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RESEARCH ARTICLE

A study on morphological variations in different population of Antheraea assamensis

Tarali Kalita and Karabi Dutta

Department of Zoology, Gauhati University Jalukbari -781014, Assam, India

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Abstract

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*Corresponding Author

Corresponding manor

Tarali Kalita

Assam and North East India hold an unique position in the global sericulture map for its monopoly in muga culture. The muga silk is the 2^{nd} most expensive silk in the world which is produced by the insect *Antheraea* assamensis of order Lepidoptera and family Saturniidae. At the present day world, phenotypic variation have been used extensively to describe the population structure in many species and is considered that it plays an important role in conservation strategies of many threatened organisms. In the present study, survey, collection and morphological characterization showed that there exist great variation in color, size and weight of various stages of life cycle of *A. assamensis* which indicates a higher genetic diversity among the population. Such studies may help to select a beneficial trait for future breeding program and to conduct *ex-situ* conservation program of the collected population in a conductive climatic condition to prevent the loss of crop failure due to adverse climatic condition.

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INTRODUCTION

Antheraea assamensis. Helfer, is endemic to Brahmaputra Valley of Assam and few places of North Eastern region of India only. It may be due to the food plant availability and requisite climatic condition of the insect (Ahmed *et al.*, 2011). Muga silkworm is polyphagous in nature and the primary food plants are *Persea bambycina* (King ex Hook. f.) Kost (Som) and *Listea monopetala* (Roxb.) Pers (Soalu) of the family Lauraceae. It is semi-domesticated as its larval stage is reared in outdoor condition. The wild counterpart of this insect is also available and found in the dense forest of Assam, Arunachal Pradesh, Meghalaya and Nagaland. The wild stocks of this insect have two generations in a year–autumn and spring while the semi domestic stocks have 5-6 generations in a year. For semi domestic stock in accordance with Assamese calendar the crops are named as follows–*Chatua* (February-March), *Jethua* (May-June), *Aherua* (June-July), *Bhodia* (August-September), *Kotia* (October-November) and *Jarua* (December-January).

It is well documented that adverse climatic condition, nutrional stress, presence of chemicals and many other factors that cause stress during development can lead to an increase in the presence of morphological asymmetries that result high intra-specific variation (Benitez, 2013). Therefore, it is obvious that when environmental condition changes, organisms have to adapt to new conditions (Clarke, 1993). Quantitative variations of morphological traits have been used extensively to describe the population structure in many organisms (Clabaut *et al.*, 2007) and is of great importance for improving the conservation strategies for imperiled species.

Studies on morphology of muga silkworm varieties was initiated by Sengupta *et al.*, (1975). Then Chowdhuri (1981) and Hampson (1982) had given some description for different stages of life cycle of *A. assamensis*. Thangavelu and Bhagowati (1984) through extensive and intensive survey in the entire North East region reported five variants of wild stocks along with the natural variants of semi domestic stocks.. Kakati (1991) studied biology and bionomics of *A. assamensis* with special emphasis on its economy in North East India. Nath (2009) and Zamal (2013) carried out studies on morphological variation of different stocks of *A. assamensis*

collected from different regions of Assam. However systematic comparative analysis on morphological variations in different stages of life cycle of different population of *A. assamensis* is very limited. Hence, the present study have been aimed to study the intra and inter population phenotypic variation in *A. assamensis*. A comparison was also made among the population and between wild and semi-domestic stocks to identify the beneficial economic characters for future breeding program.

Materials and Methods:

Extensive survey was carried out in different regions of Assam, Meghalaya, Arunachal Pradesh and Nagaland covering the autumn and spring season particularly during the year 2012-2014. The geographical details of the sites of sample collection are represented in table 1.For phenotypic study, the shape and color of egg, larvae, pupa, cocoon and moth were observed visually and documented using Sony Digital Camera. The size of egg, length of larval body, cocoon size (Length and breadth), pupal size (length and breadth), wing span of moth were measured using measuring scale and varnier caliper. The weight of egg, weight of larval body, pupal body weight, cocoon weight were recorded using electronic balance. The silk % was calculated following standard formula. Scanning Electronic Microscopic (SEM) Study was also performed following standard techniques to know about the egg surface and cocoon filament structure in detail.

Result:

During the study period a total of 7 semi domestic stocks and 4 wild stocks were collected from different regions of North East India. Being a holometabolous insect, *A. assamensis* passes through the following stages – egg, larva, pupa and adult to complete its life cycle. The observations made on phenotypic characters of various life stages of various population of *A. assamensis* are described below –

Egg : The eggs were oval shaped and the color showed variation from brown and dark brown to reddish brown and blackish brown the detail of which is represented in table 2. The size of egg ranged from 2.00-2.31 mm and weight from 1.24 no/g - 143.25 no/g (table2)

SEM studies showed that the follicular imprints of *A. assamensis* egg cell consisted of oval main cells and intracellular space on the entire egg surface expect the micropylar region. Each cell imprint contains 6-8 aeropyles on its ridges. The micropylar region was represented by the following structures-micropylar pit having micropylar canal, encircled with petal shaped primary and secondary cells. Such structures were observed same in all the population expect the number of – aeropyles, number of micropylar canal and the number of primary and secondary cells.

LARVA : The larva moves through five stages of development viz. 1st instar, 2nd instar, 3rd instar, 4th instar and 5th instar. Here, the 5th instar matured larvae were taken for its morphology study. The head capsule was observed brown, hard and roundish in all the population. The body colors were found green, blue, yellow, light green and green with black marking irrespective of population and the details of it is showed in table 4. Out of the dorsal, ventral and lateral body line, the lateral lines were only visible and it was coffee and yellowish in color in all the population. The body setae and tubercular setae were innumerable and the color appeared as yellowish and blackish. The spiracles were oval in shape, deep black in color except the accession number AW1 which showed orange color spiracles with a black marking on its periphery. There were three pairs of brown colored thoracic legs and no variation was observed among the population. The color of the abdominal legs were found to vary according to the larval body color and four pairs of abdominal legs were recorded having black spots and black hairs on each leg. The clasper lobe was found same as the larval body color with a yellowish periphery and the black clasper margin. The length of matured 5th larvae were recorded to vary between 7.32-9.88 cm in male and 8.24-10.01 cm in female (Table 4). The body weight were measured between 7.95-9.60 g in male and 10-05-12.98g in female.

Tables:

Table1: Geographical details of the sites of sample collection

Population↓	Type of stock	Geographical Details		Altitude
		Latitude	Longitude	(m)
Uzirbari,	Semi domestic	26 ⁰ 39'43.73''N	91 ⁰ 21'27.99"E	53
Baksa(AC1)				
Kaliabari, Kamrup	-do-	25 [°] 58'39.97"N	91 ⁰ 14'08.05"E	75
(Ac2)				
Ghilamara,	-do-	27 ⁰ 19'04.57''N	94 [°] 25'15.47"E	98

Lakhimpur (AC3)				
Sapekhati, Sibsagar	-do-	27 ⁰ 06'40.79''N	95 ⁰ 09'41.23"E	112
(AC4)				
Jia, Lower Dibang	-do-	28 ⁰ 03'43.94"N	95 [°] 44'23.92"E	131
Valley (AC5)				
Mokokchung,	-do-	26 ⁰ 19'19.33''N	94 ⁰ 30'48.47"E	1325
Nagaland (AC6)				
Rompara, East	-do-	25 ⁰ 53'39.28''N	90 ⁰ 36'05.97"E	468
Garo Hills (AC7)				
Ronga Reserve	Wild	27 ⁰ 09'32.5''N	93 ⁰ 51'51.56"E	114
Forest, Lakhimpur				
(AW1)				
Maithabari , Nalbari	-do-	26 ⁰ 44'43.46''N	91 [°] 25'53.00"E	50
(AW2)				
Selsela, West Garo	-do-	25 ⁰ 40'28.79"	90 ⁰ 01'40.75"E	79
Hills (AW3)				
Rongrenpal,South	-do-	25 ⁰ 1609.26"N	90 [°] 41'53.51"	467
Garo Hills (AW4)				

Table 2: Egg characters of various population of A. Assamensis

Characters→	Egg shell colour	Egg size (mm)	Egg weight (no./g)
Population↓			
AC1	Brown	2.11±0.10	140.28±6.24
AC2	Brown	2.21±0.13	139.25±6.27
AC3	Dark brown	2.22±0.10	135.25±5.89
AC4	Dark brown	2.11±0.10	140.25±7.11
AC5	Reddish brown	2.22±0.10	135.21±5.67
AC6	brown	2.20±0.15	138.25±8.85
AC7	Dark brown	2.00±0.14	143.25±9.24
AW1	Dark brown	2.23±0.10	132.85±7.27
AW2	Reddish brown	2.31±0.08	126.87±8.11
AW3	Blackish brown	2.24±0.07	131.21±9.21
AW4	Blackish brown	2.27±0.08	129.45±9.36

Table3: Larval characters of various population of A. Assamensis

Characters→	Body colour	Body length	(cm)	Body weight (g)	
Population↓		Male	Female	Male	Female
AC1	Green, Blue, Yellow	7.38±0.25	8.55±0.28	8.57±0.12	11.65±0.05
AC2	Green, Blue	7.48±0.17	8.24±0.20	8.56±0.12	10.05±0.09
AC3	Green, Blue, Yellow	8.50±0.22	8.79±0.29	9.24±0.12	11.83±0.05
AC4	Green, Blue	7.32±0.19	8.40±0.25	7.95±0.01	10.82±0.08
AC5	Green	7.34±0.18	8.47±0.28	8.48±0.13	10.95±0.06
AC6	Green	7.85±0.21	8.84±0.25	8.92±0.12	11.85±0.06
AC7	Green, Blue	8.30±0.24	8.87±0.27	9.02±0.13	12.05±0.05
AW1	Light green	8.86±0.17	9.84±0.20	9.45±0.03	12.97±0.08

AW2	Light green,	9.88±0.26	10.01±0.25	10.58 ± 0.05	12.98±0.06
	Green				
AW3	Green with black marking	9.80±0.21	10.50±0.18	10.01±0.03	13.65±0.05
AW4	Blue green	9.62±0.21	10.18±0.22	9.60±0.11	13.39±0.07

Table4: Pupal charachers of various population of A. Assamensis

Characters→	Pupal	Pupal size (Male) (cm)		Pupal size (Female) (cm)		Pupal weight(g)	
Population↓	colour	Length	Breadth	Length	Breadth	Male	Female
AC1	Light	3.30±0.20	1.57±0.13	4.07±0.14	1.79±0.05	4.18±0.04	5.02±0.17
	brown						
AC2	Light	3.43±0.16	1.56 ± 0.15	4.33±0.21	2.09 ± 0.03	4.19±0.01	5.87±0.12
	Brown						
AC3	Dark	3.72±0.14	1.60 ± 0.09	4.71±0.18	2.07 ± 0.02	5.21±0.08	6.72 ± 0.08
	brown						
AC4	Dark	3.28±0.15	1.54 ± 0.18	4.13±0.23	2.03±0.01	4.17±0.05	5.11±0.17
	brown						
AC5	brown	3.71±0.21	1.69 ± 0.11	4.82±0.15	2.11±0.01	5.11±0.09	6.95±0.12
AC6	blackish	3.27±0.15	1.53 ± 0.09	4.15±0.36	2.08 ± 0.02	4.16±0.07	5.50 ± 0.14
AC7	Copper	3.58 ± 0.28	1.50 ± 0.09	4.42±0.12	2.00 ± 0.02	5.10 ± 0.02	6.56±0.14
	black						
AW1	Copper	4.10±0.21	1.97 ± 0.09	5.00 ± 0.05	1.80 ± 0.09	5.83±0.22	7.89 ± 0.05
	brown						
AW2	Dark	4.88 ± 0.08	1.87 ± 0.03	5.10 ± 0.02	2.10 ± 0.01	6.19±0.12	8.47 ± 0.08
	brown						
AW3	Copper	4.62 ± 0.17	1.89 ± 0.03	5.14 ± 0.03	2.18 ± 0.04	6.10±0.11	8.50 ± 0.05
	black						
AW4	Blackish	4.25±0.05	1.91±0.03	4.85±0.09	2.01 ± 0.06	6.00±0.03	7.56 ± 0.02

Table5: Cocoon characters of various population of A. Assamensis

Chracters→	Cocoon	Male cocoo	n size (cm)) Female cocoon size		Cocoon weigth (g)		Silk %
	colour			(cm)				
Population↓		Length	Beadth	Length	Breadth	Male	Female	
AC1	Whitish	5.24±0.25	2.45±0.15	5.40±0.84	2.41±0.19	4.88±0.12	6.36±0.13	10.04±0.07
	yellow							
AC2	Light	5.20 ± 0.25	2.42 ± 0.19	5.50 ± 0.82	2.15 ± 0.84	4.69±0.17	6.28±0.11	11.42 ± 0.04
	golden							
AC3	Bright	5.60±0.22	2.81±0.19	5.91±0.88	2.92±0.18	4.90±0.13	7.34±0.18	11.72±0.03
	golden							
AC4	Whitish	4.91±0.25	2.33±0.16	5.41±0.84	2.40±0.19	4.54±0.17	5.67±0.14	9.47±0.04
	yellow							
AC5	Golden	5.49±0.28	2.55±0.18	5.62±0.79	2.53±0.18	4.89±0.12	7.23±0.17	12.06±0.03
	yellow							
AC6	Golden	5.64±0.28	2.18±0.19	5.80±0.91	2.52±0.18	4.75±0.10	6.24±0.17	12.21±0.07
	yellow							
AC7	Golden	5.58±0.22	2.67±0.19	5.81±0.65	2.97±0.19	4.92±0.13	7.33±0.18	10.97±0.09
	brown							
AW1	Golden	5.91±0.28	2.82±0.18	6.11±0.88	2.71±0.14	5.02±0.15	9.01±0.15	18.72±0.10
	yellow							
AW2	Golden	6.21±0.31	2.76±0.19	6.40 ± 0.81	2.91±0.17	5.08±0.16	9.57±0.16	16.14±0.06

AW3	Bright Golden	5.86±0.26	2.83±0.18	6.00±0.75	2.76±0.17	5.00±0.11	9.50±0.12	15.60±0.03
AW4	Light golden	6.06±0.23	2.23±0.19	6.43±0.81	2.93±0.27	5.12±0.17	8.45±0.13	19.33±0.08

Table 6: Adult moth characters of various population of A. Assamensis

4

Characters \rightarrow	Wing colour		Wing span (cm)	
Population ↓	Male	Female	Male	Female
AC1	Reddish brown	Light brown	11.51±0.79	14.58±1.32
AC2	Reddish brown	Light brown	12.55±0.17	15.60±1.65
AC3	Yellowish brown	Ochre brown	11.56±0.65	14.85±1.49
AC4	Brick red	Chestnut brown	11.22±0.97	14.50±1.32
AC5	Yellowish brown	Light brown	11.89±0.93	14.99±1.79
AC6	Magenta red	Chestnut brown	11.01±1.03	14.69±1.84
AC7	Brick red	Dark brown	12.97±0.43	15.17±1.41
AW1	Dark reddish brown	Reddish brown	13.95±0.59	16.63±1.95
AW2	Magenta red	Brown	13.45±0.93	16.81±2.02
AW3	Brick red	Ochre brown	13.72±0.74	16.01±1.32
AW4	Magenta red	Chestnut brown	14.18±0.85	16.33±1.88



5

6



- Fig1= Egg of A. Assamensis
- Fig2= Aeropyles in cell imprint
- Fig3= Micropylar rosette
- Fig4= Green colourmorph of A. Assamensis larva
- Fig5= Blue colourmorph of A. Assamensis larva
- Fig6=Yellow colourmorph of A. Assamensis larva
- Fig7= Pupa of A. Assamensis
- Fig8= Cocoon of A. Assamensis
- Fig9= Filament network of Semi domestic stock of A. Assamensis
- Fig10= Filament network of wild stock of A. Assamensis
- Fig 11= Female moth of Semi domestic stock of A. Assamensis
- Fig12= Male moth of Semi domestic stock of A. Assamensis

PUPA and COCOON :

PUPA : The pupa were spindle shaped and the color were observed as light brown, dark brown, dark brown, brown, blackish, copper black and copper brown in various population studied (Table: 4). The size were recorded between 3.28x1.53 cm - 4.88x1.87 cm in male and 4.07x1.79 - 5.14x2.18 cm in female. The weight of pupa were measured 4.16-6.10 g in male and 5.50-8.47 in female (Table: 4).

COCOON : The cocoons were found elliptical, rough texture and glossy nature of floss in all the population. But the color of the cocoon showed distinct variation viz. whitish yellow, light golden, bright golden, golden yellow and golden in different population (Table 5). The size of cocoons were recorded to vary between 4.91x2.33 - 6.21x2.76 cm in male and 5.40x2.30-6.43x2.93 cm in female. The cocoon weight showed the values between 4.54-5.12 g in male, 6.04-8.12 g in female. The silk% were recorded in the range of 9.47-19.33%.

SEM study of silk fibres showed that they form an intricate network in the cocoon surface and the filaments are loosely packed in the semidomestic stocks than the wild stocks. The silk fibres were consisted of fibroin brins covered with a sericin layer. It was also observed that the silk fibre surface contained some irregular shaped crystals

on it. Fourier Transform Infrared Spectroscopy (FTIR) and Energy Dispersive X ray Spectroscopy (EDX) study confirmed that the crystals were composed of calcium oxalate. The amount of crystals were found to vary in different population.

ADULT MOTH : The antennae present in the anterior portion of the head of a adult moth were bipectinate type and brown in colour. The males possessed larger antennae than females. The variation of the wing color were observed not only among the population but also between the male and female the details of which is represented in table 6. The wing span showed significant variation among the population and the value recorded between 11.01-14.18 cm in male and 14.50-16.81 cm in female.

Discussion :

Variations were observed in the color, size and weight of egg but the shape of eggs were oval for both semi domestic and wild accessions as observed by Jolly *et al.*, (1981). Tazima (1978) reported that the color variation of egg shell is due to serosa pingment. The variations in egg size and weight were also revealed by Nath (2009) and Zamal (2012). The present investigation shows oval main cells, aeropyles and micropylar rosette throughout the egg surface. Such structure of *A assamensis* was observed by Chodhury *et al.*, (2013). The aeropyles are the main root through which oxygen exchange takes place between outside and inside the egg. Dey *et al.*, (2003) depicted that the architectural feature of the chorionic surface of *A. assamensis* are different from those of the other species of *Antheraea* and this may be responsible to increase the sensitivity of *A. assamensis* egg chorion to the abiotic factors.

The larval body color showed variation among the various population. These variations are known as phenotypic plasticity phenomenon. Bhattacharya et al., (2005) commented that the color factor may be due to interaction of genes. Observation on the color of lateral body line showed transition from non prominent in the primary instars to coffee and yellowish in the final instar, indicating an increase in melanin pigment with the subsequent stages. This may be due to adaptive responses of the larvae to detrimental conditions as observed by Blest (1977). Leather et al., (1993) described that several species exhibit a large variability in the color present within the population which contribute to the morphological and physiological changes. It is also possible that the host plant may determine the color and it is a cryptic mechanism determined by the host plant to avoid predators. The color and number of thoracic leg, abdominal legs and color of anal clasper showed no variation in the various population of A. assamensis. Such similarity in characters may indicate stability in taxonomic parameters for Saturniidae as observed by Thngavelu and Bhagowati (1984). The body length and weight of 5th instar larvae showed more values in the wild accessions than the cultured accessions indicating a better quantitative character. The variations in body length and weight express the genetic and population diversity among different populations which were also observed by Thangavelu et al., (1987). The host plant has profound effect on relative survival behaviour, rate of food intake, digestion and assimilation which directly influence growth and development of silkworm (Balakrishna et al., 2005).

The spindle shape of pupa is a hereditary and established character hence no difference was observed in wild and cultured stock. The pupal color showed variation not only between the cultured and wild stocks (Zamal, 2012) but also among the various population. The size and weight pupa were found to vary among the various population and they were observed more in the wild accessions. In the present study it was also noticed that lower size and weight of pupae do not contribute to higher silk yield as described by the Sahu *et al.*, (2000).

Elliptical shape of silkworm cocoon is an established character and does not show any difference among the population. But the color of cocoon is found to vary among the various population of A. assamensis. The color variation may be due to presence of coloring pigments in the protein of fibre which is important from taxonomical point of view (Rangaswami et al., 1991). The cocoon size, cocoon weight and silk% also show variation among and within the wild and cultured stocks. The wild stocks show higher values than the cultured stocks. This is similar to views of Sahu et al., (2004) which reported that the cultivated cocoons were found to be flossy and devoid of long peduncle compared to cocoons of wild muga silkworn that have long peduncle. Singh (2012) also reported the similar findings that there exists variation between the wild and cultured stocks in case of cocoon size, cocoon weight, shell weight and silk%. Shamitha and Rao (2006) confirmed that the cocoon fibres of Antheraea mylita under SEM shows cross binding and bifurcation of filaments which form a Y shaped structures. As the filament length of wild stocks are more than semidomestic stocks the fibres of semi-domestic stocks may be loosely packed than wild stocks. Akkir et al., (2010) stated that the cocoon fibres that were tightly packed have higher silk quality. According to Shamitha and Rao (2006), the amount of structural component sericin and fibrin depends on the surrounding atmospheric condition and the sericin is deciding factor on the cocoon quality and raw silk reeled whereas filament length and quality of the shell are based on the fibroin content. The presence of calcium oxalate on the cocoon fibres in A. assamensis and the other members of saturniidae family are confirmed by Freddi et al.,

(1994). Gheysens *et al.*, (2011) also depicted that the hardness of cocoons depend on the amount of calcium oxalate on the silk fibres. More the amount of calcium oxalate results compact and hard cocoon shell.

In adult moth, the bipectinate antennae are the most important olfactory sensory organs. Sexual dimorphism is distinct in male and female. The males of wild accessions shows large size of antennae than the cultured ones indicating adaptive modification of sense organs to locate the male in a wide spread area as observed by Roth and Willis (1952). The wing color show variation among the different population. Bates (1862) depicted that the darker wing color of wild accessions may be a selective adaptation for mating behavior and mimicry in Lepidoptera. The wing span were observed more in wild accessions than the cultured accessions. This may be correlated to the more flying capacity of wild morphs (Rangaswami *et al.*, 1976).

Conclusion:

The study clearly shows that there exists distinct morphological variations in intra specific and inter specific population level of *A. assamensis* indicating a high genetic diversity in muga silkworm germplasm. Such studies are important for the selection of beneficial traits for future breeding program. Such study may also help to establish a 'Satellite Centre' in a conductive climate for *ex-situ* conservation of the collected germplasm to avoid the loss of gene pool due to adverse climatic condition.

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