

## Mating Behaviour in Mulberry Silkworm, *Bombyx mori* (L.)

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(Received 15 August 2004; Accepted 10 May 2005)

**Mating is an essential behavioural social event in the life cycle of silkworm, *Bombyx mori* (L.) for the perpetuation of population. A number of intrinsic and extrinsic factors and events of significant importance are involved in successful mating and egg deposition by an adult silk moth which besides biochemical, physiological and environmental factors also includes attraction of reproductively competent male and female moth for mating, duration and frequency of mating, age of moth at the time of mating, reuse of male moth in the production of eggs etc. An attempt has been made in this review article to elucidate briefly the behaviour of male towards female moth after eclosion, impact of duration and frequency of mating on egg deposition and oviposition, reuse of mated male moth in the production of quality and quantity eggs etc. in the silkworm, *B. mori* and its significance in silkworm seed production.**

**Key words:** *Bombyx mori*, Mating behaviour, Repeated mating

### Introduction

Silk, as one of the bio-resources for human life has always occupied a place of pride in the society. There have been incessant endeavors to improve the silk both in terms of quality and quantity and there has been a spectacular accomplishment of feats in the field of sericulture. One of the inputs that play a decisive role in the success of silkworm crops is the silkworm seed quality (Samson and Biram Saheb, 1999). Quality of seed is determined by

many factors of significant importance wherein mating plays an important basic role. Mating, an instinct and a biological obligation bring the male and female individuals together for ultimate perpetuation of the species. Being a key social event, it influences the parental behaviour, evolution of society, sex ratios and population genetics etc. It provides a stimulus for oogenesis and brings about dramatic biochemical events in the spermatophore of the silkworm (Osanai *et al.*, 1990), assures the presence of sufficient number of normal sperm and or testicular fluid in the female reproductive organs (Davey, 1985), activate ovulation (Yamaoka and Hirao, 1977), accelerate the oviposition behaviour (Leopold, 1976) and egg deposition (Shetty and Ramaiah, 1979; Ravikumar *et al.*, 1995). Age of moth on mating, frequency and duration of mating, time and number of ejaculation during mating, reuse of male moth for successive mating, impact of multiple mating on seed quality have been discussed by many workers from time to time (Sengupta *et al.*, 1973; Yamaoka and Hirao, 1981; Paul *et al.*, 1993; Suzuki *et al.*, 1996; Singh *et al.*, 2003). An attempt has been made in this review article to discuss briefly the advances achieved so far on various aspects of mating behaviour in the mulberry silk moth, *B. mori* and its significant role in egg production technology.

### Recognition of sexes for mating

Insects chiefly employ tactile, visual, auditory and chemical means to recognize the opposite sex. In *B. mori*, the communication between sexes is accomplished through chemical signals. The virgin adult females after emergence emit a sex attractant chemical substance, termed as 'Pheromones' or 'Social hormones', which is sufficiently volatile in nature, into the atmosphere for attracting the males. This liberated chemical signals the presence of female and in turn excites the males, triggers the courtship behaviour, which eventually culminates into copulation. The first insect sex pheromone to be chemically deci-

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phered is that from the female silk moth, *B. mori*. Butenandt *et al.* (1959) after extensive studies spanning over 20 years successfully accomplished the task of isolation of this olfactory sex attractant and named it 'Bombykol'. Butenandt and Hecker (1961) concluded that this natural sex attractant is 10 – 12 hexadecadien-1-ol. It is a primary alcohol with its chemical structure as  $\text{CH}_3(\text{CH}_2)_2\text{CH} = \text{CHCH} = \text{CH}(\text{CH}_2)_8 \text{CH}_2\text{OH}$ . The male and females are non-feeding in their adult life, can not fly (due to extreme domestication) and instantly anxious and eager to mate instantaneously, consequent to their escape from silk cocoons. Succeeding the eclosion, after propelling for the full expansion of wings, the female protrudes the paired scent glands from the hind most abdominal segment and exposes outside the actual odoriferous surface and then performs the task of a quick and efficient liberation of the chemical substance. The female retrieves the glands into the body immediately after being touched or contacted by an approaching male. The perception of the pheromone by the male is through the chemoreceptors located in the antennae. Though the males perceive the hormone instantly, a certain delay in the message transfer is imminent. Hence, the just emerged male after expanding its wings, remains inactive, till such time it deciphers the chemical message. Similarly, in the absence of females, which implies non-availability of the chemical in the immediate vicinity, the males remain unexcited. However, the excitation in males could be elicited at unbelievably low concentrations, as low as 100 molecules of 'Bombykol' per cubic centimeter of air (Englemann, 1970). Even a single molecule of this attractant is sufficient to trigger an impulse in the male nerve receptor cells which is due to the presence of large number of sensilla (60,000) on the bipectinate antennae of the male moth whose surface area with all the remi included is approximately 29 mm<sup>2</sup> (Obara, 1979) with approximately 3000 pores on each sensillum. Each pore is 10 – 15 nm in diameter and there are some 45 million pores on the bipectinate antennae of the moth. The information from the individual chemoreceptors concerning the perception of sex attractant is summated and integrated in the central nervous system. Arido *et al.* (1996) reported that mating duration of more than 6 hrs was necessary to permanently terminate the production of the sex pheromone and such females mated for 6 hrs, if injected with synthetic pheromotropic neuropeptide (which is known to activate pheromone biosynthesis in a virgin female) would again produce 'Bombykol'.

#### **Behaviour of the male**

Stimulated males are anaerotactically attracted towards the source of pheromone (Englemann, 1970). The

responding male exhibits typical signs of alertness- first cleans its antennae, lifts the head, vibrates wings in smaller to larger amplitudes (Schwinck, 1954) to produce a strong air current which flows parallel to the body's longitudinal axis so that it could locate the source of pheromone (Obara, 1979). Obara (1979) observed that an actively dancing male can draw air from a distance of 4 cm from the frontal part of its head, while wing vibrations draw air from the sides thus improving the efficiency of the antennae. Stimulated male, immensely active, runs in circles, semi-circles and zigzag paths, with a distinctly bent abdomen and eventually adopts an oriented turn (Schwinck, 1954) resulting in contact with the female genitalia. The excitement behaviour serves as an easy mode for identification of male while females conspicuously remain inactive. In the process, characteristic dancing movements guide the male towards the direction of a female and during the dance the male would touch any part of the female body and ultimately by trial and error establishes a contact with the female genital portion and succeeds in copulation. The copulation pattern is referred to as 'end to end pattern' (Samson and Biram Saheb, 1999). The locking of the male genitalia with the female is adequately so firm that one has to physically slide out or pull back the male from the female while decoupling. Hence, it is an established practice to lift the pairs by holding the female and slipping them into special enclosures (cellules) for stipulated mating durations.

Kellogg (1907) observed that male moths with intact antennae and blackened eyes found females immediately than males devoid of antennae. The removal of both antennae of males resulted in significant reduction in searching ability (Nakazima, 1931) and took substantially longer duration and copulates only when placed in closer proximity (Biram Saheb *et al.*, 1998). A partial amputation in one or both the antennae or total severing of any one of them in no way affected the mating capability. But the males severed with any one of the antennae, experienced a sudden shock and remained idle for a few seconds and recovered smartly only to circle repeatedly towards that side where the antenna was intact and succeeded in mating (Biram Saheb *et al.*, 1998). Sengun (1954) showed that in the absence of air movement, normal males could detect females only from a short distance of 5 cm whereas females could be traced from a distance of 25 – 150 cm with moving air. Odor-free air currents cannot elicit any searching activity. Singh and Mathur (1989) reported that under normal atmospheric conditions, the male with intact, stretched and fully expanded wings could succeed in identifying the female within a few seconds ( $25 \pm 10$ ) even from a distance of 10 cm while those possessing defective wings, consumed much longer time. The

females can attract males' up to 5 days after eclosion (Kuwahara *et al.*, 1983).

### Mating duration and its impact

The mating duration in silkworm essentially affects silkworm seed quality and quantity. Mating is not obligatory either for oviposition or egg production but insemination is essential for further development (Punitham *et al.*, 1987). However, for successful oviposition the following three aspects are of utmost importance:

- a. Participating male and female partners should be reproductively competent.
- b. Mating should be accomplished at the appropriate time.
- c. Mating should ensure transfer of sperm and the viscous heterogeneous secretions including male factor (oviposition stimulating substance or fecundity enhancing substance) into the reproductive tract of the adult female.

After mating ejaculation takes place in a long or successive mating at intervals of 60 – 90 min (Machida and Watanabe, 1927; Omura, 1939) and each ejaculation is completed in about 40 min. The male moth does not begin ejaculation immediately after the beginning of copulation and does not separate automatically from the female until the second or third ejaculation is completed. Initially during ejaculation general semen is emitted which contains spermatozoa and consume 5 – 10 min followed by ejaculation of the spermatozoa, which lasts for 10 min. Thereafter the emission of the semen without spermatozoa occurs again lasting for some 10 min (Omura, 1939). The sperm release is accomplished in a rhythmic way.

When natural copulation extends to unduly longer durations, there is a fear that females may die without laying any eggs (Tanaka, 1964). Kovalev (1970) observed that during continuous mating, sometimes-spontaneous detachment of pairs takes place and the liberated free male; in case the mated partners are not confined in special enclosures (cellules); disturb the paired female physically by its rigorous movements. Hence in seed production, it is a common practice to enclose them in cellules.

According to Tazima (1978) the first ejaculation takes place in 30 min and the second after 1 – 1.5 hrs and a three-hrs mating ensures one to two ejaculations. From the various studies it has been accepted that duration of 3 – 5 hrs is necessary for optimum fecundity and fertility (Tanaka, 1964; Tazima, 1978; Jolly, 1983; Narasimhanna, 1988; Yeole *et al.*, 1995). Petkov *et al.* (1979) recommended mating duration of 150 - 180 min as optimum for maintaining both productivity and quality of silkworm seed. They suggested that mating duration should not be

less than 150 min and 120 min for the production of reproductive and commercial seed respectively. There are numerous studies on mating duration, which have contradictory observations. A few reports project the view that even 30 min (Jadav and Gajare, 1978; Ram and Singh, 1992; Tripathi and Singh, 1995) or 45 min (Jolly *et al.*, 1964) is adequate for optimum recovery of eggs without impairing the fecundity and fertility. On the contrary, Askari and Saran (1984) found that duration of mating has a profound bearing on the recovery of eggs and increased mating duration produced higher quantity of eggs. Punitham *et al.* (1987) reported that increase in mating duration not only reduces the pre-oviposition period but also increases the egg output and enhances the hatching percentage and body weight in the succeeding generation. Duration of pairing has an inverse correlation with oviposition period. An increase in the mating duration reduces the pre-oviposition period (Narayanan *et al.*, 1964; Kovalev, 1970; Manoharan, 1983; Gowda, 1988). Manoharan (1983) and Gowda (1988) established that there is no relationship between the duration of mating and the number of viable eggs laid but stated clearly that when a minimum of one hour and a maximum of 10 hrs pairing was allowed, the speed of oviposition is altered significantly, faster in the longer duration and slower in the moths mated for shorter durations.

### Reuse of male moth for mating and its impact on fertility

A male has the inherent capacity to mate with certain number of females without affecting fecundity (Jadav and Gajare, 1978; Askari and Saran, 1984; Ram and Singh, 1992; Vijayan *et al.*, 1994) after which both fecundity and fertility are drastically reduced. The potency of male moth or its capacity to mate with different females has been extensively studied in different silkworm breeds. In order to reduce the cost of silkworm seed production it is a common practice to allow males to mate more than once. The phenomenon of re-mating has been accepted in seed production due to several reasons such as shortage of male moths, to make commercial seed production economical and viable, non-availability of fresh male moths due to failure in synchronization of emergence in the component races and prevalence of weaker males.

In hybrid egg production, where both direct and reciprocal crosses are prepared (*i.e.*, Chinese × Japanese and Japanese × Chinese) equal number of parental seed cocoons are preserved to ensure male component for every female. Well documented is the fact, that in a given batch of cocoons, the males invariably outnumber the females, more so when the crops have suffered due to diseases. Thus, in bivoltine hybrid egg production, there is

always the availability of equal or surplus males for the purpose of mating. But in countries like India, a distinct strategy is *in-vogue*, where commercially a specific hybrid, inviting a cross between an indigenous multivoltine female parent (Pure Mysore, Nistari, Tamil Nadu White etc.) and a bivoltine male parent (NB<sub>4</sub>D<sub>2</sub>, NB18, KA, NB7 etc.) is produced. It has become a rule of thumb to use the male moths more than once to make seed production viable and economical. Declared as a pragmatic approach, in reducing the cost of production, it is a practice to procure the bivoltine and multivoltine parental seed cocoons in a ratio of 1 : 2 (Singh and Saratchandra, 2004). Repeated mating is reported to result in genetic variability in the offspring by maintaining vigour in the population (Jolly *et al.*, 1964). Reports on repeated mating has shown controversial recommendations on egg production efficiency in terms of recovery %, fecundity and fertility. According to different workers who explored the possibility of reusing males reported that males are potent enough to mate 4 females in Nistari (Jolly *et al.*, 1964); 7 – 9 in Pure Mysore (Askari and Sharan, 1984; Vijayan *et al.*, 1994); 11 in SKAUST-23 (Ram and Singh, 1992) and 16 females (Thiagarajan and Govindaiah, 1988; Gupta *et al.*, 1991; Vijayan *et al.*, 1994) and 19 – 20 (Jadav and Gajare, 1978; Gupta *et al.*, 1991) females for other popular silkworm breeds.

In light of these observations, it can be inferred that mating performance is breed specific and a general recommendation cannot be professed to various silkworm breeds as also opined by Siddhu *et al.* (1967), Gowda (1988) and Narasimhanna (1988). The males of Japanese races (dumbbell shaped) are found to be more potent than those of Chinese races (oval shaped) and bivoltine males are found often more potent to the multivoltines.

Majority of these workers have converged their message pointing out that as the frequency of mating increases, it would affect viability, fecundity and egg recovery significantly (Gowda, 1988; Thiagarajan and Govindaiah, 1988; Ram and Singh, 1992; Babu, 1993), but it is rather difficult to envisage as to from which mating that the negative trend appears.

Benchamin *et al.* (1990) upon screening the comparative potency of males of the hybrids and pure races observed that fecundity was not affected in the multivoltine female parent (Pure Mysore) while other characters *viz.*, effective rate of mating, laying yield and fertility differed significantly between pure breed and hybrids. Rajanna *et al.* (1995, 1999) carried out extensive studies on the evaluation of different silkworm breeds and their possible hybrid combinations as male parents, with Pure Mysore and Nistari races serving as female parents on the performance of different quantitative characters. The out-

come of their studies indicated that the hybrids as male parents were either on par with pure breed males or surprisingly sometimes inferior in respect of certain characters. Petkov and Mladenov (1979) categorically suggested that only two mating by the male moths in commercial seed production and only one mating for reproductive seed be accepted.

When the male moth is deployed for repeated mating, an interruption through rest between two mating comprising a full day or even shorter durations of 1 – 2 hrs for the male was found to be beneficial phenomenally (Kovalev, 1970; Jolly, 1983; Narasimhanna, 1988). Such a rest is accorded by exposing them to a low temperature of 5°C / 7°C, to protect them from getting exhausted under ambient conditions, besides restricting the occurrence of unfertilized eggs. Continuous mating with no rest between matings produces significantly higher percentage of unfertilized and dead eggs (Subramanayam and Murthy, 1987). Babu (1993) carried out meticulous observations on the introduction of continuous and discontinuous multiple mating on the performance of selected economic traits and activity of the esterase isozymes in the male reproductive system. It is reported that in continuous type of mating, fertility and effective rate of rearing were affected right from the second mating onwards while fecundity, hatchability and shell ratio declined from the third mating onwards. Eggs retained inside the ovarioles increased significantly after second mating. More unfertilized eggs laid in continuous type of mating from the second mating onwards, while in the discontinuous type it was evident from the third mating. The inference drawn from his study revealed that discontinuous type of mating is better for harvesting higher egg recovery. It is also suggested to increase the duration of mating for the subsequent mating (Ming, 1994).

One of the distinct features of the silk moth is that the adult is a non-feeding stage, thus it has to depend on the stored energy reserves accumulated during the late larval instars. It is obvious that the energy reserves are limited and adequate to accomplish the lone physiological event, reproduction. Evidently the females produce eggs only once in their adult life, which stretches to about 8 – 10 days. Contrary to the females, the males have acquired inherent capabilities to mate more than one female, for they produce enormous quantity of spermatozoa (estimated to be 256 sperms / bundle × 3000 bundles × 2 testes or 15.4 × 10<sup>5</sup> spermatozoa per head) (Tazima, 1978). Even if the male ejaculates about one-lakh sperms per ejaculation, logically it can afford to mate many more females and fertilize all the eggs. Interestingly, besides the sperm, the male transfers a stream of heterogeneous viscous secretions for sperm nutrition and final stage maturation.

The contents also comprise of oviposition stimulating substance, which has been identified as 'prostaglandins' (Setty and Ramaiah, 1979). Oviposition is stimulated in the female moth following the transfer of prostaglandins during mating (Setty and Ramaiah, 1980). Thus the males should have inherent prescribed arrangements to extract the same intensity and flow of secretions from its reproductive organs and the accessory glands. Such secretions are readily available for the maiden mating while for the subsequent mating a rest ensures proper accumulation and could take care of the responsibility of fertilization of the female. Increase in the pairing duration for the subsequent mating (Ming, 1994) may indirectly help the moth to draw the available resources. The limiting factor is the depletion in the quantity and quality and hence from third mating onwards it would result in appearance of large number of unfertilized eggs, retention of eggs inside the ovarian tubules and lower fecundity. Repeated mating of the same male lead to inadequate discharge of spermatic fluid and this reduces the number of eggs laid by the subsequently mated female. Siddhu *et al.* (1967) stated that the quantity of the semen in the seminal vesicle available in the male moth is meager and sufficient only for four consecutive ejaculations. The quantity and quality of spermatozoa deposited in the bursa copulatrix and later migrated to spermatheca in the female, determines the fertility performance of the female. Mere transfer of spermatozoa from spermatophore to the female bursa-copulatrix would not suffice, but the transfer / movement of sperm via ductus-seminalis into receptaculum seminis and into the fertilization canal is vital for accomplishing fertilization of eggs. On a couple of occasions it is experienced that even the third or fourth mating by a male becomes successful. Male moth potentiality is related to the inherent physical and physiological status. It is also observed that male moths emerging out of certain breeds of parental seed cocoons are extremely potential and can successfully fertilize even 4 or more females. It is also known that all the males and females in a given population are not adequately fit and healthy to perform mating / copulation. Further with increase in the number of matings, more and more males become incompetent and fail to mate.

#### **Impact of multiple mating and sperm precedence**

Askari and Sharan (1984) reported that multiple mating results in more number of eggs and the reasons for such an increase was attributed to the additional transfer of fecundity / fertility enhancing substance from the males during copulation (Englemann, 1970). Sperm precedence / dominance is variable among species and under different conditions even in the same species. Suzuki *et al.* (1996) investigated sperm precedence and sperm movement

under different copulation intervals. When a female is copulated once, the mass of sperm begins to enter the spermatheca one hr after the copulation and attained a plateau in about 2.5 hrs after the end of copulation. They inferred that the timing of sperm transfer might cause the difference in sperm precedence in relation to copulation intervals from the spermatophore to the spermatheca and from the spermatheca to the vestibulum. In yet another study on multiple mating, Biram Saheb *et al.* (1996) observed that the sperm dominance or precedence depended on the copulation duration of the first male moth.

#### **Mating and oviposition**

It is a well-known fact that mating is not exactly obligatory for either ovulation or oviposition, but insemination is essential for securing a complement of fertilized eggs. It is also well established that brisk egg laying begins under the influence of mating. The stimuli, which are applied, to the female during mating are both direct (copulation, grasping to female by claspers and the repletion of bursa copulatrix with the seminal fluid) and indirect (migration of spermatozoa from bursa copulatrix to vestibulum via receptaculum seminis) (Omura, 1939).

Yamaoka and Hirao (1973, 1977) and Paul *et al.* (1993) observed that mating stimulates egg laying so definitively that within 24 hrs of mating, females laid most of the eggs stored inside the reproductive organs and stated that mating changed the humoral condition of the female which may stimulate the motor neurons in the 9<sup>th</sup> ganglion for activating the egg laying of mated females. It is generally recognized that the male factor or the oviposition stimulating substance derived from the male reproductive glands and transferred to the females during copulation induce brisk oviposition behaviour (Fugo and Arisawa, 1992). After mating vigorous biochemical changes take place in the spermatophore (Osanai *et al.*, 1990). The presence of sperm and seminal fluid inside the female reproductive organs, are prerequisites for oviposition (Fugo and Arisawa, 1992).

Oviposition rhythm or the peak of oviposition changes with duration of mating, time of mating, age of the female parent and the inherent capability of the parents. Narayanan *et al.* (1964) reported that the pre-oviposition period and the duration of pairing have an inverse correlation. Duration of mating influences the oviposition rhythm and number of eggs laid at different intervals. Longer duration of pairing showed highest percentage of eggs laid within 6 hrs after depairing. This is interpreted as a consequence of the interaction of factors such as increased egg maturation, successive ejaculation and optimum peak of egg laying period (Gowda, 1988).

### Impact of age on mating capability and oviposition

Kovalev (1970) showed that females mated after 24 hrs of emergence laid all the eggs within 30 min of depairing. Biram Saheb *et al.* (1998) drew an inference from their studies that when one or two days old females were utilized, which implies a significant delay in the pairing schedule, the peak of oviposition was remarkably advanced and over 70% of the eggs are recovered within a short period besides a marked reduction in the retention of eggs inside the ovarian tubules. They also observed that the female moths, which were mated immediate after emergence or within 8 hrs of eclosion, deposited 81 – 91% eggs on the first and 9 – 19% of eggs on the second day of oviposition. Quiet distinctly, the one day / two day old females upon mating for 4 hrs, oviposited more than 95% of eggs within 24 hrs and a small percent of eggs on the second day. Yamaoka and Hirao (1981) noticed that if mating occurred within the peak egg laying period at dusk; the egg laying commenced instantly after decoupling, but if the mating is scheduled to midnight, great part of the egg laying is carried over to the next day.

Higher incidence of unfertilized eggs was reported in females mated after 6 – 12 hrs of emergence and it was attributed to time lapse that existed between moth emergences and mating (Gowda, 1988). Increase in age-related mating schedule affects the mating potential, which ultimately causes a decrease in the protein and fat contents of the eggs (Haniffa and Thatheyus, 1992). They further stated that an increase in age of female or male moth causes a decrease in the total egg output and hatchability. Paul and Kumar (1995) reported that the mating capacity of the males decreased significantly with the increase of age and established a significant negative correlation ( $r = -0.097$ ) between age of males and mating capacity. The time of ejaculation and the dissociation of eupyrene sperm bundles delayed or inhibited with increase of age in the males and so also the migration of both apyrene and eupyrene sperm into the spermatheca (Kumar and Paul, 1993). Paul *et al.* (1993) have observed that the fertility percentage decreased significantly with increasing age of females and significant negative correlation between the age of females and fertility level. They did not observe any significant difference on the total egg deposition and the longevity of virgin and mated females.

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