



Comparative study (II): On the longevity of pupa and adult moths in selected few bivoltine purelines of silkworm *Bombyx mori* (L) in all the seasons of the year.

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ABSTRACT

A study utilizing the four bivoltines, in three seasons of the year compared for longevity between pupal and adult moth stage of silkworm revealed a positive significance. Pupal duration was calculated after chrysalis stage among four bivoltines recorded variable pupal duration in all the three periods. Among the four bivoltines C₁₀₈ recorded lowest duration of 214 hours in male in pre-monsoon period, where as the longest pupal duration of 312 hours was evident in the female pupae of CSR₂ breed during post-monsoon period. Based on the results from adult life span among four bivoltines in three seasons in the two sexes (male moth and female moth) it is clear that C₁₀₈ race revealed shortest lifespan where as CSR₂ breed recorded longest life span in all the three seasons. Among the two sexes, the unmated (virgin) male and females moths exhibited the longest life span than those of mated male and female moths. The seasonal influence on adult life span is very clear. The results of the study in pre-monsoon is more conspicuous (revealing short lifespan) where as in post-monsoon season the adult lifespan is longest, from the present studies it clearly demonstrate that the expression of adult longevity and healthiness is depends on the racial, sexwise differences and seasonal impact during the rearing period.

KEYWORDS: *Silkworm, bivoltine, longevity, seasons, environmental conditions, pupa, moth.*

INTRODUCTION

The term aging is commonly used by bio gerontologists and public as a synonym to the word senescence. Attempts to develop a fundamental quantitative theory of aging, mortality and life span have deep historical roots. An exponential increase in death rates with age is observed for many biological species including fruit flies *Drosophila melanogaster* (Gavrilov & Gavrilova, 1991), nematodes (Brooks *et al.*, 1994, Johnson, 1987; 1990) mosquitoes (Gavrilov, 1980), human lice (*Pediculum humanus*), (Gavrilov & Gavrilova, 1991), flour beetles *Tribolium confusum*, (Gavrilov & Gavrilova, 1991) mice (Kunstyr & Leuenberger 1975; Sacher 1977) rats (Gavrilov & Gavrilova, 1991) dogs (Sacher, 1977) horses (Strehler, 1978) mountain sheep (Gavrilov, 1980). In its broadest sense, the process of aging can be defined as the sum total of those time dependent reproducible changes both in structure and function for a given organism, species during its total life span. As for senescence, the definition of aging can be modified more specifically to include the sum total of those changes in structure and function which, by virtue of their deleterious and degradative nature, result ultimately in the failure of the individual to survive and therefore, result in its death. During the life course the organisms are running out of their cells (Gosden, 1985; Herndon *et al.*, 2002) losing reserve capacity (Bortz, 2002; Sehl & Yates, 2001), and this redundancy depletion explains the observed "compensation law of mortality" (mortality convergence at older ages) as well as the observed late-life mortality deceleration, leveling-off, and mortality plateaus. The observation that mammalian species do not demonstrate mortality deceleration at advanced ages agrees well with the prediction of reliability theory of aging

according to which more complex living systems/organisms with many vital subsystems (like mammals) may experience very short or no period of mortality plateau at advance ages in contrast to more simple living organisms (Gavrilov & Gavrilova, 2011).

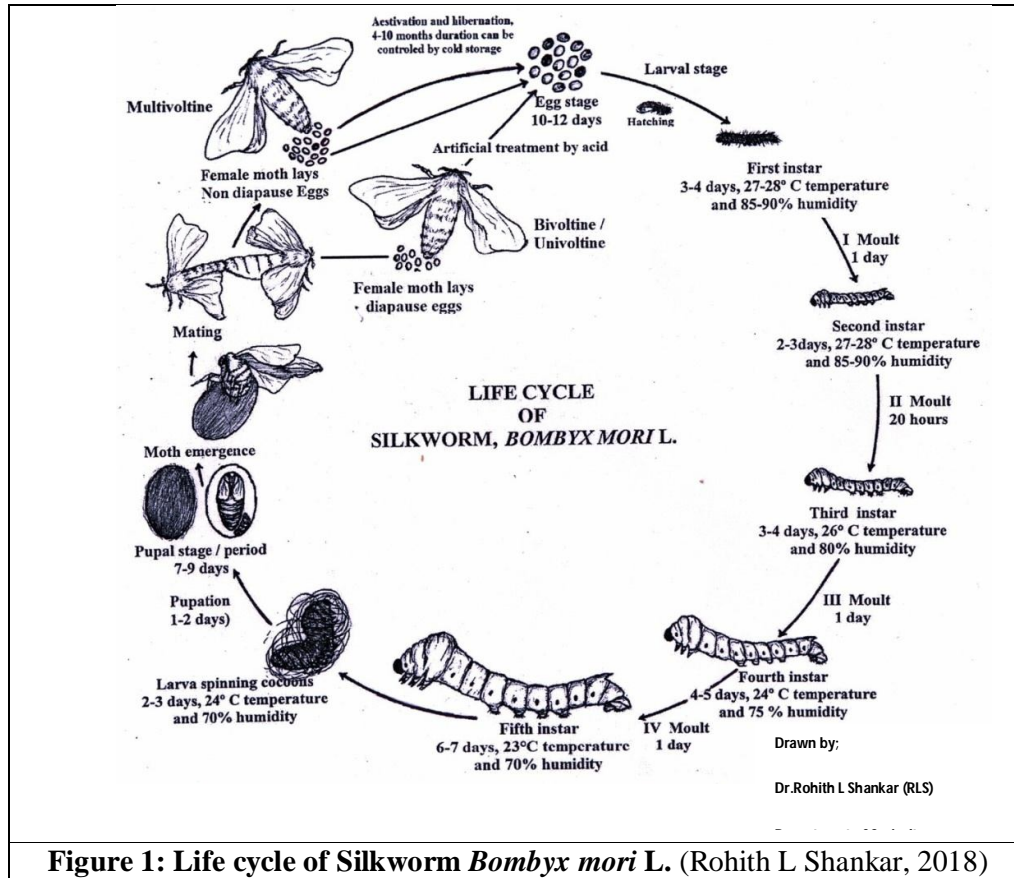


Figure 1: Life cycle of Silkworm *Bombyx mori* L. (Rohith L Shankar, 2018)

The insect, having evolved successfully from a time long before man's most primitive origin, are made up of cells indistinguishable from those of higher animals including man and which cells in turn possess the full complement of biochemical system involved in metabolism, in general and in cellular functions, in particular. Moreover, insects grow old and die and manifest aging processes exactly like those of higher vertebrates, including aging of postmitotic tissues such as striated muscle and nervous tissue. In addition, this highly diversified and ubiquitous group of distinctive animals present an unusual combination for virtually all the questions concerning the nature of the aging process. Hence, short lived species or strains of animals as insects are highly desirable for studies of Gerontology (Rockstein, 1973). In the case of house fly *Musca domestica* which complete its life span in three months and indeed several replicate generational studies can be even pursued by overlapping studies during the life span of the first generation. Accordingly, over a period of one year numerous replicate studies are possible. Similarly, spring and summer honey bees with a maximum longevity of approximately three months, as well, offer similar advantages for aging studies in insects (Clark & Rockstein, 1964). Thus, several research workers emphasized gerontological research work utilizing insect species because of high degree of reproductability of experimental data (Medawar, 1946; Charlesworth, 2001), short life span like *Drosophila melanogaster* and other Dipteran insects and relatively limited space requirement and low cost maintenance. Since reports utilizing lepidopteran pupa

and moths on aging are limited the present work is being undertaken utilizing different races of mulberry silkworms (Fig.1).

MATERIALS AND METHODS

The four bivoltine races/breed were selected for the present investigation are drawn from the germplasm bank of the Department of Studies in Sericulture Science, Manasagangothri, Mysuru. The bivoltines C₁₀₈, Kalimpong-A, (hence forth denoted as KA) NB₄D₂ are bivoltine races and CSR₂ is an evolved bivoltine breed. The description of characteristic features of the bivoltine races/breed are presented in Table-1 and Plate 1. At the end of larval stage, the spinning larvae were mounted on the mountages. Immediately after chrysalis stage Plate-2 the male and female pupae were separated and kept in perforated containers along with cocoon shell. For the experiment fifty males and fifty females were collected and kept in rearing tray and the pupal duration was recorded. After emergence of the moths, twenty five male and female virgins and twenty five male and female mated moths were covered with cellulose. The observation was carried out at intervals time of 5.30 A.M, 11.30 A.M, 5.30 P.M and 11.30 P.M respectively. The death of the adult was identified when they did not show biological response to being poked by the point of a pencil. The adult mean life span for each races/breed/hybrids for male and female was calculated following the method of Murkumi *et al.* (1989a). The life span of pupae and moth was recorded in hours in the three different seasons viz., pre-monsoon, monsoon and post- monsoon.

TABLE-1: THE MORPHOLOGICAL CHARACTERISTIC FEATURES OF FOUR BIVOLTINE PURE RACES/BREED.						
Sl. No	Races/breed	Origin	Larval markings	Cocoon colour	Cocoon shape	Voltinism
BIVOLTINE RACES						
1	C₁₀₈	China	Plain	White	Oval	Bivoltine
2	KA	India	Plain	White	Oval	Bivoltine
3	NB₄D₂	India	Plain	White	Dumbbell	Bivoltine
BIVOLTINE BREED						
4	CSR₂	India	Plain	White	Oval	Bivoltine



C₁₀₈ race



Kalimpong - A race



NB_{1D}₁ race

CSR₂ breed

PLATE 1: COCOONS OF BIVOLTINE RACES/BREED.

The data obtained on the seasonal performance of the multivoltine, bivoltine and hybrids was analyzed by employing the statistics SPSS 20.0 packages. The pupal duration was calculated with the help of the following formula (Murkami and Ohtsuki, 1989).

$$\text{Total duration from spinning to moth emergence } \left[\sum n_D \right] = \sum n_S + \sum n_C + \sum n_P$$

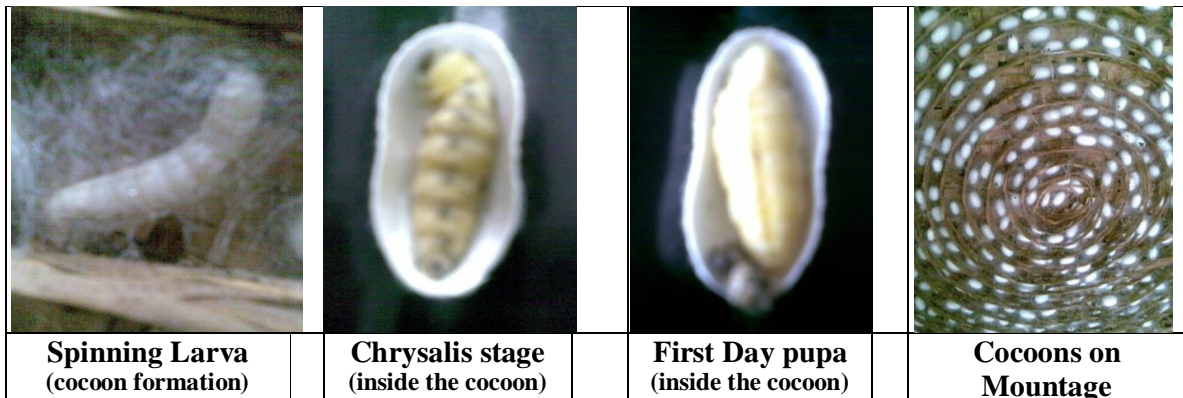
$$\text{Pupal duration } \sum n_P = \left[\sum n_D \right] - \left[\sum n_S + \sum n_C \right]$$






Where,

$\sum n_S$ = Duration of the spinning larva

$\sum n_C$ = Duration of pre-pupa (Chrysalis)

$\sum n_P$ = Duration of pupa



		
<p>Emerging moth (piercing out of the cocoon)</p>	<p>Unmated female moth (just emerged)</p>	<p>Unmated male moth (just emerged)</p>
		
<p>9th day female and male pupae (one day before emergence as moth)</p>		<p>Mated female and male moths (moths copulate just after emergence)</p>
<p>PLATE 2: DIFFERENT STAGES FROM SPINNING LARVA TO MOTH EMERGENCE(SEPARATED AS UNMATED FEMALE AND MALE AND MATED FEMALE AND MALE) IN SILKWORM, <i>BOMBYX MORI</i> L.</p>		

RESULTS

The data pertaining to the pupal duration of four bivoltine races/breed in three seasons viz., pre-monsoon, monsoon and post-monsoon are presented in tables-2 along with statistical analysis and depicted in fig.2, Similarly, the data pertaining to the adult life span in bivoltine are presented in the tables-3. The graphical representation of the data calculated based on the mean values are depicted in fig.3. The detailed results of the above experiments on pupal duration and adult life span are as follows.

Pupal longevity: The statistical data (ANOVA) with mean squares, F values and CD at 5% on the pupal duration of four bivoltine races / breed in both the sexes in three seasons and the same is depicted in fig.2. The lowest pupal duration among four bivoltine was observed in the males of C₁₀₈ race (192±3.47 hours) and a highest pupal duration was recorded in the CSR₂ (240±2.47 hours) in pre-monsoon. In monsoon season the lowest pupal duration was recorded in the males of C₁₀₈ (204±5.47 hours) where as the longest pupal duration was observed in CSR₂ breed (264±4.62 hours). In the post- monsoon season a lowest pupal duration of 246±6.23 hours was observed in C₁₀₈ race whereas, a longest pupal duration of 288±4.83 hours was noticed in CSR₂ breed. It is interesting to note that KA and NB₄D₂ recorded the pupal duration intermediary between C₁₀₈ and CSR₂ (F value was 2.66).

SEASONS	PRE-MONSOON		MONSOON		POST-MONSOON		AVERAGE	
Races/breed \ Sex	Male	Female	Male	Female	Male	Female	Male	Female
C ₁₀₈	192 ± 3.47	216 ±3.42	204 ±2.83	228 ± 6.92	246 ± 6.23	264 ± 6.13	214 ±4.17	236 ±5.49
KA	204 ± 5.47	228 ±3.83	216 ±2.99	240 ± 5.12	264 ± 6.22	288 ± 4.82	228 ±4.89	252 ±4.59
NB ₄ D ₂	216 ± 4.47	240 ±4.12	228 ±4.82	252 ± 5.03	264 ± 4.93	228 ± 2.92	236 ±4.74	240 ±4.02
CSR ₂	240 ± 2.47	258 ±4.61	264 ±4.62	282 ± 5.4	288 ± 4.83	312 ± 3.13	264 ±3.97	284 ±4.38
MSE between races	36.00	36.00	144.00	264.00	144.00	144.00	108	148
F-value	1.25	1.25	0.31	0.62	0.31	0.31	0.93	0.72
CD at 5%	5.75	5.75	11.50	15.57	11.50	11.50	9.96	11.65

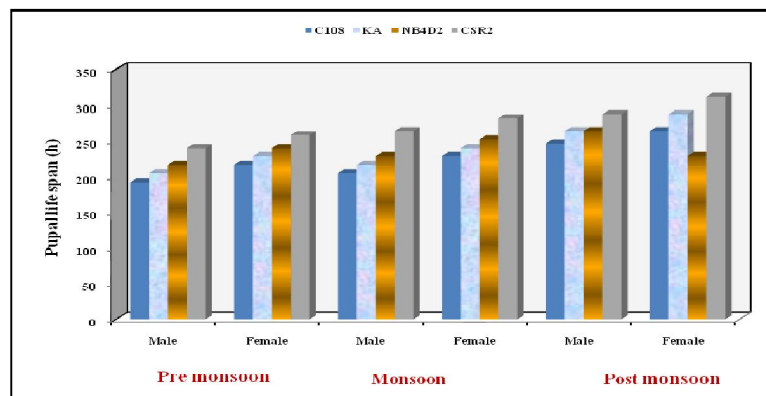


FIGURE 2: PUPAL DURATION OF BIVOLTINE RACES/BREED DURING ALL THE THREE SEASONS.

Adult moth longevity: The results of analysis of variance along with the mean values of adult life span in the four bivoltine races/breed viz., C₁₀₈, KA, NB₄D₂ and CSR₂ in pre-monsoon season are presented in Table 3 and represented in fig. 3. Based on the data it is clear that mated males of C₁₀₈ race revealed mean adult life span of 150 ± 3.14 hours, where as in all the other three bivoltines the data was 156 hours. However the unmated males of KA and NB₄D₂, exhibited a uniform adult life span of 168 hours, where as in the C₁₀₈ race and CSR₂ breed the duration of adult life span was 106 and 180 hours respectively. The data also revealed a similar trend in regard to the mated and unmated female moths, wherein unmated CSR₂ female recorded the longest life span of 240.20 ± 2.78 hours.

TABLE 3: ADULT LIFESPAN OF FOUR BIVOLTINE RACES/BREED				
(i) in Pre-monsoon season:				
Sex \ Races	C₁₀₈	KA	NB₄D₂	CSR₂
Mated male	150.00±3.14	156.00±1.85	156.00±1.95	156.00±1.95
Unmated male	169.00±3.02	168.00±2.54	168.00±2.68	180.00±1.85
Mated female	181.60±2.26	180.00±3.14	180.00±1.99	192.00±1.85
Unmated female	209.80±3.21	208.00±3.22	216.00±2.89	240.20±2.78
MSE between races	171.42	150.10	115.57	91.51
F-value	74.15	66.01	116.29	274.15
CD at 5%	12.54	11.74	10.30	9.16
(ii) in Monsoon season:				
Mated male	180.00±3.19	204.00±2.22	204.00±2.39	216.00±1.63
Unmated male	185.40±2.65	240.00±3.08	240.00±3.08	249.00±1.41
Mated female	198.00±3.16	243.00±3.96	246.00±3.14	265.20±2.37
Unmated female	222.0±3.08	258.00±3.43	258.00±4.04	275.00±3.43
MSE between races	180.85	208.80	206.52	109.89
F-value	38.63	50.49	52.29	121.56
CD at 5%	12.88	13.84	13.77	18.04
(iii) in Post-Monsoon season:				
Mated male	193.20±3.24	210.00±3.14	235.00±1.72	252.00±1.68
Unmated male	216.00±1.94	244.80±2.37	245.00±2.49	270.00±2.31
Mated female	236.40±2.83	252.00±3.02	258.00±2.47	282.00±1.95
Unmated female	252.00±3.01	261.60±3.86	270.00±2.81	288.00±4.36
MSE between races	157.26	197.05	115.51	153.47
F-value	82.66	51.29	39.99	32.84
CD at 5%	12.01	13.45	10.30	10.87
Average adult life span of six multivoltine races in all the three seasons				
Mated male	174.40±2.97	190.00±3.45	198.40±4.40	208.00±5.25
Unmated male	189.80±2.86	217.60±4.82	217.80±4.86	233.20±5.13
Mated female	205.34±3.38	225.20±4.61	228.00±4.70	246.40±5.23
Unmated female	227.94±2.91	242.54±3.76	248.07±3.56	267.74±3.32
MSE between races	169.84	185.31	145.86	115.75
F-value	64.97	55.96	74.84	131.76
CD at 5%	12.47	13.01	11.20	10.02

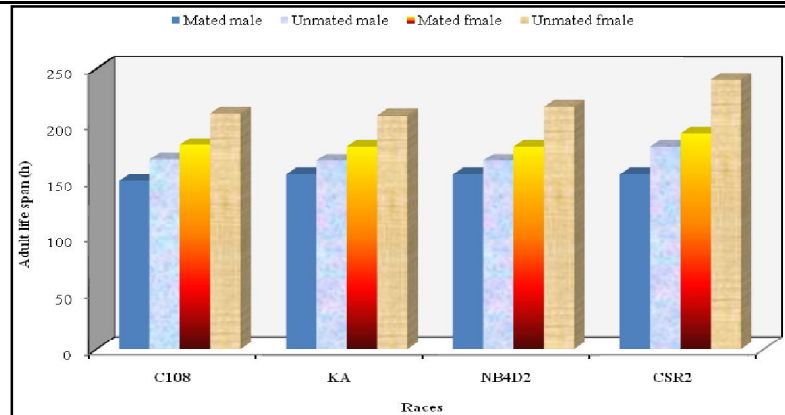


FIGURE 3: MEAN ADULT LIFE SPAN OF FOUR BIVOLTINE RACES (AVERAGE OF THREE SEASONS)

The data pertaining to the adult life span of four bivoltine races in the mated and unmated moths of both the sexes along with the results of ANOVA during monsoon season are presented in table-3. Among bivoltines C₁₀₈ race is distinct by recording the lowest adult life span of 180±3.19 hours and 185.40 ± 2.65 hours, in the respective mated and unmated males. Similarly, CSR₂ breed revealed 216 ± 1.63 hours and 249±1.41 hours which is the highest among mated and unmated males respectively.

A comparison between the races/breed from fig. 3, it is evident that statistically significant differences are noticed among all the races (P<0.05) except the races of KA and NB₄D₂. Based on the data related to the life span of mated and unmated females, it is evident that the lowest of 198 ± 3.16 hours of adult life span was recorded in C₁₀₈ race, where as an highest of 275 ± 3.43 hours was observed in CSR₂ breed. The overall picture that emerges from Table-3 is that C₁₀₈ race and CSR₂ breed revealed statistically significant differences (P<0.05) between them, where as between KA and NB₄D₂ statistically insignificant differences (P>0.05) were observed. The respective F value for four bivoltines C₁₀₈, KA, NB₄D₂ and CSR₂ was 38.63, 50.49, 52.29 and 121.56.

The data in regard to the adult life span of four bivoltine races in post-monsoon season among the mated males the data ranges from 193.20 ± 3.24 hours (C₁₀₈ race) to a highest of 252 ± 1.68 hours (CSR₂ breed). A comparative analysis of the data between mated and unmated females the C₁₀₈ race exhibited a shortest adult life span of 236.4 ± 2.83 hours, where as the lowest adult life span of 282 ± 1.95 hours was recorded by CSR₂ breed. It is also important to note that, KA and NB₄D₂ revealed intermediary adult life span duration compared to CSR₂ breed and C₁₀₈ race. The data pertaining to unmated females, it is the CSR₂ breed which ranked first not only among the mating types but also among the races by exhibiting significant longest adult life span (P<0.05) of 288 ± 4.36 hours.

From the Table, the relevant CD values for four bivoltine races/ breed viz., C₁₀₈, KA, NB₄D₂ and CSR₂ were 12.01, 13.45, 10.30 and 10.87 which are all statistically significant for adult life span among the races.

The data in regard to the overall pooled data (average life span) among all the four bivoltine races for the three seasons is presented in Table- 3 and Fig. 3. Mated moths of C₁₀₈ exhibited the lowest average adult life span of 174.40 ± 2.97 hours, where as unmated CSR₂ revealed an highest of 267.74 ± 3.32 hours among all the races which exhibited statistically significant results (P<0.05).

DISCUSSION

Sequential biological events viz., growth, aging and death are characteristic features of any bisexual organism. Narayan (1972) reported that prior to onset of spinning, the larvae take their last meal and insect development shows the dynamic unfolding of events of larval, pupal and adult transformation (L-P-A) when cells differentiate to assume newer and more complex functions. Rockstein (1972) termed these events as life span. Collatz & Sohal (1986) studied the strategies and mechanism of insect aging and highlighted how insect aging has a relevance to senescence processes in higher bisexual organisms. The silkworm, a member of the order Lepidoptera, offers an important laboratory model to understand the mechanism of adult life span because its status lies between that of bacteria and man. Further, its biomass, organismic complexities etc. are very conspicuous and easy to handle and analyze (Murakami, 1990). Clark & Rockstein (1964) in their detailed investigation on housefly and honeybee demonstrated that the adult life span in these insects are known to be greatly influenced by the environment and also other factors such as food, temperature, season and genotype of the individual. Since longevity is considered as a fitness component in all organisms, scientist are now more interested in the genetics of longevity. The information on the pupal duration in lepidopteron silkworm, *Bombyx mori* has been studied by Murakami (1990); Anantha *et al.*, (2018a, 2018b). It is well established fact that majority of the lepidopteron insects including *Platysamia* (*Hyalophora*) *secropia*, *Philosamia cynthia ricini*, *Attacus cynthia*, *Caligura japonica*, *Chilo suppressalis*, etc. are of a pupal diapause type. However, some species including *Bombyx mori* and its putative ancestor *Bombyx mandarina* exhibited the pupal duration extending to 17-18 days. Murakami (1990) demonstrated in the two important temperate races namely J115 and Ascoli that the pupal duration was extended by 17-18 days. It is important to know the relevance of short term selection for pupal weight in two Indian races namely Pure Mysore and NB18 which was reported by Puttaraju & Rajanna (1997). Added to this Rajanna & Puttaraju (1997) reported heterosis for pupal weight during interline selection. Murakami & Ninaki (1993) showed that the life span of pupae of *Bombyx mandarina* ranges from 11-45 days regardless of the season and clearly demonstrated that long lived pupal life span is dominant over short lived pupae. Based on the present studies, it is evident that pupal duration is comparatively higher in females compared to the male pupae. Similar results were observed from the data pertaining to the pupal duration in monsoon and post-monsoon season among all the races. The pupal duration is prolonged in the post-monsoon season in the females and as well as male pupae. In the monsoon season though the pupal duration is intermediary between the two seasons, the shortest pupal duration was noticed in the pre-monsoon season. Thus, it is a clear indication how the role of environmental factors play a predominant role in pupal duration. From the present studies, the four bivoltine races/breed recorded 8-12 days pupal duration. However, a detail investigation in this regard may throw light upon whether long lived pupae may show dominant over short lived pupae and has any relevance to maternal inheritance. A wealth of information is also available on the life span of various group of insects. Rockstein (1972) termed the events of aging in lepidopteron moths as “duration of adult life span”. Similarly, adult longevity and aging in silkworm have been the focus of study by many researchers resulting in substantial contribution to the longevity studies among eukaryotic system. In temperate bivoltine races, Murakami (1989) demonstrated that the aging of silk moth is one of the important biological phenomena and opined that the generally adult silkworm, *Bombyx mori* has a very short lifetime, since it lives without taking food after emergence. In an interesting findings, Osanai (1978) demonstrated that high temperature of 30-35°C results in shortening the life span by 50% where as the moths kept at 4°C maintained life approximately 5 times longer

than the moth at optimal temperature. In a historical monograph, Partridge (1986) clearly underlined how reproductive activity in insects reduces the life span of both the sexes.

Thus, based on the comparative analysis of the adult life span between bivoltines in the present study it is clear that it is always the females of bivoltine exhibited the longest adult life span (Anantha *et al.*, 2018a, 2018b). Such variations in the adult life span exhibiting the sexual differences may be due to the racial specificity and is in conformity with the observations of Murakami & Shimada (1988) demonstrated that the life span of female is 1.5 times longer than males in temperate bivoltine strain J106. Further, extending the research work on a polyvoltine race Cambodge, Murakami (1987) demonstrated that the multivoltine Cambodge lives for a shorter period than the bivoltine races. The present studies corroborates with the studies of Murakami, 1987; Murakami & Shimada, 1988; Rohith *et al.*, 2008). Because of the duration of adult life span significantly differs bivoltine strains it is possible to say that the adult life span is strain specific. It is interesting to note that the species and strain difference in longevity have also been reported in a few silkworm races. Umashankara (2004); Doddaswamy (2007) demonstrated that the average adult life spans of bivoltine races depend on the genotype of the silkworm race. Murakami (1989a,1989b); Osanai (1978); Doddaswamy & Subramanya (2007) who in their studies on multivoltine and bivoltine races showed that virgin female moths have the highest longevity and proposed that diet plays an important role in the longevity and hence the bivoltines which consume maximum leaves compared to multivoltines were found to be long lasting in the adult stage. Further, Murakami & Shimada (1988) also indicated that males are more active than females in *Bombyx mori* and hence energy consumption and metabolic rate could be the main factor for sexwise differences in adult life span. In the silkworm, males have higher oxygen consumption per body weight based on index of metabolic activity than females throughout the adult life span (Osanai, 1978). The wing beat frequencies of male moths are higher than that of female moths for first several days after eclosion and as a result the energy consumption is higher. However there are few contradictory reports where a lethal gene “sdi” quickly acts on the adult life span and results in the death of the moth within a span of 48 hours (Murakami, 1999). But, Maynard (1958), in his detailed studies using *Drosophila melanogaster* demonstrated that the longer female life span may possibly due to the presence of two ‘X’ chromosomes in the females. The second chromosomes may contribute for any lethal genes that may be present in the ‘X’ chromosomes and are expressed in males. Overall picture that emerges out from this study on adult life span is that adult life span difference observed in different races/breed may be due to in part to races/breed/genotype, and environment and in the present investigation put forth the fact that longevity may not be explained by these factors alone.

CONCLUSION

It is understood from the present study that, the pupal duration was calculated after chrysalis stage recorded variable pupal duration in all the three periods in all the four bivoltines. Among the four bivoltines C₁₀₈ recorded lowest duration of 214 hours in male in pre-monsoon period, where as the longest pupal duration of 312 hours was evident in the female pupae of CSR₂ breed during post-monsoon period. Based on the results from adult life span among the four bivoltines, C₁₀₈ race revealed shortest lifespan where as CSR₂ breed recorded longest life span in all the three seasons. Among the two sexes, the unmated (virgin) male and females moths exhibited the longest life span than those of mated male and female moths. The influence of three seasons on adult life span is very clear from the present experiment and it revealed that, the effect of pre-monsoon is more conspicuous (revealing short lifespan) where as in post-monsoon season it is

longest and the monsoon is intermediate. The results obtained from the present studies clearly demonstrate that racial differences, seasonal impact and sex wise differences are important in the expression of adult longevity.

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