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SEXUAL DIFFERENCE IN HAEMOLYMPH PROTEINS OF THE LAST LARVAL INSTAR OF *Bombyx mori* L.

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ABSTRACT

The average haemolymph protein concentration was significanly higher in females than males. The percentage increase was 13.7 %. Two isozymes were chosen to demonstrate a sex-linked different response of glucose-6-phosphate dehydrogenase (G-6-PDH) and alkaline phosphate (ALP) activites and protein concentration in both sexes. The multiple forms of both studied isozymes are pentameric and trimeric, respectively in females and males. Three isoenzyme bands of G-6-PDH are characteristic for male, while other three bands are characterisfic of females. On the other hand, the isozyme ALP has an electrophoritic band (R_F 0.58) characterizing females and a band of R_F 0.5 characterizing males,for haemolymph protein, last instar larvae, *Bombyx mori* isozymes,

***Key words**: Bombyx mori, haemolymph protein, isozymes last instar larvae,sexual differentiation.*

1. INTRODUCTION

The mulbery silkworm, *B. mori* has been intimately connected with humans, as the only truly domesticated insect, as the foundation of sericulture, and economic survival for farmer and worker in the textile industry. In the nineteenth century, *B. mori* became a model for scientific discovery in microbiology, physiology and genetics (Xu *et al.*, 2002).

The biochemical may be of one characteristics,to confirm the variability among sexes. This variability may be explained by determination of total haemolymph protein concentrations, proteins profile and regulation of proteins synthesis using isozyme technology. Minor qualitative differences in the blood protein of the two sexes in various insects was recorded by Steinhauer and Sterphan (1957). Martoja and Lauverjat (1964) found a sexual dimorphism in the fat body of *Locusta* and other Orthoptera, where females are richer in RNA, amino acids and phenolic compounds than males. In addition, Wyatt (1975) found that the activity of the fat body, which is the chief site of protein synthesis, appears to be sex and stage specific, which changes in the capacity of protein synthesis and reflects the level of RNA.

The aim of this work was to determine the total protein concentrations and to use the isozyme technique to elusidate the correlation between enzyme activity and changes in the capacity of protein synthesis in the haemolymph of male and female late instar larvae of *B. mori* L. and to evaluate the quantitative and qualitative nature of late instar larvae haemoymphprotein, amino acid concentration, protein profile and regulation of protein synthesis using isoenzyme analysis.

2. MATERIALS AND METHODS

2.1. Rearing technique

Rearing was carried out under laboratory conditions using the selected strain 380. Hatched larvae were transferred

from egg cards to the rearing trays. The rearing temperature was 28 ± 2°C, and the relative humidity was 75 – 80 %. Fresh mulberry leaves were offered to larvae 4 times daily.

Cocoons were collected after ten days of spinning. Thirty cocoons from each sex were weighted separetely, and the average weight was calculated. Pupae of each sex were removed and weighted after separating the cocoon shells and the cocoon shell ratio for each sex was calculated as follows:

$$\text{Cocoon shell ratio (\%)} = \frac{\text{Weight of cocoon shell}}{\text{Weight of cocoon}} \times 100$$

2.2.Total protein concentration

The haemolymph was collected from a punctured proabdominal leg of healthy late instar larvae of both sexes in a sterile test tube with a small crystal of phenylthiourea to prevent melanization of the sample.

The determination of total protein concentration was estimated according to the method suggested by (Henry *et al.*, 1964). The method depends on protein forms colored complex with cupric ions in an alkaline media.

2.3. Isoenzymes assay

The enzymes of G-6-PDH and ALP in blood supernatants were separated by discontinuous polyacrylamide gel electrophoresis according to Maurer and Suss (1968) and were assayed and detected by the methods of Show and Prasad (1970). Enzymatic protein bands were designated according to the system nomenclature proposed by Shaklee *et al.* (1990).

Electrophoresis was carried out conveniently in discontinuous polyacrylamide gels (stacking and tracking gels). An amount of 50 µl of protein dye (1% bromophenol blue) and 20 µl of 2 % sucrose and 30 µl of the mixture per gel slot were used to be applied per each sample for isoenzymes electrophoreses. After electrophoresis, the gel was transferred into a staining solution (50-70 ml), then replaced by destaining mixture of methanol, acetic acid and water (5: 1: 5 v / v / v). A protein gradient of 20 v / cm across the gel was applied for 4 hr at 8 °C (Heckel, 1993).

2.4. Statistical analysis

3. RESULTS AND DISCUSSION

Results in Table (1) show that the average cocoon weight (1.396 ± 0.04 gm), pupal weight (1.132 ± 0.02 gm), and cocoon shell weight (0.259 ± 0.01) in females and males (1.110 ± 0.03, 0.846 ± 0.03 and 0.247 ± 0.01) were positively correlated with each other.

The ratio of the cocoon shell weight to the total cocoon weight is a very important feature for cocoon quality and differences between females and males. This was highly significant. Heavier cocoon shell means large quantity of silk fibers. Higher fresh cocoon weight will lead to increase cocoon shell weight and pupal weight.

Table (1): Cocoon weight, pupal weight, cocoon shell weight (gram), cocoon shell ratio and haemolymph protein concentration of the final instar larvae of both sexes of *B. mori* L.

Parameter	Males mean ± S. D.	Females mean ± S. D.
Cocoon weight (grams)	1.110 ± 0.03	1.396 ± 0.04
Pupal weight (grams)	0.846 ± 0..03	1.132 ± 0.02
Cocoon shell weight (gram)	0.247 ± 0.01	0.259 ± 0.01
Cocoon shell ratio (%)	22.24	15.68
Total haemolymph protein concentration	3.72 ± 0.54	4.91 ± 0.91

In the present work, the average concentration of haemolymph protein was significantly higher in females than in males. In females, the concentration was 4.91 ± 0.91 gm protein / dl haemolymph, while in males; it was 3.72 ± 0.54 gm protein / dl haemolymph. The percent increase was 31.7 %. Statistical analysis of the protein contents of both males and females revealed significant difference ($P > 0.01$).

Electrophoresis was undertaken to determine whether the increase in haemolymph protein concentration in females, was due to an overall increase in the concentration of all haemolymph proteins or whether only certain proteins were involved. Analyses of electropherograms from the hemolymph indicated the presence of 13 negatively charged proteins in females and 11 bands in females. Of the 23 negatively charged protein bands in the haemolymph of both sexes, only one band with a molecular weight of 75.536 KDa was common and

shown to be shared in the protein profiles of both males and females.

Determination of amino acids in haemolymph shows that, the proline is the most abundant amino acid; its concentration was 45.814, and 55.254 µmol / ml in males and females, respectively (Abou El Ela, 2007).

There is no doubt that tissue and stage-specific patterns of protein synthesis are correlated with changes in gene activity at different phases of both sexes of insect's life cycle. Nucleic acids are growth factors for insects where RNA accelerates the growth rate and the capacity of the cell to synthesize protein. The content of DNA has been reported to remain constant in the cell and any increase would reflect that growth is accomplished partly by an increase in the cell number (Alonso, 1973). Burr and Hunter (1969) showed that RNA content was higher in females than in males of *Drosophila melanogaster*. This means that there is a close relationship between the synthesis of the amount of total proteins and the nucleic acid contents in the cell; thus specific patterns of protein synthesis are correlated with changes in gene activity at different phases of the male and female life cycle.

Heamolymph isozymes of the last instar larvae were detected by polyacrylamide gel electrophoresis. The number of bands recorded for G-6-PDH in the haemolymph of both sexes in *B. mori* are listed in Table (2) and the electrophoretic patterns are shown.

Table (2): Glucose-6-phosphate dehydrogenase (G-6-PDH) activity in haemolymph of last instar larvae of both sexes of *B. mori* L.

Bands	Males		Females	
	R_F	% Amount	R_F	% Amount
1	-	-	0.053	23.1
2	0.01	18.8	-	-
3	0.31	18.3	0.31	22.2
4	0.43	17.8	-	-
5	-	-	0.49	16.8
6	0.68	22.3	0.68	19
7	-	-	0.88	18.8
8	0.94	22.6	-	-
Sum	99.9		99.9	
In lane	100		100	

Eight bands were recorded. Two common bands (No. 3 and 6) are common in both females and males. Their R_F values are 0.31 and 0.68. Protein bands No.2 (R_F 0.10), No. 4 (R_F 0.43) and No. 8 (R_F 0.94) are characteristics for males, their amount are 18.8, 17.8 and 22.6 % of all proteins in males, while band No. 1 (R_F 0.053), No. 5 (R_F 0.49) and No. 7 (R_F 18.8) are charateristic for females, their amounts are 23.1, 16.8 and 18.8 % of all female protein bands. Band No. 8 in males maintained the highest average amount percent composition (22.6 %), The most predominant band is No. 1 (23.1). Only two bands No. 3 (R_F 0.31) and No. 6 (R_F 0.68) showed similar mobility for both sexes and considered as common bands shared by males and females. The other six bands exhibit different mobilites in both sexes. Thus, each sex has 3 different bands and 2 common bands *i.e.*, pentameric in males and females.

Isozymes are enzymes that have similar or identical catalytic functions, but are by one means or another identifiably different with respect to structure. Electrophoresis has proven to be a powerful tool in the analysis of allelic expression, in that the utilization of isozymes as indicators of gene expression provides an approxmation of the transcriptional and translation patterns at many gene loci, and different isozyme expressions are the result of different synthetic rates (Davis and McIntyre, 1988).

It has been pointed out that isozyme studies have given clear evidence for the regulation of protein synthesis in insects during development and metamorphosis. This regulation is probably exerted at the DNA transcription level (Pryor and Ferrell, 1981). Another aspect of the regulation of gene activity is the phenomenon of dosage compensation. An attempt has been made to gain some insight into this problem by the use of the isozyme G-6-PDH resulting from *D. Melanogaster*. A strain has the isozyme resulting from the action of the allele ZW^A and a B strain has a different isozyme associated with the allele ZW^B. Both variants of the enzymes show dosage compensation, but there is less A enzyme than B enzyme activity per milligram protein in males. These activites are equal in females. An analysis of various crosses suggests that autosomal loci influence the

level of G-6-PDH activity in male flies, and to a lesser extent in females. These autosomal loci appear to be specific for regulation of this enzyme. Also, the ZW^A allele responds less markedly to the regulator than the ZW^B. The result is that B males have more enzyme activity than A males. Hence, a possible example here of differential response of allelic genes to regulator genes existed (Bowman and Simmons, 1973). Glucose-6-phosphate dehydrogenase has an important role in fat body tissue during development and adult life, G-6-PDH is regulated through the complex interaction of hormones and dietary factor, although the precise details such interaction are not clear. Sun and Holten (1978) suggested the involvement of a modulation in the translation efficiency of the massenger RNA encoding the enzyme, whereas other workers (Miksicek and Towle; 1982 and Kletzien *et al.*, 1985) have demonstrated a linear relationship between the rate of synthesis and the massenger RNA.

Table (3): Activities of alkalin phosphate- se (ALP) in haemolymph of last instar larvae of both sexes of *B. mori L.*

Bands No.	Males		Females	
	R_F	% amount	R_F	% amount
1	0.27	30.9	0.27	27.7
2	0.51	31.1	-	-
3	-	-	0.58	19.2
4	0.83	38	0.83	53
Sum	100		99.9	
In Lane	100		100	

The amount of immunoreactive protein and enzyme activity was 2-fold greater in sexually mature female rats compared with aged matched male animals, and the administration of insulin to the normoglycaemic animal increased the level of G-6-PDH in the female, but was without effect in the male. G-6-PDH enzyme plays an important role in catalysing the frist committed step of the pentose phosphate pathway (Barton and Bailey, 1986) and is considered as key enzyme in intermediate metabolism (Surholt, 1976). It is mainly active in the fat body (Horie, 1967). Barton and Bailey (1986) showed that the level of enzyme activity and immunoreactive protein are heigher in females than males. Electrophoretic patterns of alkaline phosphatae isoenzymes in the haemolymph of fifth instar *B.mori* L. males and females larvae are shown in Fig. (2). Secretory ribonucleases are a wide group of enzyme whose molecular properties are scarcely known, probably due to the small levels found in the tissues studied. Their enzymic properties, subcellular distribution and possible functions have been reviewed in a number of articles (Roth, 1967: Barnard, 1969 and Sierakowska and Shugar, 1977). However, the biological significance of their RNA-degrading action is still uncertain. Among these ribonucleases, alkaline RNAases are a group of intracellular enzymes for which a regulatory role in protein biosymthesis has been proposed (Roth, 1967 and Brewer *et al.*, 1969). Such hypothesis is based on the existence of specific inhibitor proteins for these alkaline activities (Blackburn and Gavilanes, 1982).

The inhibitor-RNAase I system might determine the rate of RNA catabolism and, in turn, the rate of protein synthesis (Kraft and Shortman, 1970). An alkaline ribonuclease has been purified from *Ceratitis capitata* larvae, and the enzyme preparation gave a single band on SDS-polyacrylamide gel electrophoresis (Garcia – Segura and Gavilanes, 1982). ALP isozyme is the second isozyme studied in this work. Four protein banded interlocus have been observed in haemolymph of male and female late instar larvae. One band (RF 0.51) characterizes males, while another band (RF 0.58) characterizes females. Two bands (RF 0.27 and RF 0.83) are common in both sexes. Band of RF 0.83 represents 53 % of total ALP isozyme bands in females, while it represents 38 % in males. This may indicate that the catalytic power of this band is higher in females than males.

Finally, the results presented here demonstrate a sex-linked differential response of haemolymph G-6-PDH and ALP activites and protein synthesis. The differential responsiveness of the two studied isozymes in male and female late instar larvae of *B. mori* to ecdyson and juvenile hormones, may help to explain some of the contradictory evidence in the literature regarding the control of these

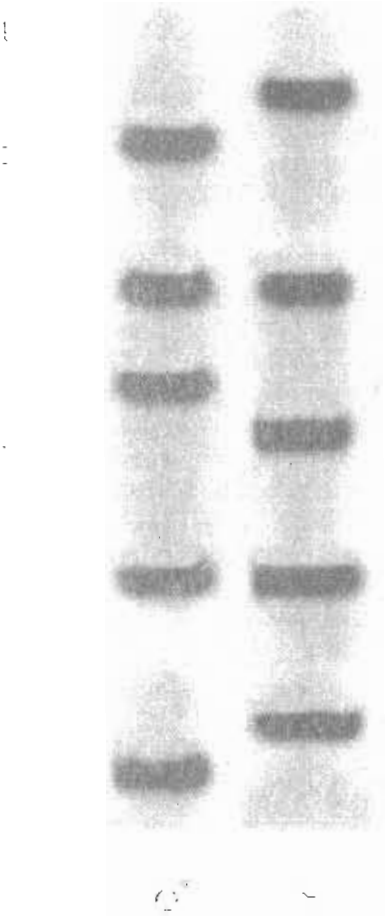

Fig. (1): Electrophoretic patterns of 6-phosphogluconate dehydrogenase isoenzymes in the haemolymph of the fifth instar male and female larvae of *B mori* L.

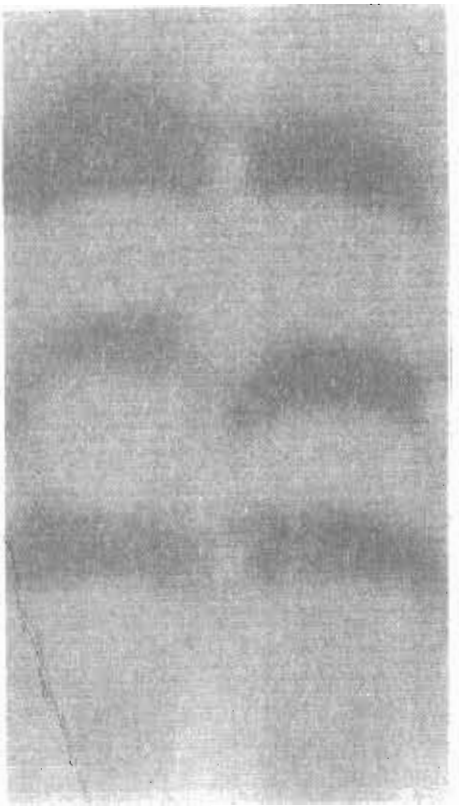

Fig. (2): Electrophoretic patterns of alkaline phosphatase isoenzymes in the haemolymph of the fifth instar male and female larvae of *B.mori* L.

enzymes and would provide an interesting model to study control of fat body gene expression by insect hormones.

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الاختلاف الجنسي في بروتينيات الدم في العمر اليرقي الأخير لديدان الحرير

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ملخص

يصل متوسط تركيز البروتين في الدم إلى أعلى درجة معنوية في الإناث عنها فى الذكور. تزداد النسبة إلى ١٣,٧%. تم اختيار اثنين من الايزوزيم, لكى تظهر الاختلافات المرتبطة بالجنس من حيث الاستجابة لجلوكوز-٦-فوسفات و الفوسفات القلوية و تركيز البروتين في كل جنس, يكون تعدد الشكل من الدراسات للايزوزيم خماسية او ثلاثية التركيب على التوالى للإناث و الذكور, يوجد ثلاث أشرطة من ايزوزيم الجلوكوز-٦-فوسفات مميزة للذكور, بينما يوجد ثلاثة أشرطة أخرى مميزة للإناث, من الجهة الأخرى, تكون أشرطة التفريد الكهربائي للايزوزيم الجلوكوز-٦-فوسفات ذات (شحنة ٠,٥٨) مميزة للإناث و أشرطة التفريد الكهربائي ذات (شحنة ٠,٥) مميزة للإناث.

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