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Intra-Populational Variation Studies in Laria - Wild Ecorace of Tropical Tasar Silkworm Antheraea Mylitta Drury

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Abstract- Laria is a wild ecorace of tropical tasar silkworm Antheraea mylitta Drury and the adaptability of this ecorace mainly on Shorea robusta (Sal). It has high economic importance is noteworthy for its small size, robust with variable cocoon colour and has low filament denier. In nature, the voltinism of this ecorace is uni-, bi-, and trivoltine. In the present study, variability in the cocoon characters and biochemical parameters was assessed to analyze the degree of heterogeneity with in the natural population of Laria in relation to the cocoon colour. Differential amount in the cocoon traits, such as cocoon weight and shell ratio were recoded with significant variability at $p<0.05$ level. Protein and reducing sugar concentrations were recorded higher in whitish grey cocoons compared to the yellow colored cocoons. This would be highly warranted to study intra-population variability for the understanding of wild tasar silkworm ecoraces like Laria. The study also facilitates to harness the potentialities of Laria ecoraces to apply in breeding prospects.

Index Terms- Laria, cocoon color, protein, reducing sugar, cocoon trait

I. Introduction

Tropical tasar silkworm *Antheraea mylitta* Drury is an ecological insect species producing the world famous tasar silk. The tropical tasar silkworm populations occupying different ecological and geographical regions show certain degree of phenotypic variability for which they are known as 'eco-races' (Srivastava *et al.,* 2003). About 44 ecoraces have been recognized out of which, eight ecoraces are being exploited for commercial tasar silk production in India (Suryanarana and Srivastava, 2005). Tasar silkworm *A. mylitta* a polyphagous insect feeds on wide range of host plants. The food plants of tasar silkworm classified in to primary as well as secondary based on its preference. Three important food plants such as *Terminalia arjuna* (Arjun), *Terminalia tomentosa* (Asan) and *Shorea robusta* (Sal) are widely utilizing for the rearing and production of commercial tasar silk. The implication of food plants on growth and development of the silkworms and subsequent productivity in terms of seed (fecundity) and silk recovery are prominent. Also it is apparent that the expression of phenotypic characters is largely depends on interaction with its host plants biochemical profile and the environment.

Laria is a *Shorea robusta* (Sal) based ecorace of economic importance distributed in various parts of Jharkhand and adjoining regions of Chhattisgarh. The ecorace is notable for spinning small size with long peduncle, varied color (Whitish grey-grey, dark & light yellow) cocoons, and robust with low filament denier. In nature the voltinism of this ecorace is not fully understood but presumed to exist as uni-, bi- and trivoltine (Suryanarana and Srivastava, 2005). In some limited areas farmers use Laria ecorace for 2nd crop (during September-October) that too in small scale. Third crop (November-February) is unstable and there is loss of biomass due to unseasonal moth emergence in unfavorable seasons. Adaptability of this ecorace is primarily on *Shorea robusta* (Sal) and this flora covers 86.9% of the total tasar flora in India as against 13.1% of *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan). The efforts for the utilization of Sal plantation are become thrust and need of the hour to enhance the total tasar silk production in India. Exploitation of Laria ecorace in this direction would pave the way to achieve the prime objectives. Thus, this would be highly warranted to study the intra-population variability for the understanding of wild tasar silkworm ecoraces its potentialities to harness for the higher tasar silk production and also to apply breeding strategies for better perpetuation and conservation.

II. MATERIALS & METHODS

Studies were undertaken at Silkworm Breeding and Genetics laboratories of Central Tasar Research & Training Institute, Ranchi, during October-2011 to February 2012. The cocoons of wild ecorace Laria collected from forest area of Peterbar, Jharkhand. The good cocoons were sorted based on the sex and the colour (whitish-grey and yellow). About 50 male and 50 female cocoons each of whitish-grey and yellow were used for the cocoon trait assessment and for the biochemical analysis.

a. *Assessment of Cocoon Characters*:

The important commercial cocoon characters such as individual cocoon weight and shell weight (for calculation of the shell ratio) were analyzed by random selection of 50 yellow colored cocoons and 50 whitish grey cocoons from the natural population of Laria which was collected from forest area of Peterbar, Jharkhand.

Preparation of Samples for biochemical studies:

Hemolymph of both whitish grey and yellow of both the sexes and male gonad were collected separately for the biochemical studies.

i. Hemolymph was collected by cutting the anterior part of the pupa and pro leg of silkworm larvae in a pre-cooled micro-centrifuge tube containing a pinch of phenylthiourea as an anticoagulant and centrifuged at 5,000 rpm for 5 min at 4°C. The supernatant collected and stored at -20°C until further use.

ii. Testes was collected in the male. Dissection was carried out in ice cold phosphate buffer. Homogenization of testis was done in a homogenizer using phosphate buffer pH 7. The homogenate was transferred to a clean centrifuge tube and centrifuged at 5000rpm for 5 min in cooling condition. The supernatant was collected in a clean test tube and were stored at -20°C until further use.

The total protein concentration and reducing sugars were estimated in both hemolymph and testes (Lowry *et al*., 1951; Sinha *et al*., 1998).

Statistical Analysis

One-way analysis of variance was used to test the significance of differences between the mean values of independent observations of proteins and reducing sugars in the haemolymph and testes of silkworm pupae. Comparisons were performed with Duncan's Multiple Range Test (DMRT) to find significance differences at $p < 0.05$ (Duncan, 1955).

III. RESULTS AND DISCUSSIONS

a. Cocoon Assessment

The study of commercially important cocoon traits such as cocoon weight and shell ratio showed variability among white and yellow color cocoons. Higher cocoon wt. and shell % was observed in the whitish-grey cocoons compared to yellow cocoons (Table. 1). Whitish grey is considered as predominant cocoon color spun by many ecoraces of the tasar silkworm and has commercially good cocoon qualities compared to other types of cocoon (Suryanarayana and Srivastava, 2005). With regard to quantitative characters, the cocoon and shell weight is variable from race to race. The *Shorea* based ecoraces shown higher shell weight and compactness compared to *Terminalia* based populations (Srivastava *et al* 2001).

b. *Biochemical analysis*:

The studies on the variability in the biochemical parameters of whitish-grey and yellow cocoons with in the natural population of Laria are recorded as follows;

i. Protein concentration:

A significant variation in the Protein concentration at $p<0.05$ was observed in whitish-grey and yellow cocoons. Higher concentration of proteins (mean± SD: 142 ± 5.00) observed in the hemolymph of female whitish-grey cocoons compared to other samples studied followed by male gonads (140 ±5.29), hemolymph of yellow female (132 ±5.00) and also significant difference in the protein concentration was observed between the different tissues studied (Table. 2).

The proteins play an important role in the haemolymph of insects not only in specific transport functions, but also in their enzyme action. Hurliman and Chen (1974) & Chen (1966) asserted that the synthesis and utilization of haemolymph proteins are conditioned by genetic and hormonal control. There is a general agreement that the fat body is a main source of haemolymph proteins and others may come from haemocytes. In silkworms the haemolymph protein fluctuates during its developmental stages and concentration was found to increase seven fold during last instar of larval life. The high protein concentration is an indication of a greater metabolic activity of the tissue. The haemolymph, the carrier of all nutrient substances distributes to each and every part of the body for cellular metabolism, wherein the micromolecules get converted into complex macromolecules like proteins and carbohydrates.

Higher concentration of the protein in the whitish-grey cocoons (pupal hemolymph) is attributed to the fact that these larvae have fed quality leaves and efficiently converted the dietary food during its feeding stage the corresponding level of proteins were maintained even in the non-feeding pupal stage (Babu *et al*. 2009). Also many authors have similarly recorded the higher proteins in the hemolymph of female cocoons earlier (Kumar *et al*., 1998; Lokesh *et al*., 2006; Lokesh *et al*., 2006; Kumar *et al*., 2011; Srivastava *et al.,* 2001). This is also corresponds with the cocoon weight recorded in the present study. The differential concentration of proteins in whitish-grey and yellow cocoons depicts the heterogeneity (Venugopala Pillai *et al*., 1987) within the population of *A. mylitta* and better quantitative characters of the whitish-grey cocoons over the yellow cocoons (Srivastava *et al*., 2002; Srivastava *et al*., 2004).

ii. Reducing sugars:

The reducing sugars recorded significantly variable amounts in the tissues studied and between the yellow and whitish-grey cocoons. Higher reducing sugars were recorded with hemolymph of female white cocoons (32 ± 1.53) compared to yellow cocoons and the lowest was recorded in the hemolymph of Male yellow cocoon (28± 1.53) represented in the Table. 2.

Carbohydrates are essential components for the energy demand during non feeding stage. Trehaloses, glucose, fructose and mannitol, these compounds are highly essential during different developmental stages of an organism (Forcella *et al.*, 2007). Silkworms utilize carbohydrate in the production of some new proteins/ biomolecules to cope with the developmental changes. The concentration of reducing sugars in different tissues is in relation to the energy requirement for the metabolism. Higher amount of reducing sugars in the hemolymph is due the release of cellular carbohydrate in to the circulatory medium this subsequently channelize to different tissues/ organs of the body (Lakshmikumari *et al*,. 1997; Sarangi, 1985). Differential carbohydrate concentration between male and female also among different tissues in the present study is in correspondence with earlier works (Sinha *et al*, 1998; Srivastava *et al*, 2001; Srivastava *et al*, 2003; Kar *et al*., 1994).

IV. CONCLUSION

From the present study, variability in the cocoon characters and biochemical parameters is apparent that, there is high degree of heterogeneity within the natural population of Laria. Differential amount in the cocoon traits, protein and sugar concentration in whitish-grey and yellow cocoons can be considered as an indicator. Since, white cocoons recorded quantitatively better this could be preferentially consider in the selection for better quantitative traits in conventional breeding.

Table 1: Study of variability in Cocoon characters in natural Laria population (Mean±SE)

	Cocoon Weight (g)	Shell Weight (g)	Shell ratio (%)
Whitish grey cocoon	11.137±0.476	1.914±0.040	17.26± 0.050
Yellow cocoon	10.241±0.702	1.668±0.078	16.373±0.114

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2287.227	5	457.445	368.642	.050
Within Groups	67.008	54	1.241		
Total	2354.236	59			

Table 2 : Biochemical variations in the white and yellow cocoon (Pupa) of Natural Laria Population (Mean±SE)

	White cocoon (Pupa)		Yellow cocoon (Pupa)	
	Proteins (mg/ml)	**Reducing sugars** (mg/ml)	**Proteins** (mg/ml)	**Reducing sugars** (mg/ml)
Male hemolymph	130±2.51	32 ±0.88	121±3.60	28 ±0.88
Female hemolymph	142±2.88	39 ±1.52	132±2.88	33±2.08
Male gonad	140 ±3.05	34 ±1.00	128 ±3.60	30 ±1.52

		Sum of Squares	df	Mean Square	F
Protein (Whitish-grey)	Between Groups	248.000	2	124.000	5.167
	Within Groups	144.000	6	24.000	
	Total	392.000	8		
Protein (Yellow)	Between Groups	186.000	2	93.000	2.709
	Within Groups	206.000	6	34.333	
	Total	392.000	8		
Reducing Sugar (Whitish-grey)	Between Groups	84.222	2	42.111	10.243
	Within Groups	24.667	6	4.111	

	Total	108.889	8		
Reducing Sugar (Yellow)	Between Groups	42.889	2	21.444	2.881
	Within Groups	44.667	6	7.444	
	Total	87.556	8		

REFERENCES

[1] Babu, K.R., S. Ramakrishna, Y.H.K. Reddy, G. Lakshmi, N. V. Naidu, S.S. Basha and M. Bhaskar, (2009). Metabolic alterations and molecular mechanism in silkworm larvae during viral infection: A review. Afr. J. Biotechnol., 8: 899-907.

[2] Chen, P. S. (1966). Amino acid and protein metabolism in insect development. In : "Advances in insect physiology". (Ed. by Beament J W L, Treherne, J. E. and Wigglesworth,

[3] Duncan, D. B. (1955). Multiple range and multiple F- tests. Biometrics, 11, 1-42.

[4] Forcella, M., E. Berra, R. Giacchini and P. Parenti, (2007). Antioxidant defenses preserve membrane transport activity in Chironomus ripariuslarvar exposed to anoxia. Archv. Insect Biochem. Physiol., 65: 181-194.

[5] Hurliman, R. F. and Chen, P. S. (1974). Ontogenetische Veranderungen des enzymmusters in der haemolymphe von Phormia rigina. Revue. Swisse. Zool., 81 : 648-654.

[6] Kar, P.K., Srivastava, P.P., Sinha, R.K. and Sinha, B.R.R.P. (1994) Protein concentration in the pupal haemolymph of different races and F1's of top crosses of *Antheraea mylitta* D. *Indian J. Seric*. 33 ; 114-175.

[7] Kumar, N.K., S.M. Ismail and A. Dutta, (1998). Differential uptake of storage protein by the fat body of Rice moth, *Corcyra cephalonica*during the larval pupal development. Entomon, 23: 83-90.

[8] Lakshmikumari, Ananthanarayana, S.R. and B., Jayaprakash (1997). Effect of radiation on the activity of digestive enzymes in the silkworm, *Bombyx mori* L., Sericologia, 37(2) : 221-228.

[9] Lokesh, G., M. Narayanaswamy and S.R. Ananthanarayana (2006). The Effect of Chemical Mutagen on Hemolymph Proteins of silkworm, Bombyx mori L (Lepidoptera : Bombycidae) in F1 Stage. J. Appl. Sci. Environ. Mgt. 10(3); 21-25.

[10] Lokesh. G., 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 (2012). Study of Phenotypic Variability in Silk Gland Characters in Three Ecoraces of Tropical Silkworm *Antheraea mylitta* Drury. *Asian Journal of Animal and Veterinary Advances*. 7(1); 80-84.

[11] Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem*., 193: 267-275.

[12] Sarangi, S. K. (1985). Studies on the silk gland of *Bombyx mori* : A comparative analysis during fifth instar development. *Ind. Acad. Sci*. **94** : 413-419.

[13] Sinha, U.S.P., A.K. Sinha, B.N. Brahmachari and S.S. Sinha, (1998). Seasonal variations in the carbohydrate content during different larval stages of Tasar silkworm, Antheraea mylitta Drury. Sericologia, 38: 385-388.

[14] Srivastava A. K., A.K.Sinha and B.R.R.P. Sinha (2003), Descriptor of tropical tasar silkworm, *Antheraea mylitta* Drury.(Lepidoptera Saturniidae), CTR&TI, Ranchi:1-14.

[15] Srivastava A. K., Sinha A.K. and Sinha B.R.R.P. (2002). Present status of tropical tasar silkworm germplasm management. Proceedings of workshop on Germplasm Management and Utilization, CSGRC, Hosur, 116-122 pp.

[16] Srivastava, A. K., A.H. Naqvi, A.K. Sinha, S.R. Vishwakarma and G.C. Roy, (2004). Genotype and environment interaction in *Antheraea mylitta* Drury and its implications. *Perspectives in Cytology and Genetics*, 11:219-224.

[17] Srivastava, A. K., P.K. Kar, A.H. Naqvi, A.K. Sinha, B.M.K. Singh, B.R.R.P. Sinha and K. Thangavelu (2001) Biochemical variation in ecoraces of *Antheraea mylitta* : A review, *Perspectives in Cytology & Genetics*, 10: 359-346

[18] Suryanarayana, N. And A.K. Srivastava, (2005). Monograph on Tropical Tasar Silkworm. Central Tasar Research and Training Institute, CSB, India.

[19] Venugopal Pillai, S., Krishnaswami, S. and Kashivishwanathan, K. (1987). Growth studies in silkworm, *Bombyx mori.* L. under tropical conditions. II. Influence of agronomical methods of mulberry on growth, cocoon crop and fecundity of silkworm. *Ind. J. Seric*. 26(1) : 38-45.

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