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A COMPARATIVE MORPHOMETRIC ANALYSIS OF DABA AND BDR-10 ECO-RACES OF TROPICAL TASAR SILKWORM *ANTHEREA MYLITTA* D

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ABSTRACT

Insects show morphometric variations associated with the environment that may be the result of phenotypic response or genetic inheritance, or both. Thereby, studies that emphasize the variability in body size are very useful for understanding the dynamics and the stability of ecological systems. In tasar silkworm *Antheraea mylitta, D* its populations occupying different ecological and geographical regions show a certain degree of phenotypic variability for which they are known as 'eco-races'. In tropical tasar grainage, quality seed production and its timely supply play an important role as the entire tasar sericulture industry depends on quality eggs for quality silk production. Hence, sex identification or gender determination is very important step in order to sort the cocoons, preserve and process the cocoons in grainage to produce the seeds/ Disease free laying (DFL). Although many studies on morphological, ecological and molecular attributes of several species of butterflies including mulberry silkworms from all over the world are available, very little is known about the tasar silkworm.In tasar silkworm, very few morphometric studies were reported. An attempt was made to establish a detailed picture of the variations in length and width of tasar silkworm cocoons and pupa of Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 and its sex identification.Among three eco-races length of cocoons with peduncle (CL+P) was found to be highest in BDR-10 female cocoons (9.4±0.988) followed by DBV (8.62±0.397) and DTV (8.24±0.825) and also results revealed that the length, breadth and weight of female cocoons was found to be highest compared to the male cocoons in all the three eco-races. Among three eco-races, the highest shell weight was observed in BDR-10 (2.23±0.280), followed by DBV (1.70±0.245) and DTV (1.23± 0.100) whereas in male cocoons same pattern was observed.

Keywords: Daba, BDR-10, Length, Breadth, DFL

Introduction

Tasar silkworm (*Antherea mylitta* D) is polyphagous in nature which feeds primarily on *Terminalia arjuna*, *Terminalia tomentosa* and *Shorea robusta* etc to form cocoons. Varying flora, ecological conditions and geographical isolation have led to a large number of variants of this species known as ecotypes or eco races. There are total of 44 eco races reported in India (Singh and Srivastava, 1997; Srivastava, 2002; Srivastava *et al*., 2003). They differ from each other with respect to various qualitative as well as quantitative traits including voltinism.

Insects show morphometric variations associated with the environment that may be the result of phenotypic response or genetic inheritance, or both. Thereby, studies that emphasize the variability in body size are very useful for understanding the dynamics and the stability of ecological systems. In tasar

silkworm *Antheraea mylitta,* its populations occupying different ecological and geographical regions show a certain degree of phenotypic variability for which they are known as 'eco-races'.

Morphometric features are considered to be the most straightforward and reliable way of identifying specimens, a process known as morphological systematics (Nayman, 1965) and developing statistical relationships between them is crucial for taxonomic work (Narejo, 2010) and taxonomic status (Ihssen *et al*., 1981). These characteristics aid in the identification and classification of species.

Morphometric approaches are used to assess size, form, and the relationship between the two (allometry) and shape was an abstraction before the so-called "revolution" (Rohlf & Marcus, 1993), a residue after scaling for size, and it was impossible to perceive the "residue." The substitution of initial variables indicating a distance between two anatomical sites with their coordinates, as well as the subsequent visualization techniques, constituted a huge step forward in the direct study of shapes.

Silkworm sex identification is one of the important processes in the sericulture industry because it can assist in effectively separating strong and healthy silkworm pupae from weak ones. To obtain high quality raw silk, male silkworms are selected via a silkworm sex identification process (Jin *et al*., 1995). The gender separation process can also assist in the search for strong and healthy silkworms. In the last 40 year, several silkworm gender separation techniques have been proposed and demonstrated. This includes a highly-accurate approach based on DNA analysis (Fujii and Shimada, 2007). However, it is hardly used in sericulture industry because it is expensive, time consuming, and destructive in nature. A common low-cost and non-destructive approach is based on the visual inspection of the silkworm pupa at the abdomen segment which essentially requires highly-skilled and well-trained officers.

In tropical tasar grainage, quality seed production and its timely supply play an important role as the entire tasar sericulture industry depends on quality eggs for quality silk production. Hence, sex identification or gender determination is very important step in order to sort the cocoons, preserve and process the cocoons in grainage to produce the seeds/ Disease free laying (DFL).

Although many studies on morphological, ecological and molecular attributes of several species of butterflies including mulberry silkworms from all over the world are available, very little is known about the tasar silkworm. In tasar silkworm, very few morphometric studies were reported. An attempt was made to establish a detailed picture of the variations in length and width of tasar silkworm cocoons and pupa of Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 and its sex identification.

Material and Methods

The study was conducted at Basic Tasar Silkworm Seed organization (BTSSO), Central Silk Board, Ministry of Textiles, Govt. of India, Bilaspur, Chhattisgarh (22.09°N 82.15°E, above mean sea level 207m).

Collection of Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 tasar cocoons

The Daba Bivoltine (DBV) and Daba Trivoltine (DTV) cocoons were collected randomly from grainage houses of Basic Seed Multiplication and Training Center, Bilaspur, and BDR-10 Cocoons were collected from Central Tasar Silkworm Seed Station, Kota, Basic Tasar Silkworm Seed organization (BTSSO), Central Silk Board, Bilaspur, Chhattisgarh**.**

Weighing of tasar cocoons

The Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 cocoons were brought to lab and weight of individual cocoon was recorded by electronic digital weighing balance.

Recording of morphometric parameters

The Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 cocoons were brought to lab and a visual inspection was made. Various morphometric parameters *viz.,* length, breadth and weight were considered for sex identification in cocoon and pupal stage. The length was measured using measuring scale, breadth using vernier calliper and weight by electronic weighing balance. The sizes, shape of cocoons were also recorded individually for all the cocoons. And also the peduncle was cut off from the cocoons and the length of peduncle was also recorded by measuring scale. The cocoon shell weight of all three races was weighed and recorded separately. The Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 cocoons were cut and the pupa was taken out and weighed individually. The length and width were recorded separately. The size and shape of the pupa were also recorded as well as color of pupa was also observed.

Results and Discussion

Length of male & female DBV, DTV & BDR-10 cocoons

The results revealed that, among three eco-races length of cocoons with peduncle (CL+P) was found to be highest in BDR-10 female cocoons (9.4±0.988) followed by DBV (8.62±0.397) and DTV (8.24±0.825) where cocoon length without peduncle (CL-P) was found to be highest in DBV (5.42±0.106) followed by BDR-10. However, peduncle length (PL) was found to be highest in BDR-10 (4.48±1.166) followed by DTV (4.08±0.771) respectively (Table 1)

In case of male cocoons the length of cocoons with peduncle (CL+P) was found to be highest inBDR-10 (8.42±1.133) followed by DBV (8.2±1.280) and DTV (8.18±0.897). Whereas, the length of the cocoon without peduncle (CL-P) was found to be highest in DBV (4.46±0.136) followed by DTV and BDR-10. However, peduncle length (PL) was highest in BDR-10 (4.08±0.937) followed by DTV and DBV (3.94±1.369) (Table 1) (Plate 1)

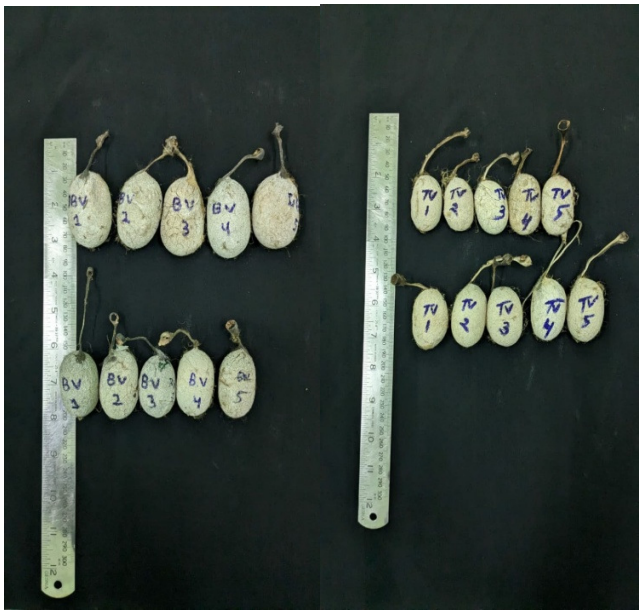

Plate 1: Length of DBV & DTV male & female cocoons

Table 1: Length of male & female DBV, DTV & BDR-10 cocoons

Eco-race	Female cocoon Parameters (Mean± SEm)			Male cocoon Parameters (Mean± SEm)		
	CL-P	PL	CL+P	CL-P	PL	CL+P
DBV	5.42±0.106	3.28±0.384	8.62±0.397	4.46±0.136	3.94±1.369	8.2±1.280
DTV	4.62±0.106	4.08±0.771	8.24±0.825	4.36±0.187	4±0.976	8.18±0.897
BDR-10	5.3±0.208	4.48±1.166	9.4±0.988	4.34±0.220	4.08±0.937	8.42±1.133

CL-P: Cocoon length without peduncle PL: Peduncle length CL+P: Cocoon length with peduncle

Breadth of male & female DBV, DTV & BDR-10 cocoons:

The cocoon breadth/width was found to be highest in BDR-10 female cocoons (3.366 ±0.208) followed by DBV (3.266 ±0.106) and DTV (2.868 ±0.063) female cocoons. Whereas in male cocoons the highest breadth in BDR-10 cocoons (2.842 ±0.092) followed by DBV (2.788 ±0.051) and DTV (2.62 ±0.071) (Table 2).

Table 2: Breadth of male & female DBV, DTV & BDR-10 cocoons

Eco-race	Female cocoon Breadth (Mean±SEm)	Male cocoon Breadth (Mean±SEm)
DBV	3.26±0.106	2.78±0.051
DTV	2.86±0.063	2.62 ±0.071
BDR-10	3.36±0.208	2.84±0.092

Weight of male & female DBV, DTV & BDR-10 cocoons:

The cocoon weight was found to be highest in BDR-10 female cocoons (11.95±1.623), followed by DBV (10.70±0.652) and DTV (8.66±0.469). Whereas, in male cocoons, the highest weight was noticed in BDR-10 (8.16±0.419), followed by DBV (8.02±0.556) and DTV (7.71±0.663) (Table 3). Among all the three races DBV, DTV and BDR-10 female cocoons weighed more compared to male cocoons.

In case of female cocoons, the highest shell weight was observed in BDR-10 (2.23±0.280), followed by DBV (1.70±0.245) and DTV (1.23± 0.100). Whereas in male cocoons same pattern was observed (Table 3).A comparison was made between male and female cocoons where female cocoons of all three eco-races contained highest shell weight. Peduncle weight was found to highest in BDR-10 female cocoons (0.23± 0.028), followed by DTV (0.2±0.054) and DBV (0.15±0.046).Whereas in male cocoons highest peduncle weight was found in DTV (0.23±0.184), followed by BDR-10 (0.16±0.059) and DBV (0.11±0.008) (Table 3).

Table 3: Weight of male & female DBV, DTV & BDR-10 cocoons

Eco-race	Female cocoon parameters(g) (Mean±SEm)			Male cocoon parameters (g) (Mean±SEm)		
	Cocoon weight	Shell weight	Peduncle weight	Cocoon weight	Shell weight	Peduncle weight
DBV	10.70±0.469	1.70±0.245	0.15±0.046	8.02±0.556	1.13±0.179	0.11±0.008
DTV	8.66±0.652	1.23±0.100	0.2±0.054	7.71±0.663	0.99±0.175	0.23±0.184
BDR-10	11.95±1.623	2.23±0.280	0.23±0.028	8.16±0.419	1.29±0.295	0.16±0.059

A study by Suryanarayana and Srivastava (2005) focused on the morphological characterization and variation in Tasar silkworm races. The authors examined the morphological traits of different Tasar silkworm races, including body size, shape, color, and markings. The study provided insights into the variations within Tasar silkworm populations, aiding in their identification and classification. Rath *et al*. 2006 also reported that, the meta physiological activity in the life cycle of the tasar silkworm including reproduction are influenced to a great extent by the climate and the feeding quality of leaves. Similarly, in the present case, different parameters studied in the silkworms of different groups revealed variations due to the influence of the biotic as well as abiotic factors (Srivastava *et al*., 2003).

The most visible aspect of an organism's phenotype is its morphological shape. It establishes a strong link between a species' genotype and its surroundings (Ricklefs & Miles, 1994). Environmental cause variations differ among individuals of the same species and are dependent on the individual's ability to defend against environmental challenges (Digo *et al*., 2015). Morphological variations in moths and butterflies are mostly associated with environmental effects (Mutanen *et al*., 2007). Variation in body size is said to be a natural feature of populations and has important implications for gaining a better knowledge of population dynamics and stability (Roonwal, 1981; Filin Iovadia, 2007) of an ecological system. Kundu *et al*. (2020) conducted a morphometric analysis of Tasar silkworm germplasm resources. The authors measured and analyzed various morphological traits, including body size, shape, and wing venation patterns, in different Tasar silkworm populations. The study provided insights into the morphological diversity and population structure of Tasar silkworms. Suryanarayana and Srivastava (2005) examined the morphological characteristics of different Tasar silkworm ecoraces in India. The authors described and compared the external morphology, body size, shape, and color patterns of Tasar silkworms from various regions of India. The findings contributed to understanding the diversity and regional variations in Tasar silkworm morphology.

Sex identification in pupal stage

Pupa is the inactive resting stage of silkworm. It is a transitional period during which definite changes take place. During this period, biological activity of larval body and its internal organs undergo a complete change and assume the new form of adult moth. The mature silkworm passes through a short transitory stage of pre-pupa before becoming a pupa. During the pre-pupal stage, dissolution of the larval organs takes place which is followed by the formation of adult organs. Soon after pupation the pupa is white and soft but gradually turns brown to dark brown, and the pupal skin becomes harder.

Visual inspection was made for the collected pupa of Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10. The color of pupa was dark brown in color in all the three eco-races. A pair of large compound eyes, a pair of antennae, fore and hind-wings, and the legs were visible. Ten segments can be seen on the ventral side, but only nine are visible on the dorsal side. Seven pairs of spiracles are present in abdominal region, the last pair being non-functional. Sex markings are prominent and it is much easier to determine the sex of pupa. The female has a fine longitudinal line on the eighth abdominal segment, whereas such marking is absent in case of male. In case of female pupa “x” marking was observed on eighth abdominal segment whereas in male pupa dot (.) was observed (Plate 2A, 2B & 2C).

Plate 2A: Male pupa

Plate 2B: Female pupa

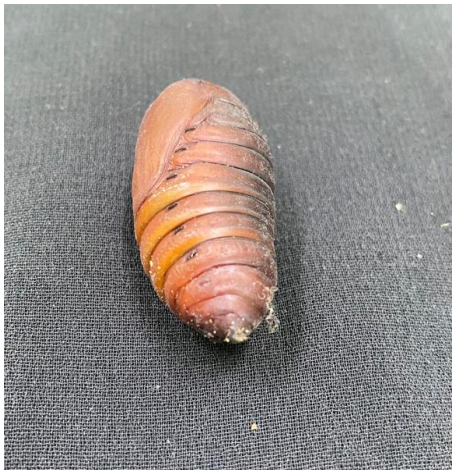

Plate 2C: Spiracles of pupa

In the present study, it was found that female tasar silkworm pupae were bigger than the male silkworm pupae in all three races i.e., Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10 cocoons. The weight of female pupa was found to be highest in BDR-10 (10.834±2.008), followed by DBV (8.844±0.255) and DTV (7.396±0.848). Whereas, in male pupa highest weight was found in DTV (6.71±1.180), followed by BDR-10 (6.65±0.615) and DBV (6.71±1.180) (Table 4)

Table 4 : Weight of male & female pupa of DBV, DTV & BDR-10 eco races

Eco-race	Weight of Female pupa (Mean±SEm)	Weight of male pupa (Mean±SEm)
DBV	8.84±0.255	6.71±1.180
DTV	7.39±0.848	6.71±1.180
BDR-10	10.83±2.008	6.65±0.615

Zhang *et al.* (2010) examined the use of morphological characteristics, including size, shape, and abdominal features, for sex identification in silkworm pupa. The researchers conducted detailed visual observations and measurements to establish reliable criteria for distinguishing between male and female pupae based on their morphological traits. When in the process of sorting, another particular aspect of the pupae which could help in identifying the gender is the rail gonad. According to Tao *et al.* (2019), the tail gonad of each pupae of the silk worms has a particular shape which helps in determining the gender; the males have a dot pattern while the females have an X pattern in the gonad. This aspect helps in easily identifying the gender of the silk worm resulting to a faster way of classifying the worms in terms of their gender. When a dot pattern is identified, the pupae is automatically considered male. On the other hand, when an X pattern is identified, the pupae is classified as female. However, this aspect is not openly realizable thus the need for the incorporation of other features of the silk worm in the classification stage (Tao *et al.*, 2019). Further a research study identifies that gender discrimination could be achieved using a computer based vision method. This is according to a paper written by Liu and Wang (2019) where they suggest application of these computer systems in identifying the gender of the silk worm as early as it is in its larvae stage. This computer system uses the Automatic Gender Recognition module in its operation when identifying the gender of the silk worm (Liu & Wang, 2019). As a result, it becomes easier for the gender of any silkworm to be identified depending on the stage of growth it is in.

Length and Breadth of pupa of DBV, DTV & BDR-10 Eco races:

Among all three races, the highest female pupal length was found in BDR-10 (4.02±0.501) followed by DBV (3.98±0.498) and DTV (3.35±0.714). Whereas, in male pupa highest length was found in DTV (3.80±0.243) followed by DBV (3.62±0.371) and BDR-10 (3.43±0.425). The highest breadth of female pupa was found in BDR-10 (2.84±1.057) followed by DBV (2.27±0.349) and DTV (1.958±0.109).In case of male pupa highest breadth was found in DBV (2.03±0.364) followed by BDR-10 (1.92±0.065) and DTV (1.87±0.062) (Table 5) (Plate 3).

The results revealed that length and breadth were found to be highest in female pupa of all three races when compared with male pupa.

Plate 3: Length of Pupa

Table 5: Length and Breadth pupa of DBV, DTV & BDR-10 eco races

Eco-race	Female pupa parameters (Mean±SEm)		Male pupa parameters (Mean±SEm)	
	Length	**Breadth**	**Length**	**Breadth**
DBV	3.98±0.498	2.27±0.349	3.62±0.371	2.03±0.364
DTV	3.35±0.714	1.95±0.109	3.80±0.243	1.87±0.062
BDR-10	4.02±0.501	2.84±1.057	3.43±0.425	1.92±0.065

The study on morphometric parameters in cocoon and pupal stage revealed that all the parameters *viz.,* length, breadth and weight of female cocoons were higher compared to male cocoons in all three eco-races *viz.,* Daba Bivoltine (DBV), Daba Trivoltine (DTV) and (BDR-10). Also, in case of pupa all the morphometric parameters were also found to be higher in female compared to male and the genital pores of male and female pupa located on eight abdominal segments was prominent and evident. These are the most reliable characters for sex identification in cocoon and pupal stage. This method of sex identification or gender discrimination in cocoon and pupal stage is found to be easy, non-destructive and economical which helps in sorting cocoons and process in grainage for quality seed production.

Silkworm gender separation process can be achieved with precise distinction when checking their physical characteristics. Gender separation is achieved through various characteristic identification. Aspects such as the shape, weight and volume are the most common measures of separable applied commonly in the gender distinctions of cocoon (Mahesh *et al*., 2017). However, these characteristics could be observed from the use of technology, initially, manual separation resulted to lower yields in egg collection compared to electronic separation techniques. To avoid gender discrimination in silk worm gender separation, Mahesh *et al*. (2017) have suggested the incorporation of electronic identification media and systems in most silk culturing laboratories. They suggest the use of the ToF camera which is useful in identifying and classifying the silk worms according to the gender they pose in terms of weight, length, shape and volume. They identify it as the most particularistic solution ever invented with the purpose of making the entire process easier (Mahesh *et al*., 2017).

Conclusion

Gender classification or sex identification in the cocoon stage and of the pupa is an important task in the tasar sericulture industry. Because in tropical tasar grainage cocoon garlands will be prepared and cocoon garlands will be preserved for 6-7 months in grainage house as the tasar pupa undergoes diapause for about 6-7 months, also the phenomenon of moth emergence takes place early in case of male cocoons compared with female. Hence, the cocoons have to be sorted out and a perfect ratio of male and female cocoons has to be maintained in the garland to make male moths available for coupling or pairing with female moths which emerge quite later than male moths and the female moth which is coupled or paired effectively with male moth can only lay the fertile and quality eggs or seeds. As seed or Disease free laying (DFL) is the whole and sole of the sericulture industry the utmost care has to be taken during the sex identification in the cocoon stage. Hence, sex identification at cocoon stage plays a vital role in tropical tasar grainage.

The present study revealed that the sex of male and female cocoons can be identified by visual method and based on the morphological characteristics of cocoons where the size of female cocoons is found be larger compared with male cocoons and the weight of female cocoons was also found to be higher in all the three eco-races *i.e.,* Daba Bivoltine (DBV), Daba Trivoltine (DTV) and BDR-10. Further, in order to re-confirm the results obtained based on morphometric parameters *viz.,* weight, length and breadth of cocoons, the sorted cocoons were cut with a sharp knife and the pupa was taken out and recorded the morphometric parameters of pupa as well where the 99 percent accuracy was found.

The visual and morphometric methods are found to be an easy, non-destructive, rapid, reliable and economical method for gender discrimination or sex identification at cocoon stage which plays a vital role in tropical tasar grainage in order to produce quality DFLs.

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Conflict of Interest

The authors' declare no conflict of interest.

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