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Male silk moths for the post-mating activities of female Silkmoth *Bombyx mori* L.

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Abstract
Male contribution of lipids to the female at the time of copulation was evaluated by measuring the lipid content in reproductive tissues of virgin and mated, male and female moths of *Bombyx mori* L. The lipid content was also measured after egg laying in both virgin and mated female moths. Lipid content decreased in the reproductive tissues of male moth after mating. In female contrary to male, a significant rise was observed in all the tissues. Following oviposition the lipid content decreased in all the tissues of both virgin and mated females, however, the loss was more in mated moths.

Keywords: *Bombyx mori*, mating, oviposition, lipid

Introduction
Studies on various insects have shown that males not only contribute sperm to the female during mating but also some substances secreted by different tissues of reproductive system (Leopold, 1976; Friedel and Gillott, 1977) [15, 12] and nutrients via spermatophore and seminal fluid (Thornhill, 1976; Boggs and Gilbert, 1979; Rutowski, 1984) [25, 5, 23]. This is of great importance to the reproductive success, since seminal fluid components perform diverse functions, like, induction of oogenesis, enhancement of fertility and fecundity, suppression of remating, stimulation and acceleration of oviposition etc. in the female and benefits both the male and its mate (Pickford *et al*., 1969; Engelmann, 1970; Gillott, 1988; Bali *et al*., 1996; Eberhard, 1996; Wolfner, 1997) [19, 8, 11, 2, 7, 27].
In many insects, females make use of the seminal fluid as nutrients for somatic maintenance or egg production or egg deposition (Boggs and Watt, 1981; Marshall, 1985) [4, 17]. Among nutrients, lipids are of vital importance to many insects as metabolic reserves especially for those insects, which exhibit, prolonged periods of metabolic activity like embryogenesis, metamorphosis and flight (Gilbert, 1967; Van Heusden, 1993) [10, 26], diapause (Downer, 1985) [6] and mating (Ranganathan and Padmanabhan, 1994) [20]. There exists a lacuna in understanding the nutrient utilization especially the lipids and their source at the time of mating and egg laying in silkmoth *Bombyx mori* L. Present investigation quantify lipids in various reproductive tissues of male and female moths before mating, after mating, and after oviposition to analyze their utilization for the processes of mating and oviposition, as these processes are quite elaborate and occur at the expense of enormous energy. In silkmoths mating is not an absolute necessity for egg laying. As virgins also lay eggs, it is interesting to analyze the nutrient level in these moths after egg laying.

Material and Methods
Silkworm seed cocoons (NB4 D2) were purchased from the Government Bivoltine Cocoon Market, Bangalore, India. The pupae were removed from the cocoons, sexed and maintained in separate cages to avoid copulation immediately after emergence. The moths were allowed to mate for 6 hours as soon as they emerged. The female moths were divided into two sets. The first set was allowed for egg laying along with virgins. The female and male moths of second set were used immediately for the assay of lipid. After egg laying the virgins and females of first set were also used for the assay. The virgin male and virgin female before egg laying served as control.
For lipid assay by Handel (1985) [13] method, the moths were dissected in silkmoth saline (Yamaoka, 1977) [28] and different tissues of reproductive system were pooled from ten months.

The data was generated by repeating the experiment thrice and subjected to statistical analysis (Snedecor and Cochran, 1994) [29]. The parameters were analyzed by student's 't' test.

The depletion of lipid in oviposited moths was calculated using the following formula:

$$\text{Percentage Depletion} = \frac{\text{Lipid content before egg-laying in virgins/ *mated moths} - \text{Lipid content after egg laying}}{\text{Lipid content in virgins before egg laying}} \times 100$$

*In mated moths the loss was calculated considering the gain after mating.

Results

In virgin male moths the lipid content was more in testes followed by seminal vesicle, vas deferens, accessory glands and ejaculatory duct. After mating it decreased in all these tissues (Table 1). The maximum loss of lipid was in testes (57%), followed by accessory reproductive glands (42%) and the least was in seminal vesicle (24%) (Fig.1).

Table 1: Lipid content (mg/g) in tissues of male silkmoths before and after mating

Reproductive tissues	Virgin moths Mean ± S.E	Mated moths Mean ± S.E
Testes	78.85 ± 1.04	33.55 ± 1.12 *
Vas deferens	64.57 ± 0.37	40.20 ± 0.33 *
Seminal vesicle	69.19 ± 1.09	52.80 ± 1.06 *
Ejaculatory duct	32.41 ± 0.67	24.35 ± 0.66 **
Accessory gland	48.59 ± 0.37	28.50 ± 0.31 *

Significant at the level of * 0.001, ** 0.005

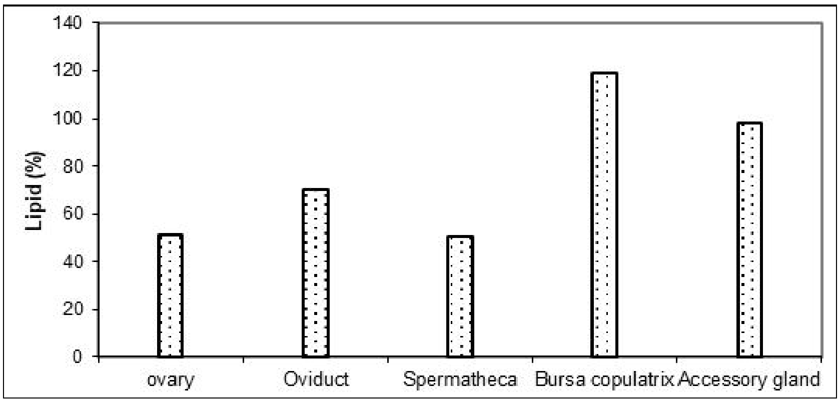

Fig 1: Gain of lipids in reproductive tissues of female moths after mating

In female moths before mating the lipid content was maximum in ovary followed by oviduct, accessory reproductive glands, bursa copulatrix and spermatheca. Following mating it increased significantly in all the tissues (Table- 2). Relatively the gain was more in bursa copulatrix (119%) followed by accessory reproductive gland (98%) where as spermatheca recorded the least (Fig. 2).

Table 2: Lipid content (mg/g) in tissues of female silkmoths before and after mating

Reproductive tissues	Virgin moths Mean ± S.E	Mated moths Mean ± S.E
Ovary	43.60 ± 0.69	65.90 ± 1.04 *
Oviduct	14.79 ± 0.75	25.22 ± 0.88 *
Spermatheca	3.99 ± 0.01	6.01 ± 0.11 *
Bursa copulatrix	7.25 ± 0.02	15.90 ± 0.23 *
Accessory gland	11.31 ± 0.51	22.39 ± 1.01 *

Significant at the level of *0.001

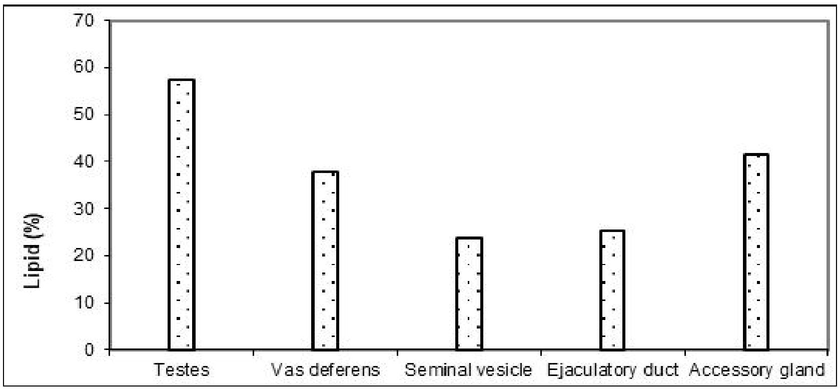

Fig 2: Loss of lipids in reproductive tissues of male moths after mating

Following oviposition the lipid content decreased in all the tissues of both virgin and mated moths (Table 3). In oviduct, bursa copulatrix and accessory gland of mated moths, the loss exceeded the initial content i.e., before egg laying. In virgins the loss was very small compared to the mated moths. (Fig.3).

Table 3: Lipid content (mg/g) in tissues of female silkmoths after egglaying

Reproductive tissues	Virgin moths Mean ± S.E	Mated moths Mean ± S.E
Ovary	17.87 ± 0.28	23.31 ± 0.36 *
Oviduct	11.09 ± 0.56	10.10 ± 0.51 NS
Spermatheca	3.78 ± 0.03	2.09 ± 0.06 *
Bursa copulatrix	6.86 ± 0.02	5.53 ± 0.08 *
Accessory gland	8.57 ± 0.38	8.39 ± 0.38 NS

Significant at the level of *0.001
NS: Not Significant

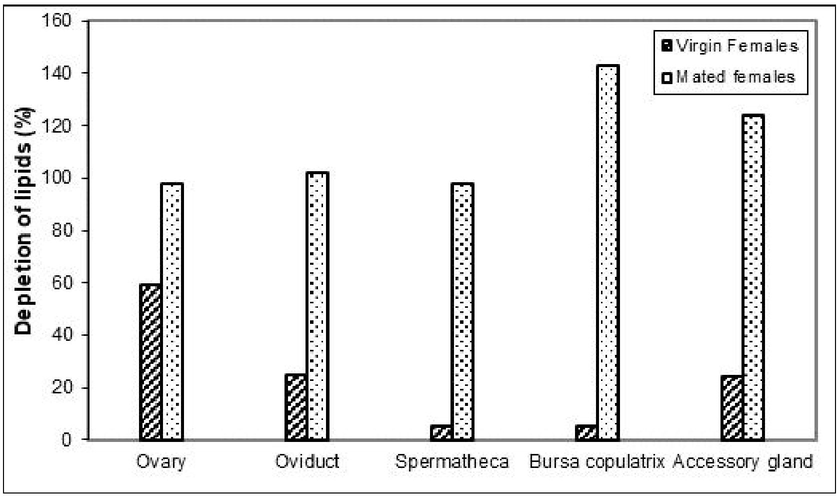

Fig 3: Depletion of lipids in the tissues of virgin and mated female moths after egg laying

Discussion

In insects mating not only alters the behaviour of female but also provide nutrients, as some of the substances are transferred from male to female during mating (Leopold, 1976) [15]. It is very much evident from the present investigation in which the lipid content of the different reproductive tissues in both the sexes of *Bombyx mori* was measured. The reproductive tissues of male moth lost considerable amount of lipids whereas the female tissues gained considerably after mating.

In male moth the loss is due to the seminal flow into female during copulation. Testis being the major loser, the loss may be attributed to the outflow of sperms from the testis. Vas deferens and ejaculatory duct transport the sperms from testis to seminal vesicle and from there to bursa copulatrix of female respectively exhibit peristaltic contractions to propel the sperms down (Romoser, 1973) [22]. Ejaculatory duct also secretes some of the substances, which are introduced into the female (Bairati, 1968; Gillott and Friedel, 1977) [1, 12]. In these tissues the loss may be due to lipolysis as to provide energy for muscle contraction and may also aid in secretion. The seminal vesicle store the sperms until they are delivered to female (Romoser, 1973) [22]. The nourishment of sperms in seminal vesicle may be supported by the lipids. The accessory gland is a site of many functions like activation of sperms, production of spermatophores, nourishment of sperms etc. which may utilize the lipids for these functions. Accessory glands are also known to secrete some of the substances, which are transferred to female where they perform several functions (Rockstein, 1964; Leopold, 1976; Thornhill, 1976; Boggs, 1981; Wolfner, 1997) [21, 15, 25, 3, 27]. In Odontopus varicornis the tri-acyl glycerol a lipid from the accessory gland contributes to the energy needs for mating especially in prolonged mating (Ranganathan and Padmanabhan, 1994) [20]. This may be true in *Bombyx mori* as well because the lipid depletion was more in the accessory glands.

In most of the insects the nutrient supplement to the female seems to be the second most priority of the male next to sperm contribution as the nutrient level increases in females following mating. This increase is a result of male contribution either directly or indirectly by mobilization of nutrients from other reproductive tissues as well as non reproductive tissues (Niemierko *et al*., 1956; Boggs and Gilbert, 1979; Marshall, 1985; Soller *et al*., 1997) [18, 5, 17, 24].

In *Bombyx mori* also the lipid content increased by one and a half to two times in female tissues following mating. This clearly indicates that the loss of lipid in male is not totally to meet the energy needs of various reproductive tissues for their respective functions in that moth but also due to the transfer of lipids from male to female at the time of copulation. Irrespective of mating or not, the female lay eggs, but, there is a lot of difference in the egg laying behaviour of virgin and mated females. Mated moths lay more eggs than virgins and at a faster rate laying full quota of their egg complement within 46 hours against virgins, which lay about 75% of the eggs that are laid by mated moths over a period of 101 hours (Manjulakumari and Geethabali, 1993) [16]. The loss of lipids was more in mated moths following oviposition compared to oviposited virgins as, mated moths exhibit vigorous oviposition.

The loss in some of the tissues like oviduct, bursa copulatrix and accessory glands of mated moths was more than that was available in virgins before egg laying. Oviduct facilitates passage of ovum by peristaltic waves naturally requires more energy. Bursa copulatrix stores the sperms for considerable time before passing them on to spermatheca. The accessory glands secrete adhesive material and serve to cement eggs to the substratum as in case of *Bombyx mori* or hold them together in masses as in case of cockroach where the secretion surrounds the eggs to form a capsule or ootheca (Romoser, 1973; Hinton, 1981) [22, 14]. These tissues require lot of energy especially in mated moths as they lay about 76% of their eggs within six hours after the initiation of oviposition whereas the virgins lay only 5% over the same period (Manjulakumari and Geethabali, 1993) [16]. Lipids may serve these tissues as energy source in their various functions at the time of oviposition in silkmoth *Bombyx mori.*

The mated moths could afford to spend more lipids than the amount that was present in them prior to mating to meet the energy requirement of oviposition process as they could gain some from male moths. In providing nutrients the male actually makes an investment on female to ensure the viable progeny of its own. It is of significance especially in some of the lepidopteran species such as *Bombyx mori*, which do not feed during adult life.

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