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Study of Phenotypic Variability in Silk Gland Characters in Three Ecoraces of Tropical Tasar Silkworm *Antheraea mylitta* Drury

G. Lokesh, Putkho Paul Pao, K.N. Madhusudhan, P.K. Kar, A.K. Srivastava, M.K. Sinha, R. Manohar Reddy, P.M. Muniswamy Reddy and B.C. Prasad
Silkworm Breeding and Genetics, Central Tasar Research and Training Institute, Central Silk Board, Piska Nagari, Ranchi-835303, India

Corresponding Author: G. Lokesh, Silkworm Breeding and Genetics, Central Tasar Research and Training Institute, Central Silk Board, Piska Nagari, Ranchi-835303, India

ABSTRACT

The wild silkworm, tropical tasar *Antheraea mylitta* Drury being mainly culturing in central and eastern parts of India and produces famous *tasar* silk. This insect species expresses divergent phenotypic characters in response to varying ecological and climatic conditions thus exists as ecoraces. Hence, in the present study, three ecoraces viz., Daba, Sukinda (Semi-domesticated) and Sarihan (wild) have been selected for the assessment of the variability in silk gland related traits. The parameters of the silk glands studied like comparative length, weight and silk gland to larval body mass index. Besides, some biochemical contents of the silk gland tissue were also studied in three ecoraces. The results showed significant variations at p<5% in all the parameters among the three ecoraces studied, also showed the sexual dimorphism in expression of silk gland features and economic traits. The semi-domesticated Daba ecorace showed higher performance in most of the parameters with comparatively greater in silk gland weight (22%) and in body mass index (>16%). Apart from this, wild ecorace Sarihan cocoons showed higher shell ratio (>10% over the value of other ecoraces). The study infers that, there is wide variability in the expression of phenotype with respect to silk gland related traits among the different ecoraces of tropical tasar silkworm *A. mylitta*. Since the silk glands are site of biosynthesis of silk proteins, these may be considered for the assessment of diversity or characterization of different ecoraces of tropical tasar silkworm.

Key words: Daba, fibroin, Sarihan, Sukinda, silk gland protein

INTRODUCTION

The tropical tasar silkworm, *Antheraea mylitta* D. has encountered diverse ecological conditions in diverse ecopockets and formed into ecoraces with wide variations in phenotypic, physiological and behavioural characters (Suryanarayana and Srivastava, 2005). Presently 44 ecoraces have been identified in tropical India with significantly divergent in phenotypes, breeding potential and silk production. The variability in the different ecoraces of *A. mylitta* have been studied for the selection of better parent in breeding with respect to its behaviour, productivity and other morphological, physiological and biochemical traits (Suryanarayana and Srivastava, 2005; Srivastava *et al*., 2001; Reddy, 2010).

The natural silk synthesized by the silkworm and spun in the form of a silk cocoon is originally synthesized in the silk gland. Silk gland of *A. mylitta* is a typical exocrine gland secreting large

amount of silk proteins. The silk glands of *A. mylitta* are classified as Z-type which is characterized by its overall shape and tracheal distribution (Mondal *et al.*, 2007). According to its morphology and function, the silk gland can be divided into three regions namely Anterior Silk Gland (ASG), Middle Silk Gland (MSG) and Posterior Silk Gland (PSG). MSG and PSG are more important since these glands secretes actual silk proteins i.e., sericin and fibroin, both these proteins mix in the ASG while silkworms release silk proteins during the construction of cocoon (Mondal *et al.*, 2007). The secretion of the silk proteins in the silk gland is largely depended on the genetic basis of the silkworm and the quality and quantity of food intake . The concentration and secretion of fibroin in the silk gland is prominent to understand the total silk output of silkworms (Miao and Nair, 2003; Maity *et al.*, 2010; Collin *et al.*, 2010). In the present work, the expression of different silk gland related traits in three important ecoraces of *Antheraea mylitta* was analyzed.

MATERIALS AND METHODS

The present study was undertaken at Central Tasar Research and Training Institute, Ranchi, Jharkhand State, India, during August-December 2010. Three different ecoraces of tasar silkworm *A. mylitta* viz. Daba, Sarihan and Sukinda were selected for the study. The ecoraces reared separately in Arjun (*Terminalia arjuna*) plants of identical age and similar inputs. The silkworm larvae of above said three ecoraces were collected on the day before spinning, separated the larvae based on the sex, recorded the body mass of the larvae and used for silk gland related studies. Remaining larvae were allowed to spin cocoons. Subsequently, the cocoon characters of different ecoraces were recorded by random selection of cocoons of both the sexes separately.

The individual larvae were used for dissection of silk glands in a dissection tray containing ice cold Bodenstein's Ringer solution prepared with Tris buffer, pH 7. The dissected silk glands were allowed for 5-7 min in the buffer. Later transferred to thin and neat blotting paper to dry excess moisture on the surface of gland and immediately morphological features like length, colour and weight of the silk glands were recorded. Silk glands of both the sexes were transferred to a sterilized glass homogenizer. The tissue was homogenized with 20% (w/v) 50 mM Tris-HCl buffer (pH 7). The homogenate was transferred to a clean centrifuge tube and centrifuged at 10000 rpm for 30 min in cooling condition. The supernatant was collected in a clean glass test tube and used for protein assay by method of Lowry *et al.* (1951).

The fibroin percentage from the silk glands was assessed following the method of Tashiro *et al.* (1968).

Statistical analysis: Data were analyzed using one-way ANOVA of SPSS 10, when significant differences were found, means were separated. Comparisons of the treatment means were performed with Duncan's Multiple range test (DMRT, $p = 0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

The study of silk glands related traits and biochemical constituents of different ecoraces were recorded as follows.

Morphological studies of silk glands: The measurement in the length and weight of the silk glands showed a variation among the ecoraces studied and also between the sexes. Daba silkworms showed significantly higher larval weight (male 33.92 g, female 36.18 g) compared to Sukinda (male 30.12 g, female 32.18 g) and Sarihan (male 24.89 g, female 26.68 g). This trend is also

Table 1: Morphological parameters of silk glands of three ecoraces

	Daba		Sukinda		Sarihan	
Parameter	Male	Female	Male	Female	Male	Female
Larval weight (g)	33.92±0.32^{b}	36.18±0.60^{a}	30.12±0.65^{d}	32.18±0.19^{c}	24.89±0.43^{f}	26.68±0.30^{e}
Silk gland weight (g)	8.14±0.10^{b}	8.32±0.19^{a}	6.32±0.10^{d}	6.43±0.12^{c}	4.97±0.23f	5.00±0.20^{e}
Silk gland/ Body mass index (%)	24.00±0.24^{a}	23.00±0.12^{b}	21.00±0.20^{c}	20.00±0.34^{d}	20.00±0.39^{d}	18.75±0.36^{e}
Length of silk gland (cm)	76.67±0.28^{b}	78.33±0.35^{a}	70.8±0.41^{d}	71.9±0.32^{c}	67.5±0.27^{f}	68.3±0.31^{e}

The values represent the mean of three replications with standard error. Values with different small alphabets are significant among values mentioned in the columns, according to Duncan's multiple range test ($p<0.05$)

Table 2: Total proteins and fibroin concentration in silk glands of different ecoraces

	Daba		Sukinda		Sarihan	
Parameter	Male	Female	Male	Female	Male	Female
Proteins (mg g^{-1})	412±8.18^{b}	512±5.85^{a}	280±4.72^{f}	315±5.56^{e}	360±7.37^{d}	395±9.45^{c}
Fibroin (%)	62±4.72^{c}	65± 4.35^{b}	58± 4.04^{e}	60±4.04^{d}	65±3.60^{b}	68±2.51^{a}

The values represent the mean of three replications with standard error. Values with different small alphabets are significant among values mentioned in the columns, according to Duncan's multiple range test ($p<0.05$)

repeated even in silk gland weight and length of the silk gland. All these characters are reflected in the sexual dimorphism where higher weight of the larva and silk gland showed in female than in males. But this was appeared reverse in case of percentage of silk gland weight over larval weight (silk gland-larval body mass index) in all the ecoraces (Table 1).

Daba is a semi-domesticated ecorace and acclimatized to West Singhbhum district of Jharkhand, grows richly on Arjun and Asan (*Terminalia tomentosa*) leaves thus this ecorace has showed higher performance over other ecoraces in the parameters studied. This result corroborates with earlier reports (Suryanarayana and Srivastava, 2005). Though, there is higher silk gland weight in females corresponding to larval body weight, the silk gland ratio to the larval body weight recorded higher in case of males. This is obvious that the budgeting of energy and the protein allocation towards the development of its reproductive structures is more in case of female silkworms during final larval stage. Thus, the silk gland ratio in the female larvae is lower, this is clearly pronounced in the shell ratio of the cocoons (Lokesh *et al.*, 2006; Tazima, 1978; Mondal *et al.*, 2007). The length of the silk glands is also in correspondence to the weight of the larvae and silk gland weight. The average length of the silk glands are recorded in the order as Daba> Sukinda> Sarihan (Table 1).

Proteins in silk glands: The analysis of protein concentration in the silk glands of three different ecoraces carried out in the present study resulted with significant variations. Higher protein concentration was recorded with silk glands of Daba ecorace (male-412 mg g^{-1}, female- 512 mg g^{-1}) and lower concentration recorded with Sukinda (male-280 mg/g, female-315 mg/g) ecorace. Also sexual dimorphism in protein concentration was well pronounced with higher in females compared to males in all the ecoraces (Table 2).

Daba ecoraces recorded highest concentration of silk protein in its silk glands compared to other ecoraces studied. The higher consumption of dietary food and corresponding higher metabolic activities leads to better conversion of leaf proteins in to silk proteins. As mentioned earlier this

Table 3: Cocoon characters of different ecoraces of tasar silkworm

	Daba		Sukinda		Sarihan	
Parameter	Male	Female	Male	Female	Male	Female
Cocoon weight (g)	11.58 ±0.27^{b}	12.79± 0.37^{a}	10.88±0.62^{e}	11.59±0.17^{b}	9.29±0.62^{e}	9.92±0.43^{d}
Shell ratio (%)	16.23±0.37^{c}	15.21±0.57^{d}	16.00±0.35^{c}	15.08±0.29^{d}	17.52±0.32^{a}	16.88±0.72^{b}

The values represent the mean of three replications with standard error, values with different small alphabets are significant among values mentioned in the columns, according to Duncan's multiple range test ($p<0.05$)

ecorace have been well-adapted to the present experimental ecological region. Similarly the concentration of the protein was recorded higher in case of females compared to males in all the ecoraces (Srivastava *et al*., 2001; Kumar *et al*., 2011).

Fibroin percentage from the silk glands: Fibroin percentage showed a contrasting trend to total protein concentration, where higher fibroin concentration was recorded with Sarihan (Male-65%, female-68%) compared to Daba (Male-62%, female-65%) and Sukinda (Male-58%, female-60%) (Table 2). It is possible that both polygenic and environmental consideration influence normal protein synthesis that bring about the population variability in a species.

Cocoon characters: The cocoon characters showed significant variations among the ecoraces. Daba cocoons weigh higher and Sarihan cocoons showed higher shell ratio, may be because of compactness of these cocoons compared to other ecoraces (Table 3). But the shell ratio was recorded higher in case of males compared to females. The quantitative characters like cocoon weight in most of the ecoraces is highly variable from race to race. The Sal based ecoraces have shown higher shell weight and compactness as compared to *Terminalia* based populations (Sinha and Sinha, 1994; Sinha *et al*., 1995).

It is also accepted that a widely distributed species occupy quite heterogeneous environment and are exposed to disruptive selection. This type of selection can bring about an increase in genetic variability and genetic adaptability also establishes polymorphism (Sinha *et al*., 1995; Reddy *et al*., 2010). The variability in these populations reflects their genetic flexibility, high recombination indices. The variability among the ecoraces can be considered as adaptive divergence while similarities are adaptive convergence at phenotypic level. The possible genetic variations need to be introspected with modern biological tools. Moreover, the silk conversion, secretion of silk proteins in the silk glands can be taken as an index during characterization.

CONCLUSION

Silk glands are site of biosynthesis of silk proteins, the amount of secretion of silk is based on the genetic background of the individual insect. Also this is corresponding to the size of the silk glands as perceived from the present study. Thus, silk gland related traits could be used as one of the parameters to study the phenotypic diversity and characterization of different ecoraces. Also this can be useful information for selection of breeding components for developing high silk productive breeds of tropical tasar silkworm *A. mylitta*. This would also provide validation for present knowledge of variability in these populations.

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