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Research Article

A STUDY ON QUANTITATIVE ESTIMATION OF PROTEINS OF NEW BREEDING LINES OF BOMBYX MORI L

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A R T I C L E I N F O

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A B S T R A C T

Background: Knowledge on biochemical studies in silkworm is very meagre indicating the type and amount of work to be done in future. At the same time, it is interesting to note that different silkworm races reared in laboratory offer an important testing ground for the application of biochemical methods to taxonomic problems
Objective: The present study was designed to conduct with the main purpose to quantitative estimation of proteins in new breeding lines and races *viz.*Kalimpong-A (KA), B_{18}, Pure Mysore (PM), evolved R1 and R2 of *Bombyx mori* L.
Methods: The modified method of Lowry was used for the quantitative estuation of proteins during the different developmental stages of KA, NB_{18}, PM, evolved R_1& R_2 races, and the protein concentration was expressed as µg/ml to weight of the tissue.
Results: Results revealed that The soluble proteins in different developmental stages of KA, NB_{18}, PM, R_1 and R_2 have revealed comparatively a low protein content in eggs which however reaches a peak in female pupae. Then declines slowly. The soluble protein content in eggs is high in 24h embryos, later decreases and still decreases in 216 h embryos and in instar larvae suddenly increases and reaches a peak in female pupae of 288 h. Highest quantity of protein was noticed in KA, and is followed by NB_{18}, R_1 and R_2 and PM.
Conclusion: The quantitative analysis of proteins revealed low protein content in eggs, reaches peak in female pupae. The bivoltine parents revealed high protein followed by the hybrids and the Pure Mysore.

INTRODUCTION

Any change in the protein/enzyme pattern during the development can be considered as being directly determined by gene function. This reflects on alteration in the metabolism of the developing organism. [1] Analyses of different gene enzyme system have revealed differential regulation of gene activation during metabolic changes. Isozymes can be considered to be ideal gene products for investigating the patterns of gene expression during development. They offer a potential tool and a biochemical index to assess the genetic variability in natural populations. [2-5] These studies have quite often been used to follow the developmental variations in various enzymatic patterns as well as in species comparision.[6] At the same time, analysis made on isozyme differentiation are systems during ontogeny of wide biological value.[7] The isozymes present during different phases of development can be studied in two ways. One is to understand the differential gene action during development and the other is to compare the manifestation of homologous enzymes during developmental stages.

Though the subject of silkworm genetics, cytogenetics and breeding has gained its importance in Rand D programmes of several laboratories, the literature available with reference to isozyme studies is not sufficient to warrant any plaurable line of explanation regarding recognition of different races and the process of speciation in the evolution of silkworm fauna. Furthemrore, Considerable data is available at present on

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aspects of genetics, cytogenetics, breeding, physiology, pathology and embryology of silkworm Bombyx mori. [8] However, our knowledge on biochemical studies in silkworm is very meagre indicating the type and amount of work to be done in future. At the same time, it is interesting to note that different silkworm races reared in laboratory offer an important testing ground for the application of biochemical methods to taxonomic problems. Withthis viewpoints, the present study was undertaken with the main aim to estimate quantitatively proteins in new breeding lines and races of *Bombyx mori* L.

MATERIALS AND METHODS

Silkworm Varieties and Rearing

The pure races of bivoltine Kalimpong-A (KA) spinning oval white cocoons, New Bivoltine-18 (NB_{18}) spinning dumbbell white cocoons and multivoltine Pure Mysore (PM) spinning pointed yellow cocoons of mulberry silkworm Bombyx *mori* L. were selected for the present breeding programme. These races were obtained from their respective seed areas and are reared in cytogenetics laboratory, Jnana Bharathi, Bangalore University.

The disease free layings were prepared as described by [9], and were incubated at 25°C and relative humidity of 60-70%. On 8th day composite layings were prepared (10-20 layings were prepared 100-200 eggs were collected from each laying). The hatched worms were reared according to the method described by [10] MS variety of mulberry leaves were used in rearing. The worms were reared in mass upto III instar, after III moult 300 worms were collected in three replicates in order to evaluate the rearing performance. Standard temperature and humidity were maintained in the rearing house.

Breeding

Single and three way crosses were made by using the above said three races. The first single cross involved KA females and PM males. The second single cross involved NB_{18} females and PM males. During the course of breeding selection was made at the egg, larva, pupa and cocoon stages to fix the desirable traits. F_5 progenies of the respective crosses were back crossed to their respective bivoltine males to improve commercial characters.

Evolutions of New Lines R_1 and R_2

Females of KA and NB_{18} were crossed with males of PM. The composite layings of F_1 hybrid were brushed and reared under standard laboratory conditions. The selection parameters explained earlier were applied to choose the seed cocoons for the preparation of F_2 layings. The replicates showing higher pupation rate were selected for intra family selection of cocoons. Further, segregation with respect to cocoon colour and built was noticed. Only white oval in case of KAxPM and dumbbell white in case of NB_{18}xPM qualifying the parameter of selection were choosen for breeding in subsequent generations. The females of F_5 were backcrossed to the males of KA and NB_{18} respectively in both the lines and reared up to 11 generations. At the end of the 11th generation the lines R_1 and R_2 were extracted with higher ERR than their respective better parents, with shorter larval period and with moderate cocoon productivity character in case of R_1 and R_2.

Breeding Plans I and II

	KA	O +	O i-	I x	PM	Cto		NB18	O +	O +	II x	PM	Cfo			
				F1							F1					
				F2							F2					
				F3							F3					
				F4							F4					
F5	x	KA	O	→	er'		F5x	NB18	Cta	+						
				F1							F1					
				F2							F2					
				F3							F3					
				F4							F4					
				F5							F5					
				F6	(R1)						F6	(R2)				

Quantitative Analysis of Proteins

The modified method of Lowry et al was used for the quantitative estuation of proteins during the different developmental stages of KA, NB_{18}, PM, evolved R_1 and R_2 races. The protein was expressed as µg/ml to weight of the tissue.[11]

RESULTS AND DISCUSSION

The soluble protein concentration in any developing organism has been shown to depict marked changes. The present observations confirm the same. The soluble proteins in different developmental stages of KA, NB_{18}, PM, R_1 and R_2 have revealed comparatively a low protein content in eggs which however reaches a peak in female pupae. Then declines slowly. The soluble protein content in eggs is high in 24h embryos, later decreases and still decreases in 216h embryos and in I instar larvae suddenly increases and reaches a peak in female pupae of 288 h. Highest quantity of protein is noticed in KA, and is followed by NB_{18}, R_1 and R_2 and PM (Figs. 1-4).

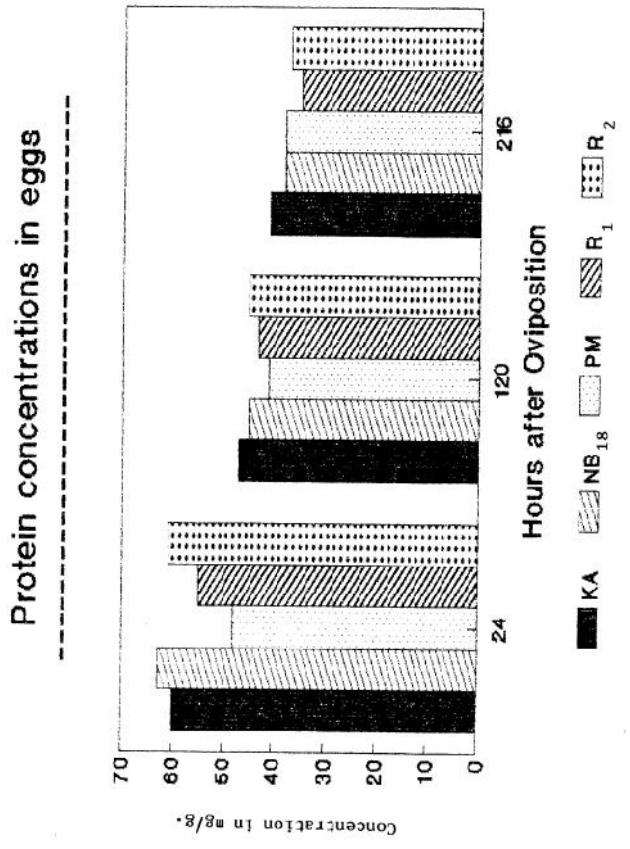

Figure 1

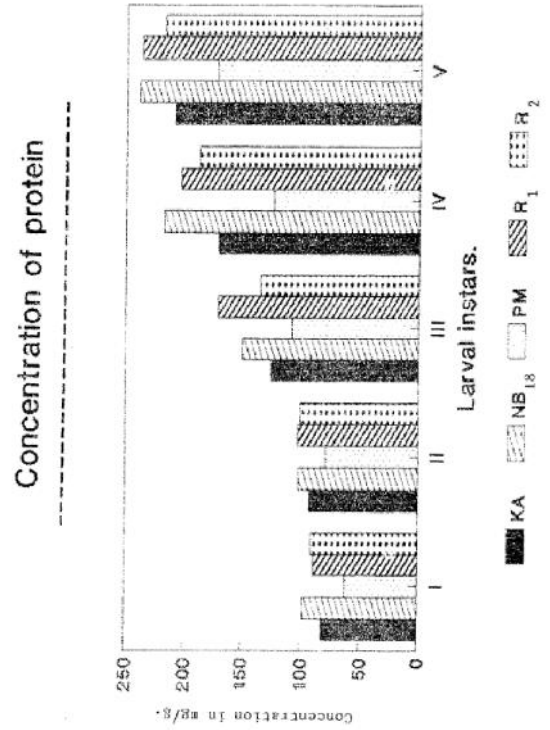

Figure 2

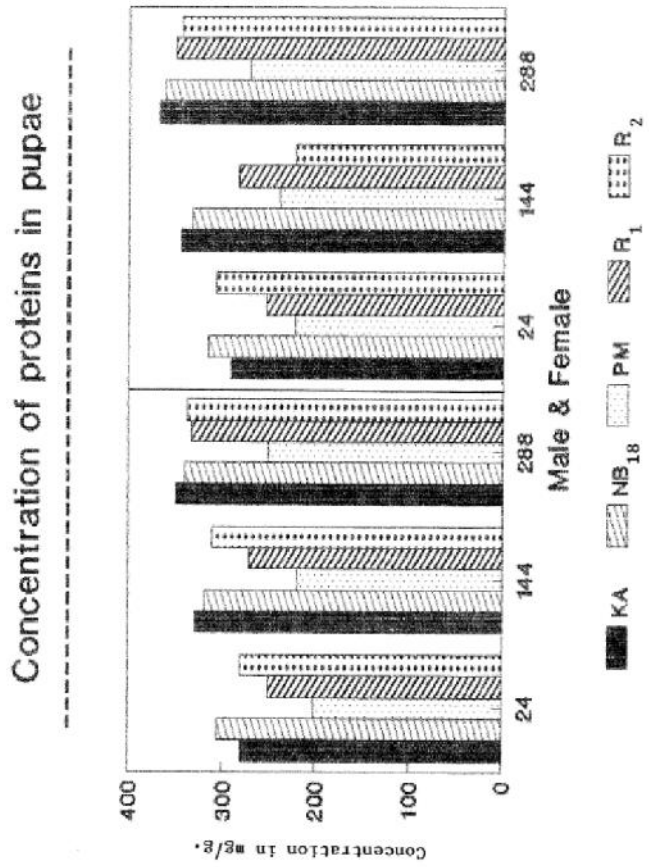

Figure 3

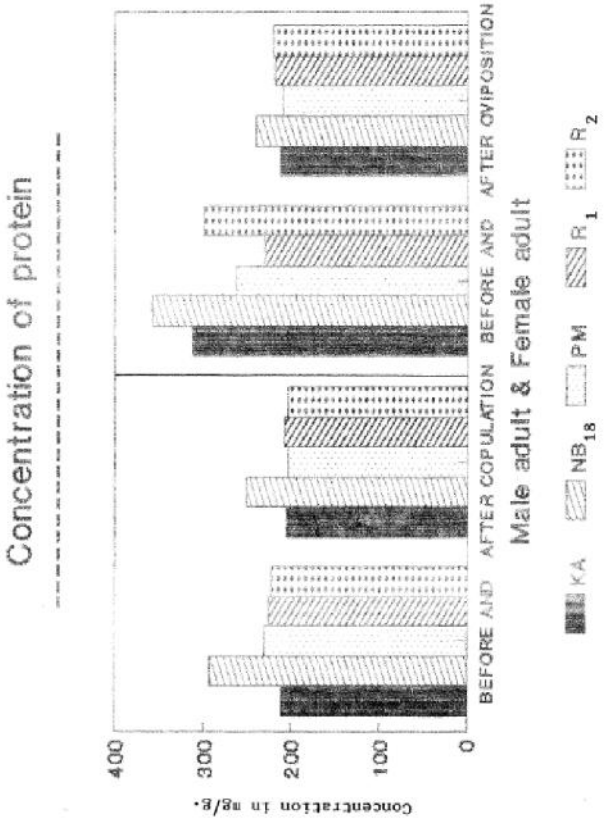

Figure 4

The results of quantitative estimation of protein of new breeding lines and races of *Bombyx mori* L viz. KA, NB_{18}, PM, R_1 and R_2 are presented in figures 1-4. The results revealed that that protein contents in *Bombyx mori* vary according to the race, sex and developmental stage. This is in agreement with similar research involving other species of insects. [12-14] As presented in Figs. 1-4 they are relatively gradual, yet dramatic quantitative changes during the larval and pupal stages and reached lower but relatively constant level of activity in the adult insects. The protein activity in the eggs is high in 24 hours of development whereas it has slightly decreased in 120 and 216 hours. This may be due to the degradation of yolk proteins. Embryogenesis which occurs depend on the degradation of the yolk proteins. [15-19] In larval instars it increases and is shown to be high in pupae which is due to the formation of protein granules in the fat body of an insect.[20]

Many of the quantitative changes that we observe in developing Bombyx follow the same trend as reported by some other worker.[21,22] That is, the concentration rises in the growing larvae, reaches peak at some points in the pupal and then taper off to a lower concentration in the adult insect.

CONCLUSION

In conclusion, quantitative analysis of proteins revealed low protein content 1n eggs, reaches peak in female pupae. The bivoltine parents revealed high protein followed by the hybrids and the Pure Mysore.

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