Int. J. Indust. Entomol. Vol. 16, No. 1, 2008, pp. 1~5

International Journal of *Industrial Entomology*

Impact of Egg Laying Duration on the Occurrence of Fertilized and Unfertilized Eggs of the Newly Evolved Race of *Bombyx mori*, L.

Vinod B. Mathur* and Kunal Sarkar

Central Sericultural Research and Training Institute, Mysore-570 008 India

(Received 26 December 2007; Accepted 30 January 2008)

The present study was carried out with the aim to evaluate the viability of layings (delivery of fertilized and unfertilized eggs) with egg laying duration of elite bivoltine races. The temporal aspect of mating in terms of egg layings duration may also have impact on the number of eggs laid, pattern of egg laying and their viability. After different interval of egg laying duration, moth of *Bombyx mori* are removed from oviposition site after they have completed egg laying. Present investigation confirmed that there are no significant difference in relation to the occurrence of viable (fertilized) and non viable (unfertilized) eggs and duration of oviposition. Percentage of unfertilized eggs varied from 2.41 to 3.42% in case of CSR-3, 2.59 to 3.62% in case of CSR-6, 2.82 to 3.66% in case of CSR-16 and 2.58 to 3.40% in case of CSR-17 in different treatments. The occurrence of unfertilized eggs are also not significant in the different treatments specially in those eggs which were laid 24 hours after oviposition and kept for 4 months hibernation schedule.

Keywords: Fertilized and unfertilized eggs, moth, egg laying duration, silkworm

Introduction

Silk moths have a tendency to pair immediately after emergence. The pair came together immediately and copulate. In case of *Bombyx mori* it was found that three hours of mating is sufficient (Krishnaswami *et al.*, 1973 and Ullal and Narsimhanna, 1981). The production of silkworm seed involves a long chain of interdependent and highly specialized conditions. The moths of *Bombyx. mori* are removed from oviposition site after they have completed egg laying (approximately 24 hours after oviposition) (Krishnaswami *et al.*, 1973). During this period, at least two ejaculations occurred, the first during the first 30 minutes and second after one and one half hour. During this time the male has ejaculated sufficient seminal fluid and sperms to fertilize 400 - 500 and more than 500 eggs in the female of new elite bivoltine races *i.e.* CSR breeds. The temporal aspects of mating in terms of duration may also have impact on the number of eggs laid, pattern of egg laying and their viability (Mathur and Sarkar, 2006). It is generally recognized that the male factors or the oviposition stimulating substance derived from the male reproductive glands and transferred to the females during copulation induce brisk oviposition behaviour (Fugo and Arisawa, 1992 and Osavi *et al.*, 1990). The angle of inclination during egg laying also has a definite influence on the number of eggs laid by a moth (Jolly *et al.*, 1966).The substratum for oviposition considerably influences the egg laying behaviour (Manjulakumari and Geethabali, 1991and Majula ,1993). Besides that environmental conditions like light, temperature, humidity also influence the rate of oviposition. Usually dark condition favours rapid oviposition (Meenal *et al.*, 1994). Temperature and relative humidity influence ovulation and fecundity significantly. At higher temperature (30°C and above) both fecundity and fertility are severely affected (Annual Report, SSTL, 1994-95). Humidity can play an important role in egg production. The rate of oviposition is sometimes sensitive to changes in humidity and in addition may affect mating (Rockstein, 1974). Fecundity of *Bombyx mori* is also influenced by nutritional quality of leaves (Himanthraj *et al.*, 1988).

There is limited information on record concerning the periodicity of egg laying (fertilized and unfertilized) eggs in case of *Bombyx mori* from other parts of the country

*To whom the correspondence addressed
Central Sericultural Research and Training Institute, Mysore - 570 008 India ; Tel: +91-821-2362802 : Fax: +91-821-2362845 : E-mail : vinodmathurpulkit@rediffmail.com

and abroad. Therefore, present study was undertaken to study the impact of time duration on the production of viable (fertilized and unfertilized) with egg laying duration of four elite bivoltine pure races *viz.* CSR3, CSR6, CSR16, CSR17 and also to assess whether oviposition during early hours and late hours have any impact in the occurrence of fertilized and unfertilized eggs.

Material and Methods

The present study was carried out at the Central Sericultural Research and Training Institute, Mysore, India to assess the occurrence of fertilized and unfertilized eggs with egg laying duration of elite bivoltine races. The detail methodology adopted during the course of investigation is given below:

Experimental races

The CSR series is evolved in 1992-1993 under Japan International Cooperation Agency (JICA) project of Central Sericultural Research and Training Institute, Mysore, India. Four races of elite CSR series viz., CSR3, CSR6, CSR16 and CSR17 were used for the present study.

Rearing procedure

To assess the oviposition rate of egg layings of elite CSR bivoltine races the rearing of CSR3, CSR6, CSR16 and CSR17 races were conducted utilizing V1 variety of mulberry leaves maintained under irrigated condition. The rearing was conducted as the procedure laid by Krishnaswami in 1978. As prophylactic measure and control of diseases bed disinfectant was dusted as per the recommendations (Baig and Kumar, 1987). The rearing of above races were conducted in three different seasons *i.e.* May-June (Summer), November (Autumn) and January-February (Winter).

Experimental design

The cocoons of different races were harvested on the seventh day after the mounting of spinning silk worms. By shaking the cocoons gently the number of live and dead pupae of all the cocoons of different races was assessed and the percentage of pupation was calculated. Defective and deformed cocoons from these batches were removed and cocoons which were uniform in shape, size and confirming to racial traits, were retained for conducting the grainage operation and experiments.

Grainage operation

The selected cocoons of all the four races were cut to remove pupae from them. Male and female pupae were separated based on genital markings. The separated pupae of different races were preserved separately in mating room where recommended temperature and relative humidity was maintained at 25°C ± 1°C and 80% ± 5%. respectively. On the expected day of moth emergence, light was put on at 5 to7 am and moths were allowed to emerge in this period. The emerged moths were collected and allowed to copulate. Light was again provided on the next day from 5 to 7 am. This process was repeated till the completion of moth emergence (Krishnaswami *et al.* (1973) and Narasimhanna, 1988).

Duration of coupling

Three hours coupling was given (as per the procedure laid by Krishnaswami *et al.* (1973) and Ullal and Narasimhanna (1981). Excess males were collected and preserved at 5°C with 75 - 80% relative humidity.

Treatment details

Decoupled females were kept in egg sheet at 10 am and cellules were kept over it and female were allowed to deposit eggs with time duration as detailed below:

T1→4 hours after decoupling (upto 2 pm)
T2→8 hours after decoupling (upto 6 pm)
T3→12 hours after decoupling (upto 10 pm)
T4→16 hours after decoupling (upto 2 am) (next day)
T5→20 hours after decoupling (upto 6 am)
T6→24 hours after decoupling (upto 10 am) (control)
T7→32 hours after decoupling (upto 6 pm)
T8→40 hours after decoupling (upto 2 am) (next day)
T9→48 hours after decoupling (upto 10 am)

Calculation of fertilized and unfertilized eggs

Twenty replications were maintained of each treatment and females were allowed to deposit the eggs. Periodicity of egg laying with different time duration were recorded. Eggs deposited by the female after 24 hours of decoupling were considered as control. Eggs laid by each female were recorded replication wise for all the treatments as mentioned above. Fertilized (viable) eggs were calculated along with unfertilized (inviable) eggs in different time duration to know the numbers and percentage of fertilized and unfertilized eggs at different time duration. This is done to assess whether oviposition during early hours has any impact in the occurrence of more fertilized or unfertilized eggs. Data obtained were statistically analyzed for calculating the fertilized or unfertilized eggs at different time duration.

Results

The present study was undertaken to know the rate of ovi-

Table 1. Delivery of fertilized and unfertilized eggs (numbers and percentage) with time duration in CSR-3 race of *Bombyx mori* L.

Treatments	Eggs laid upto mean number	Unfertilized eggs		Fertilized eggs	
		Mean number	Mean percent (%)	Mean number	Mean percent (%)
T-1	336	10	2.97	326	97.03
T-2	442	12	2.71	430	97.29
T-3	502	15	2.98	487	97.02
T-4	518	17	3.28	501	96.72
T-5	528	13	2.46	515	97.54
T-6	539	13	2.41	526	97.59
T-7	555	19	3.42	536	96.58
T-8	569	19	3.33	550	96.67
T-9	584	20	3.42	564	96.58
C.D at 5% : 1.51		C. D at 1% : 1.97		N. S. : (Non-significant)	

Table 2. Delivery of fertilized and unfertilized eggs (numbers and percentage) with time duration in CSR-6 race of *Bombyx mori* L.

Treatments	Eggs laid upto mean number	Unfertilized eggs		Fertilized eggs	
		Mean number	Mean percent (%)	Mean number	Mean percent (%)
T-1	309	10	3.23	299	96.77
T-2	410	13	3.17	397	96.83
T-3	455	14	3.07	441	96.93
T-4	487	15	3.08	472	96.92
T-5	498	16	3.21	482	96.79
T-6	501	13	2.59	488	97.41
T-7	520	18	3.46	502	96.54
T-8	540	19	3.51	521	96.49
T-9	551	20	3.62	531	96.38
C.D at 5% : 1.54		C. D at 1% : 2.02		N. S. : (Non-significant)	

position with time duration of egg layings of elite bivoltine races viz. CSR-3, CSR-6, CSR-16 and CSR-17 and its impact on the occurrence of fertilized and unfertilized eggs. The moths were allowed to lay eggs at different time durations after decoupling and data of fertilized eggs and unfertilized eggs at different time duration of laid layings were recorded and analyzed. The result obtained in different races of different treatments is given in Tables (1-4).

To calculate the numbers and percentage of fertilized (viable) and unfertilized (inviable) eggs, the eggs which were laid in different treatments (T1 to T6) upto 24 hours were acid treated and the eggs laid after 24 hours in three different treatments *i.e.* T7, T8 and T9 were kept for 4 months hibernation schedule.

CSR-3

It is observed that average number of fertilized eggs were found in the different treatments are as followed *i.e.*, 326 in T1, 430 in T2, 487 in T3, 501 in T4, 515 in T5, 526 in T6, 536 in T7, 550 in T8 and 564 in T9 (Table - 1). Least percentage of unfertilized eggs are found in T6 (2.41%) followed by T5 (2.46%), T2 (2.71%), T1 (2.97%), T3 (2.98%), T4 (3.28%), T8 (3.33%), T7 (3.42) and T9 (3.42) (Table 1).

CSR-6

Average number of fertilized eggs were recorded in the different treatments are *i.e.*, 299 in T1, 397 in T2, 441 in T3, 472 in T4, 482 in T5, 488 in T6, 502 in T7, 521 in T8 and 531 in T9. Least percentage of unfertilized eggs were found in T6 (2.59%) followed by T3 (3.07%), T4 (3.08%), T2 (3.17%), T5 (3.21%), T1 (3.23%), T7 (3.46%), T8 (3.51%) and T9 (3.62%). (Table 2)

CSR-16

Average number of fertilized eggs were found in the different treatments are *i.e.*, 301 in T1, 400 in T2, 431 in T3, 465 in T4, 475 in T5, 482 in T6, 487 in T7, 500 in T8 and 518 in T9. Least percentage of unfertilized eggs are found in T6- control (2.82%) followed by T1 (2.90%), T5

Table 3. Delivery of fertilized and unfertilized eggs (numbers and percentage) with time duration in CSR-16 race of *Bombyx mori* L.

Treatments	Eggs laid upto mean number	Unfertilized eggs		Fertilized eggs	
		Mean number	Mean percent (%)	Mean number	Mean percent (%)
T-1	310	9	2.90	301	97.10
T-2	413	13	3.14	400	96.86
T-3	445	14	3.14	431	96.86
T-4	480	15	3.12	465	96.88
T-5	490	15	3.06	475	96.94
T-6	496	14	2.82	482	97.18
T-7	504	17	3.37	487	96.63
T-8	519	19	3.66	500	96.34
T-9	537	19	3.53	518	96.47
C.D at 5% : 1.57		C. D at 1% : 2.06		N. S. : (Non-significant)	

Table 4. Delivery of fertilized and unfertilized eggs (numbers and percentage) with time duration in CSR-17 race of *Bombyx mori* L.

Treatments	Eggs laid upto mean number	Unfertilized eggs		Fertilized eggs	
		Mean number	Mean percent (%)	Mean number	Mean percent (%)
T-1	343	11	3.20	332	96.80
T-2	465	13	2.79	452	97.21
T-3	505	16	3.16	489	96.84
T-4	519	16	3.08	503	96.92
T-5	529	17	3.21	512	96.79
T-6	542	14	2.58	528	97.42
T-7	556	18	3.23	538	96.77
T-8	571	19	3.32	552	96.68
T-9	587	20	3.40	567	96.60
C.D at 5% : 1.48		C. D at 1% : 1.960		N. S. : (Non-significant)	

(3.06%), T4 (3.12%), T2 (3.14%), T3 (3.14%), T7 (3.37%), T9 (3.53%) and T8 (3.66%). (Table 3)

CSR-17

The obtained results were recorded and presented in (Table 4).Fertilized eggs were recorded in the different treatments are *i.e.*, 332 in T1, 452 in T2, 489 in T3, 503 in T4, 512 in T5, 528 in T6, 538 in T7, 552 in T8 and 567 in T9. Least percentage of unfertilized eggs are found in T6 (2.58%) followed by T2 (2.79%), T4 (3.08%), T3 (3.16%), T1 (3.20%), T5 (3.21%), T7 (3.23%), T8 (3.32%) and T9 (3.40%) respectively (Table 4)

Discussion

Like other insects pattern of egg laying of *Bombyx mori* is also influenced by circadian rhythm (Kovalev, 1960). According to Trag *et al* (1988) egg deposition commenced immediately when the moths were placed on egg cards and maximum number of eggs were laid in the first 4 hours after of oviposition and which formed 51.07% of total eggs laid upto 24 hours. Rajanna *et al* (1996) observed that 58-74% of the eggs were laid in different races during the first 4 hours. Our results are also in confirmity with the above observations as delivery of eggs was recorded upto 63% within 4 hours after decoupling in all the races.

Occurrence of fertilized eggs varied from 96.58 to 97.59 % in case of CSR-3, 96.38 to 97.41 % in case of CSR-6, 96.34 to 97.18 % in case of CSR-16 and 96.60 to 97.42 % in case of CSR-17 in different treatments. (Tables 1-4). Significant difference in the different treatments was not observed which is similar to findings of Gowda and Jolly (1988). Who stated that 98.6, 99.1, 99.3 and 98.8% viable eggs are found in case of PM Race, HM race, NB7 race and NB18 race respectively in all the given treatments related to time intervals *i.e.* 0-6 hours, 6-12 hours, 12-18 hours and 18-24 hours. The reason behind the occurrence of almost similar percentage of viable eggs in different

treatments is that in all the different treatments three hours of mating was given and three hours of mating is sufficient to fertilize almost all the eggs in *Bombyx mori* (Krishnaswami *et* al 1973 , Ullal and Narasimhanna, 1981). Percentage of unfertilized eggs varied from 2.41 to 3.42% in case of CSR-3, 2.59 to 3.62% in case of CSR-6, 2.82 to 3.66% in case of CSR-16 and 2.58 to 3.40% in case of CSR-17 in different treatments. (Table 1-4) .The occurrence of unfertilized eggs are also not significant in the different treatments specially in those eggs which were laid 24 hours after oviposition and kept for 4 months hibernation schedule. The result clearly indicates that the occurrence of fertilized and unfertilized eggs is not affected by periodicity of egg laying.Similar observation was also made by Kovalev (1960) who observed that 85 % of the eggs were laid in first 24 hours in *Bombyx mori.* Trag *et al* (1988) also informed that about 9 % of eggs were laid between 24 to 48 hours, which confirms our findings. The findings of present investigation is very important for sericulture industry while producing layings keep for different hibernation schedule especially for autumn/ late autumn crop, female may be allowed for prolonged oviposition to get 8 - 10% more fecundity (without much unfertilized eggs) and farmer may fetch 8 - 10 kg. more cocoon yield/100dfls with more profit margin.

References

Annual Report, (1994-1995): Silkworm Seed Technology Laboratory, Bangalore. Published by Dr. M. V. Samson, Director, SSTL, Bangalore.

Baig, M. and Kumar, P (1987): Silkworm diseases in appropriate Sericulture Techniques (ed. Dr. M. S. Jolly) CSR&TI Mysore, India, 108-116.

Fugo, H. and Arisawa, T. (1992): Oviposition behaviour of the moth which mated with males sterilized by high temperature in the silkworm *Bombyx mori.* J. Seric Sci. Jpn., **61**(2), 110-115.

Gowda, P and Jolly, M. S. (1988): Studies on some aspects of egg production in silkworm Bombyx mori L. in tropics. Thesis, Submitted in fulfillment of the requirements for the degree of Doctor of Philosophy of the University of Mysore, India.

Himantharaj, M. T. and Reddy, Sreerama, G. (1988): "Studies on the influence of nutrition on fecundity in Silkworm *Bombyx mori* L: Thesis submitted to the University of Mysore, India for the award of Doctor of Philosophy.

Jolly, M. S., Subba Rao, S. and Krishnaswami, S., (1966): Effect of plane of inclination on egg laying by *Bombyx mori* L. Indian J. Expt. Biol., **2**(3), 165-166.

Kovalev, P. A. (1960): Silkworm Breeding Stock (in Russian). English translation By Central Silk Board, Bombay 400002, India, pp. 133.

Krishnaswami, S., Narasimhanna, M. N., Suryanarayan, S. K., Kumaraj, S. (1973): Sericulture manual 2- Silkworm rearing, FAO agricultural Service Bulletin, and Rome. pp. 47-48.

Krishnaswami, S. (1978): New Technology of Silkworm Rearing. Central Silk Board, Bangalore. Bull, No. 2, pp. 1-23

Manjula, A., (1993): Oviposition behaviour in silkworm moths under tropical conditions. Indian Text. J., 103 (4): 102-104.

Manjula Kumari, D. and Geethabali. (1991): Ovipositional response of *Bombyx mori* L. to different textures of the substratum. Entomol, **16**(1), 11-15. (English).

Mathur, V. B. and Sarkar, K. (2006) Impact of mating duration on the fecundity of newly evolved races. Int J. Indst. Entamol. **12**(2), 81-86.

Meenal, A., Mathur, V.B. and R.K.Rajan (1994) Role of light during incubation of silkworm eggs & its effect on rearing performance & diapause. Indian J. Seric., **33**(2),139-141.

Narasimhanna, M. N. (1988): Mannual on silkworm egg production. Central Silk Board, Bangalore, India. pp. 142.

Osavi, M., Kasuga, H. and Aigaki, T., (1990): Physiology of sperm maturation in the spermatophore of the silkworm, *Bombyx mori.* In: Advances in invertibrate reproduction (Eds. Hoshi, M. and Yamashita, O.), **5**, 531-536. Elsevier Science, Publishers.

Rajanna,K. L., Biram Saheb, N. M., Chandrasekharaiah and Samson, M. V. (1996) : Oviposition rhythm in the silkworm *Bombyx mori.* Linnaeus under tropical conditions. Ann. Entomol. **14**(2), 31-35.

Rockstein, M. (1974): The physiology of insecta . (Vol II) Academic Press, New York and London.

Trag, A., Nabi, G. H., Fayaz, A and Bindroo, B. B. (1988): Studies on the oviposition rate in fertilized univoltine females of *Bombyx mori* L. Proceedings of the International Congress on Tropical Sericulture Practices. (Feb18-23). pp. 7-10.

Ullal, S. R. and Narasimhanna, M. N. (1981): Hand book of practical sericulture. Central Silk Board, Bangalore, India. pp. 61-82.