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# Male contribution of nutrients to female at the time of mating: A token of love

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**Abstract**
Nutrients are transferred from male to female during copulation and are important to silkmoth, which does not feed during its adult life. The present study examined the protein content as nutrient source and its utilization by the females that were allowed to mate for different durations. It was observed that there was a significant increase in protein content in female moths after mating, which subsequently decreased on oviposition and this decrease was more in mated females when compared to oviposited virgins. Increased mating duration reduced more protein in male reproductive tissues and increased the gain in female moths. Protease activity remained the same in all the tissues of male and female moths before and after mating, except in male accessory glands, and female bursa copulatrix and spermatheca, suggesting that the transferred proteins do not serve as the main source of energy for mating or post mating activities of female moths.

**Keywords:** *Bombyx mori*, mating, oviposition, protein, protease

## Introduction
The reproductive success of a male not only depends on the delivery of sperm to female but also on the transfer of seminal fluid, which is packed within a spermatophore, produced by one or more reproductive tissues of the male. The seminal fluid transferred to the female during mating can enhance the reproductive success of males by inducing many behavioral and physiological changes in females [1]. The seminal fluid components may induce un-receptivity in females [2]; prevent further mating by introducing a mating plug [3]; or influence egg maturation and fecundity [4]. In some insects species it is shown that the male spermatophore provides nutritional benefits that increase female reproductive output (egg number or size) or contribute to female somatic maintenance while such effects are lacking in other species [5].
The reproductive phase of an insect involves mating and egg-laying that occurs at the expense of enormous energy. In some of the Lepidopterans like silkmoth, adult stage is a non-feeding stage. In such insects, probably the seminal fluid transferred to female plays a major role in providing the nutrients.
Even though proteins are transferred to the female during copulation, their role as energy source is not well understood. Enormous data on the changes in protein content derived from male accessory glands before and after mating is available, but a lacuna exists concerning proteins from other reproductive tissues. Also, copulation duration has a significant effect on the number of eggs laid and the pre-oviposition period in silkmoth [6].
Therefore, the aim of our study was to investigate the causal relationship between copulation duration and firstly, the amount of protein transferred and secondly, the protease enzyme activity – a marker for protein utilization. Due to availability of resources we extended the study to evaluate the effect of oviposition on protein content and enzyme activity in mated and virgin female moths.

## Material and methods
Silkworm seed cocoons ($NB_4D_2$) were purchased from the Government Bivoltine Cocoon market, Bangalore. The pupae were sexed, and maintained in separate cages as the moths begin to copulate immediately after eclosion. The moths were allowed to mate for different durations: 2hours (2h), 4 hours (4h), and 6hours (6h) as soon as they emerged. The 6h-mated females were divided into two groups. One group was used to quantify the protein and

enzyme activity immediately after mating along with 2h and 4h mated moths. The second group and virgin females were allowed to lay eggs and later used for quantification. Just emerged virgin male and female moths served as controls. Each experimental group contained ten moths.

Moths of all the groups were dissected in ice cold silkmoth saline[7] and different tissues of the reproductive system were pooled and assayed immediately. The total protein content was estimated by Lowry's method[8] and the protease activity was measured by Kunitz method[9]. All the experiments were repeated thrice and the resultant data was subjected to statistical analysis using Student paired samples *t*- test.

## Results

### Copulation duration and Protein content

Protein content decreased in male moths after mating (Table-1). In testes the decrease was 11% at the end of 2 hours mating and increase in the mating duration up to 4 hours did not show any decrease. But at the end of 6 hours mating the total loss was 26%. In vas deferens also the decrease was 11% at the end of 2 hours mating, but with increase in the mating duration the protein content slightly increased . In seminal vesicle the decrease was only in 2 hours mated moths. Increase in mating duration to 4 hours did not cause further loss in protein content, but an increase of 30% compared to 4 hours mated moths was observed in 6 hours mated moths. The protein content decreased with increase in mating duration upto 4 hours in ejaculatory duct. When compared to 4 hour mated moths, a slight increase was recorded after 6 hours of mating. Whereas in accessory glands with 4 hours of mating a slight increase was observed. Compared to 2 hour mated moths. However in all these tissues increase is in comparison with the loss occurred in moths with short mating duration but not with the virgin moths and this increase was not significant. Irrespective of mating duration the protein levels always remained lower than that of virgins.

**Table 1:** The protein content (mg/g) in different tissues of male reproductive system after mating

| Rep. tissues | Virgins Mean ± S. E | 2 H Mated Mean ± S. E | 4 H Mated Mean ± S. E | 6 H Mated Mean ± S. E |
|---|---|---|---|---|
| Testes | 30.88 ± 0.86 | 27.55 ± 0.77**** | 27.77 ± 0.97***** | 22.55 ± 0.93* |
| Vas deferens | 33.77 ± 0.908 | 30.08 ± 0.808**** | 31.06 ± 0.83***** | 31.31 ± 0.941 NS |
| Seminal vesicle | 43.54 ± 2.03 | 25.26 ± 1.17**** | 25.33 ± 1.18* | 33.94 ± 1.58*** |
| Ejaculatory duct | 34.48 ± 2.85 | 29.29 ± 2.42 NS | 20.47 ± 1.89*** | 21.25 ± 1.75*** |
| Accessory gland | 50.44 ± 2.77 | 26.06 ± 1.43* | 33.30 ± 2.83*** | 25.10 ± 1.38* |

(Significant at the level of * 0.001, ** 0.005, *** 0.01, **** 0.02, ***** 0.05 , NS: Not significant)

In female moths the protein content increased gradually with increase in mating duration (Table-2). In ovary, oviduct and accessory glands it increased by 64%, 89% and 55% respectively after 6h mating, whereas in spermatheca there was a 3-fold increase and in bursa copulatrix there was a 5-fold increase when compared to virgin controls after 6h mating.

**Table 2:** The protein content (mg/g) in different tissues of female reproductive system after mating

| Rep. tissues | Virgins Mean ± S. E | 2 H Mated Mean ± S. E | 4 H Mated Mean ± S. E | 6 H Mated Mean ± S. E |
|---|---|---|---|---|
| Ovary | 31.46 ± 0.92 | 36.61 ± 1.08*** | 41.49 ± 2.22*** | 51.45 ± 1.51* |
| Oviduct | 7.47 ± 0.90 | 9.38 ± 1.13 NS | 12.56 ± 1.82***** | 14.15 ± 1.71**** |
| Spermatheca | 12.4 ± 0.57 | 20.19 ± 0.92* | 29.99 ± 1.87* | 39.49 ± 0.81* |
| Bursa copulatrix | 8.78 ± 0.63 | 28.63 ± 2.07* | 32.57 ± 2.85* | 47.59 ± 2.44* |
| Accessory gland | 42.60 ± 0.98 | 46.86 ± 1.08**** | 58.78 ± 1.35* | 66.16 ± 0.52* |

(Significant at the level of * 0.001, ** 0.005, *** 0.01, **** 0.02, ***** 0.05 , NS: Not significant)

### Oviposition and Protein content

Following oviposition the protein content decreased in all the tissues of both virgin and mated female (Table-5). Protein depletion was more in all the tissues of mated female. In bursa copulatrix and oviduct the depletion exceeded the protein content that could have been available without mating. In virgins the depletion was comparatively more in ovary and accessory glands, but in all the tissues the depletion was less when compared to mated moths.

**Table 5:** The protein content in different tissues of female reproductive system before and after egg laying

| Rep. Tissues | Virgins Mean ± S. E | VAE Mean ± S. E | 6 H Mated Mean ± S. E | MAE Mean ± S. E |
|---|---|---|---|---|
| Ovary | 31.46 ± 0.92 | 12.52 ± 0.36 | 51.45 ± 1.51* | 15.73 ± 0.46** |
| Oviduct | 7.47 ± 0.90 | 6.14 ± 0.74 | 14.15± 1.71**** | 7.22 ± 0.87NS |
| Spermatheca | 12.4 ± 0.57 | 11.35 ± 0.52 | 39.49 ± 0.81* | 19.19 ± 0.88* |
| Bursa copulatrix | 8.78 ± 0.63 | 8.62 ± 0.62 | 47.59 ± 2.44* | 34.16 ± 2.47* |
| Accessory gland | 42.60 ± 0.98 | 17.31 ± 0.39 | 66.16 ± 0.52* | 21.21 ± 0.48* |

Note: VAE: Virgin moths after egg laying, MAE: Mated moths after egg laying

(Significant at the level of * 0.001, ** 0.005, *** 0.01, **** 0.02, ***** 0.05 , NS: Not significant)

### Copulation duration and Protease activity

The enzyme activity in all the tissues of male reproductive system showed no significant difference with the increase in mating duration except in accessory glands of 6 hours mated moths (Table-3).

**Table 3:** The protease enzyme activity (mg/g/min) in different tissues of male reproductive system after mating

| Rep. tissues | Virgins Mean ± S. E | 2 H Mated Mean ± S. E | 4 H Mated Mean ± S. E | 6 H Mated Mean ± S. E |
|---|---|---|---|---|
| Testes | 0.102 ± 0.08 | 0106 ± 0.07 NS | 0.107 ± 0.09 NS | 0.136 ± 0.07 NS |
| Vas deferens | 0.066 ± 0.03 | 0.07 ± 0.01 NS | 0.072 ± 0.02 NS | 0.079 ± 0.03 NS |
| Seminal vesicle | 0.048 ± 0.02 | 0.071 ± 0.03 NS | 0.072 ± 0.02 NS | 0.055 ± 0.03 NS |
| Ejaculatory duct | 0.15 ± 0.02 | 0.136 ± 0.06 NS | 0.20 ± 0.03 NS | 0.18 ± 0.07 NS |
| Accessory gland | 0.176 ± 0.04 | 0.28 ± 0.03 NS | 0.23 ± 0.04 NS | 0.32 ± 0.02**** |

(Significant at the level of * 0.001, ** 0.005, *** 0.01, **** 0.02, ***** 0.05 , NS: Not significant)

In female tissues the enzyme activity showed significant decrease only in spermatheca and bursa copulatrix of all the mated groups where 3-5 fold increase in protein content was found. (Table-4)

**Table 4:** The protease enzyme activity (mg/g/min) in different tissues of female reproductive system after mating

| Rep. tissues | Virgins Mean ± S. E | 2 H Mated Mean ± S. E | 4 H Mated Mean ± S. E | 6 H Mated Mean ± S. E |
|---|---|---|---|---|
| Ovary | 0.067 ± 0.02 | 0.058 ± 0.03 NS | 0.051 ± 0.03 NS | 0.042 ± 0.02 NS |
| Oviduct | 0.15 ± 0.08 | 0.131 ± 0.06 NS | 0.11 ± 0.08 NS | 0.12 ± 0.04 NS |
| Spermatheca | 1.05 ± 0.12 | 0.71 ± 0.07***** | 0.05 ± 0.08*** | 0.4 ± 0.06** |
| Bursa copulatrix | 1.67 ± 0.06 | 0.546 ± 0.09* | 0.50 ± 0.06* | 0.35 ± 0.05* |
| Accessory gland | 0.026 ± 0.01 | 0.024 ± 0.01 NS | 0.02 ± 0.01 NS | 0.019 ± 0.01 NS |

(Significant at the level of * 0.001, ** 0.005, *** 0.01, **** 0.02, ***** 0.05 , NS: Not significant)

Oviposition and protease activity:

Following oviposition the enzyme activity significantly decreased only in bursa copulatrix and spermatheca when compared to 6h mated moths before egg laying, whereas in virgins there was no significant difference in enzyme activity before and after egg laying (Table 6).

**Table 6:** The protease enzyme activity in different tissues of female reproductive system before and after egg laying

| Rep. tissues | Virgins Mean ± S. E | VAE Mean ± S. E | 6 H Mated Mean ± S. E | MAE Mean ± S. E |
|---|---|---|---|---|
| Ovary | 0.067 ± 0.02 | 0.11 ± 0.04 | 0.042 ± 0.02 NS | 0.035 ± 0.02 NS |
| Oviduct | 0.15 ± 0.08 | 0.14 ± 0.05 | 0.12 ± 0.04 NS | 0.165 ± 0.02 NS |
| Spermatheca | 1.05 ± 0.12 | 1.03 ± 0.07 | 0.4 ± 0.06** | 0.57 ± 0.08*** |
| Bursa copulatrix | 1.67 ± 0.06 | 1.57 ± 0.07 | 0.35 ± 0.05* | 0.39 ± 0.03* |
| Accessory gland | 0.026 ± 0.01 | 0.057 ± 0.03 | 0.019 ± 0.01 NS | 0.036 ± 0.02 NS |

Note: VAE: Virgin moths after egg laying, MAE: Mated moths after egg laying

(Significant at the level of * 0.001, ** 0.005, *** 0.01, **** 0.02, ***** 0.05 , NS: Not significant)

## Discussion

The transfer of seminal fluid from male to female and its effect on female behavior and physiology has been documented in several insects [10, 11]. For example, some chemicals in the seminal fluid transferred to female at the time of mating induce acceleration of egg laying [12, 13] and/or unreceptivity of the female to male [14, 15]. The males actually maximize their reproductive success by inducing monogamy in females. They can also maximize their reproductive success by transferring the nutrients to females [16-18]. In many insects it is shown that mating depletes a lot of substances from male reproductive system where as females gain many of these substances after mating [19]. In silkmoths we have analyzed the transfer of proteins at the time of mating.

It was found that the male moths lost lot of protein from their reproductive tissues after mating irrespective of mating duration. On the other hand the reproductive tissues of females gained proteins after mating indicating a transfer of proteins from males to females at the time of mating. A slight increase in protein content in some tissues of 4hr and 6hr mated moths after an initial loss with 2hr mating may be due to the residual flow of sperm and other secretions, especially to the seminal vesicle. The protease enzyme activity is more in accessory glands of 6hr-mated moths may be indicating activity level of these fluids.

In female moths the enzyme activity was found to decrease but was significant only in spermatheca and bursa copulatrix, indicating that protein is not the major source of energy for mating activities particularly in the spermatheca and bursa copulatrix, which act as storehouses. Either these two tissues need less energy or they depend on either carbohydrates or lipids but not on proteins.

In some insects carbohydrates or lipids serve as main sources of energy [20, 21]. It is clear from the enzyme activity of male and female moths that proteins are transferred to female but they are not the main source of energy for their mating activities but for ovipositional activities in females as it is a lengthy process taking place for several days. It is evident from the present studies, as the tissues of females are depleted of protein to a greater extent. In mated moths the protein depletion exceeded the virgin level in ovary, spermatheca and accessory glands, suggesting not only the transfer of nutrients from male to female but also that they serve as source of energy for ovipositional activities. Where as in virgin moths though the proteins are depleted after egg laying, the depletion is negligible in all the tissues except ovary and accessory glands as ovary releases eggs and accessory glands coat each egg with a material, which glues it to the substratum. In silkmoth B.mori the egg laying behavior of virgins and mated moths vary to a great extent. The virgin lays less number of eggs, at a slower rate, and initiation of

egg laying is also delayed compared to the mated moths [22, 23] of male origin, but the less number of eggs is probably due to the nutritional level.

The oocytes, like muscle, fat body and other tissues, not only have specific function but also could be called upon as reservoir of food. Sometimes they are reabsorbed in the ovarioles of insects and utilized for general metabolic activities [24] in silkmoth virgins, as they do not lay their full quota of eggs, some of them may be reabsorbed, hence the enzyme activity after egg laying is more in ovary of virgins compared to mated moths. Hence virgins live longer. This holds good even with accessory glands where enzyme activity is more. Since the number of eggs laid is less, much of accessory glands secretions are not used to glue the egg.

The whole purpose of transferring nutrients from male to female is to see that females (non-feeding) are not deprived of nutrients to lay eggs. Males achieve their goal of maximizing their reproductive success by donating nutrients to female.

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