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Effect of Mating Delay and Mating Duration on Reproductive Performance of *Antheraea mylitta*

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Tropical tasar silkmoth *Antheraea mylitta* D. is an economically important insect. The wild nature and above all unsynchronized emergence of moths lead to impairment in reproductive activity. The mating in moths is low (64%-70%), leaving about 30-36% of potential females unused for seed (=egg) production. Delay in mating adversely affects the reproductive performance such as mating behaviour, fecundity and fertility. Females are more severely affected by mating delay than males. Therefore, a study was under taken to develop a method to overcome the problem so that the production of layings can be increased to meet the ever increasing demand. It was revealed from the study that out of 16 possible age combinations (from the age groups of day 0 to day 3), female and male of day 0 (4-8 hours old) excel in their reproductive performance than others (with 94.4% mating success, 284 fecundity and 91.5% hatching). Reproductive performance declined significantly even if one of the parents was older. When either of the mating pair was 1 day old, the mating declined by 28%; fecundity by 32.8 %; and hatching by 40.8%. The insemination of eupyrene sperm was declined by 35.2% when both the parents are 1 day old. Mating duration of 8 hours resulted in insemination of 8.3×10^5 eupyrene sperms, which was declined by 31.6%, 48% and 55% upon lowering of mating duration to 6 hr, 4 hr and 2 hr were maintained respectively.

Key words: *Antheraea mylitta*, Eupyrene sperm, Mating, Mating duration, Reproductive performance.

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Introduction

Mating and oviposition are two important behavioural aspects of insect reproduction. As the physiology of both the sexes change over time, the age of the insect when they mate influence their reproductive performance and population growth. Delay in mating generally shortens the oviposition period and reduces fecundity and fertility. This reduction in fertility and fecundity may be the result of oviposition of unfertilized eggs by virgin females, affecting their overall fecundity (Foster *et al.*, 1995; Rath *et al.*, 1997), or decline in mating aptitude by older ones (Lingren *et al.*, 1988; Haniffa and Thatheyus, 1992; Paul *et al.*, 1993; Spurgeon *et al.*, 1995; Proshold, 1996; Rath *et al.*, 1997, 2002; Rath, 2000) or reception of sperm of low quantity and quality from old males (Unnithan and Paye, 1991, Roger and Marti, 1994; Vickers, 1997). A number of deleterious changes such as, low mating ability in old virgin males, decline in total number of mature spermatocytes and insemination rate, change in ductus ejaculatorious simplex, testes and ovary are the major factors for decline in reproductive performance (Fonesca and Buhrnheim, 1984; Chien and Chow, 1985; Verhoek *et al.*, 1989; Mahmood and Reisen, 1994). The parental age influence the reproductive performance of the mating pair through mating behaviour, fecundity and fertility of gametes (Clark and Rockstein, 1964; Rogers and Marti, 1994).

Tropical tasar, *Antheraea mylitta* moth is wild and behaves differently in captivity. Emerged adult moths are sexually mature and mate with in 2-3 hours of emergence (Jolly *et al.*, 1979). After natural mating inside the grainage (64-70%) about 30-36% of potential females remains virgin that lay good number of eggs (20-60 eggs) unfertilized. Decline in mating success, fecundity and fertility with advancing age in *A. mylitta* have been reported (Rath *et al.*, 1997, 2002; Rath, 2000). Literatures revealed that no work was attended in the past on change in the quan-

tum of insemination with advancing age of the mating pair and their subsequent movement in the female tract and at different hours mating duration and its impact on reproductive performance which is the aim of the present study.

Materials and methods

Antheraea mylitta Drury (Lepidoptera: Saturniidae, eco-race: Daba bivoltine) moths were collected soon after eclosion in the night and kept separately in closed bamboo basket at room temperature. A total of 1200 individual pairs of moths of different age combinations (Table 1) were collected and mating were affected for 8 hours (Rath *et al.*, 2002). After the desired period of mating each female was provided with an earthen cup for oviposition. Egg masses were collected after 72 hours and fecundity and fertility were recorded. As the age combinations beyond day 1 did not gave good results (*i.e.*, the mating success and fertility remained below 50%) and hence were not considered for further analysis to draw the results. Single egg weight, larva weight at hatching and their survival till I moult were recorded to find out the effect of mating delay.

The selected pair combination F × M: 0 × 0, 0 × 1, 1 × 0 and 1 × 1 were further used for eupyrene sperm counts in the female tract (spermatheca and bursa copulatrix). After 8 hours of mating, the spermatheca and bursa copulatrix were dissected out and eupyrene sperm were counted in an improved Neubaur hemocytometer (as described by He *et al.*, 1995). There were 5 replications with sample size of 7 in each.

In an another experimental set up we have studied the effect of different mating durations (2 hr, 4 hr, 6 hr, 8 hr and 10 hr) on oviposition and fertility in the age combination of day 0 to assess the extent of effect. Eupyrene sperm counts for different period of mating were also recorded to correlate the effect. There were 5 replications with a sample size of 10 in each.

The collected data were subjected to statistical analysis using ANOVA, regression and correlation coefficient for significance.

Results

Effect of mating delay on reproductive performance and eupyrene sperm count in female reproductive tract

The probability of successful mating significantly decreased as the age of both the sexes increased ($r=-0.8773$, $P<0.001$). For example 94.4% mating success was observed when both male and female were 0 day old, and declined to 73.3% when 1 day old male was used, and to 71.7% when the female aged 1 day; while it was 67.9% when both the pairs were 1 day old (Fig. 1, Table 1). Fecundity was significantly decreased as the age of either sex increased ($r=-0.9622$, $P<0.001$), so also fertility ($r=-0.9803$, $P<0.001$). The numbers of eggs laid by the female of the mating pair of 0 day old were decreased by 93 (32.8%) eggs when the male and female moth of the mating pair was 1 day old. Fertility was declined by

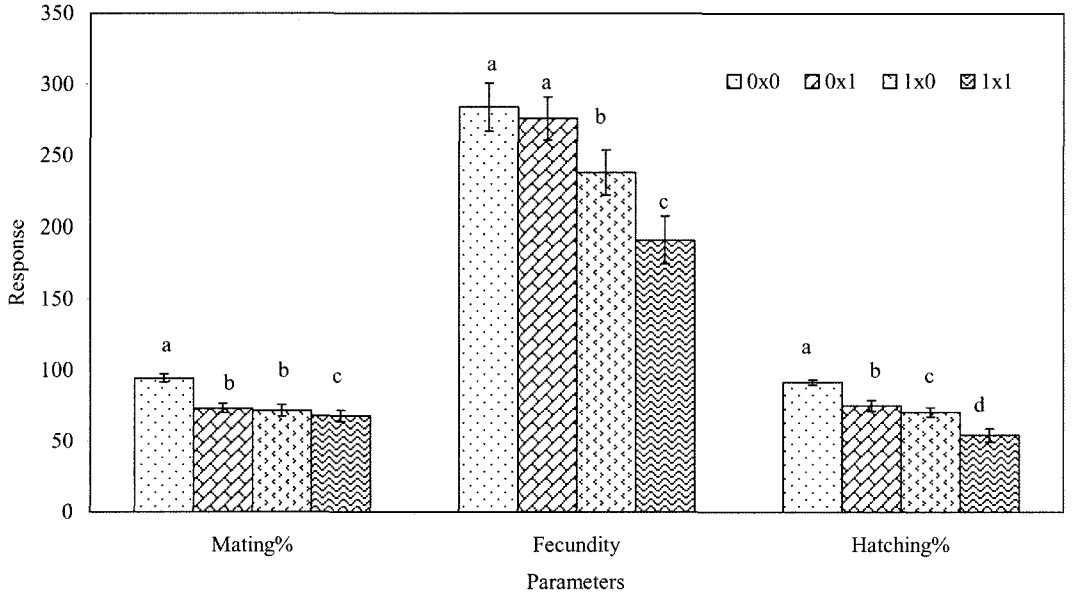

Fig. 1. Effect of age of the mating pair (days, F × M) on reproductive performance in *A. mylitta.* Values are means ± SD. Different alphabets show significant difference at 5% level (one-way ANOVA) among age groups.

Table 1. Age combination of mated pairs and sample size to assess the effect of mating delay on reproductive performance in *Antheraea mylitta* (weight of female moth 6.5–7.2 g, mating duration 8 hours, n = 50, values are mean ± SD).

Age (days)		Mating (%)	Fecundity	Hatching (%)
Female	Male			
0	0	94.40 ± 2.87	284 ± 17	91.53 ± 1.78
0	1	73.27 ± 3.41	276 ± 15	75.14 ± 3.72
1	0	71.73 ± 4.18	238 ± 16	70.46 ± 3.36
1	1	67.93 ± 4.11	191 ± 17	54.23 ± 5.71
0	2	38.00 ± 5.13	191 ± 13	41.74 ± 3.66
2	0	36.07 ± 7.96	131 ± 11	41.01 ± 3.31
0	3	8.80 ± 1.97	137 ± 15	15.78 ± 3.68
3	0	15.87 ± 4.29	75 ± 10	9.03 ± 1.20
2	2	12.20 ± 3.23	73 ± 12	32.96 ± 4.90
3	3	6.73 ± 1.75	24 ± 7	5.67 ± 1.05
1	2	22.07 ± 2.55	155 ± 10	22.96 ± 6.79
2	1	14.07 ± 3.49	97 ± 11	35.35 ± 5.13
2	3	8.00 ± 1.69	60 ± 12	6.74 ± 0.79
1	3	20.20 ± 3.08	111 ± 12	11.45 ± 4.07
3	2	7.40 ± 1.80	28 ± 7	6.07 ± 1.22
3	1	7.60 ± 1.30	55 ± 10	6.47 ± 1.36

40.8% (from 91.5% to 54.2%) in older pairs of 1 day (Fig. 1). It was evident from the results that the reproductive performances of females were more severely affected by mating delay than males. Further, the reduction in fertility was associated with aging in females was significantly greater than in males (Table 1). Coefficient of determination revealed that increased age of either sex of the mating pair was the main factor contributing towards the decline in reproductive performance in *A. mylitta* (77% for mating, 92.6% for fecundity and 96% for hatching). The overall reproductive performance resulted from the present study in relation to the age (days) of the mating pair (F × M) was in the order of 0 × 0 > 0 × 1 > 1 × 0 > 1 × 1.

With the advancing age of the mating pair the eupyrene sperm count in spermatheca declined significantly ($r=-0.9721$, $P<0.001$), so also the total (eupyrene sperms in spermatheca + eupyrene sperms in bursa copulatrix) eupyrene sperm count ($r=-0.9731$, $P<0.001$), but the quantitative changes of eupyrene sperms in bursa copulatrix did not affected ($r=0.4703$, P, NS). The eupyrene sperm count in spermatheca was declined by 22%, 26% and 46.2%; and the total (spermatheca + bursa copulatrix) eupyrene sperm count by13.9%, 17.5% and 35.2% in the mating pair (F × M) of 1 × 0, 0 × 1 and 1 × 1 respectively (Fig. 2). On the basis of eupyrene sperm count in spermatheca and total, the mating pairs were categorized as best mating pair (grade I) that includes 0 × 0; while both 1 × 0 and 0 × 1 constitute as grade II mating pair and 1 × 1 as grade III mating pair. The age of the mating pair contributed 94.7% towards the decline in total eupyrene count responsible for fertilization of eggs ($y=-0.906x+9.185$, $R^2=0.947$, $P<0.0001$).

Egg weight, larva weight and larval survival

Age of the mating pair had a significant effect on single

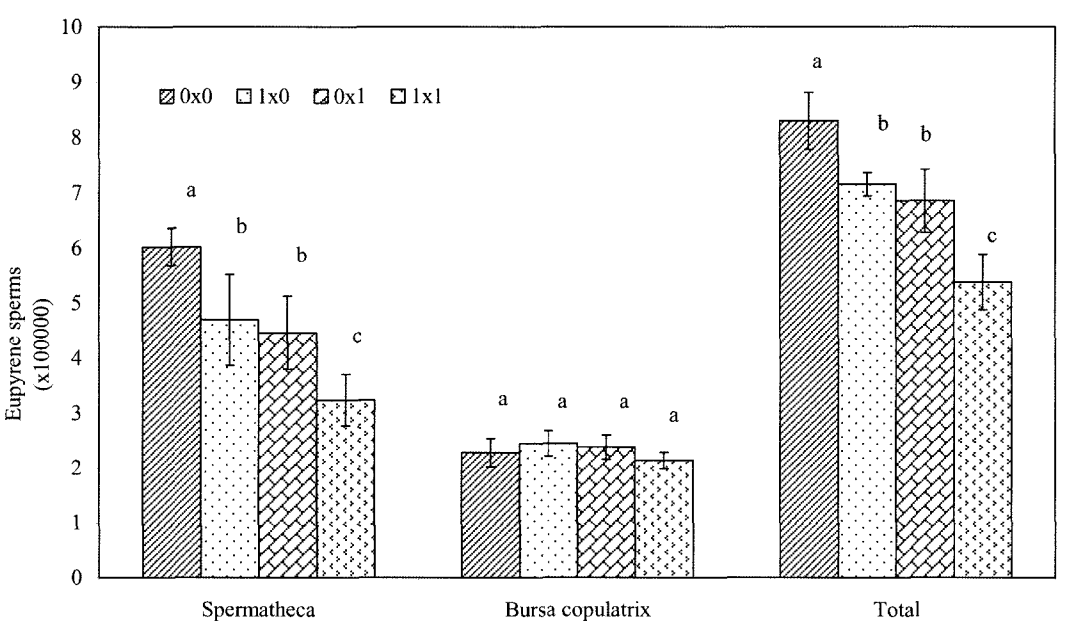

Fig. 2. Effect of age of mating pair (days, F × M) on eupyrene sperm count in the female of *A. mylitta*. Values are means ± SD. Significant differences between the age groups are indicated by different alphabets (one-way ANOVA at 5% level)

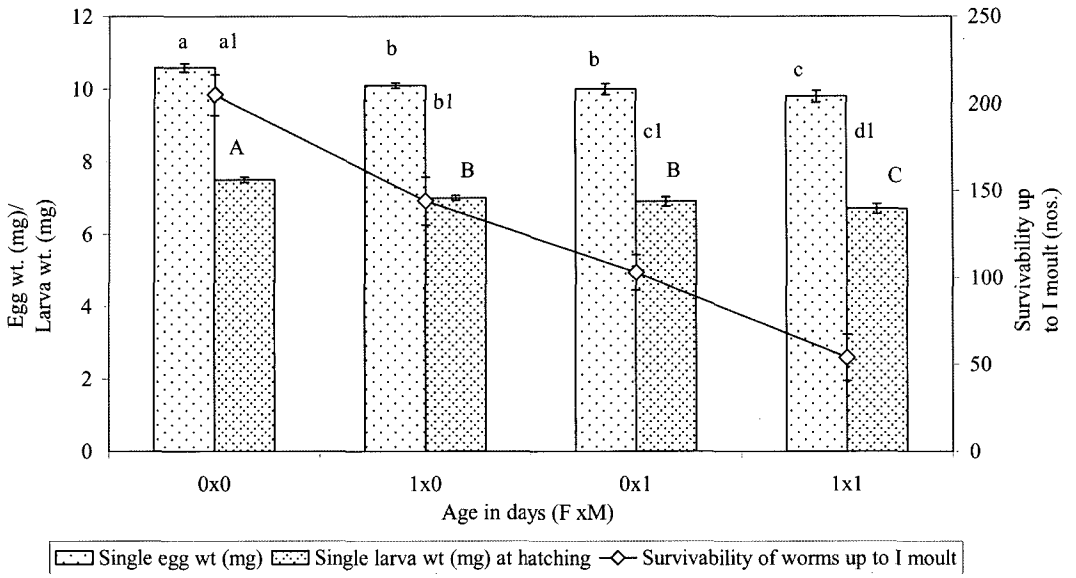

Fig. 3. Effect of age of the mating pair on egg weight, larva weight at hatching and survivability in *A. mylitta.* Values are means ± SD. Significant difference between the age groups are inidcated by different alphbets (one-way ANOVA at 5% level).

egg weight, weight of the larva hatched out and its survival up to I moult. The single egg weight was higher (10.6 mg) for fresh mating pair (each 0 day old) which was declined to 9.8 mg when the mating pair was 1 day old ($y=-0.25X+10.75$, $R^2=0.8993$, $P<0.0001$). The weight of newly hatched out larva from the eggs laid by the mating pair of 0 day old were found significantly high and then declined with the increase in the age of mating pair ($y=-0.25X+7.65$, $R^2=0.8993$, $P<0.0001$). Larval survival up to I moult was recorded highest for the young mating pair (0 day old) and declined thereafter with the age of the mating pair ($y=-49.4X+250$, $R^2=0.9939$, $P<0.0001$). Thus, the survival of the larvae declined by 29.8%, 49.8% and 73.7% in the mating pair (F × M) of 1 × 0, 0 × 1 and 1 × 1 respectively (Fig.3).

Effect of mating duration on eupyrene sperm count, oviposition and hatching

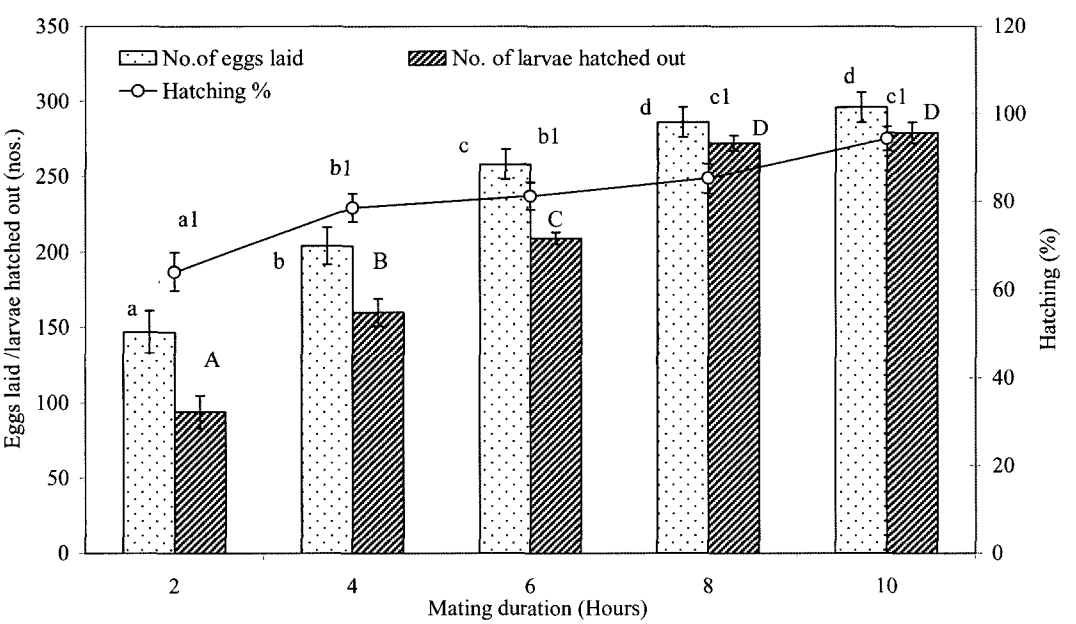

Fig. 4. Effect of mating duration on egg laying, larvae hatched out and hatching in *A. mylitta.* Values are means ± SD. Significant differences are indicated by differenct alphabets (one-way ANOVA at 5% level).

Mating duration had a significant effect on eupyrene sperm count, oviposition and hatching. With increasing mating duration the eupyrene sperm count increased significantly in spermatheca ($F=161.99$, $P<0.0001$, $R^2=0.876$), bursa copulatrix ($F=39.053$, $P<0.0001$, $R^2=0.629$), and total (spermatheca+bursa copulatrix) eupyrene count ($F=160$, $P<0.0001$, $R^2=0.8796$) (Fig. 4). Increase in mating duration also significantly increased fecundity ($F=210$, $P<0.0001$, $R^2=0.901$), number of larvae hatched out ($F=404.7$, $P<0.0001$, $R^2=0.946$) and hatching percentage ($F=124$, $P<0.0001$, $R^2=0.843$) (Fig.5). The results indicated a continuous rise in the parameters up to 8 hours of mating duration after which the parameters did not get affect significantly. Based upon the eupyrene sperm count the mating duration can be sorted out in to three distinct groups, such as, the lower mating constitute 2 hr and 4 hr groups, 6 hr forms the middle, and 8 hr and 10 hr together constitute the super groups.

Discussion

Previous studies show that mating delay substantially reduces the reproductive potential in insects including sericigenous species (Haniffa and Thatheyus, 1992; Paul *et al.*, 1993; Foster and Ayres, 1996; Knight, 1996, Rath *et al.*, 1997, 2002; Vickers, 1997; Knight and Turners, 1999; Rath, 2000; Jimenez-Perez and Wang, 2003). The present study revealed that out of 16 possible age combinations the performance level of only 4 combinations of mating pair (days, F×M) such as 0×0, 0×1, 1×0 and 1×1 remain above 50%. The mating success declined from 94.4% in mating pair of 0 day old to 67.9% in 1 day old, a decline of about 28%, the fecundity declined by 32.8% and hatching by 40.8%. Such type of reduction in the reproductive performance have been reported earlier confirms our results (Clark and Rockstein, 1964; Haniffa and Thatheyus, 1992; Paul *et al.*, 1993; Rogers and Marti, 1994; Rath *et al.*, 1997, 2002; Rath, 2000; Jimenez-Perez and Wang, 2003). The insemination and movement of sperms as recorded in the present study by the counting of eupyrene sperms in the female tract further confirm the decline in reproductive performance. The eupyrene count in spermatheca declined by 46.2%, while the total eupyrene count declined by 35.2% in mating pair of 1 day old over 0 day old. This might be due to decline in insemination rate or any deleterious changes in reproductive system in insects with advancing age (Mahmood and Reisen, 1994; Chien and Chow, 1985; Verhoek *et al.*, 1989). Aged *Bombyx mori* male moths fail to maintain the ejaculations and the separation of spermatozoa from sperm bundles are affected (Kishore Kumar and Paul, 1993). Reduction in eupyrene count in the female *A. mylitta* might be due to reception of sperms of low quantity from old males as reported for other insects (Unnithan and Paye, 1991; Rogers and Marti, 1994; Vickers, 1997) or might due to diminish females' ability to transport or store sperm during aging (Proshold, 1996). Age related decline in eupyrene bundles in the testes of adult male moth, *S. litura* are in accordance with our findings (Seth *et al.*, 2002).

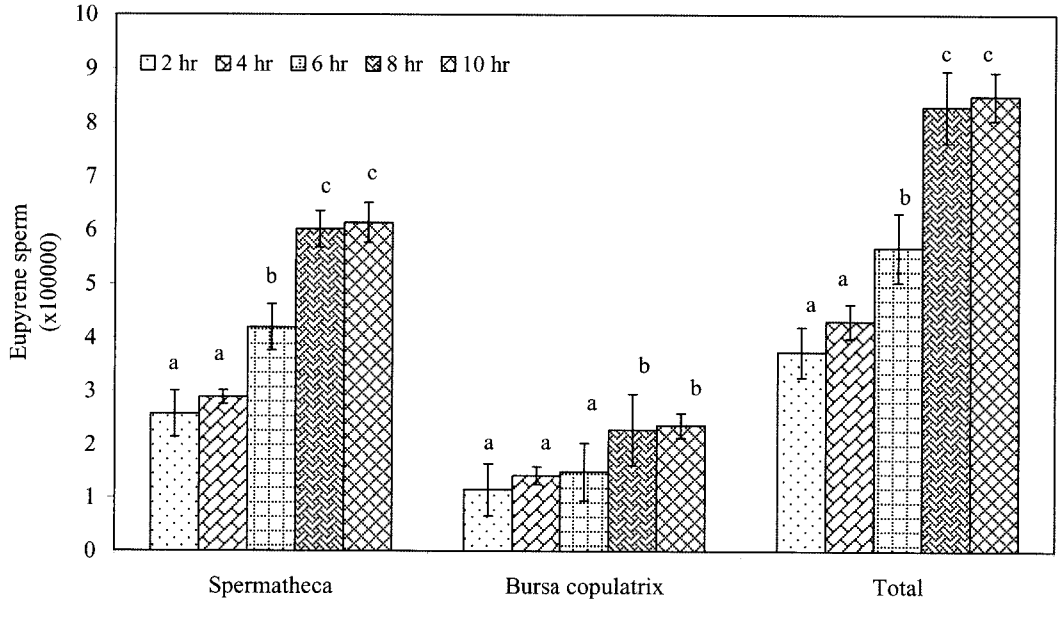

Fig. 5. Effect of mating duration on eupyrene sperm count in female reproductive organs of *A. mylitta*. Values are means±SD. Signficant differences are indicated by different alphabets (one-way ANOVA at 5 % level).

Single egg weight and the weight of the larva hatched out declined as the female moth ages and also get influenced to some extent by delay in mating in both the sexes. The single egg weight laid by the female of mating group of 0×0 was 10.6 mg and declined significantly to 9.8 mg in 1×1, so also the larva weight declined from 7.5 mg to 6.7 mg. Haniffa and Thateyus (1992) had also reported similar results in *B. mori* which might be due to the non-feeding status of the moth. Variation in egg weight, development and hatching are functions of the mother's age have been reported (Richards and Kolderie, 1957) also corroborates our results. The larval survivability also gets affected following mating delay. In the fresh pair maximum larvae survived up to I moult, but the survival of the larvae decline with delay in the mating, even if one partner is aged it had a substantial effect on the survival. In the mating pair of 1 day old the survival was declined to the tune of 73.7%. Earlier findings reveal that progeny from mated group immediately after eclosion lives longer corroborates our results (Clark and Rockstein, 1964).

Mating provides stimulus for oviposition in *A. mylitta* and other insects (Yamajashetty and Ramaiah, 1979; Ravikumar *et al.*, 1995a, b: Rath *et al.*, 1997). In Lepidoptera, mating stimulates oviposition as egg maturation is completed by the time of adult emergence, which is also seems to be true for *A. mylitta*. The numbers of eggs laid increased from 147 to 286 and number of larvae hatched out increased from 101 to 272 with the increase in mating duration from 2 hr to 8 hr. The longer mating duration enables the male to inseminate sufficient sperms to increase fertility which is evident from our present study. Mating duration of 8 hours is treated as the standard mating duration for *A. mylitta* (Rath *et al.*, 2002) that yields 286 eggs per moth with a hatching of 95.2%. Similar findings were also reported in *B. mori* and *P. ricini* when 6 hr mating duration was maintained (Roy Chowdhury, 1974; Singh *et al.* 1994.). With increase in mating duration the eupyrene bundles increase in female reproductive tracts of *B. mori* supports the results of the present study (Kishore Kumar and Paul, 1993). Eupyrene count in spermatheca of female *A. mylitta* declines to a level of 57.1%, 52% and 30.4% when the mating duration was lowered to 2 hr, 4 hr and 6 hr respectively over 8 hr, similar trend in total eupyrene count was also observed due to lower mating periods. This is corroborated with reduction in hatching by 27.4%, 17.3% and 14.1% in females mated for 2 hr, 4 hr and 6 hr respectively.

Physiologically the aged moths are less energetic and hence could not contribute substantially towards population growth. Delayed mating resulted in failure in mating success, low egg recovery and hatching and lower insemination. Even when one of the mating pair is aged 1 day it has a significant effect on reproductive performance. As *Antheraea mylitta* reproduces once in their life (although the male some times mate more than once) and because of its economic importance proper care should be taken to mate the moths on the day of their emergence in order to get higher mating success, higher fecundity and fertility, higher quality of egg and larval survivability. The delayed mating should be avoided in the interest of viable seed (=egg) production.

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