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Mating and reproductive performance of silk moth, *Bombyx mori* L.

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Abstract
The effect of mating duration and multiple use of male on fecundity and fertility was investigated in the hybrids of Silkworm, *Bombyx mori* L. One hour to five hours of mating duration did not affect egg laying (fecundity) significantly, while fertilization affected significantly. Below 50% fertility was noticed when mating duration was reduced to below two hours. Similarly multiple use of male caused significant reduction in fertility but not fecundity. Till fourth mating no significant reduction in fertility was observed, thereafter fertility reduced considerably.

Keywords: Mating, fecundity, fertility, *Bombyx mori*

Introduction
Quality silkworm seed is vital for viable sericulture industry. Quality refers to richness of layings, viability, uniform hatching and subsequently good rearing performance of the progeny (Ullal and Narashimhanna, 1981) [24]. Timely supply of adequate quality and quantity of disease free silkworm eggs to the sericulturists is crucial for successful harvest of cocoon crops. The production of silkworm seed involves a long chain of interdependent and highly specialized conditions. Since egg production of the silkworm is managed by seed producers, various processes such as procuring the quality cocoons, emergence of moths, mating, egg laying, preservation and hatching of eggs are all important from the point of maximizing viable egg production (Ayuzawa *et al*., 1972; Jolly, 1983; Kovalev, 1960; Yokyoma, 1962) [2, 10, 13, 29]. Duration of coupling assumes great importance in commercial egg production. In case of *Bombyx mori* it was found that three hours of mating is sufficient (Krishnaswami *et al*., 1973; Narasimhanna, 1988) [14, 17] to get complete fertility. During this period, at least two ejaculations occurred, the first during the first 30 min and second after one and half hour. Generally during commercial silkworm egg production, seed producer's cold storage the cocoon (pupae) or moths in order to synchronize mating for preparation of multi x bi or multi x multi eggs. This in turn causes irregular emergence and leading to reduce the duration of coupling. Similarly potentiality of male moth is another important factor to prepare quality eggs. If male moth can be used again and again cost of seed cocoon production can also be reduced. Hence a systematic study was conducted with some popular bivoltine and multivoltine breeds being practiced in tropical parts of India especially in Eastern India to elucidate the role of different coupling duration and repeated uses of male moths on reproductive performance.

Material and Methods
The seed cocoons of Nistari, PM (Multivoltine) and NB7, NB4D2 (Bivoltine) were raised by adopting standard rearing method for preparation of three hybrid combinations i.e., Nistari × PM (Multi x Multi), Nistari × NB7 and Nistari × NB4D2 (Multi x Bi]. The male and female cocoons of each breed were separated and kept at 25~26 °C and 80±5% RH. On the expected day of moth emergence, moths were allowed to emerge and moths of different races were collected. Mating duration The female moths of Nistari and male moths of PM, NB4D2 and NB7 were collected and three hybrid combinations were prepared. The moths from each combination were subjected to different mating duration (see treatment details) and after specific mating duration, moths were decoupled and the females moths were kept for

oviposition and the males were disposed. The entire process was done at 25 °C±1 °C and 75%~80% relative humidity. In each treatment, five replications were made.

Treatment details

T1 - Mating duration - 1 h (9 am ~ 10 am)
T2 - Mating duration - 1.30 h (9 am ~ 10-30 am) T3 - Mating duration - 2 h (9 am ~ 11 am)
T4 - Mating duration - 2.30 h (9 am ~ 11-30 am)
T5 - Mating duration - 3 h (9 am ~ 12 Noon) (Control) T6 - Mating duration - 4 h (9 am ~ 1 pm)
T7 - Mating duration - 5 h (9 am ~ 2 pm)

Multiple use of male moth

The female moths of Nistari and male moths of PM, NB4D2 and NB7 were collected and three hybrid combinations were prepared. The same male was used for repeated mating as described in treatment details, after giving different periods of resting time. Every time fresh female was used. The Decoupling is done after 3 hr of mat- ing. After decoupling females were kept for oviposition. During the time of coupling, decoupling and oviposition 25 °C±1 °C and 75%~80% relative humidity was maintained. In each treatment, five replications were made.

Treatment details

T1 - Fresh males are used (9am ~ 12pm)
T2 - Male used in T1 were used after 1 h rest at 5 °C (1.00pm ~ 4.00pm)
T3 - Male used in T2 is used after 16~18 h rest at 5 °C (9am ~ 12 pm in next day)
T4 - Male used in T3 is used after 1 h rest at 5 °C (1.00 pm ~ 4.00 pm in same day)
T5 - Male used in T4 is used after 16~18 h rest at 5 °C (9 am ~ 12 pm in next day)
T6 - Male used in T5 is used after 1 h rest at 5 °C (1.00 pm ~ 4.00 pm in same day)
T7 - Male used in T6 is used after 16~18 h rest at 5 °C (9 am ~ 12 pm in next day)

Results

The present study was undertaken to know the impact of different mating duration and repeated use of male moth on the reproductive performance in the three different cross breeds of Silkworm, *Bombyx mori* L.

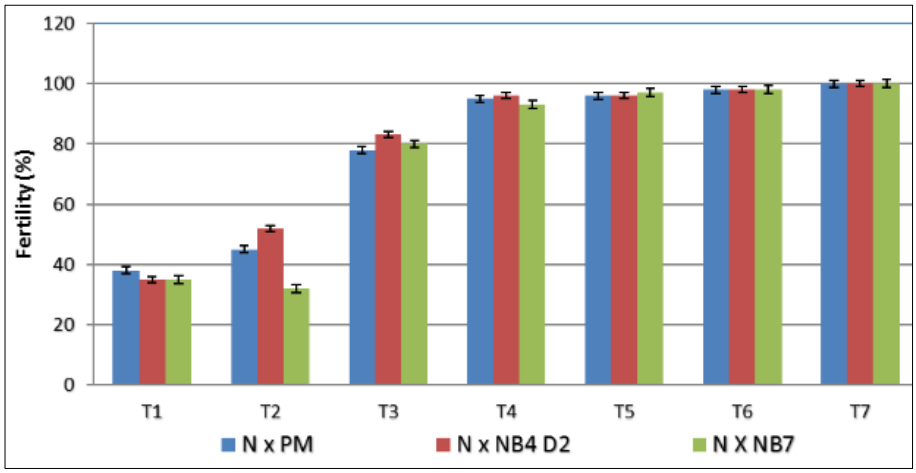

Fig 1: Effect of mating duration on fertility (%) in three hybrid combinations. TI-T7 are different treatments. N × PM is Multi × Multi hybrid, N × NB4D2 and N × NB7 are the Multi × Bi hybrids. Vertical bars represent the standard error of the mean

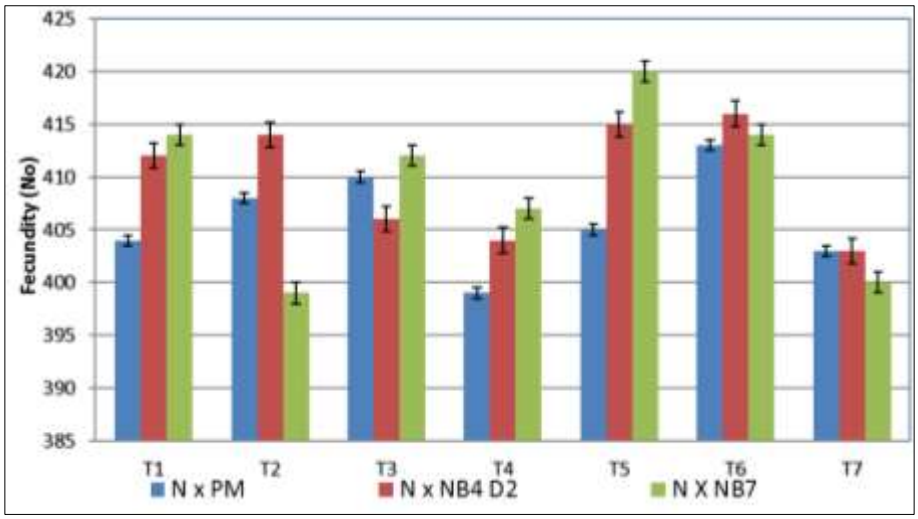

Fig 2: Effect of mating duration on fecundity (No.) in three hybrid combinations. TI-T7 are different treatments. N × PM is Multi × Multi hybrid, N × NB4D2 and N × NB7 are the Multi × Bi hybrids. Vertical bars represent the standard error of the mean.

Mating duration

Results on the impact of different mating duration on fertility and fecundity studied in three different combination of silkworm hybrids [N × PM, N × NB4D2 and N × NB7] is presented in the Fig. 1 and 2.

Fertility

Significant differences were observed among different treatments in each of the hybrids studied. In N×PM, fertility varied largely between 38.8 to 100%. Higher fertility was observed in T7 (100%) and lower in T1 (38.8%). In N×NB4D2, fertility was varied between 35~100%. Highest observed in T7 (100%) and lowest in T1 (35). In N×NB7, it ranged between 33 to 100%. Highest noticed in T7 (100%) and lowest in T1 (33.1) (Fig. 1)

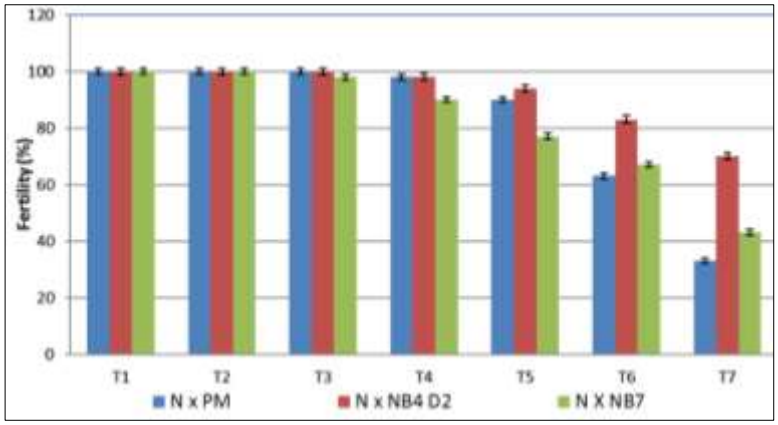

Fig 3: Effect of multiple use of male on fertility (%) in thre hybrid combinations. TI-T7 are different treatments. N × PM is Multi × Multi hybrid, N × NB4D2 and N × NB7 are the Multi × Bi hybrids. Vertical bars represent the standard error of the mean.

Fecundity

In case of fecundity, no significant variation was observed among different treatments in each of the hybrids. Fecundity ranged between 403~410, 403~416 and 399~420 in N × PM, N × NB4D2 and N × NB7 respectively. In N×NB7, higher fecundity was observed in T2 (419) and low in T5 (395) (Fig. 2)

So it is observed from all the combinations, different types of coupling duration have no significant effect on total fecundity, but has significant effect on fertility of eggs.

Multiple use of male moth

Effect of repeated use of male moth on fertility and fecundity in different combinations is represented in Fig. 3 and 4 respectively.

Fertility

No significant variation in fertility was observed in the treatments T1~T4 in the hybrid N × PM and more than 90% fertility (99.5~96.3) was observed in these treatments. Whereas treatment T7 showed lowest fertility (33%) followed by T6 (63%). In N × NB4D2, variation in fertility was observed among different treatments. Higher fertility % was observed in T1-T5, it showed 99.7~92.7% fertility. T6 and T7 showed lower level of fertility i.e., 83.6 & 70.8% respectively. The fertility of the eggs was decreased about 16.37% in T6 and 29.13% in T7. In N X NB7, more than 90% fertility was observed in T1-T4 (99.75~99.42), whereas treatment T7 showed lowest fertility (43%) followed by T6 (67%). The fertility of the eggs was decreased about 33.09% in T6 and 56.32% in T7 (Fig. 3).

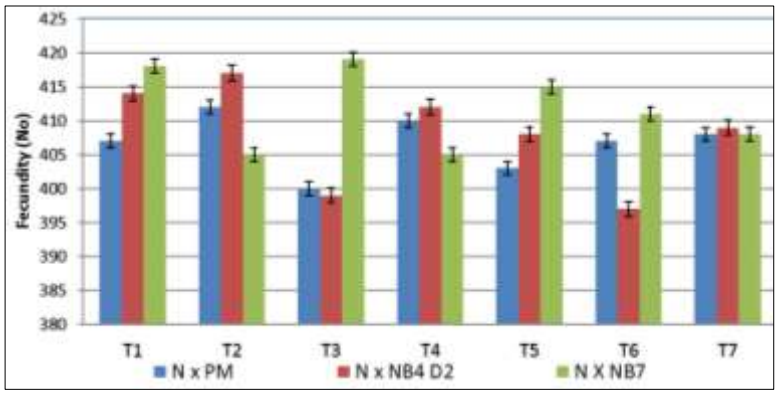

Fig 4: Effect of Multiple use of male on fecundity (No.) in three hybrid combinations. TI-T7 are different treatments. N × PM is Multi × Multi hybrid, N × NB4D2 and N × NB7 are the Multi × Bi hybrids. Vertical bars represent the standard error of the mean.

Fecundity

Fecundity did not show much variation among different treatments in the hybrids studied. In N × PM, fecundity ranged between 400~412, in N × NB4D2 between 399~417 and in N×NB7, it was between 405~419. In N × PM, the higher number of egg (Fecundity) was observed in T-2(412) followed by T4 (410) and T7 (408). Low fecundity was noticed in T3 (400). In N × NB7, higher number of egg (Fecundity) was observed in T-3(419) followed by T1 (418) and T5 (415) (Fig. 4).

Discussion

Mating frequency and duration have an important impact on reproductive fitness in insects. The age of mating individuals is apparently a key factor in reproduction with effects on their sexual performance and progeny production (Ahmed *et al*., 2004) [1]. Narayanan *et al*. (1964) [30] have

reported that in the mulberry silk moth (*Bombyx mori*.) 1 to 2 h of mating is enough for normal oviposition. Jadav and Gajare (1978) [9] reported that maximum hatchability of eggs in B. mori occurred after 4 hours of mating. However, Punitham *et al*. (1978) [18] reported that an increase in mating duration of B. mori from 3 h to 9 h enhanced the hatching percentage of eggs from 83% to 97%. On the other hand 4 h of mating is optimal for normal oviposition in eri silk moth Samia ricini Hutt (Behura and Panda, 1978) [3] and in A. mylitta (Dash *et al*., 1993) [5]. Our study indicates no significant variation among different treatments in different hybrids in case of fecundity is concerned, but fertility greatly varied between 33 to 100%. In *Bombyx mori* within two hours of coupling at least two ejaculations occurred, the first during the first 30 min and second after one and one half hour. During this time the male has ejaculated sufficient seminal fluid and sperm to get more than 80% fertile and lay optimum number eggs. Gillot and Friedel (1977) [8] have suggested that the egg production depends on the secretion of fecundity-enhancing substances by male insects at the time of mating. Moreover it is reported in many insects that oviposition–stimulating substances (OSS) derived from the male reproductive tract are transferred to the females during mating have the ability to accelerate the oviposition behaviour (Leopold, 1976; Raabe, 1986) [15, 19]. The males mated for prolonged durations produce large ejaculates containing nutrients that are used by females for reproduction and somatic maintenance (Eberhard, 1996) [7]. We observed even one hour coupling duration have not shown any significant variation in fecundity, it might be due to use of fresh males every time. This findings corroborating with earlier report of Roy chowdhury *et al*. (1992) [21], they reported 60 min of coupling is enough for maximum egg laying, but they have not mentioned about the fertility status. The possible reason may be since fresh male were used every time, sufficient male secretions was passed to female, which in turn might induced normal oviposition. Reduction of fertility in the treatments (i.e., mating duration is less than two hours) is might be due to reduced quantity of spermatic fluid having less sperm.

Multiple mating of both males and females copulate with two or more partners, is widely documented in numerous species (Keller and Reeve, 1995; Ridley, 1990) [11, 20]. Mating is one of the decisive factors which not only influence the total number of eggs a species lays but it is also important in the inducement of the regular oviposition (Ridley, 1990) [20]. From a male perspective ejaculate (Dewsbury, 1982) [6] production is costly. Such evidence includes a decline across multiple mating in male performance measured in terms of sperm numbers, spermatophore size, stimulation of female egg production rate, and inhibition of female remating (Simmons, 2001) [22]. Results of this study revealed, male mated for five times within a span of 3 days caused reduction in fertility, but no significant variation was observed in fecundity. Vemanantha Reddy *et al*. (2002) [26] reported decrease in fertility was occurred in Silk- worm *Bombyx mori*, when cold stored male was used for 3rd pairing. They also reported, effect of cold storage was more pronounced in multivoltine (Pure Mysore) than bivoltine (NB4D2). In our study also we observed 63% fertility on 6 pairing when multivoltine male [PM] used, where as in bivoltine (NB4D2) it was 80%. It indicates the bivoltine male has more potency than multivoltine male and can withstand low temperature longer time.

Studies on T. castaneum have shown that males can successfully inseminate as many as three different females in rapidly sequential copulations, and that the number of sperm transferred generally declines across consecutive matings (Bloch Qazi *et al*. 1996) [4]. Similarly Lewis (2004) [16] found in Tribolium castaneum, that males are capable of mating with as many as seven different virgin females within 15 min, however, when males copulated with previously mated females, there was a significant decline in male paternity success across sequential copulations, possibly due to male sperm depletion. Torres Vila *et al*. (2002) [23] observed in Lobesia botrana that delayed mating did not affect female mating success but fertilization was reduced, but more number of day's delays of mating substantially affected daily oviposition pattern and resulted in a significant reduction of both fecundity and fertility. Wang *et al*. (2005) [27] in their study in diamondback moth (DBM), Plutella xylostella (Lep., Plutellidae) reported, males could mate for five times with virgin females during scotophase, but the copulation rates, fecundity of female, and longevity of both females and males decreased when male mating times increased, whereas copulation duration increased. Knight (2007) [12] in their study in Cydia pomonella L. observed, sequential mating by male moths had no effect on the fecundity of female moths or egg fertility, however, male moth age did impact on fertility. So decrease in fertility after 4th pairing might be due to aging effect of male as well as depletion of sperm.

So it can be inferred from this study that 2.30 h mating is essential to get more than 90% fertility and single male can be used for four times without affecting fertility pro- vided sufficient rest at low temperature (5~7 °C).

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