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Egg laying beahaviour in silkmoth *Bombyx mori*

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Abstract
Although, the virgin as well as mated silkmoths lay eggs, the egg laying behaviour was found to be quite different in these two with respect to the duration of pre-oviposition period, the total number of eggs laid, the time taken to complete egg laying and the temporal pattern of egg laying i.e., the number of eggs laid at different intervals of egg laying period.

Keywords: silkmoth, oviposition, temporal pattern of egglaying, virgin, mated moths

Introduction
Insects occupy a unique position in the animal kingdom with respect to the diversity. This is especially true when we consider the behavioural traits. Among invertebrates, complexity in behaviour seems to have attained its culmination in insects. Concomitantly the regulatory mechanisms underlying these behavioural traits are also highly complex.

Two important phases of reproduction namely mating and egg laying are very interesting in insects, especially in silkworm, as it is of commercial importance. Since the early stages of human civilization silkworm has been used as a source of silk for producing exquisite textiles. Because of its industrial importance extensive scientific studies have been carried out on silkworm to support the development of sericultural technology.

Studies are mainly aimed at improving the economic characters such as yield, and quality of silk focussing on the larvae and pupae, whereas adult moths received very little attention though reproduction involving both male and female adult moths is a critical phase for continuation of one's own progeny.

Review of Literature
Studies on adult silkmoth mainly deals with the understanding of the behavioural aspects of mating and egg laying (Yamaoka and Hirao, 1981; Manjulakumari, 1991) [55], but not so much, the physiology of mating and egg laying, the two most important aspects of reproduction.

In insects, mating is not a mere process of inseminating the female but, has a profound influence on the reproductive behaviour and physiology of female. The post-mating effect of male on females include induction of oogenesis (Pickford *et al.*, 1969) [37], enhancement of fecundity (Danthanarayana and Gu, 1991) [13], suppression of remating (Shirk *et al.*, 1980) [46], stimulation of oviposition (Watanabe, 1988; Bali *et al.*, 1996) [51, 3], acceleration of egg laying (Herndon and Wolfner, 1995; Soller *et al.*, 1997) [25, 48], and so on.

Male is actually the source of compounds which modulate the physiology of female in several ways (Chen, 1984; Gillott, 1988; Chen *et al.*, 1988; Aigaki *et al.*, 1991; Kalb *et al.*, 1993; Herndon and Wolfner, 1995; Chapman *et al.*, 1995; Kubli, 1996; Wolfner, 1997; Shu-Xia Yi, Gillott, 1999) [8, 21, 1, 25, 7, 27, 47]. During insemination, male introduces not only gametes but also some of the substances secreted by different tissues of the reproductive system viz., testes (Smith, 1956), ejaculatory duct and accessory gland (Bairati, 1968; Gillott and Friedel, 1977) [2, 22]. These substances may be proteins (Rockstein, 1964; Ranganathan, 1982; Chen *et al.,* 1988; Basker, 1988; Wolfner, 1997) [52, 41], carbohydrates (Chino, 1958; Blum *et al.*, 1962; Ranganathan, 1970, 1973; Baumann, 1974; Pant and Sharma, 1976; Muse and Balogun, 1993;) [39, 40, 4, 36, 33] or lipids (Ranganathan and Padmanabhan, 1994) [42] entering the female and influencing its physiology. Such substances have been termed differently as fecundity enhancing substances, receptivity inhibiting substances, oviposition stimulating substances (Gillott and Friedel, 1977; Shu-Xia Yi, Gillott, 1999) [47, 22] etc. In addition to this males supplement zinc (Engebretson and Mason, 1980) [18] and sodium requirements of the female (Pivnik and McNeil, 1993) [38].

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The male derived substances also play an important role in successful transfer of sperms (Gracia and Bellido, 1964; Lefevre and Moore, 1967; Chen, 1971; Fowler, 1973) [23, 30, 9, 19], sperm storage (Gilbert, 1981; Neubaum and Wolfner, 1999; Uyen Tram and Wolfner, 1999) [20, 34, 50] and utilization (Kalb *et al.*, 1993). If female does not receive adequate quantity of these substances, it fails to store as many sperms as it could have otherwise (Kalb *et al.*, 1993). In *Drosophila melanogaster* seminal fluid molecules from the male accessory glands are responsible for initial behavioural changes like an elevation of egg laying but persistence of these changes require stored sperm (Neubaum and Wolfner, 1999) [34].

The transfer of seminal fluid molecules along with sperm during mating is of great importance to the reproductive success of male. Since seminal fluid components perform diverse functions in the female, it benefits both the male and his mate (Gillott, 1988; Eberhard, 1996; Wolfner, 1997) [21, 17, 52].

In most of the insects egg laying can take place irrespective of mating. Though virgins could lay eggs in some insects (Hinton, 1981) [26], generally they lay fewer eggs when compared to mated females. The increased egg laying capacity can be stimulated by copulation in insects (Ridley, 1988; Danthanarayana and Gu, 1991) [43, 13].

Oviposition in insects is an interesting phenomenon with lot of diversity - diversity in selecting the oviposition site, in the pattern of laying eggs, rate of egg laying etc. In silkworm, due to its domestication over several years and human intervention in selecting specific characters, the behavioural traits that can be observed are limited to the act of feeding and reproduction. One such interesting behaviour is the laying of eggs in a monolayer. The adult female lays eggs packed close to one another and never one above the other except under a certain pathological condition. Egg laying is a multi step process, it begins with oocyte release by the ovaries followed by egg movement down the oviducts and the deposition of eggs on to the substratum. Yael Heifetz (2000) [53] reported that the Acp 26Aa stimulates the first step in egg laying release of oocytes by the ovary. During mating Acp 26Aa begins to accumulate at the base of the ovaries, a position consistent with action on the ovarian musculature to mediate oocyte release. For successful insect reproduction there should be synchronization of copulation with a number of factors including the presence of mature or nearly mature gametes in both sexes, ability to produce the secretions necessary for sperm transfer in male and availability of nutrients for egg maturation in female. Generally mated females lay more eggs than virgins (Bali *et al.*, 1996) [3]. In virgins oviposition may be delayed or even prevented and egg maturation is slower.

In *Rhodnius*, the rate of egg laying is greatly increased by mating, but this has little effect on the rate of egg formation (Coles, 1965) [11]. Mating and producing hatchable eggs are two different, although connected events. Roth and Willis (1956) [44] in *Blatta orientalis,* reported that mating stimulates egg laying and showed that mated females, on an average laid 160 eggs, while unmated lay only 114. Similar results were reported in *Haematosiphon inodorus* (Lee, 1954) [29], *Hesperocimex sonarensis* (Ryckman, 1958) [45] and *Cimex lectularis* (Davis, 1964, 1965a, 1965b) [14, 15, 16]. A virgin moth lays fewer than 10% of the eggs on the first day and continues egg laying for 4 to 5 days without increasing the rate of oviposition. After mating, over 90% of eggs are laid at one time during the first day. The mechanism that controls acceleration of egg laying is obscure despite many studies to clarify it. Mating may be immediately followed by an outburst of egg laying, but in many species the manner of laying by mated and virgin females is very different. Thus, even when the total number of eggs laid is the same for both mated and virgin females, virgins may delay laying or lay with an irregular periodicity as compared with mated females, e.g. in the fly *Cochliomyia hominivorax* (Crystal and Meyners, 1965) [12] and the acridids *Gamphocerous rufus* (Loher and Huber, 1964) [31] and *Locusta migratoria* (Mika, 1959) [32]. In the bug *Oncopeltus faciatus* a single mating with a normal male has at most a minor and temporary effect on the rate of egg production even when enough sperms are transferred to make the production of the some fertile eggs possible for several weeks. In silkworm, so far, not many attempts have been made to closely examine the role of mating on egg laying behaviour. The few attempts which have been made (Omura, 1936, 1938; Yamaoka and Hirao, 1977; Thomas punitham *et al.*, 1987) [35, 55, 49] have failed to demonstrate the effects quantitatively. To understand the mechanisms which control and regulate egg laying behaviour, it is very essential to understand these aspects mentioned above. During the current investigations, the egg laying behaviour was examined closely in virgins as well as in mated moths and the results were compared quantitatively with one another.

The stimulus to egg laying is provided by mating via chemical substances secreted by different tissues of male reproductive system. These substances may pass through the wall of the tract in a modified or unmodified form and stimulate the female tract (Baumann, 1974) [4] to bring about the necessary changes to initiate egg laying.

Pickford *et al.*, (1969) [37] working with the migratory grasshopper, *Melanoplus sanguinipes,* showed that an egg laying stimulant was produced by the male accessory reproductive glands. In *Schistocerca gregaria,* Leahy (1973a) [28] implanted male accessory glands in virgin females and found their oviposition rate to be increased. Lange and Loughton (1985) showed that injection of mature male accessory gland extracts of *Locusta migratoria* stimulated an increase in oviposition rate of virgin females comparable to that of mated females.

Material and Methods

Silkworm seed cocoons of bivoltine race $NB_4 D_2$ were collected from Government bivoltine cocoon market and maintained in the laboratory at room temperature. The males and females were segregated at the pupal stage itself and maintained in separate cages to avoid copulation.

The newly emerged silkmoths were allowed to copulate for 6 hours. The females were then transferred on to the egg cards. By placing a plastic cellule around them, the space of egg laying was restricted. A few moths were left free without placing a cellule for better observation of their movements during oviposition. The following parameters were recognized to quantify the egg laying behavior.

The pre-oviposition period – Period between termination of copulation and initiation of egg laying.

The oviposition period – Period between initiation of egg laying and the completion of egg laying.

The temporal pattern of egg laying – Number of eggs laid at different intervals of the oviposition period.

Total number of eggs laid.

The egg cards were carefully changed every 24 hours after the initiation of oviposition with minimal disturbance to animal, to record the temporal pattern. Similarly the egg laying behaviour of virgin females were quantified.

Egg laying behaviour of silkmoths injected with the extract of whole and different tissues of reproductive system was studied by injecting the extract into haemocoel of female moths. The different organs of male reproductive system were pooled from 40 unmated freshly emerged male moths. The tissues were homogenized separately in 2 ml silkmoth saline, (Yamaoka, 1977) [54] centrifuged at 10000_g for 10 minutes. The crude extract was injected in to the virgin females around 6 AM in the early morning soon after their emergence. The dosage injected was equivalent to that of one male moth (i.e., 50μl/moth) and the egg laying behaviour was studied by considering the parameters mentioned earlier. Normal mated, virgin and the saline injected moths served as controls.

Results and Discussion

Although, the virgin as well as mated silkmoths lay eggs, the egg laying behaviour was found to be quite different in these two with respect to the duration of pre-oviposition period, the total number of eggs laid, the time taken to complete egg laying and the temporal pattern of egg laying i.e., the number of eggs laid at different intervals of egg laying period.

Table 1: Egg laying behaviour of Virgin and Mated female silkmoths

Moth sample	Pre-oviposition period (hrs) Mean ± S E	t-value	Oviposition period (hrs) Mean ± S E	t-value	Total number of eggs laid Mean ± S E	t-value
Virgins	7.65 ± 0.081	14.498*	226.56 ± 5.89	17.221*	399.00 ± 11.00	7.819*
Mated	5.84 ± 0.095		92.16 ± 5.12		537.00 ± 13.00	

* Significant at the level of 0.001

The pre-oviposition period lasted for about 5.84 ± 0.095 hrs in mated moths. In virgin moths it lasted for about 7.65 ± 0.081 hrs. The pre-oviposition period in case of virgins was taken into account from the time of termination of copulation in mated moths as they emerged around the same time in order to compensate for mating duration. The oviposition duration was longer in virgins lasting for about 226.56 ± 5.89 hrs. Whereas in mated moths it was about 92.16 ± 5.12 hrs. There was a significant difference in the number of eggs laid by mated and virgin moths. The total number of eggs laid by mated moths (537.00 ± 13.00) was found to be significantly higher when compared to that laid by virgin moths (399.00 ± 11.00). The virgin laid only 74.42% of the eggs laid by mated moths, a decrease of about 25.58%.
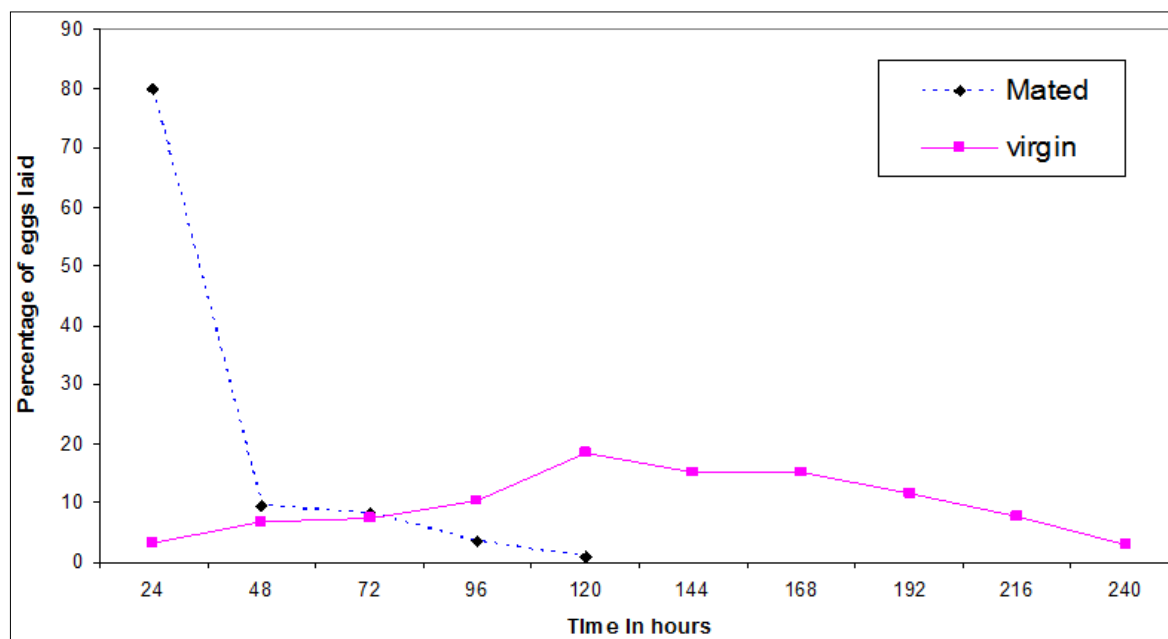

Fig 1: Temporal pattern of egg laying in virgin and mated silkmoth *Bombyx mori*

The egg laying was much faster in mated moths than in virgins. The mated moths laid about 80.01% of the total eggs within the 24 hrs after the initiation of oviposition, whereas the egg laying was very slow in virgin moths and they laid only 3.21% of the total eggs in the same period. The rate of egg laying declined with the advance of time in mated moths, whereas in virgins it increased with time to reach a maximum of 20% on the fifth day and started declining thereafter.

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