

ELECTROPHORETIC HAEMOLYMPH PROTEIN PATTERN IN A FEW BIVOLTINE RACES OF THE SILKWORM, *BOMBYX MORI*

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ABSTRACT

The objective of this study is to understand the genetic difference between six bivoltine races useful for silkworm hybridization. The haemolymph proteins of V instar 2nd day larvae were analyzed utilizing six bivoltine races by SDS-PAGE. Based on the commassie brilliant blue pattern, the protein fractions were classified into three groups, I, II and III. The high molecular weight fractions were grouped under I, whereas II and III were included under moderate and low molecular weight fractions respectively. All the three groups fall in the range of 29 kDa to 97.4 kDa. The results have clearly indicated, the differentially expressed protein banding pattern in the six bivoltine races with highest number of 31 bands were observed in P₃₁ race (Bione, European race), whereas 24 bands were evident in C₁₀₈ race (Chinese race). Based on the UPGMA dendrogram the genetic distance between the races was established.

INTRODUCTION

Mulberry silkworm, *Bombyx mori* is one of the popular beneficial insect for the production of sleek sensuous silk fibre, which is considered as “Queen of Textiles”. The silk fibre is produced from the spinnert of the silkworm having two kinds of polymorphic proteins the fibroin and sericin. The synthesis of fibroin and sericin is one of the major physiological phenomenon, which involved sequential interlinked mechanism of silk production from mulberry as food to protein and protein into silk fibre. Further, haemolymph of V instar mainly contributes for silk protein biosynthesis in the different compartments of the silk gland. Perusal of literature have clearly demonstrated that the two main final product of silk proteins the fibroin and sericin though form the main component of silk fibre, yet there are racial difference in the total silk produced. Hence, silkworm breeders mainly concentrating on protein polymorphism in different races so as to provide insight into genetic variability between races to step up further hybridization programme to better the best (Gamo, 1976).

The Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is one of the powerful tools which has helped in understanding allelic variations through separation of protein bands among different genotypes. Hubby and Lewontin (1966) explained the importance of protein polymorphism in *Drosophila melanogaster* in the light of speciation and evolutionary mechanism. Thus, the importance of the study of polymorphic proteins through SDS-PAGE added

a new dimension in plant and animal breeding (Frankel and Brown, 1983).

The protein polymorphism has been observed in silkworm *Bombyx mori* by their differential protein mobility. As a result, Perusal of literature indicated haemolymph protein as one of the most extensively studied banding profiles and with wide overlapping substrate specificities and pattern of inhibition and protein polymorphism occurs in numerous forms expressed by distinct gene loci that generally have a high degree of genetic variability (Takasusuki *et al.*, 2006). The importance of similar study relevant to animal and plant breeding (Frey *et al.*, 1983) and conservation of genetic resource (Zeng *et al.*, 2003), genetic variability in mosquitoes (Pushpalatha and Vijayan, 1999) and in silkworms (Hegde and Krishnamurthy, 1980; Sreerama Reddy and Subramanya, 1982; Somasundaram *et al.*, 2004; Doddaswamy and Subramanya, 2007) are clearly established. However, the present report is aimed to assess the protein polymorphism in three oval and three dumb-bell cocoon producing bivoltines having different genetic background and through statistical analysis dendrogram was formed and the six genotypes of silkworm races are clustered into three groups by applying Unweight Pair Group Method Analysis (UPGMA).

MATERIALS AND METHODS

The six bivoltine races selected in the present experiment are C₁₀₈, Kalimpong-A, NB₄D₂, CSR₂, P₃₁ and NB₁₈ and the characteristic features of the above six races are presented in Table 1. Among them, NB₄D₂, P₃₁ and NB₁₈ are dumb-bell

cocoon producing races, whereas remaining three are oval cocoon producing races. The V instar 2nd day old larvae of the above six bivoltines were chosen from the germplasm bank of the Department of Studies in Sericulture Science, Manasagangotri, University of Mysore, Mysore, which were reared during the favorable rearing period of monsoon season of 2008-09 following rearing method of Krishnaswami *et al.*, (1978) and subsequently, the haemolymph was extracted from larvae of six bivoltines following standard procedure described by Tazima, (1978) as detailed below.

The abdominal legs were punctured and the haemolymph was collected in pre-chilled eppendorf tubes containing 1mM thiourea- crystal to prevent melanization. The samples for the protein assay were prepared by using V instar larvae haemolymph and all the six races were selected for biological assay. Subsequently, the 8.25% of Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method was followed to achieve the high resolution of protein banding profile. The haemolymph sample extracted from silkworm was preserved in 4°C, meanwhile, at the time of sample loading, the sample was dissolved by using sample buffer with 1:10 ratio (*viz.*, 1 μ L was nothing but haemolymph sample and 10 μ L was sample buffer respectively).

The mixed samples in eppendorf tube were kept in a float of water bath at 95°C for 3 minutes. The 8.25% SDS-Polyacrylamide gel was prepared and the 10 μ L samples were loaded into each slot with bromophenol blue as the indicating dye. Electrophoresis was carried out at 20 V for stacking gel and 80 V for separation gel in a buffer solution containing 0.025 M Tris (Running/Tray buffer) until dye front head migrated to within 2 mm end of the gel. The gel, after electrophoresis was processed to localize the proteins by 0.5% coomassie brilliant blue (CBB) R-250 staining (CBB was prepared with Glacial Acetic Acid: Methanol: Distilled water in ratio of 3:22:25 respectively by shaking for two hours) for overnight. Subsequently, the destained gel was scanned and analyzed the number of bands in each particular races and their respective molecular weight and finally photographed in gel documentation unit with Alpha Innotech, USA.

RESULTS AND DISCUSSION

The protein pattern of six bivoltine races after SDS-PAGE is shown in Fig. 1. A close scrutiny of the banding pattern, which is measured with the help of Alpha Innotech (USA) Gel Documentation System along with marker proteins revealed differential expression of proteins among the six bivoltines.

Table 1: Characteristic features of six bivoltine races

Races	Voltinism	Larval pattern	Cocoon colour	Cocoon shape	Origin
C ₁₀₈	Bivoltine	Plain	White	Oval	Chinese
Kalimpong-A	Bivoltine	Plain	White	Oval	Indigenous
NB ₄ D ₂	Bivoltine	Plain	White	Dumb-bell	Indigenous
CSR ₂	Bivoltine	Plain	White	Oval	Indigenous
P ₃₁	Bivoltine	Marked	White	Dumb-bell	European
NB ₁₈	Bivoltine	Plain	White	Dumb-bell	Indigenous

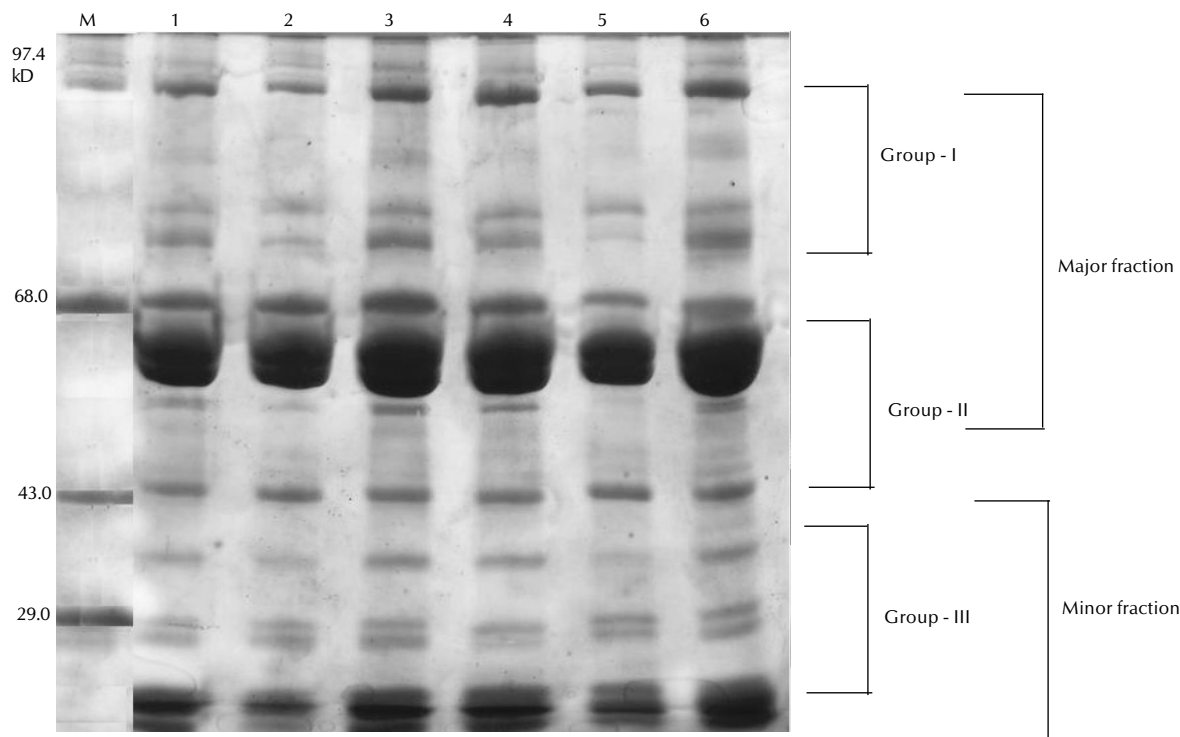
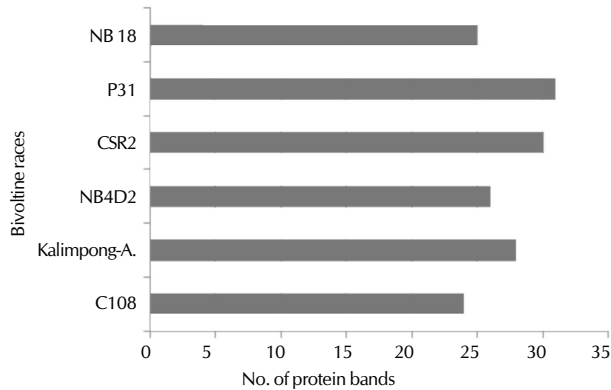


Figure 1: Haemolymph protein profile of V instar second day of six bivoltines

M = Marker protein; 1 = C₁₀₈, 2 = KA, 3 = NB₄D₂; 4 = CSR₂, 5 = P₃₁ and 6 = NB₁₈

Table 2: Haemolymph protein banding pattern (numbers) in six bivoltine races

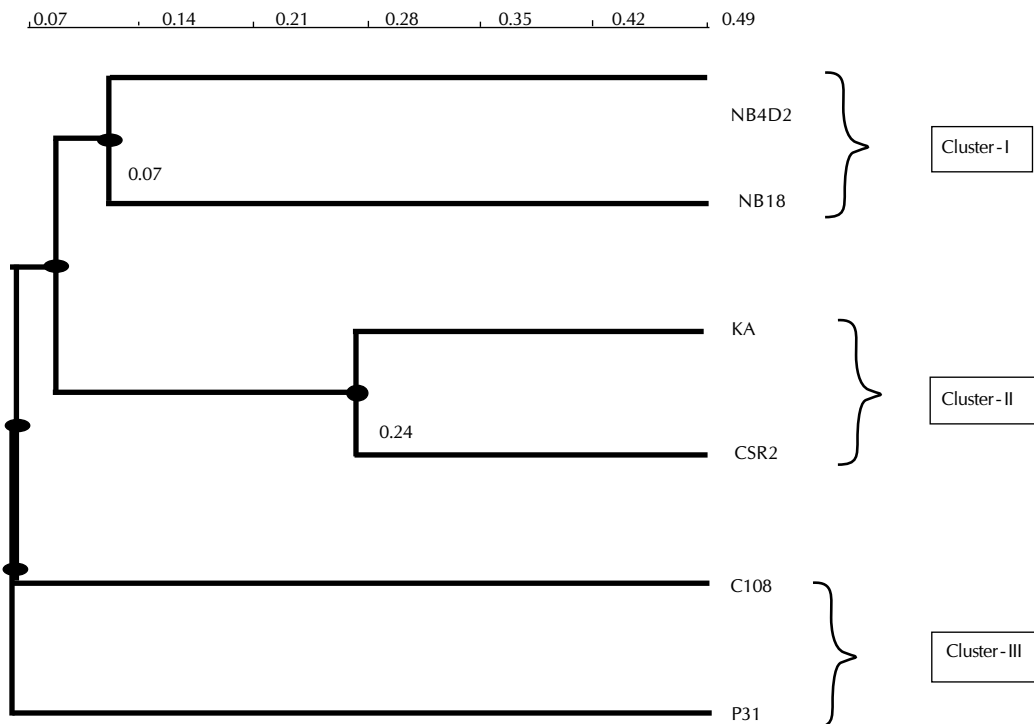
Races	Total no. of bands
C ₁₀₈	24
Kalimpong-A	28
NB ₄ D ₂	26
CSR ₂	30
P ₃₁	31
NB ₁₈	25

**Figure 2: Graphical representation of haemolymph protein bands in the six bivoltine races**

Based on the electrophoretic protein pattern and molecular weight, it is possible to arbitrarily classify the bands into three fractions namely major, intermediary and minor which were denoted as Group-I, II and III respectively. All the three groups were observed in the range of 29 kDa to 97.4 kDa, wherein higher molecular weight bands falls under Group-I and lower molecular weight under Group-III. A maximum of 31

differentially expressed protein bands with their differential intensity was observed in P₃₁ race and a least of 24 bands in C₁₀₈ race. However, in the races of CSR₂, KA, NB₄D₂ and NB₁₈ the protein bands recorded were 30, 28, 26 and 25 respectively (Table 2 and Fig. 2).

Perusal of the literature in regard to the studies on protein polymorphism in plants and animals are well documented (Pasteur and Kastiris, 1971). Protein variation in different species form the basis to understand the phylogeny and to establish the genetic basis of taxonomic relationships between species and enzyme polymorphism are known to be extremely common and easy to find in most species of *Drosophila* (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966 and Ayala *et al.*, 1974). In lepidopteran silkworms, the polymorphic esterases groups are reported by Hegde and Krishnamurthy, (1980); Sreerama Reddy and Subramanya, (1982); Somasundaram *et al.*, (2004) and in mosquitoes by Pushpalatha and Vijayan, (1999). Further, the importance of polymorphic proteins in silkworm is also well demonstrated (Gamo, 1977; Kumar and Dandin, 2003). In addition Somasundaram *et al.*, (2005) and Vasudha *et al.*, (2006) demonstrated heat shock protein expression in the haemolymph of mulberry silkworms. Thus, the present studies supports the findings of the above authors in various animals indicating genetic diversity. Three group of clusters obtained from the dendrogram representation of the six bivoltine races has clearly established their genetic identity (Fig. 3). The NB₄D₂ and NB₁₈ races were genetically close relatedness fall in one group, whereas Kalimpong-A and CSR₂ races fall under another cluster followed by C₁₀₈ and P₃₁ races which were fall in other cluster interlinked to each other respectively. The branches in the UPGMA dendrogram reflect the genetic distance differentiation among populations of different races.

**Figure 3: Dendrogram showing cluster relationship of six bivoltine races**

The populations of above pure races covered a broad geographical range and thus protein polymorphism is expected to exist among them. An understanding of the genetic diversity and population genetic structure is not only important for the conservation of many species of plants and animals but is also essential for maintenance of genetic diversity within the populations (Millar and Westfall, 1992). The reason for the variations in the protein fractions was due to post-translational modifications (Dunbar, 1987) and change in the sequence of single amino acid (Noel *et al.*, 1979 and Seeberg *et al.*, 1984). They have underlined the importance of genetic diversity and protein patterns in different organisms. But in a pilot experiment the role of co-dominant allelic genes in the expression of lipoprotein in silkworms was demonstrated by Gamo, (1977). The reports of the above author in silkworms clearly indicated the existence of protein polymorphism during the larval development of the silkworm, *Bombyx mori*. From the foregoing discussion on the proteins, it is clear that, these studies helps us to understand the nature and number of genes involved even though it is difficult to understand their detection in polygenic systems.

The haemolymph composition of insects reflects the nature and degree of the protein metabolism may be due to synthesis and breakdown of specific proteins which are due to differential racial features. Thus, the understanding of such variations not only helps in the identification of races in the germplasm station but also unveil the loci governing the changed phenotype of the pure races.

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