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RESEARCH ARTICLE

AGGLOMERATIVE HIERARCHICAL CLUSTERING TO STUDY THE DIVERSITY IN *BOMBYX MORI L.*, RACES ON THE BASIS OF WEIGHT GAIN CHARACTERISTICS

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ABSTRACT

Sericulture is a tradition in the lives of Indian people. Bivoltine silk is known for its good quality. J & K state is known for producing bivoltine silk of international quality, it is the only state in India which produces only bivoltine silk from *Bombyx mori* L. The present study involved morphological studies on 3 Jam and 3 Pam races of *B.mori* at egg, larval and cocoon level. Clustering was done on the basis of quantitative character i.e. larval weight gain of V instar from day 1 to day 5 before cocooning. The weight of worms is directly related to the yield of silk, higher larval weights leads to higher silk production. The results indicate that the clusters can be realized based on larval development parameters; further sub-grouping under the groups highlights the genetic differences associated with different races as well as their significance for silkworm breeding.

INTRODUCTION

The silkworm *Bombyx mori* L. is an insect of immense importance to mankind; it spins valuable silk fibre making it one of the most beneficial insects. It has its use in textile industry as well as non- textile uses as it has become a model organism for studying other lepidopteran insects that cause serious agricultural damage and also an important model for scientific discovery in the areas of microbiology , physiology and genetics.(Nagaraju and Goldsmith, 2002)

A rich diversity of *B.mori* exists globally which is derived from Japanese, Chinese, European and Indian strains, which have distinct traits. A recent compilation of silkworm genetic stocks indicate that there are around 3000 genotypes of *Bombyx mori* at global level, which includes mutants, parthenoclones, polyploids, and geographical races (Nagaraju *et al* 2001). These geographical strains with distinct traits are very valuable genetic stocks for further improvement of silkworm strains (Goldsmith 2005).

Several studies have been done to determine the best strain for silkworm breeding programs (Raju and Krishnamurthy 1993, Porto and Okamoto 2003, Porto *et al* 2004, Rao *et al* 2006, Zanatta *et al* 2009) thus, it is necessary to study all the important sericultural characteristics related to every silkworm life cycle stage. During the different developmental stages of the silkworm, life traits affect the qualitative aspects of silk yield (Ohio *et al* 1970), Chatterjee *et al* 1993 reported 21 traits of *B.mori* that contribute to silk yield either quantitatively or qualitatively. Larval characters have significant correlation with silkworm economical performance and farmer benefits.

India is the second largest producer of silk in the world next to china with annual production of 23,679 MT's of raw silk, annual consumption of 23,202 MT's and an annual demand of 28,801MT's.(International Sericultural commission, statistics) Therefore, it has to be imported from other countries mainly from China. J & K state is known for producing bivoltine silk of international quality; however, production of quality bivoltine silk is still a challenge in J & K having enormous potential to produce bivoltine silk of international grade and reduction in import of raw silk.

The present study involves the study of phenotypic characters of *B. mori* L. races at egg, larval and cocoon level. The relationship of Jam and Pam races evolved in J & K is also done based on larval weight gain during fifth instar by clustering them on the basis of Euclidean distances in order to classify them on the basis of phenotypic similarity and their use in silkworm breeding programs.

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MATERIAL AND METHODS

The study was conducted in Regional Sericulture Research Station, MiranSahib (RSRS, Miran Sahib) and Department of Zoology, University of Jammu simultaneously during Feb-Apr 2013. For the present study, two disease free layings (DFL's) each of six races (Jam2, Jam11, Jam18, Pam101, Pam 102, Pam103) evolved in Jammu and Pampore resp. were obtained from RSRS, MiranSahib, Jammu and CSR&TI, Pampore resp. and incubated for 9-12 days in a neat and clean, disinfected room at 80-85 % Humidity and 24-25ºC Temperature with 18 hrs light till pin head stage, at this stage black–boxing was done to ensure maximum hatching on exposure to bright light. The hatched larvae were reared separately under uniform laboratory conditions as described by Yokoyama (1963) and Krishnaswami (1978).

During the entire period of research, same micro-climate and feeding conditions were ensured as per the larval stage (Table 1). The whole evolutive period was studied for Egg, Larval and Cocoon characters as follows: **At egg stage:** egg shape, egg colour, hatching percentage and average fecundity per female moth were studied. **At larval stage:** larval colour, markings, mean weight of 10 larvae on each day of V instar were studied and analyzed. The statistical analysis was done with the help of software PAST 3. **At cocoon stage:** cocoon shape, cocoon colour and cocoon grain were noted.

Table 1 Temperature and Humidity conditions at various instars of Silkworm life cycle.

Instar	Temperature (℃)	Humidity (%)
I	26-28	85-90
II	26-28	85-90
III	24-26	80
IV	24-25	70-75
V	23-24	65-70

Classical clustering was done using software PAST 3 based on single linkage, wards method and UPGMA method. The method of average linkage between groups (Romesburg, H.C.,1984) under UPGMA (Unweighted Pair-Group Method using Arithmetic average) was used for Data conclusion and the resulting clusters were expressed as Dendrograms. The clustering was based on the squared Euclidean distance.

Table 2 Biological characters of eggs of different races.

Race	Colour of hibernating eggs	Shape	Average fecundity per moth	Hatching percentage(%)
Jam 2	Granite grey	Ellipsoid	522	93.96
Jam 11	Granite grey	Ellipsoid	416	93.79
Jam 18	Granite grey	Ellipsoid	355	93.83
Pam 101	Granite grey	Ellipsoid	462	93.99
Pam 102	Nut brown	Ellipsoid	468	89.31
Pam 103	Steel grey	Ellipsoid	469	93.39

RESULTS AND DISCUSSION

Egg Stage

Analyzing the biological parameters of eggs, colour of the eggs of different races being same i.e. granite grey, except nut brown in Pam 102 and steel grey in Pam 103, and shape being ellipsoid in all the races. The highest average fecundity per moth was 522 in Jam 2 and lowest was 355 in Jam 18. The lowest hatching percent 89.31% was obtained in Pam 102 race and the highest was 93.99% obtained in Pam 101 race, with a mean of 93.04% in all studied races. (Table 2)

Table 3 Biological characters of larvae of different races.

Race	Total larval duration(days)	V age larval duration(days)	Larval colour Vage 5 day	Larval marking(V age)
Jam 2	24.33	5.21	Marble grey	Semi-plain
Jam 11	24.04	6.35	Steel grey	Marked
Jam 18	26.16	6.62	Marble grey	Marked
Pam 101	24.08	5.83	Marble grey	Semi-plain
Pam 102	23.11	5.54	Marble grey	Plain
Pam 103	23.30	6.00	Steel grey	Marked

Table 4 Weight of different races of V instar larvae from Day 1 to Day 5 in g (Mean±S.D).

	Jam 2	Jam 11	Jam 18	Pam 101	Pam 102	Pam 103
Day 1	1.287±0.16	1.503±0.33	1.538±0.23	1.693±0.08	1.449±0.20	1.309±0.16
Day 2	1.778±0.27	2.100±0.53	2.252±0.35	2.018±0.09	2.077±0.25	1.908±0.22
Day 3	2.458±0.32	2.770±0.51	2.743±0.37	2.580±0.27	3.095±0.16	2.585±0.43
Day 4	3.430±0.63	3.748±0.37	4.280±0.51	3.663±0.19	3.637±0.32	3.183±0.27
Day 5	3.751±0.45	4.108±0.33	4.510±0.50	3.825±0.21	3.861±0.24	3.296±0.25

Larval stage

The biological characters of larval stages were recorded as given (Table 3). Under ideal conditions, it has been reported that the total larval duration is 25-30 days (Raina, 2000). The total larval duration in the studied races was 24.17 days with maximum in Jam 18 (26.16 days) and minimum in Pam 102 (23.11 days). The V age larval duration was also maximum in Jam 18 (6.62 days) and minimum in Jam 2 (5.21 days) . The V age larval colour was marble grey in all studied races except in Jam 11 and Pam 103 which was steel grey in colour. All the larvae were marked with semi-plain in Jam 2, Pam 101and Plain in Pam 102. Measurements performed for the determination of weight of 10 larvae, were done at each day of V instar larvae at 11:00 a.m. for five days before cocooning (Table 4). The results obtained show high homogeneity in different races. The larval weight from day 1 to day 5 of V instar larvae was subjected to cluster analysis and similarity matrix was obtained (Table 5).

Table 5 Similarity and distance indices based on weight of different races of V instar larvae from Day 1 to Day 5 in g (Mean±S.D).

	Jam 2	Jam 11	Jam 18	Pam 101	Pam 102	Pam 103
Jam 2	0					
Jam 11	0.69011376	0				
Jam 18	1.2913106	0.68533641	0			
Pam 101	0.54505504	0.40772295	0.97737608	0		
Pam 102	0.75918575	0.42708313	0.99854895	0.5746425	0	
Pam 103	0.54913295	1.0427339	1.6949767	0.81842654	0.91301807	0

The races were clustered into different groups on the basis of Euclidean distances according to grouping from UPGMA method, ward's method and single linkage using PAST 3 and relationships were represented as Dendrograms.(fig. 1).

Cocoon stage

Of these few phenotypic characters studied, all the Jam races have peanut shaped cocoons and Pam 101 having elongated constricted, Pam 102 having constricted and Pam 103 having

oval shape., cocoon colour being white as of bivoltine races and cocoon grain medium to coarse (Table 6).

Table 6 Biological characters of cocoon of different races.

Breed	Shape	Colour	Grain
Jam 2	Peanut	White	Coarse
Jam 11	Peanut	White	Medium
Jam 18	Peanut	White	Coarse
Pam 101	Elongated Constricted	White	Medium
Pam 102	Constricted	White	Medium
Pam 103	Oval	White	Fine

The Cluster analysis (UPGMA) divided the 6 strains into 3 groups as shown in (fig.1a). All races were grouped together and 3 was far from other silkworm strains, indicating it might be suitable for future crossings, maintenance of germplasm so as to maximize heterosis and to avoid inbreeding depression. As studied by Peters *et al* 1989 UPGMA yields more accurate results for classification purposes than other hierarchial methods.

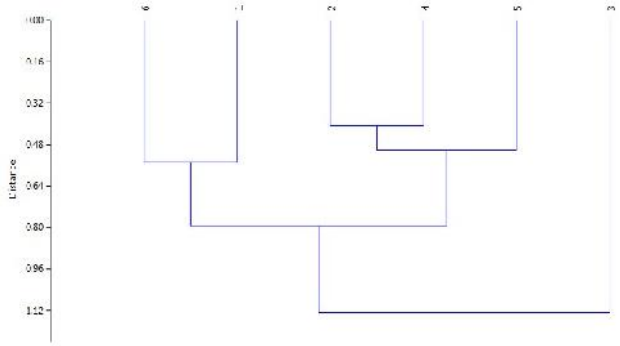

Fig 1 a

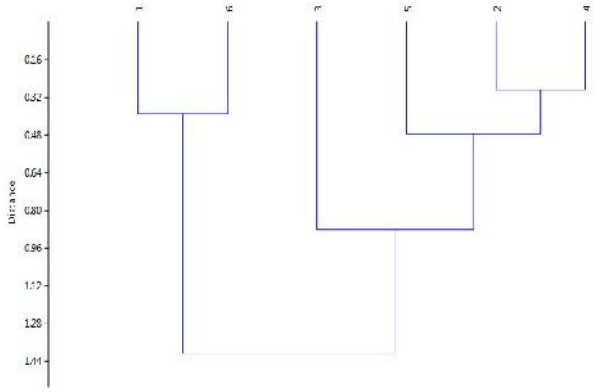

Fig 1 b

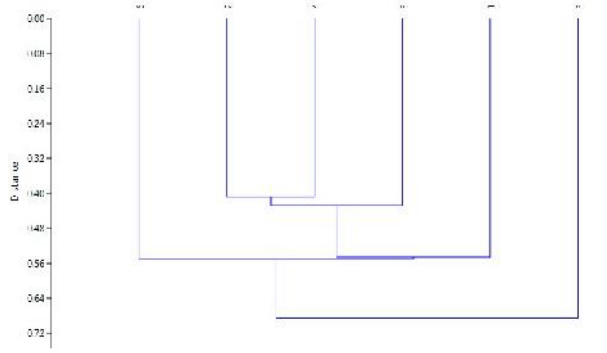

Fig 1 c where , 1=Jam 2 ; 2= Jam 11; 3= Jam 18; 4= Pam 101; 5= Pam 102 6= Pam 103.

Fig. 1 Cluster analysis based on all five studied larval weight gain traits for 6 silkworm races according to grouping from (a)UPGMA (b) Wards methd (c) Single linkage method using PAST.

Systematic studies of resource material are very important for the classification and characterization of varieties and also or the selection of promising parents to be utilized in genetic breeding programs.

Therefore, characterization of each germplasm bank and access to the maximum amount of information is essential for their appropriate utilization in future. (Zanatta *et al* 2009). The cluster analysis provides scope for adopting a recombinational breeding program using distant cluster members. Thus, the sub-grouping of high yielding bivoltine strains offers an opportunity to exploit the genetic differences between high yielding strains. The clustering also indicates the possibility for recombining low and high-yielding members from genetically distant clusters. The results presented here establish its usefulness in realizing a better projection of the genetic difference between silkworm strains of different yield potentials.

Mohammadis and Prasanna 2003 stated cluster analysis refers to "a group of multivariate techniques whose primary purpose is to group individuals based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster" (Hair *et al* 1995). The resulting clusters of individuals should then exhibit high internal (with in cluster) homogeneity and high external (between cluster) heterogeneity. Thus, if the classification is successful, individuals within a cluster shall be closer when plotted geometrically and different clusters shall be farther apart.

Researchers emphasized that the high genetic variation might not give always a high genetic diversity in the inbreeding population of same species. This further confirmed the earlier report that the genetic diversity is not always related with geographical diversity. (Ramamohana Rao and Nakada 1998). It is obvious that the silkworm germplasm contributes the potential raw materials for breeding having wide genetic variation in their genotypic expression besides additive effect due to inbreeding (Kumaresan *et al* 2007).

The obtained data showed that there are highly significant differences among the genotypes for all the studied characters. Varietal differences for studied traits in *B.mori* has been reported by Ahsan *et al* 2000, Li *et al* 2001; Furdui *et al* 2010. Similar studies on varietal diversity have also been sustained by the findings of (Reza *et al* 1993, Mistri and Jayaswal, 1992; Ahsan *et al* 1999; Umashankara and Subramanya, 2002; Nezhad *et al* 2009; Nguku *et al* 2007; Nguku *et al* 2009; Zannata *et al* 2009; Pal and Moorthy 2011).

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