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# Biochemical Attributes of Mature Female Gonads of Different Strains and Hybrid of Mulberry Silkmoth, *Bombyx mori* L.(Lepidoptera : Bombycidae)

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## ABSTRACT

**One indigenous polyvoltine strain (Nistari) and two bivoltine strains viz. $P_5$ and $NB_{18}$ along with one bivoltine hybrid ($P_5 \times NB_{18}$) were subjected for studies on the differences on some physiologically important biomolecules like protein, nucleic acids and cholesterol. Ovarian protein and RNA content remained significantly high in the bivoltine races and their hybrid over multivoltine breed, while, DNA and cholesterol content remained significantly low in all the breeds and the hybrid as compared to Nistari strain. However, the ovarian weight was higher in both the bivoltine breeds and their hybrid than that of Nistari. Higher ovarian weight together with more protein and RNA concentrations reflect the preparatory phase for production of diapausing eggs by the bivoltines. This is indicated by the production of more number of eggs by the bivoltine silkmoths and their hybrid. The variations in biochemical parameters studied herein, thus establish a distinct difference in the overt reproductive physioiogy between multivoltine and bivoltine silkworms.**

**Key words : *Bombyx mori*, Ovary, Diapause, Protein, Nucleic acids, Breeds**

## INTRODUCTION

The reproductive system of *Bombyx mori* has physiological and biochemical significance with special reference to racial differences. It is the function of the female insects to produce the eggs and to deposit them at an appropriate time and in an appropriate place. Eggs are generally the largest and most complex cells produced by a given species (Margaritis, 1985), and insects have become particularly adept at manufacturing large number of oocytes. Eggs are closed cleidoic systems in which no exchanges of materials except gases occurs and embryogenesis takes place without any intake of material from the environment. Diapause at the egg stage is thus supposed to be more advantageous for insects to survive challenging forces.

Incidentally, special attention has been paid to the egg diapause of silkworms, since, in a given strain, silkworms programmed to lay diapause eggs are superior to those that lay non-diapause eggs. Thus, the programming to diapause is desirable for increased silk production but the resultant diapause eggs are not suitable for continuous rearing of larvae throughout a year. Some studies have been made with respect to phenotypic and biochemical diversity between different races of *Bombyx mori* (Sengupta *et al.*, 1974; Ghosh *et al.*, 1993; Chattopadhyay *et al.*, 1996; Chatterjee *et al.*, 1992; Chatterjee *et al.*, 1993). The information concerning the racial differences inthe biochemical constituents in the ovary of non-diapausing and diapause destined silkworms, *Bombyx mori* is scanty. Considering this lack of knowledge, a detailed study has to be undertaken on the developmental profile and biochemical differences of the female reproductive organs together with the

function(s) of accessory glands of different breeds (including diapausing and non-diapausing races) of *Bombyx mori*. This work was undertaken as a first step in the elucidation of the biochemical differences existing in the biochemical constituents in mature ovary of multi and bivoltine silkworms *B. mori* and their possible physiological significance.

## MATERIALS AND METHODS

One multivoltine (Nistari), two bivoltine ($P_5$ and $NB_{16}$) and their hybrid ($P_5 \times NB_{18}$) of *Bombyx mori* were selected for biochemical study of female reproductive system. The silkworms were reared on fresh mulberry leaves (fed 4 times a day).

Nistari strain took about 30 days while diapausing breeds / hybrid took about 37 days to emerge as adult moth in this experiment. The average body weight of the female moths were recorded to be 0.580 g in Nistari, 0.746 g in $P_5$, 0.7 g in $NB_{18}$ and O.883 g in $P_5 \times NB_{18}$.

The ovaries were quickly dissected out in ice cold 0.65% insect saline solution within 30 minutes after the emergence of moths. A minimum of five individuals (moth) were sacrificed to dissect out the total ovary from each race and weighed individually in a Sartorius digital monopan balance. Tissues considered for estimation of various biomolecules were uniformly collected from the polytrophic ovarioles. A measured amount of ovarian tissue from the proximal (terminal filament), middle (maturing ovum) and distal (matured ovum) were used for estimation of protein and nucleic acids. The protein was estimated by the method of Lowry *et al* (1951) while RNA and DNA were determined using the method of Munro and Fleck (1966) as modified by Abalain *et al* (1981). The ovarian cholesterol was estimated following the method of Kabara (1962). The ovarian protein content was expressed in mg/100 mg tissue and mg/total amount of ovary (wet weight) in each breed, while in case of RNA, DNA and cholesterol, the total amount of the biomolecules was expressed in µg/100 mg tissue and µg/total ovary. For counting the number of eggs laid (fecundity) the female moths were allowed to lay the eggs after three hours of coupling. Data were statistically analyzed using student-'t' test.

## RESULTS

### 1. Ovarian weight and number of eggs laid (fecundity)

The ovarian weight (wet) in moth was recorded to be significantly high (32.05%/0, 19.67% and 62.14% in diapause destined breeds and their hybrids viz. $NB_{18}$, $P_5$ and $P_5 \times NB_{18}$ respectively over non-diapausing breed Nistari ($p<0.05$-$p<0.001$). However, the hybrids ($P_5 \times NB_{18}$) ovarian weight was recorded to be higher than the ovarian weight of all the breeds studied in this experiment (Fig. 1). The number eggs laid by the moths of non-diapausing strain (Nistari) remained less ($p<0.001$) when compared to that of diapausing breeds and hybrid. The hybrid $P_5 \times NB_{18}$ showed the highest fecundity among all other breeds studied (Fig. 1).

### 2. Protein

Ovarian protein was found to be significantly higher ($p<0.001$ in all the three breeds/hybrids over Nistari. Higher percentage of ovarian protein was recorded to be to the tune of 88.97%, 77.85% and 113.89% in $NB_{18}$, $P_5$ and $P_5 \times NB_{18}$ respectively when saidmacromolecules was expressed in mg/total ovary. This significant difference ($p<0.0$) was also observed when protein content was calculated and expressed in

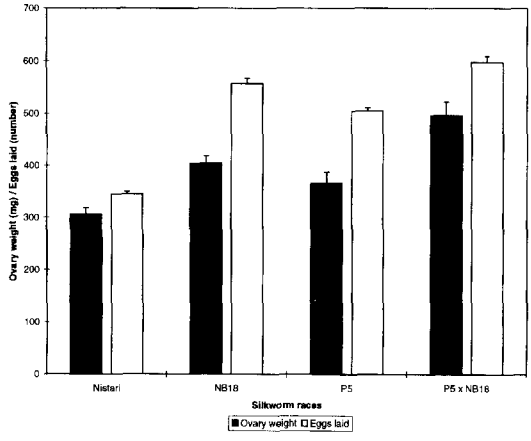


**Fig. 1.** *Variations in weight of mature ovary and the fecundity in different breeds and hybrid of silkworm Bombyx mori L. Each mean value is the average of 5 individuals (for ovarian weight) and 10 observations (for fecundity). Vertical bars represent standard error of the means.*

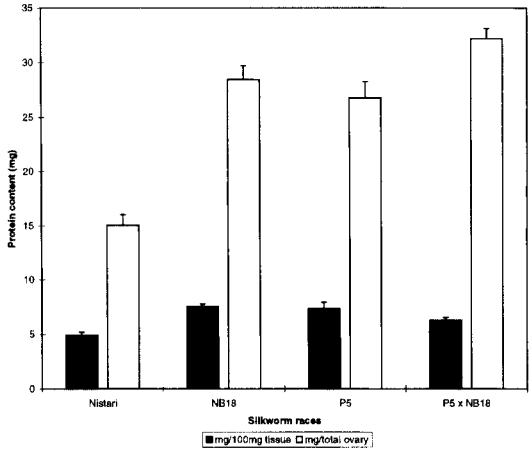


**Fig. 2.** *Variations in protein content of mature ovary in different breeds and hybrid of silkworm Bombyx mori L. Each mean value is the average of 5 replications and vertical bars represent standard error of the means.*

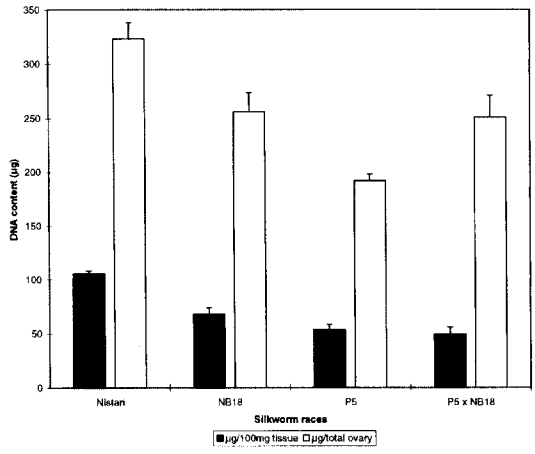


**Fig. 4.** *Variations in DNA content of mature ovary in different breeds and hybrid of silkworm Bombyx mori L. Each mean value is the average of 5 replications and vertical bars represent standard error of the means.*

mg/100 mg tissue. In addition, the bi×bi hybrids contained more ovarian protein ($p<0.05$-$p<0.01$) than their parents. However, no significant difference was observed in the protein content between two ovaries of the two bivoltine breeds ($P_5$ and $NB_{18}$) (Fig.2).

### 3. Nucleic acids

RNA and DNA content was calculated on the basis of both µg /100 mg tissue and µg/total ovary and results are presented in Fig. 3 and 4 respectively.

Ovarian RNA concentration followed the same trend like that of protein, where the biomolecules were found to be significantly higher ($p<0.001$) in all the diapause destined breeds and hybrids. The percentage was recorded to be higher(187.32%, 155.18% and 196.37% in $NB_{18}$, $P_5$ and $P_5 \times NB_{18}$ respectively), as found in the case of total protein. Interestingly, it was observed that in case of DNA, the trend was just the reverse. Nistari the multivoltine race was observed to have more DNA content ($p<0.01$-$p<0.001$) than that

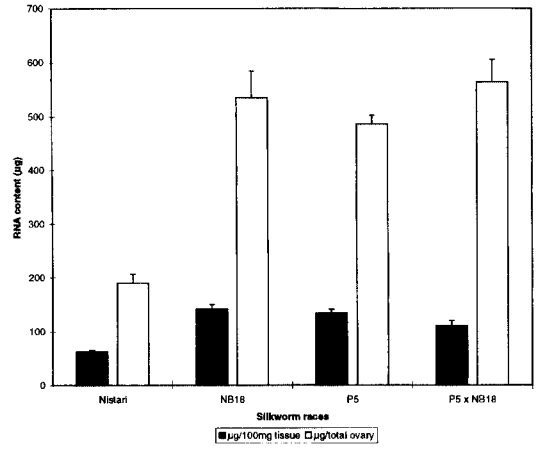


**Fig. 3.** *Variations in RNA content of mature ovary in different breeds and hybrid of silkworm Bombyx mori L. Each mean value is the average of 5 replications and vertical bars represent standard error of the means.*

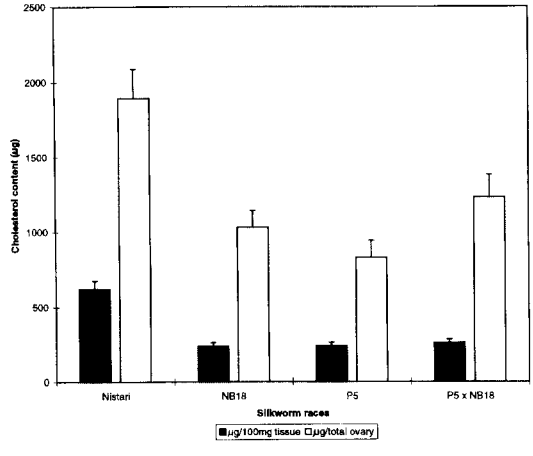


**Fig. 5.** *Variations in cholesterol content of mature ovary in different breeds and hybrid of silkworm Bombyx mori L. Each mean value is the average of 5 replications and vertical bars represent standard error of the means.*

of diapausing ovary of $NB_{18}$, $P_5$ and $P_5 \times NB_{18}$

**4. Cholesterol content**

Ovarian cholesterol concentration in freshly emerged moth was observed to be significantly lower ($p < 0.05$-$p < 0.001$) in the diapausing breeds and their hybrid (45.50%, 56.22% and 35.02% in $NB_{18}$, $P_5$ and $P_5 \times NB_{18}$ respectively) when compared to that of Nistari (multivoltine non-diapausing breed). In this case too the data was expressed in μg/100 mg tissue and μg/total ovary of silkmoths (Fig. 5).

## DISCUSSION

The results revealed that there was a definite variation, specific for a particular constituent viz. protein, RNA, DNA and cholesterol in the ovary of normally developed multi and bivoltine silkmoth *Bombyx mori*. Proteid yolk represents the major component of insect eggs and may account for upto 30% of the egg volume or upto 90% of the total protein content. The typical yolk protein known as vitellins are usually stored in the egg in the form of proteid yolk bodies which may have a crystalline structure (Zissler *et al.*, 1985). In our study, the bivoltine breeds contain more protein in the mature ovary than the multivoltine one, which may be due to more synthesis or transport of yolk/yolk precursor protein in the ovary for diapause development. However, other physiological and biochemical factors are also responsible for diapause development. Diapause is characterized by diapause development. Diapause is characterized by diapause development-physiogenesis analogous to morphogenesis-which is a prerequisite to the resumption of development Andrewartha, 1952). The variation observed in the structural features among the species is related to functional diversities; for instance, the *B.mori* eggs undergo long diapause and in that respect, the existence of very narrow aeriopyles, while providing sufficient surface area for gas exchange, keeps the eggs safe against excessive desiccation (Margaritis, 1985).

The breed specific differences in ovarian protein concentration in multivoltine (Nistari) and bivoltine ($NB_{18}$, $P_5$) silkworms is genetic and has pertinent physiological significance. Diapause occurs at embryonic, larval, pupal or adult reproductive stages of the life cycle and the stage is characteristically fixed in each species (Yamashita and Hasegawa, 1985). Further, changes in proteins will lead to changes in structure and function, in other words to development. During early embryogenesis, genetic information is expressed in newly synthesized proteins,"embryonic proteins", and is most likely maternal in origin (Sander *et al.*, 1985).

The racial differences in ovarian RNA concentrations in *B.mori* studied are indicative of marked genetic variations between diapausing and non-diapausing broods depending upon the brood specific demands of synthesis and storage of RNA in the oocytes and nurse cells of ovarian follicle. Pagtia *et al.*, (1976a, b) studied the synthesis, transport and storage of messenger RNA in the ovarian follicles of *Antheraea polyphemus*. Further, electrophysiological studies have shown that the polarized movements of macromolecules which take place within the egg chambers of the *Hyalophora cecropia* moth are caused by an electric current generated by potential differences between the occyte and the nurse cells (Woodruff and Telfer, 1973; 1974). These studies clearly point to the fact that protein synthesis/storage and RNA concentration are closely linked and are found to be in higher concentration in diapausing breeds and hybrid of *B.mori*, which may reflect the preparatory phase for production of diapausing eggs by the bivoltines.

The differences in mature ovarian DNA content may be due to variations in sequence homologies in the rDNAs of ovarian nurse cells of bivoltine and multivoltine breeds as found in *Drosophila* which again strongly establishes the racial variations in *B. mori*. In *Drosophila melanogaster* no synthesis of DNA occurs in the 16-cell clusters during their passage through region 2 of the germarium, but studies on uptake of [$^3$H] thymidine indicate that it occurs in region 3 (Cahrez, 1979). The oocyte nuclei on the other hand maintain their DNA level at the 4C value throughout oogenesis (Mulligan and. Rasch, 1980). Further, during the first 6 cycles the DNA levels observed are those expected assuming 100% replication of the pseudo-nurse cells of *D.melanogaster* (King and Buning & 1985). In our study the higher con-

centration of DNA in ovaries of multivoltine silkworms compared to bivoltines remans unclear at this stage. Further studies on more strains in both multi and bivoltine silkworms are being considered in our laboratory to speculate and generate more information on these variations at the molecular level.

The experimental results of the cholesterol content in the mature ovary of Nistari is higher than the bivoltine races and the hybrid. Cholesterol, a major zoosterol, plays an important role in insects influencing many vital activities like growth, development, molting, oogenesis, egg production and hatching(Levinson and Bergmann, 1957; Monroe, 1959, 1960; Kaplanis *et al.*, 1960; Robbins and Shortino, 1962). The high level of cholesterol in non-diapausing brood may be due to immediate demand/requirement of the biomolecules for embryogenesis and hatching. The diapausing egg undergoes a prolonged period of embryonic diapause, hence, the lower level of cholesterol in comparison to the multivoltine race Nistasi may be due to the reduced requirement of cholesterol for late hatching.

In conclusion, it is interesting to note that all the biochemical components in the ovary of $NB_{18}$ is higher than its $P_5$ counterpart, a bivoltine race, which ultimately reflects in the higher number of eggs laid by female moth in this breed. Further, all the breeds and the hybrid studied have adequate phenotypic diversity (Sengupta *et al.*, 1974; Ghosh *et al.*, 1993; Chattopadhyay *et al.*, 1996). The biochemical differences in the female ovaries of these races once again depict the racial differences which exists with respect to reproductive physiology and biochemistry of *Bombyx mori*.

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