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Biomolecular Variations in Poly and Bivoltine Strains and Their Hybrids of *Bombyx mori* during Embryonic Development

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Biometabolic assessment was made in early and late embryonic stage (just before hatching) of one poly, one bi and their hybrids (DP, YPe, DP×Ype, and YPe× DP respectively) of *Bombyx mori* to observe the racial differences. Protein and nucleic acid (RNA and DNA) concentrations were recorded to be significantly higher in bivoltine breed (YPe) and also in the hybrid than the polyvoltine (DP) strain in both the stages of embryonic development. The single egg weight of polyvoltine race was lower as compared to that of bivoltine and the hybrid studied. Age specific changes in all the biomolecules were evident where protein and RNA concentrations were elevated sharply in pre-hatched larvae while in case of DNA it was observed to be just reverse. The differences in protein, RNA and DNA composition between breeds and hybrids reflect the racial variations in biometabolic demands responsible for differential growth and development of the breeds and hybrids.

Key words : *Bombyx mori*, Hybrids, Protein, Nucleic acids, Embryo, Strain

Introduction

The cellular contents viz. protein, cholesterol, glycogen etc. are in a constant state of turnover, being synthesized and degraded continuously in all living organisms. The function of an organ depends upon the mass of functioning cellular components available and also upon the circulating factors and regulatory mechanisms, which in turn, determine the level of performance of individual cells. The breeds and their hybrids of the silkworm, *Bombyx mori* having adequate phenotypic diversity (Sengupta *et al.*, 1974; Ghosh *et al.*, 1993; Chattopadhyay *et al.*, 1996). Genotypic and phenotypic variability of some quantitative characters in *Antheraea mylitta* D. have also been reported (Sen *et al.*, 1976; Siddiqui *et al.*, 1983; 1988; Siddiqui and Sengupta 1994).

Chatterjee *et al.* (1992) established the genetic variability of amylase activity in tropical races / breeds of *B. mori* and its relevance to the geographic distribution. The racial differences in seven enzymes and their relationship with quantitative and qualitative traits along with survival has also been reported in *B.mori* (Chatterjee *et al.*, 1993). Further, racial variation in the protein pattern in the hemolymph of wild silkworm, *A. mylitta* has been observed (Kar *et al.*, 1994). Earlier studies on different biochemical parameters viz. trehalose, cocoon shell lipids and hemolymph volume to pupal weight ratio etc. have recorded significant variations among races (Kar *et al.*, 1993; Sinha *et al.*, 1993).

The present study deals with the racial evaluation of polyvoltine, bivoltine and their hybrids with respect to biochemical constituents in eggs and freshly hatched larvae of the silkworm. Hence, the protein and nucleic acid content studied in the present investigation was also found to be an equally potent tool as a first step for characterization purposes.

Materials and Methods

Selection of breeds and hybrids

One multivoltine (DP), one bivoltine (YPe) mulberry silkworm *Bombyx mori* L. and their reciprocals (DP×YPe and YPe×DP) were selected for biochemical experi-

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ments. The larvae were reared on fresh mulberry leaves at 25 - 27°C with suitable humidity (70 - 90%). After adult emergence, the healthy male and female moths were collected for coupling. After three hours of coupling, the females were allowed to lay the eggs.

Extraction and assay of protein, RNA and DNA

Silkworm eggs of 48 hrs and 240 hrs old embryos (pre-hatched larvae) of each breeds and their reciprocals were subjected to biochemical assay. A measured amount of tissue were taken separately to prepare 5% tissue homogenate in ice cold 0.65% saline using a Potter-Elvehjem all glass homogenizer and then precipitated with 0.3 N perchloric acid (PCA). Protein, RNA and DNA were extracted following the method of Chaudhuri and Medda (1987). Protein estimation was made following the method of Lowry *et al.* (1951), while RNA and DNA were measured by the method of Munro and Fleck (1966) as modified by Abalain *et al.* (1980). The results were statistically analyzed using student 't' - test. Each mean value was an average of 5 replications.

Results and Discussion

Our study revealed that 48 hrs old egg and pre-hatched (240 hrs old) larval weight (Fig. 1) remained significantly higher ($p < 0.05$ to $p < 0.001$) in bivoltine (YPe) breeds compared to polyvoltine one (DP) and their reciprocals (i.e. DP×YPe and YPe×DP). Among the hybrids, YPe ×DP (bivoltine mother) showed higher weight (16.22% in 48 hrs old and 9.75% in 240 hrs old embryo respectively) than DP×YPe (multivoltine mother). The higher weight in bivoltine breeds is due to the diapause characters. The diapausing eggs reserve / store nutrients to overcome unfavorable conditions (Yamashita and Hasegawa, 1985) besides producing healthy progeny than the polyvoltine races. Higher protein, RNA and DNA content (µg/egg) in early and late age embryos (Fig. 2, 3 and 4) in bivoltine breed (YPe) compared to that of polyvoltine (DP) supports this view and thus establishes a distinct racial variation among the diapausing and non-diapausing breeds. Reproductive output of the female have a significant correlation with the vigor of mother moths in silkworm and other insects (Crawford, 1971; Jennings, 1974; Samachary and Krishnaswami, 1980; Miller *et al.*, 1982, Gowda *et al.*, 1988; Kasuli, 1991; Ghosh *et al.*, 1996). It

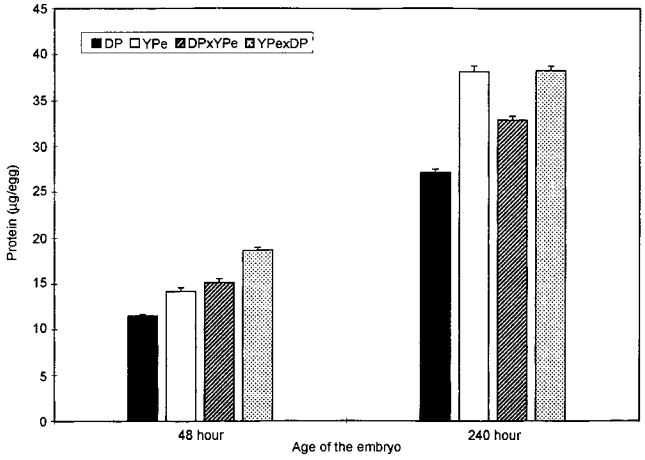

Fig. 2. Variations in protein content of eggs (48 and 240 hrs old embryos) of breeds and hybrids of silkworm *B. mori*. Each mean value is the average of 5 replications and vertical bars represent standard error of the means.

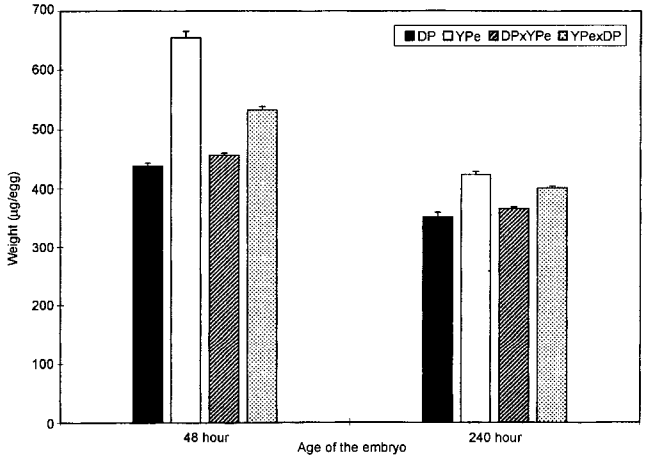

Fig. 1. Variations in weight of eggs (48 and 240 hrs old embryos) of breeds and hybrids of silkworm *B. mori*. Each mean value is the average of 100 individuals and vertical bars represent standard error of the means.

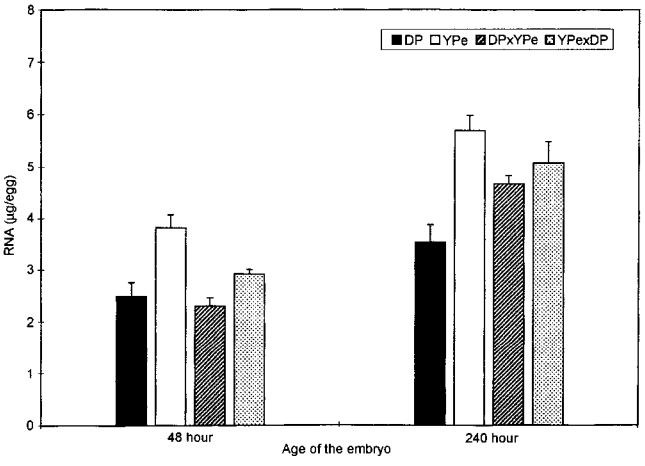

Fig. 3. Variations in RNA content of eggs (48 and 240 hrs old embryos) of breeds and hybrids of silkworm *B. mori*. Each mean value is the average of 5 replications and vertical bars represent standard error of the means.

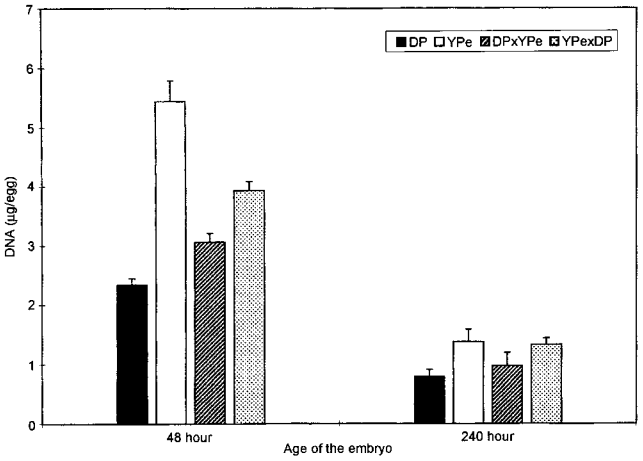

Fig. 4. Variations in DNA content of eggs (48 and 240 hrs old embryos) of breeds and hybrids of silkworm *B. mori.* Each mean value is the average of 5 replications and vertical bars represent standard error of the means.

is expected, therefore, that the egg production, its size, shape and biochemical constituents all will be dependent on the vigor of the silkworm breeds and accordingly differ among the breeds and hybrids.

Single embryo of diapause destined race YPe showed more protein, RNA and DNA content (23.36% , 53.01% and 132.45% respectively) in 48 hrs old eggs and 63.94%, 60.73% and 74.97% respectively in pre-hatched larvae over non-diapausing breed (DP). Higher concentration of these biomolecules in YPe may be explained due to higher biometabolic demands by the insect for producing diapause destined generations. This may also be due to biochemical adaptations under genetic control. To consider protein metabolism in relation to egg diapause, it is advisable to distinguish the proteins stored as nutrients from those that function as enzymes. Since vitellin is the predominant protein component in insect eggs accounting for 50-90% of the egg proteins, the metabolism of this protein seems to be closely associated with the physiological events of embryogenesis. However, little information is available on the metabolic fate of vitellin during embryogenesis including embryonic diapause. Immunotitration of vitellin in *B. mori* eggs showed that vitellin degradation occurred only at the later stage of embryogenesis and about 40% of the initial amounts remained unused at larval hatching (Irie and Yamashita, 1980), indicating no utilization of protein throughout the period of diapause. Hence, higher protein concentration in diapausing embryos over non-diapausing individuals studied by us may be due to the aforementioned reason.

The early and late embryo of the hybrids of multi x bi and bi x multi showed higher protein and nucleic acid contents than that of multivoltine strains (Fig. 2, 3 and 4) but it was found to be less than bivoltine race with a few exceptions in case of protein concentration. Proteins have a pertinent role in the development, morphogenesis and almost in all the intermediary metabolic pathways of the insect. The striking differences in the protein concentrations between the breeds / hybrids may be due to differential physiological demands of yolk protein by the different races / hybrids to maintain their progeny. Accordingly, the yolk precursor protein vitellogenin is synthesized and secreted by the fat body cells of the insects and subsequently deposited in the mature oocytes depending on specific biochemical requirements. It was reported that female specific protein, vitellogenin, are being synthesized by the fat body cells and are transported into the ovary which is under hormonal control (Ono *et al.*, 1975; Tojo *et al.*, 1981; Bradley, 1983). It should be pointed out that late embryo (240 hrs old) contain more protein and RNA than that of 48 hrs old eggs. This may be due to more synthesis of protein during the course of embryogenesis and / or organogenesis. It was reported that stages of embryonic and subsequent larval differentiation during egg life are thought to depend upon the synthesis of new proteins, for which an intensive degradation of stored proteins has first to occur to provide substrates. The utilisation of vitellin seems to be an inductive step in protein metabolism for embryonic development (Irie and Yamashita, 1980). It is generally believed that vitellogenin is an essential protein for egg formation and serves as the main source of nutrients for the ensuing embryonic development (Engelmann, 1979; Wyatt and Pan, 1978).

However, the cause of decrease in DNA concentration in hatched larvae in the breeds and hybrids studied so far remains unclear. More studies in this line are needed to explore the physiological and / genetic background in this aspect. Hence, all these striking differences in basic macromolecular constituents in eggs and pre-hatched larvae of different breeds and hybrids of *B. mori* evinced marked racial differences due to differential race / breed specific metabolic demands for growth and development of diapausing and non-diapausing individuals.

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