[Review]

Physiological and Biochemical Modulations during Oviposition and Egg laying in the Silkworm, *Bombyx mori* (L.)

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Oviposition and egg laying is an important physiological and behavioural event in the life cycle of the silkworm, *Bombyx mori* (L). Oviposition and egg laying is dependent on a number of intrinsic and extrinsic factors *viz.*, neural, hormonal, environmental, physical, behavioral etc for the perpetuation of population. Although, the virgin female moths have fully developed embryos but active egg laying begins under the influence of mating which provides essential copulation stimulus for oviposition. After mating drastic biochemical changes occurred that incites egg laying under the influence of optimum environmental conditions. Weight of pupae as well as larval density has significant role on oviposition and egg laying behaviour in the silkworm wherein high pupal weight and inadequate rearing space affects not only the biology, morphology and physiology but also the oviposition and egg laying. Surface topography, plane of inclination, mating length, age of male moth, temperature and photoperiod etc has significant effect on reproductive physiology of silkworm. An attempt has been made in this review article to elucidate briefly the works carried out on mating behaviour, direct and indirect copulation stimulus, vitellogenesis, influence of environmental factors besides effect of weight of pupae and or pharate adult and larval density on oviposition and egg laying behavior in the silkworm, *B. mori* and its significance in silkworm seed production.

Key words: *Bombyx mori*, Oviposition, Egg laying

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Introduction

Oviposition and egg laying behaviour has been at the centre of many major debates since long. Ever since, Ehrlich and Raven's (1964) report on co-evolution of butterflies and plants, a large number of species belonging to the order Lepidoptera has probably been studied for various aspects of oviposition behaviour (Singh, 1998; Samson and Biram Saheb, 1999). The biological factors depending upon behavioural patterns and physiological conditions of male and female members of insects searching each other for mating and reproduction have been reported to be responsible for perpetuation of the population. Oviposition and egg laying is an important physiological and behavioural aspect in the life cycle of the silkworm, *B. mori*. It is an act of reproduction through which viable eggs are laid by fertilized female moths. A number of factors and events of significant importance are involved in successful egg deposition which include neural, hormonal, chemical, environmental, physical and behavioural (Yamaoka and Hirao, 1975, 1977, 1981; Raabe, 1986; Singh, 1998). Besides these, successful and viable egg deposition by an adult female moth also depends upon various events of significance importance of reproductive physiology *viz.*, mating (Petkov *et al.*, 1979), vitellogenesis, ovulation (Fugo and Arisawa, 1992), oviposition (Davey, 1985), environmental conditions (Singh, 1998), weight of pupae and or pharate adult (Govindan *et al.*, 1990), surface texture and plane of inclination (Legay, 1989; Fuzimoto, 1951). An attempt has been made in this review article to discuss briefly the works carried out on factors influencing oviposition and egg laying behaviour in the silk moth, *B. mori* (L) and its significant role in egg production technology.

Mating behaviour

Developmental events that occur once in the life cycle of an insect have long been known to express at specific part

of the day to manifest a population rhythm (Saunders, 1982). A range of adult behaviour is exhibited in succession after eclosion in the silkworm. The eclosion occurs during the opening of a particular gate. The insects that had completed development are able to emerge at this gate. If the insects are not competent enough to emerge from pupal case, must wait for the next open gate. The term gate is actually applied to those events, which occur only during a limited span of time. Thus, gate is being opened for a specific period of time once each day. In silk moths, the communication between sexes is accomplished through chemical signals after emergence. Butenandt *et al.* (1959) after extensive studies stated that substance secreted to out side by an individual and received by another individual of the same species, in which that releases a specific reaction is pheromone. The female moth secretes sex pheromone from the lateral glands of the last abdominal segment to attract male moth for mating. This pheromone is identified and named as Bombycol (10-12-hexa-decadien-1-ol) with a chemical structure of CH_3 $(CH_2)2$ $(CH = CH)2$ $(CH_2)8$ CH_2OH.

The male moths after eclosion stay ideal for a few minutes, and only upon the perception and deciphering the chemical by the sensory cells present in the antennae, a change in the excitory level of the receptor cells is brought about and then they exhibit the characteristic dancing movements (Samson and Biram Saheb, 1999). The scales of silk moth also serve as a releaser of copulation attempt followed by wing vibration and mating dance by male moths (Obara, 1979). Mating dance helps in determining the location of the conspecific female (Singh and Mathur, 1989). The chemoreceptive abilities of male to bombycol produced by female are very high. Males react to bombycol at a concentration as low as 100 molecules of attractant per cubic centimeter of air. Even a single molecule of female sex pheromone is sufficient to trigger an impulse in the male receptor cells. This is due to presence of large number of sensilla (60,000) on the bipectinate antennae of moth, whose surface area with all the remi included is approximately 29 mm^2. During mating dance, male produces a strong air current with its wings, which flows parallel to bodyís longitudinal axis and traces the pheromone source by testing the air. Further, dancing male can draw air from in front of its head while wing vibrations draw air from the sides of its area, thus improving the efficiency of antennae (Singh, 1998).

The response of male moths to the female secretion pheromone is not just a simple attraction but also a complex sequential series of events by anemotaxis, which occurs, in zigzag stages (Singh and Mathur, 1989). The stimulated males clean their antennae, lift head, vibrate wings gradually from lower amplitudes to higher ampli-

tudes, resort to unoriented movements such as straight lines, circles, semi-circles, zigzags with their abdomen bent till they locate the conspecific female and starts mating. The nature of mating involves meeting of abdominal ends, which is also known as ëend-to-end patterní mating. For successful oviposition three factors or events are of utmost importance *viz.*, the mating partner should be reproductively competent, the mating should be at right time and the mating should be followed by transfer of sperm and male factor (oviposition stimulating substance or fecundity enhancing substance) into the reproductive tract of the adult female. The mating lengths in silkworm essentially affect silkworm seed quality and quantity. Various reports are available on the duration of mating and its effects on oviposition and egg production efficiency (Petkov *et al.*, 1979; Tripathi and Singh, 1995; Samson and Biram Saheb, 1999). In *B. mori* natural copulation continues for 6 – 12 hrs and sometimes because of such prolonged copulation female moth dies without laying any eggs (Tanaka, 1964). Although the number of eggs laid by a female moth depends on its genotype and development besides environmental conditions but the duration of mating affect it considerably (Askari and Sharan, 1984). Punitham *et al.* (1987) stated that mating duration up to 6 hrs increased the total egg output and reduced pre-oviposition period but subsequent increase in duration resulted in negative effects.

Moreover, mating lengths of 150 – 180 minutes is as optimal variants for enhancing productivity and quality of silkworm seed (Petkov *et al.,* 1979). Further, it is stated that for production of reproductive seed, mating for less than 120 – 150 minutes should not be allowed, while for production of commercial seed, it should not be less than 90 – 120 minutes. A minimum period of 45 minutes mating is adequate to ensure normal egg laying with regard to both fertility and number of eggs laid and four mating can easily be adopted without affecting any of the commercial traits, such as number and viability of eggs, cocoon weight, shell weight and yield of good cocoons (Jolly *et al.,* 1966). The re-use of male moths is a common practice in commercial egg production. Male has the inherent capability to mate with certain number of females without affecting fecundity (Jadav and Gajare, 1978; Askari and Sharan, 1984; Ram and Singh, 1992; Vijayan *et al.*, 1994) after which both fecundity and fertility are drastically affected. The repeated mating performance of male is race specific and hence a general recommendation cannot be made for various silkworm races as also opined by Sidhu *et al.* (1967) and Samson and Biram Saheb (1999). However, Petkov and Mladenov (1979) observed that male silk moth could successfully mate with 8 females of the same origin. Ram and Singh (1992) stated that a single male

could mate with at least 11 females in bivoltine silk moths but higher percentage of viable eggs can be obtained only from the first mating. Gupta *et al.* (1986) stated that male of multivoltine and bivoltine can mate with 16 and 18 females respectively.

Mating capacity of males decreases significantly with increase of age and a significant negative correlation between the age of males and mating capacity have been established (Paul and Kishor Kumar, 1995). Further, the mating capacity of males of *B. mori* pure breeds as compared with hybrids was studied by Benchamin *et al.* (1990) with repeated mating (6 times a day) for six consecutive days (with rest periods at 5°C between mating) and stated that fecundity in multivoltine female parent was not influenced by the male but other characters including effective rate of mating, fecundity and fertility differed significantly among different crosses. All quantitative characters seem to decrease significantly with the increase in the number of mating. Though a male moth can mate to a large number of females but many fold use of male silk moths for mating, results in decrease in the basic biological characters, which includes not only cocoon technological qualities but also the fertility of resulting moths. In case two consecutive mating are contemplated on the same day, besides intermittent rest of 2 hrs between the two mating, a pairing duration of 3 hrs and 5 hrs is adopted for the 1st and 2nd pairing respectively (Wang, 1994). Further, mating from 3rd fold onwards results in decrease of hatchability, viability, cocoon yield, cocoon weight, silk yield, filament length etc and hence future technologies should not allow more than two fold mating for the production of silkworm seed to obtain better results not only in fertility but also for stable cocoon crop. Babu (1993) while evaluating the effects of continuous and discontinuous mating of the male moths reported a general trend in reduction of fecundity after first mating but significant decrease in fecundity from 3rd mating onwards. Further, more unfertilized eggs are laid in continuous type of mating from 2nd mating onwards while in the discontinuous type it is evident from 3rd mating onwards. Therefore, intermittent rest is dispensable to enable the moths to draw the requisite content of energy during each mating and hence it is imperative to preserve the male moth for 2 hrs at 5 ± 2°C for successful second fold re-use (Subramanyam and Murthy, 1987). Repeated mating enhances fecundity only in those species where the insects feed at the time of mating, but in mulberry silkworm, a single mating is sufficient to produce a full complement of fertile eggs (Singh *et al.*, 1994). However, contradictory reports exist regarding the duration of mating and increased egg recovery (Gowda, 1988; Jadav and Gajare, 1978; Ram and Singh, 1992). It is also known that

despite adequate pairing duration of 3 – 4 hrs, a few males fail to ejaculate probably due to their weak physiological status (Samson and Biram Saheb, 1999).

Copulation stimulus

Mladenov (1990) stated that ejaculation occurs after 10 minutes of mating. At a shorter than 10 minutes mating duration, female moths could be utilized for production of hybrid silkworm seed with no risk of pure race contamination. Krishanaswamy *et al.* (1973) and Tripathi and Singh (1995) reported that first ejaculation starts about 9 minutes after copulation and completed after 25 minutes of mating and hence recommended optimum mating duration of 30 minutes to obtain desired number of viable eggs which is sufficient to provide copulation stimulus essential for egg laying without impairing the percentage of fertility. Tazima (1978) observed that the 1st ejaculation might take place during the first 30 minutes and second only one to one and half hrs later. Although in silkworm *B. mori*, the virgin female moths have fully developed eggs (Eidmann, 1931) whose development takes place during pupal stage itself but lack of copulation reduces the number of eggs laid. The copulation provides some essential stimulus for oviposition and in *B. mori*, the stimulus which incites egg laying is not the direct stimulus of copulation but is the stimulus by spermatozoa moving from the recepticulum seminalis to the vestibulum (Omura, 1939). Drastic biochemical changes occurred in the spermatophore of the silkworm after mating (Osanai *et al.*, 1990). This assured the presence of normal sperm and or testicular fluid in the female reproductive organ (bursacopulatrix / spermatheca) in sufficient number to induce the ovipositional behaviour (Davey, 1985; Raabe, 1986). On an average, normal male moth have 15.4 × 10^5 spermatozoa per head (256 sperms / bundle × 3000 bundles × 2 testis). Besides the sperms, a lot of viscous secretions are supplemented along with sperm for their maturation and nutrition. It is generally recognized that the male factors for the oviposition derived from the male reproductive tract are transferred to female during mating and these factors have the ability to accelerate the oviposition behaviour (Davey, 1985; Raabe, 1986). The male factor has been identified chemically in some insect order including Lepidoptera and is being termed as 'fecundity enhancing substance' (FES) or 'oviposition stimulating substance' (OSS). These factors in the silkworm (*B. mori*) have been identified as 'Prostaglandins' (Setty and Ramaiah, 1979). Oviposition is stimulated in the female moth following the transfer of prostaglandins during mating. Hence, level of prostaglandins increases in mated females. Brady (1983) stated that prostaglandin increases egg laying in the silk moth by stimulating muscles in the oviduct

in the same way as is being reported in egg laying vertebrates (Wechsung and Houvenaghel, 1976). The mated females lay majority of eggs on the 1st day of oviposition and frequency of eggs laid decreases gradually in subsequent days (Gowda, 1988).

Vitellogenesis

It is a process through which the terminal oocytes grow up to final size before ovulation and or oviposition. This is achieved by incorporation of female specific protein ëvitellogeniní via haemolymph. The fat body and follicle cells of the ovary synthesize the vitellogenin at a specific age in the insect life cycle. These proteins have been found to be precursor of the egg yolk protein. The vitellogenin are termed 'vitellin' when deposited in the eggs. The vitellin and other similar yolk protein make up approximately 80% of the yolk proteins. The synthesis of vitellogenin is under the control of juvenile hormone and ecdysteroids. The juvenile hormone (JH) is secreted by corpora allata (CA) and is regulated (stimulated or inhibited) by neurosecretory cells of the brain and some other factors. The ecdysone secreted from prothoracic gland responsible for moulting, also plays a major role in reproduction and follicle cell epithelium is the exact site of ecdysone biosynthesis. These ecdysteroids are either secreted into haemolymph or retained and accumulated in oocytes to play a major role in vitellogenesis. Juvenile hormone secretion causes the ovary to become prepared to respond to a neurohormone, termed as 'Egg Development Neurohormone' (EDNH). EDNH triggers the synthesis and release of ecdysone. Ecdysone, after its hydroxylation to 20 - hydroxy ecdysone, stimulates vitellogenin synthesis.

Oviposition

Ovulation is the process by which eggs passes from the ovary into the oviduct and oviposition is the passage of the eggs from oviduct through external genital opening of the female to the substratum / egg laying site. Ovulation and oviposition are related at least to the extent that ovulation is a pre-requisite for oviposition. In other words, oviposition will not occur unless ovulation has taken place and in that oviposition frequently follows ovulation. Oviposition in silkworm is dependent on a number of intrinsic and extrinsic factors *viz.*, neural, hormonal, environmental, physical, behavioural etc. It is a complex phenomenon involving multiplicity of coordinated events and such interaction between internal and external genetalia and the abdominal muscular system must be integrated in an orderly manner for the systematic oviposition (Yamaoka *et al.*, 1971).

The mechanism controlling the switch from virgin to mated behaviour is not exactly known. Giebultowicz *et al.* (1990) reported that in *Lymantria* moth, the switch from virgin to mated behaviour can occur spontaneously in senescing virgin females. The same holds true also in *B. mori* (Fugo and Arisawa, 1992). The bursa copulatrix and the spermatheca seem to be involved in the behavioural switching pattern from virgin to mated condition (Davey, 1985; Raabe, 1986). It is established that active egg laying begins under the influence of mating. The stimuli which are applied to the mated females are both direct stimuli *viz.*, insertion of external genetalia, grasping by claspers and the repletion of the bursa copulatrix with the seminal fluid, and indirect namely migration of spermatozoa from bursa copulatrix to vestibulum via receptaculum seminis (Omura, 1939). Mating provides a stimulus for activating ovulation. Information regarding mating is conveyed to the brain through 'spermathecal factor' released from spermatheca of mated females. This stimulation regulates the brain to release 'myotropic peptide' or 'ovulation hormone' or 'oviposition stimulating substance' (OSS), which affect the activity of the last abdominal ganglion of female moths for stimulation of oviposition (Yamaoka and Hirao, 1973, 1977, 1981). An actively ovipositing moth touches with its caudal tip the surface of egg card or margin of the previously laid eggs turning its abdomen from right to left or left to right. On the caudal tip a pair of anal papillae is present and sensory hairs on them serve as mechanoreceptors to perceive the textural conditions of the oviposition substratum (Yokoyama *et al.*, 1971). The duration of mating has immense effect on the oviposition rhythm. Longer duration of mating showed highest % of eggs laid within 6 hrs of decoupling (Samson and Biram Saheb, 1999). This is interpreted as a consequence of the interaction of factors such as increased egg maturation, level of humoral factors, inhibition of egg laying and strong operation of an endogenous rhythm during egg laying period (Gowda, 1988). Oviposition is faster in Chinese races followed by Japanese and European races (Wang, 1994). After mating, the female moths started to look for oviposition site. Female moths start to oviposit their eggs mostly at dusk and about 90% of the eggs that had matured are being deposited in a short span of time during night. Hardly 9.00% egg laying took place from 24 hrs to 48 hrs, while less than 1% eggs in each hour are being laid from 16 hrs to 24 hrs of decoupling.

Environment

Virgin female requires optimum condition of environment for oviposition, which must be met otherwise it will lay only few eggs or none. Bliss (1927) was apparently the first to distinguish between the effect of temperature on the development of oocytes and its effect on oviposition

behaviour. The rate of oviposition varies with temperature, which can be accelerated up to a point and then falls off rapidly. But the temperature limits between which oviposition can occur are often much narrower than the range of temperature over which the other activities of the same species remains normal. The male seems often more sensitive than female to abnormal temperature. When 5th instar larvae were exposed to high temperature (32°C) for 72 hrs, the emerging males showed complete sterility (Sugai and Takahashi, 1981). Maximum ovulation and oviposition with minimum retention of egg can occur at 25.36 ± 0.17°C (optimum) temperature and 80 ± 5% relative humidity and any fluctuation of temperature from optimum level leads to decreased ovulation, oviposition rate, fecundity besides increased retention of eggs.

Usually dark conditions favour rapid oviposition. Maximum fecundity and minimum egg retention were recorded when the females were exposed to fluorescent light (80 lux) during day and darkness at night (Mathur et al., 1995). Temperature and RH both influences ovulation and fecundity significantly (Samson and Biram Saheb, 1999). They stated that at higher temperature (30°C and above) both fecundity and fertility are severely affected. The peak of oviposition changes with duration and time of mating. Usually two peak oviposition rhythm, the first one before and after dusk and the second one between midnight and dawn is noticed. Yamaoka et al. (1976) stated that mated females showed involvement of a photoperiodic control of a circadian rhythm in egg laying. Photoperiod in insects, serves as a clock indicating the seasonal changes and influencing its life cycle, distribution and abundance. Several workers have demonstrated the influence of photoperiod on the behaviour of silkworm. Photoperiod plays an important role on oviposition (Wang, 1994) and in that, dark condition favours rapid oviposition, while bright has opposite effect (Akhundov, 1968).

Surface texture

Texture of the substratum for oviposition considerably influences the egg laying behaviour in the silk moth, *B. mori* (Yamaoka et al., 1971; Gupta et al., 1990; Nangia and Ramkumar, 1997) which includes the number of eggs laid, total time taken for complete egg laying and the rate of egg deposition. The sensory hairs (sensory receptors) on the ovipositor and on the anal papillae in silk moth influences oviposition through the sensory input received by these hairs. Maximum number of eggs is reported to be oviposited on a smooth surface (Gupta et al., 1990) and this decrease with the increase in roughness of the substratum (Legay, 1989; Manjulakumari and Geethabai, 1991). Responses to surface topography or texture by ovipositing females are mediated by tactile setae. In *B. mori*,

tactile setae on a pair of anal papillae are innervated from the last abdominal ganglion. Silkworm normally lay eggs in a monolayer and the ovipositing female keeps touching the surface or margin of the group of previously laid eggs with its anal papillae turning its abdomen from side to side. If the tactile hairs on the papillae are first cut and then burnt with acid, so that dendrites are destroyed, oviposition is disorganized and the eggs are often laid in piles instead of monolayer. Besides surface topography, the angle of inclination also played an important role in oviposition (Fuzimoto, 1951; Legay, 1989) and in that slightly inclined plane of oviposition site leads to higher fecundity (Jolly et al., 1964).

Weight of pupae or pharate adult

The number of eggs in the body of female silk moth depends on its genotype and development. Highly significant positive correlation of fecundity in some sericigenous moths, *Antheraea mylitta* (Badhera, 1992), *Philosamia ricini* (Singh and Prasad, 1987), *Samia cynthia ricini* (Kotikal et al., 1989; Govindan et al., 1993) with pupal weight have already been established. High level of linear relationship between female pupal weight and potential fecundity as well as pupal weight and eggs laid has also been studied in various insects. In the silkworm (*B. mori*) the heaviest pupae resulted in highest fecundity (Govindan et al., 1990). Further, the maximum larval weight, cocoon, shell and pupal weights, moth emergence and fecundity in succeeding generation were also higher in the progeny resulted from the heaviest female pupae. Highly significant positive correlation (Table 1) between female pupal weight and fecundity has been demonstrated in *B. mori* (Jayaswal et al., 1991; Singh, 1994; Singh and Kumar, 1995). Though the selection of individuals for higher fecundity depends on its pupal weight but extreme heavy weight should be discouraged as it may sometimes lead to bottle-neck phenomenon (Singh et al., 1998).

Table 1. Correlation between female pupal weight and fecundity in the silkworm *Bombyx mori* (c.f. Singh, 1994)

Female pupal weight (gm)		Average fecundity	Correlation coefficient
Range	Average		
1.000 – 1.200	1.12308	443.600	+0.8720**
1.201 – 1.300	1.24280	468.040	+0.9209**
1.301 – 1.500	1.41356	487.880	+0.8646**
1.501 – 1.700	1.54728	535.360	+0.8456**
1.701 – 1.800	1.74440	559.280	+0.8366**
Pooled data			
1.000 – 1.800	1.41422	498.831	+0.7820**

**Significant at 1% level of significance.

Therefore, moderate pupal weight should only be encouraged for egg production as it determines increased larval weight, survival rate, filament length etc in the successive generations (Gowda *et al.*, 1989). Female pupal weight, which is positively correlated with fecundity, has also been reported positively correlated with larval weight and shell ratio (Gowda *et al.*, 1989), cocoon weight (Singh, 1994) and moth weight (Gupta *et al.*, 1991).

Larval density

Insect growth and development proceed optimally under certain population density. Larval population density has a great impact on biology, morphology and physiology of insects (Peters and Barbosa, 1977). Larval crowding due to inadequate rearing space has also been found to increase the larval duration and mortality; reduce the larval, pupal and imaginal weights and affects morphology, longevity, fecundity and fertility of the resulting moths in several representatives of the order Lepidoptera (Hinton, 1981). In silkworm, *B. mori* the larval behaviour, yield of cocoons, cocoon features and resultant effect on fecundity are determined by the space provided to the larvae especially during rearing of 5th instars. The space requirement for the larvae during rearing is maximum a day or two before spinning. At this stage, larvae increases by about ten thousand times in weight, seven thousand times in volume and four hundred times in body surface in comparison to newly born larvae to become full grown mature larvae. Under these circumstances, it becomes essential to provide adequate spacing in rearing bed to enable the larvae to eat enough actively in accordance with their growing stage to ensure successful harvest of bumper cocoon crop.

As the weight of female moth is directly related to its weight as a pupa and as a larva, and there is therefore usually a close relation between the fecundity of the adult and the weight of its pupa and larva. Positive effect of wider rearing space on larval growth, fecundity, hatchability and also on various economic characters of cocoons have been established (Roychoudhury *et al.*, 1991; Rahman *et al.*, 1991; Singh, 2001) from which it can be inferred that an increase in population density in rearing bed decreased fecundity and hatchability of *B. mori*. Rearing space adopted in different countries indicates wider spacing under Chinese schedule than Japanese and Indian for rearing of same number of larvae of any stage. The recommended Indian spacing for rearing of different stages of silkworm larvae is in between Chinese and Japanese schedule (Roychoudhury *et al.*, 1991; Singh, 2001). Studies conducted and comparison made for three kinds of spacing schedule under Indian agro-climatic conditions reveals that wider spacing as adopted in Chinese schedule

Table 2. Correlation between various economic parameters in silkworm *Bombyx mori* influencing egg production

Correlation between	Type of correlation	References
Fecundity & productivity	Negative	Kasivishwanathan (1976)
Fecundity & robustness	Negative	Gowda *et al.* (1989)
Fecundity & female pupal weight	Positive	Jayaswal *et al.* (1991)
Fecundity & moth weight	Positive	Gupta *et al.* (1991)
Pupal weight & larval weight	Positive	Gowda *et al.* (1989)
Pupal weight & shell ratio	Negative	Singh *et al.* (1992)
Pupal weight & cocoon weight	Positive	Singh *et al.* (1998)
Larval density & fecundity	Negative	Kasivishwanathan (1976)
Larval density & pupal weight	Negative	Kasivishwanathan (1976)
Larval duration & survival	Negative	Kasivishwanathan (1976)
Larval weight & fecundity	Positive	Singh and Kumar (1995)
Cocoon weight & egg weight	Positive	Singh *et al.* (1998)
Cocoon weight & fecundity	Positive	Singh and Kumar (1995)

holds better than both Japanese and Indian schedules. Talukdar *et al.* (1991) stated that increase in larval density had adverse effect on larval and cocoon characteristics besides oviposition and egg laying behaviour. Shivaprakasam *et al.* (1997) suggested adopting optimal larval density to obtain higher effective rate of rearing and better qualitative and quantitative parameters. Studies conducted reveals that larval crowding has negative correlation with fecundity and pupal weight and its duration with survival, reelability and neatness (Kasivishwanathan, 1976) (Table 2).

It could be inferred from the above discussion that reproductively competent mating partner should only be allowed for perpetuation of population. Mating duration should at least be 3 hrs for optimum productivity and quality of seed. Future technologies should not allow more than two fold mating for production of seed. Moreover, male moths should be preserved at 5 ± 2°C for at least 2 hrs for successful second fold reuse. Optimum temperature (25 ± 1°C), photoperiod and relative humidity (80 ± 5%) during oviposition should be maintained. Any fluctuation from optimum level affects ovulation, oviposition rate and fecundity. Dark conditions should be maintained during oviposition. Texture of the ovipositing site

should be somewhat smooth. Too much high pupal / adult weight should not be allowed for egg production but moderate weight should only be encouraged. Wider spacing of rearing should be followed which have positive influence on various economic characters of paramount importance.

References

Akhundov, Z. I. (1968) Effect of light factors on the oviposition and quality of silkworm eggs of *Bombyx mori*. *Dokl. Akad. Nauk. Azerb. SSR*. **24**, 70-75.

Askari, S. and R. K. Sharan (1984) Studies on different copulating duration on pre-oviposition, fecundity and fertility of mulberry silkworm, *Bombyx mori* L. (Bombycidae: Lepidoptera). *J. Adv. Zool*. **5**, 114-119.

Babu, G. K. S. (1993) Studies on some aspects of industrial seed production in silkworm, *Bombyx mori* L. Ph. D. Thesis, Univ. Mysore, India.

Badhera, S. (1992) Relationship between pupal dimensions and fecundity in tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *Uttar Pradesh J. Zool*. **12**, 37-39.

Benchamin, K. V., S. B. Magdum and N. Shivashankar (1990) Mating capacity of male moths of pure breeds and hybrids in silkworm, *Bombyx mori* L. *Indian J. Seric*. **29**, 182-187.

Bliss, C. I. (1927) The oviposition rate of the grape leafhoppers. *J. Agric. Res*. **34**, 847-852.

Brady, U. E. (1983) Prostaglandin in insects. *Insect Biochem*. **13**, 443-451.

Butenandt, A., R. Bechmann, D. Stamm and E. Hecker (1959) Uber den Sexuallockstoff des seidenspinners *Bombyx mori*. Reinderstellung und Konstitution Z. *Naturforsch B*. 14, 283.

Davey, K. G. (1985) The female reproductive tract; in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Vol. I. Kerkut G. A. and L. I. Gilbert (eds.), pp.15-36, Pergamon Press Oxford.

Eidmann, H. (1931) Female reproductive system in Lepidoptera. *Z. Angew Entomol*. **18**, 57-112.

Ehrlich, O. P. and P. H. Raven (1964) Butterflies and plants; a study in co-evolution. *Evolution* **18**, 586-608.

Fugo, H. and N. Arisawa (1992) Oviposition behavior of the moths which mated with males sterilized by high temperature in the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn*. **61**, 110-115.

Fujimoto, N. (1951) Laying of eggs on the inclined plane in the silkworm moth. *J. Seric. Sci. Jpn*. **20**, 258.

Giebultowicz, J. M., R. A. Raina and E. C. Uebel (1990) Mated like behavior in senescent virgin females of Gypsy moth, *Lymantria dispot*. *J. Insect Physiol*. **36**, 495-498.

Govindan, R., T. K. Narayanaswamy and J. Ashoka (1990) Influence of pupal weight in multivoltine silkworm, *Bombyx mori* (L.) on some metric parameters. *Mysore J. Agric. Sci*. **24**, 499-502.

Govindan, R., T. K. Narayanaswamy and J. Ashoka (1993) Effect of female pupal weight in the eri silkworm, *Samia*

cynthia ricini on quantitative traits. *Bull. Seric. Res*. **4**, 77-79.

Gowda, B. L. V., T. K. Narayanaswamy and R. Munirajappa (1989) Impact of pupal weight on growth and development of the following generation in the silkworm Indian race NB7 (*Bombyx mori*). *Sericologia* **29**, 481-489.

Gowda, P. (1988) Studies on some aspects of egg production in silkworm, *Bombyx mori* L. Ph. D. Thesis, Univ. Mysore, India.

Gupta, B. K., A. K. Sinha and B. C. Das (1991) Studies on egg yielding capacity of different races of silkworm (*Bombyx mori* L.). *Geobios* **18**, 173-176.

Gupta, B. K., A. K. Sinha, S. Biswas and B. C. Das (1986) Assessment of number of viable sperms of male moth of silkworm (*Bombyx mori* L.). *Geobios New Reports* **5**, 193-195.

Gupta, B. K., V. K. Kharoo and N. K. Sahani (1990) Effect of different laying sheets on number of eggs laid by silkworm (*Bombyx mori*). *Bioved* **1**, 79-80.

Hinton, H. E. (1981) Biology of Insect Eggs. Pergamon Press, Oxford, Vol. I, pp.413.

Jadav, L. D. and B. P. Gajare (1978) Studies on the effect of mating durations on the viability of silkworm (*Bombyx mori* L.) eggs. *Indian J. Seric*. **17**, 28-32.

Jayaswal, K. P., T. Singh and G. Subba Rao (1991) Effect of female pupal weight on fecundity of mulberry silkworm *Bombyx mori*. *Indian J. Seric*. **30**, 141-143.

Jolly, M. S., S. Subba Rao and S. Krishnaswamy (1964) Studies on the mating capacity of male of mulberry silkworm and the possibility of utilizing polygamy in sericulture. *Indian J. Seric*. **1**, 25-32.

Jolly, M. S., S. Subba Rao and S. Krishnaswamy (1966) Effect of plane of inclination on egg laying by *Bombyx mori* L. *Indian J. Expt. Biol*. **2**, 165-166.

Kasivishwanathan, K. (1976) Silkworm breeding and genetics. *Indian Silk* **14**, 13-21.

Kotikal, Y. K., D. N. R. Reddy, A. S. Prabhu, G. G. Bhat and S. Pushpalatha (1989) Relationship between pupal size and egg production in eri silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae). *Indian J. Seric*. **28**, 80-82.

Krishnaswamy, S., M. N. Narasimhanna, S. K. Suryanarayana and S. Kumararaj (1973) Sericulture Manual, Vol. II., Silkworm rearing, FAO Agriculture Services Bulletin, Rome.

Legay, J. M. (1989) Contribution to the typological analysis of the laying behaviour in *Bombyx mori* L. *Sericologia* **29**, 163-166.

Manjulakumari, D. and M. Geethabali (1991) Ovipositional response of *Bombyx mori* (L.) to different textures of the substratum. *Entomon* **16**, 11-15.

Mathur, S. K., A. K. Roy, S. K. Sen and G. Subba Rao (1995) Fecundity and *Bombyx mori* L. ñ Light effects on silkworms. *Indian Text. J*. **106**, 74-76.

Mladenov, G. (1990) A study of the ejaculation of the silkworm, *Bombyx mori*. *Zhivotnov'd Nauki* **27**, 77-79.

Nangia, N. and S. R. Ramkumar (1997) Ovipositional response of two new breeds of mulberry silkworm to substratum.

Indian J. Seric. **26**, 167-168.

Obara, Y. (1979) *Bombyx mori* mating dance: An essential in locating the females. *Appl. Entomol. Zool.* **14**, 130-132.

Omura, S. (1939) Oviposition mechanism of the silkworm moths. I. Stimulation to elicit the oviposition. *J. Seric. Sci. Jpn.* **10**, 47-49.

Osanai, M., H. Kasuga and T. Aigaki (1990) Physiology of sperm maturation in the spermatophore of the silkworm, *Bombyx mori. Adv. Invertebr. Reprod.* **5**, 531-536.

Paul, D. C. and C. M. Kishor Kumar (1995) Influence of male age on mating capacity, fecundity and fertility of mated female silk moth, *Bombyx mori* L. under high temperature and high humidity conditions. *Entomon* **20**, 253-255.

Peters, T. M. and P. Barbosa (1977) Influence of population density on size, fecundity and developmental rate of insects in culture. *Rev. Entomol.* **22**, 431-450.

Petkov, N. and G. Mladenov (1979) Study on the multiple use of silk moth (*Bombyx mori*) male individuals in the production of pedigree and commercial silkworm seed. *Anim. Sci.* **16**, 107-115.

Petkov, N., A. Yolov, G. Mladenov and I. Nacheva (1979) Influence of mating length of silk moths of some inbred silkworm (*Bombyx mori*) lines on silkworm seed quality and quantity. *Anim. Sci.* **16**, 116-122.

Punitham, M. T., M. A. Haniffa and S. Arunachalam (1987) Effect of mating duration on fecundity and fertility of eggs in *Bombyx mori* (Lepidoptera: Bombycidae). *Entomon* **12**, 5-8.

Raabe, M. (1986) Insect reproduction: regulation of successive steps. *Adv. Insect Physiol.* **19**, 20-154.

Rahman, S. M., M. A. S. Raza, M. A. Salem and A. Bari (1991) Effect of larval population density on the fecundity and hatchability of silkworm, *Bombyx mori* (L.). *Bull. Seric. Res.* **2**, 7-12.

Ram, K. and D. Singh (1992) Role of mating disruption in the production of viable silkworm (*Bombyx mori*) eggs. *J. Entomol. Res.* **16**, 206-210.

Roychoudhury, N., D. C. Paul and G. Subba Rao (1991) Growth, fecundity and hatchability of eggs of *Bombyx mori* in relation to rearing space. *Entomon* **16**, 203-207.

Samson, M. V. and N. M. Biram Saheb (1999) Silkworm egg: key to silk industry; in *Advances in mulberry sericulture*. Devaiah, M. C., K. C. Narayanaswamy and V. G. Maribashetty (eds.), pp. 243-292, C. V. G. Publications, Bangalore, India.

Saunders, D. S. (1982) Insect clocks, Pergamon Press, Oxford.

Setty, B. N. Y. and T. R. Ramaiah (1979) Isolation and identification of prostaglandins from the reproductive organs of the male silkworm, *Bombyx mori* L. *Insect Biochem.* **18**, 613-617.

Shivaprakasam, N., S. Jayarani and R. J. Ravindra (1997) Influence of spacing on incidence of grasserie in silkworm, *Bombyx mori* L. *Indian J. Seric.* **36**, 72-73.

Siddhu, N. S., R. Sreenivasan and A. Shamachary (1967) Fertility performance of female moths depends on their male moths. *Indian J. Seric.* **1**, 77-84.

Singh, G. P. and V. Kumar (1995) Correlation of larval and cocoon weight with fecundity in silkworm, *Bombyx mori* L. *Ann. Entomol.* **13**, 15-17.

Singh, R., H. K. Chaturvedi and R. K. Datta (1994) Fecundity of mulberry silkworm, *Bombyx mori* L. in relation to female cocoon weight and repeated mating. *Indian J. Seric.* **33**, 70-71.

Singh, T. (1994) Correlation between pupal weight and fecundity in *Bombyx mori* (L.). *Annl. Ent.* **12**, 5-7.

Singh, T. (1998) Behavioral aspects of oviposition in silkworm *Bombyx mori. Indian J. Seric.* **37**, 101-108.

Singh, T. (2001) Influence of rearing space on economic traits in the silkworm, *Bombyx mori* L. *Bull. Ind. Acad. Seric.* **5**, 111-113.

Singh, T. and S. K. Mathur (1989) The dancing of male silk moths to the perfume to female moth. *Indian Silk* **28**, 17-18.

Singh, T., Chandrashekharaiah and M. V. Samson (1998) Correlation and heritability analysis in the silkworm, *Bombyx mori* (L). *Sericologia* **38**, 1-15.

Singh, T., K. P. Jayaswal and G. Subba Rao (1992) Correlation studies between some economic characters in silkworm, *Bombyx mori* (L.). *J. Zool. Res.* **5**, 47-50.

Singh, Y. R. and B. Prasad (1987) Correlation among body weight, pupal weight and fecundity in eri silkworm, *Philosamia ricini* (Lepidoptera: Saturniidae). *Sericologia* **27**, 49-60.

Subramanayam, K. and N. C. V. Murthy (1987) Effect of rest on the efficiency of male moth in relation to fecundity and hatching behaviour of silkworm eggs. *Sericologia* **27**, 677-680.

Sugai, E. and T. Takahashi (1981) High temperature environment at the spinning stage and sterilization in the males of silkworm, *Bombyx mori. J. Seric. Sci. Jpn.* **50**, 65-69.

Talukdar, F. A., M. Hossain, M. Shahjahan, A. K. M. M. Rahman and A. B. Khan (1991) Effect of larval population densities of silkworm on larval and cocoon characteristics. *Bangladesh J. Agric. Sci.* **18**, 15-18.

Tanaka, Y. (1964) Sericology. Central Silk Board, Bangalore, India.

Tazima, Y. (1978) Silkworm Eggs. Central Silk Board, Bangalore, India.

Tripathi, P. M. and T. Singh (1995) Studies on mating duration and its effect on fecundity and fertility in mulberry silkworm moth *Bombyx mori* (Lepidoptera: Bombycidae). *Bioved* **6**, 15-18.

Vijayan, V. A., G. Subramanya and N. B. Krishnamurthy (1994) Differential capacity of male silk moths in six races of silkworm, *Bombyx mori* L. *Bull. Seric. Res.* **5**, 77-79.

Wang, S. M. (1994) *Silkworm Egg Production,* Vol. III., Food and Agriculture Organization, Agricultural Services Bulletin, United Nations, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India (English translation).

Wechsung, E. and A. Houvenaghel (1976) A possible role of prostaglandins in the regulation of ovum transport and oviposition in the domestic hen. *Prostaglandins* **12**, 559-608.

Yamayoka, K., M. Hashino and T. Hirao (1971) Role of sensory hairs on the anal papillae in oviposition behaviour of *Bombyx mori*. *J. Insect Physiol.* **17**, 871-879.

Yamayoka, K. and T. Hirao (1973) Releasing signals of oviposition behaviour in *Bombyx mori*. *J. Insect Physiol.* **19**, 2215-2223.

Yamayoka, K. and T. Hirao (1975) Circadian rhythm of ovipositional behavior in *Bombyx mori* - ovipositional rhythm in virgins. *J. Seric. Sci. Jpn.* **44**, 212-219.

Yamayoka, K. and T. Hirao (1977) Stimulation of virginal oviposition by male factor and its effect on spontaneous nervous activity in *Bombyx mori*. *J. Insect Physiol.* **23**, 57-63.

Yamayoka, K. and T. Hirao (1981) Mechanisms of ovipositional behaviour in *Bombyx mori*: time gating and accumulation of the internal factor. *Intl. J. Invertebr. Reprod.* **4**, 169-180.

Yamaoka, K., T. Hirao and N. Arai (1976) Circadian rhythm of ovipositional behavior in *Bombyx mori* - ovipositional rhythm in mated females. *J. Seric. Sci. Jpn.* **45**, 365-374.