor effects the insects mainly and indirectly. Like temperature, humidity affects the developmental rate at which the insects complete their development (Bursell, 1970). Pittendrigh (1966) viewed that insects follow the phenomenon of minimizing the risk of desiccation, as demonstrated in *Drosophila* eclosion. The significant effects of both temperature and humidity and their interaction effects on pupation patterns strongly support that temperature and humidity seems to play a synergic impact on the pupation patterns in the silkworm in general or at least in NB4D2, though not on the rhythmic pattern in the marker events of pupation pattern.

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