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Effect of Multiple Matings on Longevity in Multi-bi Hybrids of the Silkworm, *Bombyx mori* L. in Three Seasons

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ABSTRACT

Sericulture is production of silk by rearing of silkworms on large scale. Silkworm is one of the important laboratory model to study the longevity or adult life span among insects. In silkworm, the total life span from egg to moth is known as ontogeny which varies based on the voltinism it belongs to, and is a genetically controlled biological phenomenon. In the present study longevity duration of unmated and mated female and male moths of PM x CSR_2 and PM x FC_2 in three seasons was calculated following the standard statistical procedure. At present adult lifespan or longevity is an important fitness parameter of silkworm breeding. It is clear from the present findings that there is sexual differentiation in the longevity of adults based on continuous and discontinuous mating. It is observed that the heterogametic sex i.e., females (ZW) exhibited shorter duration of survival compared to homogametic sex i.e., males (ZZ). Among the crosses, unmated moths show longer duration of survival than mated moths; of the three seasons longevity was higher in post-monsoon season followed by monsoon and pre monsoon. PM x CSR_2 showed higher longevity hours over PM x FC_2 among the hybrids for all the three seasons understudy.

***Key words*:** Silkworm, *Bombyx mori,* Multiple mating, Unmated, Mated, Moths, Longevity

The silkworm *Bombyx mori* is the popular beneficial insect that has been utilized as one of the genetical model to investigate various aspects of biological, genetical, molecular, physiological, biochemical and nutritional studies in insect system [1-2]. There are several species of the silkworm, while *Bombyx mori* is the most widely used and intensively studied. Longevity is a phenomenon where the durability including the feeding and non-feeding stages helps us understand its application in genetical studies as a fitness trait [3-4]. Further, several group of lepidopteron insects' exhibit different lifespans including the silkworm *Bombyx mori* which possess egg, larva, pupa and adult stages as their developmental stages during its life cycle [5-6].

In silkworm *Bombyx mori* the moth stage is sexually active and hence considered as a mature aging adult [7]. Longevity may be expressed in different meaning in different situations, few reports suggest that by focusing primarily on above said phenomenon, involved in trying to understand the changes that take place in cells, tissues and different organs over the time and situation [8-9]. Among the insects such as silkworm *Bombyx mori*, which is almost similar to the *Drosophila melanogaster* for some aspects being utilized as an excellent model system for aging studies also popularly known as gerontological studies in the various well-equipped laboratories. In this context, many reports [10-14 utilizing the silkworm as a model organism for the study of ageing related aspects.

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MATERIALS AND METHODS

The disease free layings (dfls) of the silkworm *Bombyx mori* namely multivoltine race Pure Mysore (PM) and popular bivoltine breeds CSR_2 and FC_2 were procured from the Silkworm Seed Production Centre (SSPC) of Chinthamani, Chikkaballapura district. These dfls were incubated at a temperature of 25±1°C and relative humidity of 80±5% during embryogenesis. The hatched larvae were reared by feeding the mulberry leaves of S_{36} variety to 1st and 2nd instar larvae followed by V_1 variety to 3rd, 4th and 5th instar larvae until maturity/spinning of cocoons was initiated.

The silkworm rearing was conducted as per the standard methodology suggested by Dandin and Giridhar [15] (2010). Out of the harvested cocoon lot only uniform and healthy cocoons were selected and deflossed, whereas the defective cocoons were rejected. The selected cocoons were cut opened for sex determination and the male and

female pupae were separately preserved at a room temperature of 25±1°C and relative humidity of 80±5%. The male and female moths of silkworm after emergence were utilized for the present experimental studies. The mating was allowed using multxbi hybrid combinations viz. PM × CSR_2 and PM × FC_2. After each mating, the moths were depaired and the females under experimental study were individually allowed to lay the eggs on the craft paper sheets at room temperature of 25±1°C and relative humidity of 80±5% in complete dark condition. The census of the total number of eggs laid was recorded for all sets of batches/treatments under study. In all the three seasons, a total of 75 moths were randomly selected from the freshly emerged healthy moth population in each race / breed and divided into three batches for treatment, with 25 couplings each in three replicates. The moths in different groups were allowed to mate in separate cages by giving different resting period to male moths, after which the moths were decoupled manually. The moths were allowed to mate in different durations (treatments) as given below:

Treatment details

Continuous mating of males without rest

MM0 – Multiple mating – 3 h. duration (8.00 am – 11:00 pm; 11.00 am - 02.00pm & 2.00 pm -05.00 pm).

Discontinuous mating of males with rest

a) *Multiple mating with 1 h. rest*:

MM1 – Multiple mating – 3h duration each (8.00 am – 11:00 pm mating; 11.00 pm– 12.00 pm rest; 12.00 – 3.00 mating; 3.00 pm - 4.00 pm rest; 4.00 pm – 7.00 pm mating).

b) *Multiple mating with 2 h. rest*:

MM2 – Multiple mating – 3h duration each (8.00 am – 11:00 am mating; 11.00 pm– 01.00 pm rest; 01.00 pm – 4.00 pm mating; 4.00 pm - 6.00 pm rest; 6.00 pm – 9.00 pm mating).

During the resting period of 1h. and 2h. duration the male moths were preserved in 5 - 7°C for further utilization in mating with an unmated/unmated female moths.

The mated males and females in the above mentioned treatments after mating (25 moths each) and unmated males and females (25 moths each) of both bivoltine hybrids and double hybrids were used for longevity studies to correlate the results among the experimental batches with the multiple mating/frequency. Mean evaluation index was recorded and analysed statistically (ANOVA) in three seasons namely pre-monsoon (March-June), monsoon (July-Oct.) and post-monsoon (Nov.-Feb.) to understand the season-wise variations and also inter-hybrids between the multivoltine race and bivoltine breeds/hybrids. The mortality parameter for each experimental batch were checked and recorded twice a day, i.e., once in the morning (10.00 AM) and once in the evening (4.00PM) and the data was used for calculating the total duration of survival of all the experimental batches of silk moths in hours.

RESULTS AND DISCUSSION

Longevity of silk moth is an important biological factor which is influenced by the multiple mating using male moth with and without rest. This parameter is considered as a fitness parameter in the breeding programme to evolve new breeds/hybrids. The present study was undertaken to know the impact of different/varied multiple mating on the longevity of two crosses involving multi × bi hybrids of mulberry silkworm (PM × CSR_2 and PM × FC_2). The results from season-wise data on longevity in two crosses for different treatments of multiple mating of male moth i.e., continuous mating for three hours without giving rest and also providing one hour and two hours of rest to male moth is presented in hours (Fig 1-2).

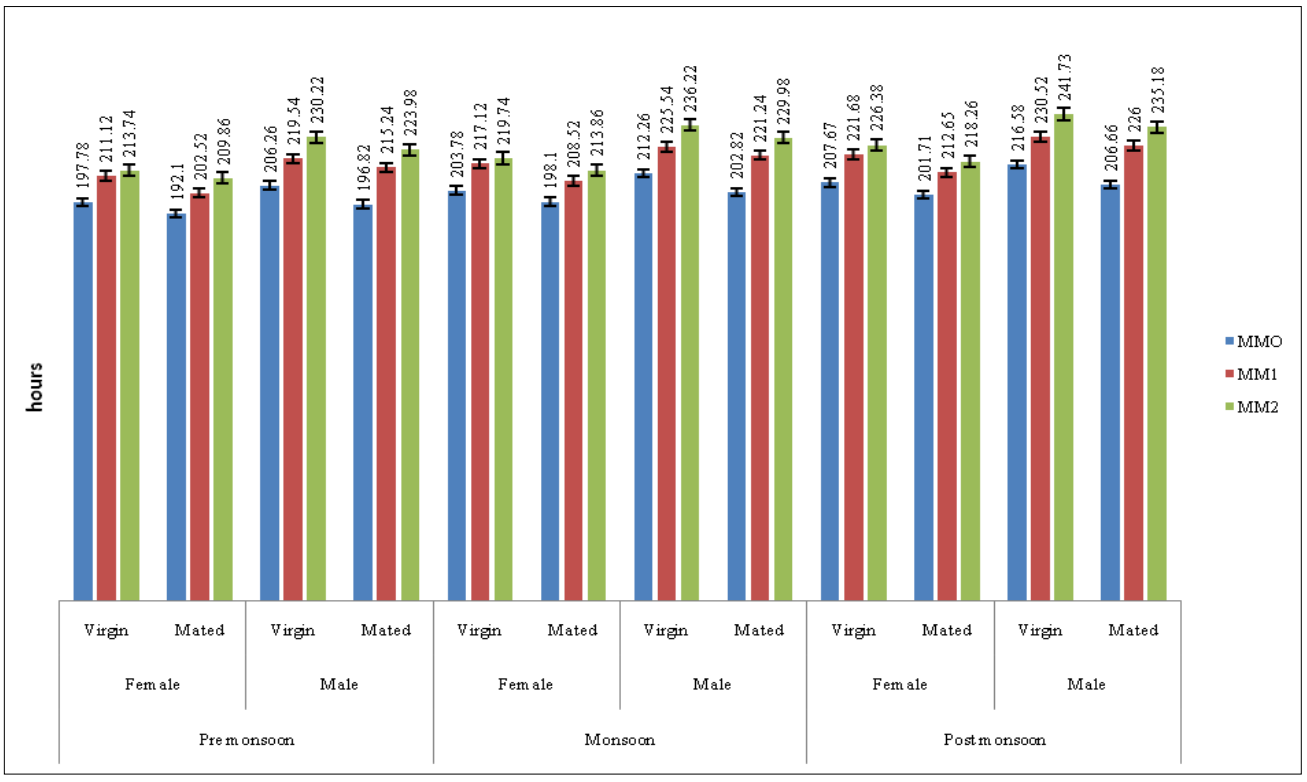

Fig 1 Mean values of longevity (h.) in the cross PM × CSR_2 of mulberry silkworm by multiple mating in three seasons (mean ± SD)

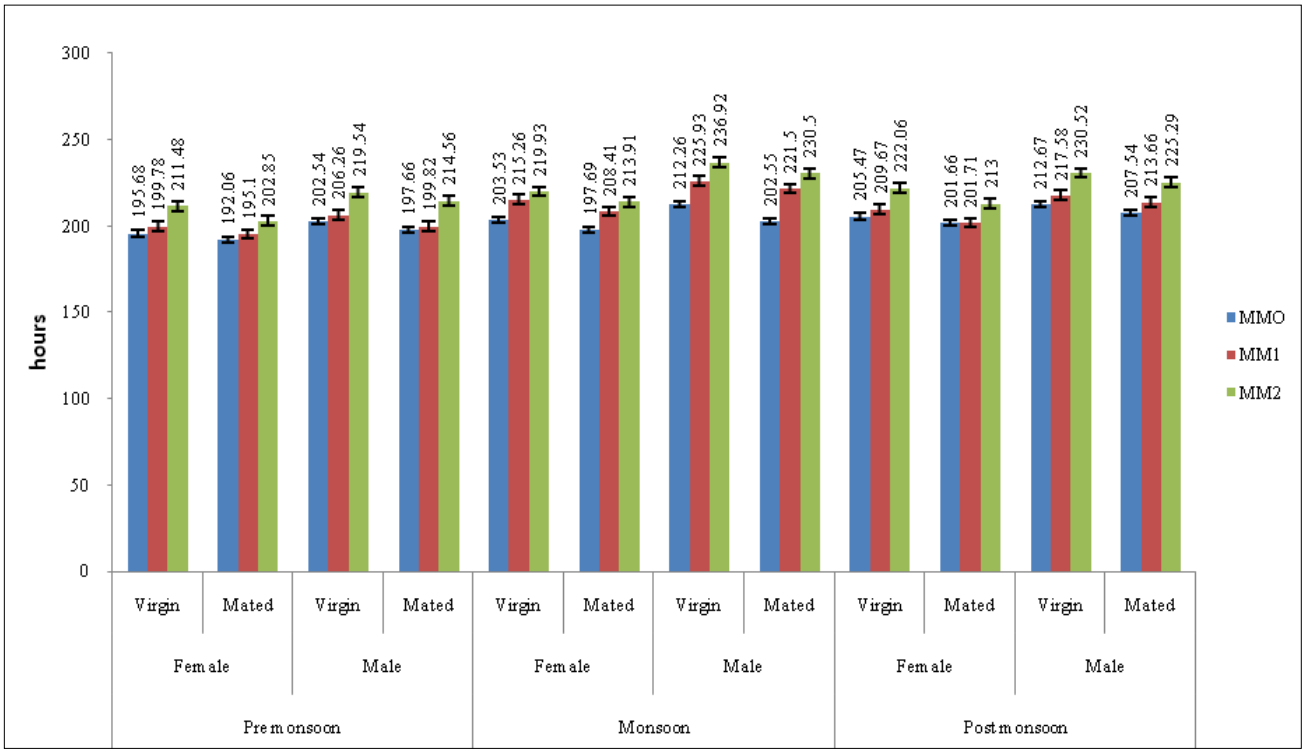

Fig 2 Mean values of longevity (hours) in the cross PM × FC_2 of mulberry silkworm by multiple mating in three seasons (mean ± SD)

The results depict that the higher duration of longevity was recorded in unmated and mated males than unmated and mated females in PM × CSR_2 over the other cross PM × FC_2 under study. Among the three seasons, higher duration of survival was observed during the post monsoon season followed by monsoon and pre-monsoon. The unmated and mated male moths live longer than unmated and mated female moths, while male moths with two-hour rest survive for longer duration over those with one and zero hour rest for all the three seasons under study.

The higher longevity duration was recorded in unmated male moths compared to mated ones as reported by [16] and the results from the present study is in agreement of the same. While the findings of [17] who in his studies on temperate bivoltine breeds and hybrids showed that the unmated female moths have the higher duration of longevity due to high fat-body accumulation compared to the males. Based on the results obtained it is proposed that the diet plays an important role in longevity of moths and hence the bivoltine breeds/hybrids which consumes higher amount of mulberry leaf compared to multivoltines were found to be long lasting in the adult/moth stage. Further, as males are more active than the females in silkworm *Bombyx mori* the energy consumption due to its hyperactive behaviour and loss of energy during copulation, the mated males live shorter compared to the unmated males. Another important point to highlight is the metabolic efficiency may be another responsible factor for sex-wise difference in survival of adults [18]. It is clear from the findings of silkworm breeding studies that there are some silkworm genotypes where such correlation related to adult moth longevity is difficult to draw. As reported by [19] the mortality of moths occur quickly (3-4 days) in Diazo strain and the short longevity in adults is due to a single recessive gene located on autosomes [20].

Based on the analysis of data recorded from the present study, it is clear that male moths' live longer than females and unmated male moths have maximum duration of longevity than the mated ones which is a clear indication of sexual differentiation along with the mating process. The mated males exhibit shorter duration of longevity as they lose the stored energy due to fluttering of the wings in search of the female mate and also during the copulation as transfer of sperms to the female moth occurs [21-22]. Also, the variations in adult longevity is observed, makes it easy to understand that it may be due to male lepidopteran insect is a homogametic and female is heterogametic [23]. Also, variations observed in longevity of moths may be because of the racial differences and inherent or acquired character. The duration of longevity is also related to the accumulation of fat bodies (weight-wise) which serves as the store house of energy and this stored energy is utilized in the non-feeding stages and moth stage is one among them. Further studies will surely throw more light on sex-wise, breed-wise, voltine-wise and season-wise differences reported by various workers in the field of gerontology, which can be of immense help in developing new hybrids either by conventional or contemporary breeding methods.

CONCLUSION

Longevity duration of unmated and mated female and male moths of PM × CSR_2 and PM × FC_2 in three seasons was calculated. It could be concluded that there is sexual differentiation in the longevity of adults based on continuous and discontinuous mating. The heterogametic sex like females exhibited shorter duration of survival compared to homogametic sex like males. Among the crosses, unmated moths show longer duration of survival than mated moths; of the three seasons longevity was higher in post-monsoon season. PM × CSR_2 showed higher longevity hours over PM × FC_2 among the hybrids for all the three seasons understudy.

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