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# Analysis of variability in Hemolymph protein pattern in four populations of wild and semi-domestic ecoraces of *Antheraea mylitta* Drury (Lepidoptera: Saturniidae)

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## Abstract

*Tropical tasar silkworm Antheraea mylitta Drury is an important wild sericigenous insect species encountered diverse tropical ecological conditions in different eco-pockets and evolved as ecoraces with wide variations in phenotypic and behavioural characters. Four populations from two important ecoraces were analysed for variability in the pupal hemolymph electrophoretic protein pattern. The hemolymph from Daba wild and semi-domestic population as well as wild and reared populations of Laria ecorace was drawn and used for the study. Protein concentration was highly variable and significant (at p<0.05) between the different groups studied. Higher protein concentration was recorded in the hemolymph of Daba (wild) female pupae (235.3 ± 1.72 mg/ml) followed by Daba (Semi-domestic) female hemolymph (225.4 ± 3.04 mg/ml) was recorded. Variations observed in the number of protein bands in SDS-PAGE analysis of male and female pupal hemolymph of different populations. Appearance of 35 kDa Protein band was significant in the female hemolymph samples of all the populations. The protein bands 98 kDa (storage proteins in lepidopterons), 45 kDa, 43 kDa, 20 kDa and 14 kDa were found to be common in all the hemolymph samples. More number of protein bands was observed in the hemolymph samples of Daba between 20 kDa to 98 kDa compared to the samples of Laria populations. The greater variability in number of bands observed between 96 kDa to 99 kDa. This indicates high heterogeneity with respect to the food plant, region and also in sexual dimorphism.*

**Keywords:** *Antheraea mylitta,* SDS-PAGE, ecorace, variability, Daba, Laria.

## Introduction

The wild sericigenous insect species tropical tasar silkworm *Antheraea mylitta* is a polyphagous in nature has rich genetic resources with forty four races acclimatized to diverse ecological zones. Distribution of ecoraces in relation to the forest type indicates that the races are restricted mainly in the tropical moist deciduous forest area. Ecoraces of *A. mylitta* have shown variation for many qualitative and quantitative traits of basic biological and economic interests, such as silk quality, fecundity, disease resistance and tolerance to various environmental entities[1]. Due to its importance, diversity study in this silkworm species has a valuable role in improving commercially important traits through selection of divergent parents. The study of genetic diversity in the silkworm is important for selection of useful parents and specific traits. As such many studies related to genetic diversity in silkworms considered mainly on the protein polymorphism this has given further impetus to provide insight into genetic variability between different races[2]. The electrophoretic variability in the protein pattern of different races has been observed in silkworm *Bombyx mori*[3-5] as well as in *Antheraea mylitta* [6,7]. It was noted that, insect hemolymph protein as one of the most extensively used for electrophoretic analysis of protein banding pattern to represent the protein polymorphism expressed by distinct gene loci that generally have a high degree of genetic variability[8]. A relevant studies also reported on plant and animal breeding[9], genetic resource conservation[10], and many have reported in silkworms[11-14]. Different methods of electrophoretic techniques including paper, starch, agar and polyacrylamide gel have been employed to separate the hemolymph proteins, which are both species and stage specific in insects[15]. Polyacrylamide gel electrophoresis (PAGE) in combination with SDS (Sodium Dodecyl Sulphate) is the widely used method for resolving the protein mixtures and the total proteins of hemolymph were resolved into their components is one of the potent tools which have been used in understanding allelic variations through separation of protein sub units among different genotypes. The importance of protein polymorphism is explained in relation to speciation and evolutionary mechanism in *Drosophila melanogaster*[16].

However, the present study is aimed to analyze the variation in the quantitative and electrophoretic protein pattern and probable

genetic variability in four different populations belonging to two important ecoraces such as Daba and Laria of tropical tasar silkworm *A.mylitta*.

## Material and Methods

Experiments were carried out at Silkworm Breeding and Genetics laboratory of Central Tasar Research and Training Institute, Ranchi. The cocoons of four populations such as semi-domestic Daba and Laria collected from harvested cocoons at CTRandTI field Germplasm and cocoons of wild population *ie.*, Daba and Laria were collected from West Singhbum forest area and Peterbar forest area of Jharkhand respectively, subsequently preserved in the Grainage. Some important characteristic features of Daba and Laria ecoraces of *A.mylitta* is given in the table-1. The hemolymph samples from male and female pupae of different populations were collected separately for further biochemical analysis.

**Preparation of Samples for biochemical studies:** Hemolymph was collected following the method of by cutting the pupa at head region and collected in a pre-cooled micro-centrifuge tube containing 0.025% of phenylthiourea as an anti-melanisation[17]. Further, it was centrifuged at 5,000 rpm for 5 min at 4°C. The supernatant was collected and stored at -20$^{o}$C until further use.

**Protein estimation:** The total protein was estimated in hemolymph of pupa following the method of with Bovine Serum Albumin (BSA) as standard[18].

**SDS - PAGE analysis of total proteins:** Comparative protein polymorphism in the hemolymph of different populations was analyzed using SDS-PAGE with slight modification[19]. The hemolymph protein samples were subjected electrophoresis in 4.5 % stacking gel initially and protein separation was done in 12 % resolving gel. The resolved proteins were visualized by Coomassie Brilliant Blue (CBB-250) and documented.

**Statistical analysis:** The data of quantitative estimation of total proteins in the pupal hemolymph of different populations was analysed. One-way ANOVA was used to test the significance of differences between the mean values of independent observations. Comparisons were performed with *WINSTAT* statistical package.

## Results and Discussion

**Quantitative assay of total proteins:** Protein concentration was recorded highly variable between the different groups studied (table-2). Higher protein concentration was recorded in the hemolymph of Daba (wild) female pupae (235.3 ± 1.72 mg/ml) followed by Daba (Semi-domestic) female hemolymph (225.4 ± 3.04 mg/ml) and the lowest concentration (140.30± 1.22 mg/ml) were recorded in the male Laria (reared). A significant variation in the protein concentration at $p<0.05$ level was recorded. Also significant (at $p<0.05$) variability between the sexes was observed (table-2a).

**Table-1**
**Characteristic features of Daba and Laria ecoraces of A.mylitta**

| Ecorace | Native | Food Plant | Cocoon traits | Voltinism |
|---|---|---|---|---|
| Daba (Semi-domestic) | West Singhbum, Jharkhand Reared in 9 States in India | Terminalia arjuna, T. tomentosa | Large size shell with short peduncle, weighs 10-12g, shell wt.1.8-2.0 g. medium denier (10) filament | Bivoltine trivoltine |
| Laria (wild) | Peterbar forest area, Jharkhand | Shorea robusta | medium size shell, long peduncle and robust cocoons with low denier silk filament | Uni, bi and trivoltine |

**Table-2**
**Comparative quantitative analysis of protein concentration in the pupal hemolymph of different population of *Antheraea mylitta* D (Mean ± S E)**

| | Daba (Semi Domestic) | Daba (Wild) | Laria (Wild) | Laria (Reared) |
|---|---|---|---|---|
| Male | 150.8±2.96 | 180.4± 0.90 | 145.8± 0.55 | 140.3± 1.22 |
| Female | 225.4±3.04 | 235.3± 1.72 | 220.9± 1.69 | 215.5± 1.17 |

**Table-2a**
**Analysis of Variance (*ANOVA*) of pupal hemolymph protein concentration**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 32876.76 | 7 | 4696.68 | 448.6381 | 3.83 | 2.6572 |
| Within Groups | 167.5 | 16 | 10.46875 | | | |
| Total | 33044.26 | 23 | | | | |

**Qualitative profile of total proteins by SDS-PAGE:** The SDS-PAGE studies for the qualitative analysis of hemolymph total proteins in the pupa of different populations of tasar silkworm was recorded and presented in the figure-1.

Polymorphic variations were noticed with regard to the number of protein bands in the hemolymph samples of male and female pupae of different batches. Appearance of 35 kDa Protein band was significant in the female hemolymph samples of all the populations; this might be the sex specific marker. The protein bands 98 kDa (storage proteins in lepidopterons), 45 kDa, 43 kDa, 20 kDa and 14 kDa are found common in all samples. More protein bands were observed in the hemolymph samples of Daba between 20 kDa to 98 kDa compared to the samples of Laria populations. The greater variability in number of bands observed between 96 kDa to 99 kDa. Over all, an apparent polymorphic electrophoretic protein bands were observed among the hemolymph samples of different populations.

**Discussion:** In insects, proteins have been widely used as biochemical tool by many insect biochemists because of their pertinent role in the development, morphogenesis and almost all intermediary metabolic pathways. Proteins act as an indicator of an expression of the gene because all gene expressions resulted with synthesis of one or more proteins. Apart from this the variation in protein in different genotypes forms the basis to understand the genetic distance, phylogeny and establish taxonomic relationship between species[20,16,21].

The different races of silkworm of Indian origin, exotic, multivoltine, bivoltine and their hybrids have been tested for protein polymorphism and few enzyme systems which bring about certain groups of chemical reactions among silkworm races, in which amylases, proteases, esterases and others have been detected by a technique specially developed for the purpose [22].

The synthesis and utilization of hemolymph proteins are under genetic and hormonal control. The fat body is the main source of hemolymph proteins and others may come from hemocytes. The high protein concentration is an indication of a greater metabolic activity of the tissue[23]. The hemolymph acts as the carrier of all nutrient substances transports to each and every part of the body for cellular metabolism, wherein the micro molecules get converted into complex macromolecules like proteins and carbohydrates.

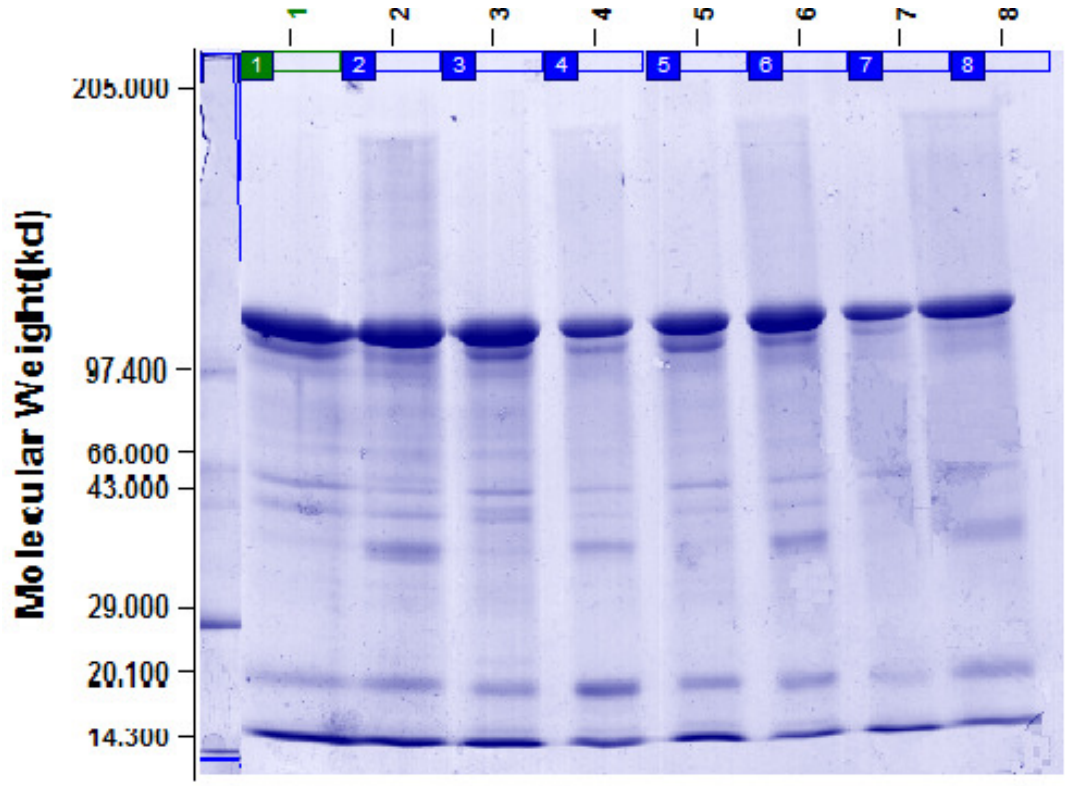


**Figure-1**
**SDS-PAGE hemolymph total protein profile of diapause pupae of different population of Tropical Tasar silk worm *Antheraea mylitta* D.**
**Lane: 1**- Daba Male, **2**- Daba Female, **3**- Daba wild Male, **4**- Daba Wild Female, **5**- Laria Wild Male, **6**- Laria Wild Female, **7**- Laria Reared Male and **8**- Laria Reared Female

Higher concentration of the protein was recorded in the hemolymph of Daba (wild) female, is attributed to the fact that these populations were developed in its natural habitat and thus acquired robustness and fed higher quantity and quality leaves during its feeding stage and subsequently it is converted as storage protein. Also many authors have earlier recorded similar observations in the hemolymph of female silkworm[24-27]. The differential concentration of proteins in different groups depicts the differential feeding rate and physiological activity among different ecoraces and adaptability for the geographical region and food plant during feeding stage[28]. Laria, being the wild ecorace of tropical tasar silkworm *A. mylitta,* mostly adapted to Sal food plant. Lower concentration of hemolymph protein in the Laria over Daba, shows the differential genetic capacity of different ecoraces of *A. mylitta*. It was reported that, the rearing performance, and expression of phenotypic characters of tasar silkworm largely depends on type of the food plant, geographical attribute such as altitude, latitude and other environmental factors[29,30].

The SDS-PAGE protein profile in the hemolymph of pupae of different groups observed that the variation in protein bands and specificity of bands between the groups and also between male and female. The appearance of few protein bands at specific site indicates the synthesis of a particular protein to meet the physiological need.

The protein bands 98Kda (storage proteins in lepidopterons), 45 Kda, 43 Kda, 20Kda and 14 kda are found common in all the hemolymph samples of all the groups. This could be species specific proteins, presumably found in all the ecoraces of the *A.mylitta*. The protein bands of 36 and 64 Kda are constitutive in nature and appeared in all the tissues of *A. mylitta*[7]. Significant appearance of 35 Kda Protein bands in the female hemolymph samples of all the population could be the sex specific protein of W- chromosomes appears in female silkworms. In most of the insect species, females are heterogamatic in nature (ZW). *Bombyx mori* serum protein (BmLSP), assumed to be located at 29kDa region. It was demonstrated that BmLSP occurs as a major protein during larval stage of *B. mori* in the early instars until the initial day of last instar and gets decreased towards larval maturity[31]. Also, reported that BmLSP was identified as a protein with a molecular weight 30,000[32]. On the contrary, in the present study, very sparsely stained band was appeared near the 29Kda region in the hemolymph samples of male and female presumed that the expression of the serum protein during pupal stage of *A. mylitta* may be low.

From the present observations it is indicated that there is an apparent variability in different populations of *Antheraea mylitta*, expressing high heterogeneity with respect to the food plant, region and also between the sex. Biochemical profiling such as proteins and enzymes can be used as a tool for the characterization of different populations of *Antheraea mylitta.* Besides, proteins found to be a suitable marker to investigate population diversity, inter-ecoracial polymorphism and also to explore different physio-genetic properties linked to quantitative traits in silkworms.

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