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Effect of Multiple Mating on Adult Life Span in Two Popular Bivoltine Hybrids of the Silkworm, *Bombyx mori* L. in Three Seasons

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ABSTRACT

Sericulture is large scale rearing of silkworms to produce cocoons utilized for silk reeling. Silkworm is an important laboratory model to study the longevity or adult life span among insect system. In silkworm, the total life span from egg to adult (silk moth) commonly called as ontogeny varies based on the voltinism the silkworm belongs to; and it is a biological phenomenon controlled genetically. In the present study, lifespan of silk moths using popular bivoltine hybrids FC₁ and FC₂ of the mulberry silkworm, *Bombyx mori* L. during three seasons of the year was calculated following the standard procedure in vogue. The importance of adult lifespan or longevity is an important parameter and fitness index of silkworm breeding which is the new field of study. Based on the results obtained, the heterogametic sex i.e., females (ZW) exhibited shorter duration of survival compared to homogametic sex i.e., males (ZZ). In all the crosses, virgin moths of both sexes recorded longer duration of survival than mated moths, among the three seasons of the year, the longevity was higher in post monsoon season followed by pre-monsoon and monsoon. FC₁ X FC₂ showed higher longevity hours over FC₂ X FC₁ among the hybrids for all treatments.

Key words: Silkworm, *Bombyx mori*, Multiple mating, Virgin moths, Mated moths, Longevity

The silkworm *Bombyx mori* is the popular laboratory model with commercial importance 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. Even though there are several species of silkworm, *Bombyx mori* is the most widely used and intensively studied. Adult lifespan or longevity studies is a phenomenon where the durability of non-feeding stage (silk moth) helps us understand its application in genetical studies as a fitness parameter which is an important trait in the breeding programs.

The silk moth of *Bombyx mori* is sexually mature used for reproduction and multiplication, hence it is considered as a mature aging adult. Longevity may be expressed in different meaning in different situations, few reports have suggested that by focusing primarily on above said phenomenon, helps us to understand the physiological, biochemical, biological, genetical and molecular level and changes that take place in cells, tissues and different organs over the time and situation. Among the insects, the silkworm *Bombyx mori* which is almost similar to

the *Drosophila melanogaster* for few aspects as said above, being utilized as an excellent model system for aging studies also called as gerontology being studied in the various laboratories and few reports were made by [1-7] utilizing silkworm as a model organism for the study of adult life span and its importance as a trait in genetics and breeding of silkworm, *Bombyx mori*.

MATERIALS AND METHODS

The disease free layings of the two popular bivoltine hybrids FC₁ × FC₂ and FC₂ × FC₁ of the silkworm *Bombyx mori* were procured from the Silkworm Seed Production Centre (SSPC) at Chinthamani, Chikkaballapura district. These dfls were incubated at a temperature of 25±1°C and relative humidity of 80±5%. The hatched larvae were reared by feeding the mulberry leaves of S₃₆ variety to 1st and 2nd instar larvae followed by V₁ variety to 3rd, 4th and 5th instar larvae until maturity/spinning of cocoons started.

The silkworm rearing was conducted as per the methodology suggested by Dandin and Giridhar [8] at GKVK, Chintamani. From the harvested cocoon lot only uniform and healthy cocoons were selected and deflossed, whereas the defective cocoons were rejected. The selected cocoons were cut open for sex determination, the sexed pupae were preserved separately at a room temperature of 25±1°C and relative humidity of 80±5%. The adult moths emerged on the tenth day,

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the males and females were utilized as the experimental material as discussed below. The mating was allowed using multixbi combinations of $FC_1 \times FC_2$ and $FC_2 \times FC_1$. After each mating, the moths were depaired and the females as per the experimental design were individually allowed to lay eggs on the egg sheets at a room temperature of $25 \pm 1^\circ\text{C}$ and relative humidity of $80 \pm 5\%$. The census of the total number of eggs laid was recorded for all sets of batches/treatments. Healthy and freshly emerged moths of both sexes were selected at random in equal proportions during each rearing season. A total of 75 moths were randomly selected, out of which healthy moths were selected 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 moth, after which the moths were decoupled manually. The moths were allowed to mate in different durations (treatments) as mentioned below:

Treatments followed

1. 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)

2. Discontinuous mating of males with rest

a). Multiple mating with 1 hour 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 hour 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 $5 - 7^\circ\text{C}$ for further utilization for mating with an unmated/virgin female moths).

The mated males and females in the above-mentioned treatments after mating (25 numbers each) and unmated males and females (25 numbers each) of two bivoltine hybrids were used for longevity studies to correlate among them with the multiple mating treatments. Mean evaluation index was recorded and analyzed statistically (Mean \pm SD) in three seasons namely pre-monsoon (March-June), monsoon (July-Oct.) and post-monsoon (Nov.-Feb.) to understand the season-wise variations and also hybrids prepared using the bivoltine hybrids commonly known as foundation cross (FC). The mortality of the moths of each treatment/experimental batch were checked and recorded twice a day i.e., 10.00 A.M. in the morning and 4.00 PM in the evening. The data recorded was used to calculate the total duration of survival of the silk moths is expressed in hours.

RESULTS AND DISCUSSION

Gerontology is a new field of study to understand the longevity of adults which serves as a fitness parameter. Longevity of silk moths is an important biological factor which is influenced by the multiple mating using male moth with and without rest known as continuous and discontinuous mating. The present study was undertaken to know the impact of different/varied multiple mating treatments on the adult life span of two bivoltine hybrids of mulberry silkworm ($FC_1 \times FC_2$ and $FC_2 \times FC_1$). The season-wise data on the life span in two crosses using different treatments of multiple mating of male moth i.e., continuous mating for three hours without giving rest i.e., zero hour (continuous mating) and also giving one hour and two hours of rest (discontinuous mating) to male moths which was mated with fresh female moths is presented graphically (Fig 1-2).

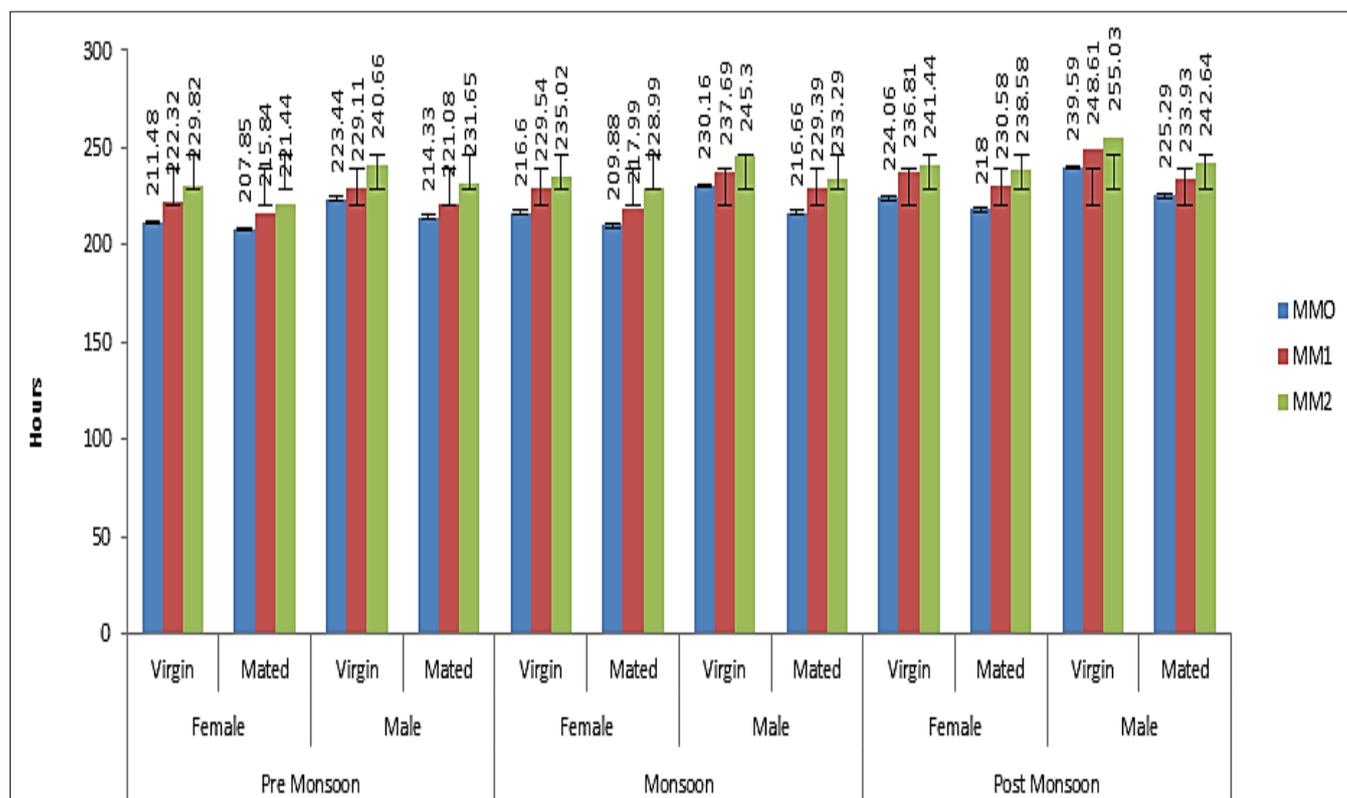


Fig 1 Mean values of longevity duration (hour) in $FC_1 \times FC_2$ of mulberry silkworm used for multiple mating treatment in three seasons (Mean \pm SD)

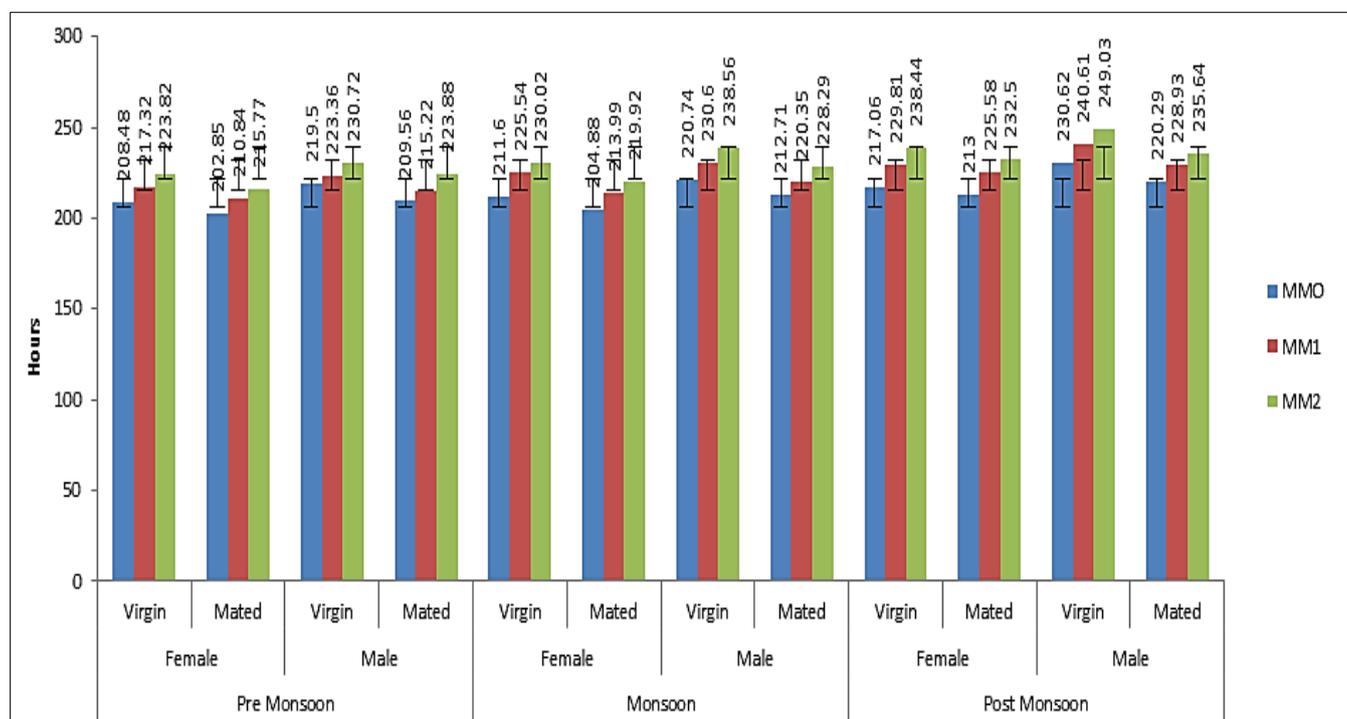


Fig 2 Mean values of longevity duration (hour) in $FC_2 \times FC_1$ of mulberry silkworm used for multiple mating treatment in three seasons (Mean \pm SD)

The results obtained exhibits in both hybrids, $FC_1 \times FC_2$ and $FC_2 \times FC_1$ the longevity is higher in the MM2 (two hours rest) than MM1 and MM0 (with one and zero hour rest respectively). Among the two hybrid crosses $FC_1 \times FC_2$ has recorded higher longevity period, followed by $FC_2 \times FC_1$ for all the treatments. Among the three treatments of the study higher longevity was recorded during post monsoon season followed by monsoon and pre monsoon for both male and female for all the three multiple mating treatments under study. The results show that the longevity recorded is higher in virgin females and males than mated females and males for both hybrids $FC_1 \times FC_2$ and $FC_2 \times FC_1$. The male moths lose their energy as they are hyper active, continuously fluttering their wings in search of female mates soon after emergence from the cocoons. Also, the transfer of the sperm and other chemical substances during mating gets added to the females by male moths [9]. The resting period during multiple mating is an important feature which helps the male to recover before it is used for next mating with the fresh / virgin female moth. The zero-hour rest will affect the performance and also the quantity and quality of sperms transferred to female moth affects the fertility as well as number of eggs laid. The number of unfertilized eggs may be more in number and the fertilized eggs may not hatch due to lesser quantity of sperms deposited in the female reproductive system affecting the fertility levels and also affects the embryogenesis in zero-hour rest treatment (MM0).

The higher longevity was recorded in virgin male moths compared to mated ones as reported by Gowda *et al.* [10] and the findings is similar to the findings of the present study. Murakami [11] in his studies on temperate bivoltine breeds and hybrids has showed that the virgin female moths have the highest longevity. Based on the results obtained it is proposed that the diet plays an important role in life span of silk moths and hence the bivoltine breeds/hybrids consuming higher leaf compared to multivoltines were found to be long lasting in the adult stage. Further, as males are more active than females in *Bombyx mori* and hence energy consumption and metabolic rate could be the main factor for sex-wise difference in survival of adults and this finding is in accordance with the observations of

Murakami and Shimada [12]. It is clear from the findings of silkworm breeding studies that there are some silkworm genotypes where such correlation related to adult life span is difficult to draw. As reported by Subramanya and Murakami [13] the death 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 [11].

Analysis of data recorded from the present study, it is clear that male moths lives longer than females and virgin male moths have maximum longevity than the mated males which is a clear indication of sexual differentiation as there is loss of energy in mated male moths during the process of mating affecting the life span. Many workers have reported that females are less viable than males in sex-limited breeds [14], even in normal strains females are less viable than males in all stages of development and the decreased viability especially in poor ecological conditions [15]. The variations in adult life span observed makes it easy to understand that it may be due to male lepidopteran insect is a homogametic (ZZ) and female is heterogametic (ZW) [10]. Also, the variations observed in moth longevity may be because of the racial differences and inherent or acquired character based on the voltinism or race of the silk moth. The longevity also is related to the accumulation of fat bodies 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. Hence the studies on adult life span is of great importance as a fitness parameter that can be used during the genetical studies and breeding programs of the silkworm.

CONCLUSION

The study on the adult life span or longevity duration of unmated and mated female and male moths of $FC_1 \times FC_2$ and $FC_2 \times FC_1$ in three seasons were conducted to understand the differences sex-wise, season-wise and duration wise. Based on the results, it is 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 i.e., males.

Among the crosses, virgin moths of both sexes recorded longer duration of survival than mated moths, among the three seasons of the year the longevity was higher in post monsoon season

followed by pre-monsoon and monsoon. $FC_1 \times FC_2$ showed higher longevity hours over $FC_2 \times FC_1$ among the hybrids used for the study for all treatments.

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