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Genetic divergence in some bivoltine silkworm (*Bombyx mori* L.) breeds

A. Maqbool[1*], H. U. Dar[1], M. Ahmad[2], G. N. Malik[1], G. Zaffar[2] and S. A. Mir[3]

[1]Division of Sericulture Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar campus, Srinagar -191 121, India.
[2]Division of Pant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar campus, Srinagar -191 121, India.
[3]Division of Economics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar campus, Srinagar -191 121, India.

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Twenty-eight genotypes of bivoltine silkworm (*Bombyx mori* L.) were studied for genetic divergence using Mahalanobis D^2 statistic. Based on fifteen important metric traits, D^2 values were obtained and the genotypes were grouped into five clusters using Tocher's method. The clustering pattern indicated a mixed trend. The silkworm genotypes originating/evolved from different geographical regions fell in the same cluster. The inter-cluster distance ranged from 6142.45 to 19605.60. The genetic divergence was maximum between Clusters 5 and 2 followed by that between Cluster 3 and 2. Fifth age larval duration (19.56%), Total larval duration (15.93%), weight of mature silk gland (16.73%), Single cocoon weight (11.29%), Cocoon yield/10,000 larvae by No. (22.78%) and Denier (13.11%) contributed maximum towards the total genetic divergence. The results reveal that while identifying parents for hybridization programme, genetic distances and not the geographic diversity are to be considered. The choice of characters is also important while planning the cross breeding programmes.

Key words: Cluster, genetic divergence, heterosis, silkworm.

INTRODUCTION

In order to synthesize high yielding silkworm hybrids it is important to have a collection of varied gene pool of silkworm races, because variability is the basic requirement for the genetic improvement of any crop. Therefore, to create new reservoirs of genetic variability cross breeding strategies have been extensively used as a means of harnessing heterosis in the bivoltine silkworms (Narayanswamy et al., 2002). One of the challenges in the silkworm breeding is the selection of suitable parental lines with which to develop heterotic combinations. Determining genetic divergence among the available lines has been seen to facilitate this task. Genetic diversity is critical to success in any crop breeding and it provides information about the quantum

*Corresponding author. E-mail: asmaskuastk@rediffmail.com

Table 1. Representation of genotypes in different clusters.

Cluster No.	No. of genotypes	Genotypes
1	24	J_{122}, NCD. Sannish, JA_1, NB_{18}, CSGRC-5, Jam_{21}, Jam_{18}, CSR_2, JD_6, YS_3, A, SRC, New race, $Pure_{81}$, J_2M, Sheiki II, Pampore-5, Meigitsu, 14M, CSR_4, NB_4D_2, Belkokona II and SPJ_2.
2	1	SH_6
3	1	B_{38}
4	1	NJ_3
5	1	JBEL

of genetic divergence and serves a platform for specific breeding objectives. Genetic diversity is a particular concern because greater genetic uniformity in silkworm can increase vulnerability to pests and diseases. Hence, maintenance of genetic diversity is a fundamental component in long-term management strategies for genetic improvement of silkworm (Bindroo and Moorthy, 2014).

Crossing of genetically diverse parents helps in the recombination of genes from diverse resources (Siddiqui et al., 1992) producing high heterotic effects and more variability in segregating generations. Hence selection of genetically pure and divergent parental strains is critical to the success of a hybridization programme in silkworm (Nagaraju and Goldsmith, 2002). Since a great deal of variability exists among various silkworm breeds it is imperative for a breeder to have a knowledge on the genetic distances and the nature and magnitude of genetic diversity among the available silkworm breeds as it is a pre-requisite for choosing parents for hybrids which in turn results in the elevation of quantitative and qualitative aspects of cocoon production (Sen et al., 1996). Various statistical tools are available to measure quantitatively the genetic divergence existing among populations. Mahalanobis (1936) developed a statistical model to study the genetic divergence among populations. This technique has been widely copied by various researchers over the years in choosing parents for hybridization in crop plants (Gupta et al., 1991; Vijayan et al., 1999). Of late, this technique has also been used to ascertain the magnitude of genetic divergence in silkworm, *Bombyx mori* L. (Farooq et al., 2005). Since all the silkworm genotypes have not been ascertained, the investigation was, therefore, undertaken to study the genetic divergence among some more silkworm *B. mori* L. genotypes.

MATERIALS AND METHODS

The present investigation was carried out at the Division of Sericulture, SKUAST (K), Mirgund during 2007-2009.The material for the present investigation comprised 28 bivoltine silkworm lines viz., New Race, $Pure_{81}$, Pampore-5, J-122, Meigitsu, JA_1, 14M, SPJ-2, J_2M, B_{38}, CSGRC-5, Belkokona II, Sheiki II, Sannish, A, Jam $_{18}$, Jam $_{21}$, JD_6, YS_3, NJ_3, NCD, NB_{18}, NB_4D_2, CSR_2, CSR_4, SH_6, SRC, JBEL, of different origin obtained from the germplasm bank maintained at Division of Sericulture, Mirgund, germplasm bank maintained at SKUAST (J), Udaiwala, Jammu and germplasm bank maintained at Central Sericultural Germplasm Resource Centre, Hosur. The silkworm genotypes were reared consecutively two times in a Completely Randomised Block Design with three replications for each treatment; each replication comprised 250 worms after 3rd moult. The rearing were conducted following methods suggested by Dar and Singh (1998).

The data pertaining to the following parameters were recorded and subjected to analysis of variance: 5th age larval duration, total larval duration, weight of mature larvae, weight of mature silk gland, single cocoon weight, single shell weight, shell ratio, cocoon yield/10,000 larvae (by number and weight), pupation rate, filament length, denier, raw silk percentage, fecundity and hatching percentage. The data was put to Mahalanobis D^2 analysis for assessing the genetic divergence among the populations and the contribution of individual characters towards divergence. Clustering was done by Tocher's method as described by Singh and Chaudhary (1977). Relative contribution of each character towards genetic divergence and average intra and inter-cluster distances were also estimated as described by Singh and Chaudhary (1977). Significance of differences among genotypes was tested using Wilk's criterion.

RESULTS AND DISCUSSION

The silkworm genotypes, their place of origin and source are presented in Table 1. On the basis of D^2 values, which were computed for each pair of genotypes, the twenty eight genotypes were grouped into 5 clusters (Table 1). The first cluster included twenty four genotypes viz., J_{122}, NCD, Sannish, JA_1, NB_{18}, CSGRC-5, Jam $_{21}$, Jam_{18}, CSR_2, JD_6, YS_3, A, SRC, New race, Pure 81, J_2M, Sheiki II, Pampore-5, Meigitsu, 14M, CSR_4, NB_4D_2, Belkokona II and SPJ_2. The remaining four silkworm genotypes viz., SH_6, B_{38}, NJ_3 and JBEL constituted second, third, fourth and fifth cluster, respectively.

The cluster mean for fifteen economic traits is presented in Table 2. There was a wide range of variation in some of the characters. The 5th age larval duration ranged from 165.26 h in Cluster 1 to 177.35 h in Cluster 5 whereas, minimum total larval duration was found in Cluster 4 (636.40 h) and maximum in Cluster 2 (653.88 h). The maximum weight of mature larvae was found in Cluster 2 (49.43 g $larva^{-10}$) and minimum in Cluster 3

Table 2. Cluster means for different characters.

Cluster No.	5th age larval duration (hr.)	Total larval duration (hr.)	Weight of mature larvae (g.larva-10)	Weight of silk gland (g.)	Single cocoon weight (g.)	Single shell weight (g.)	Shell ratio (%)	Cocoon yield/ 10,000 larvae by no.	Cocoon yield/ 10,000 larvae by wt. (kg.)	Pupation rate (%)	Filament length (m.)	Denier	Raw silk (%)	Fecundity	Hatching (%)
1	165.26	645.25	43.74	1.50	1.88	0.34	18.29	8610	16.17	84.93	827	2.82	13.61	564	93.38
2	173.88	653.88	49.43	1.53	2.19	0.37	17.05	9149	20.03	86.29	870	2.68	13.43	609	97.65
3	165.28	645.28	41.17	1.99	1.76	0.30	17.04	6872	12.09	85.62	883	2.78	12.04	608	93.99
4	166.40	636.40	41.77	1.25	1.65	0.31	18.54	8860	14.65	83.96	627	3.32	14.38	580	90.05
5	177.35	641.35	44.63	1.39	1.81	0.32	17.68	8338	15.09	83.97	893	2.27	12.68	559	95.64

(41.77 g larva^{-10}). Weight of mature silk gland was minimum (1.25 g) in Cluster 4 and maximum (1.99 g) in Cluster 3. The maximum single cocoon weight (2.19 g) and maximum single shell weight (0.37 g) were revealed by Cluster 2 whereas minimum single cocoon weight (1.65 g) and minimum shell weight (0.30 g) were recorded in Clusters 4 and 3, respectively. However, Cluster 4 exhibited highest shell ratio (18.54%) and Cluster 3 the lowest (17.04 %). The maximum cocoon yield/10,000 larvae by number (9149) and by weight (20.03 kg) were found in Cluster 2 whereas minimum cocoon yield/ 10,000 larvae by no. (6872) and by wt. (12.09 kg) were found in Cluster 3. Pupation rate ranged from 83.96 % in Cluster 4 to 86.29% in Cluster 2. Cluster 5 revealed longest filament (893.67 m) whereas, Cluster 4 exhibited shortest filament (627.33 m). Maximum denier (3.32) was found in Cluster 4 and minimum denier (2.27) in Cluster 5. Raw silk percentage ranged from 12.04 in Cluster 3 to 14.38 in Cluster 4. The highest fecundity (609) and hatching percentage (97.65%) was revealed by Cluster 2 whereas minimum fecundity (559.33) was found in Cluster 5 and minimum hatching percentage (90.05%) was found in Cluster 4. Cluster 2 comprising of SH_6 only had the highest values for nine characters viz., Total larval duration, weight of mature larvae, single cocoon weight, single shell weight, cocoon yield/10,000 larvae by number and by weight, pupation rate, fecundity and hatching percentage. However, Cluster 3 was poorest in six characters viz., weight of mature larvae, single shell weight, shell ratio, cocoon yield/10,000 larvae by number and by weight and raw silk percentage and Cluster 4 was poorest in other six characters viz., total larval duration, weight of mature silk gland, single cocoon weight, pupation rate filament length and hatching percentage.

The average intra- and inter-cluster distances are presented in Table 3 and Figure 1. The inter-cluster distances ranged from 6142.45 to 19605.60. The genetic divergence was maximum between Clusters 5 and 2 (19605.60) followed by that between Clusters 3 and 2 (17109.14), Clusters 5 and 3 (16227.71), Clusters 5 and 1 (14675.27), Clusters 4 and 3 (14343.42), Clusters 4 and 2 (13484.62), Clusters 4 and 1 (8281.53), Clusters 2 and 1 (8062.63), Clusters 3 and 1 (7424.25) and between Clusters 5 and 4 (6142.45). The inter-cluster distances were greater than the intra- cluster distance indicating the presence of high degree of genetic divergence. While analyzing the contribution of various characters towards the expression of genetic divergence (Table 4), it was found that 5th age larval duration (19.56%), total larval duration (15.93%), weight of mature silk gland (16.73%), single cocoon weight (11.29%), cocoon yield/10,000 larvae by number (22.78%) and denier (13.11%) contributed maximum towards the total genetic divergence. These characters accounted for 99.40% of the total genetic divergence in the material. The least contribution to genetic divergence was made by weight of mature larvae, pupation rate and filament length (0.20% each). The characters single shell weight, shell ratio, cocoon yield/10,000 larvae by weight, raw silk percentage, fecundity and hatching percentage did not contribute to genetic divergence in the present set of materials.

The twenty-eight silkworm genotypes formed five distinct clusters. All the silkworm genotypes originating from Japan fell in Cluster 1 except B_{38} which constituted a single solitary cluster. Among seven genotypes evolved in Mysore five viz., NCD, NB_{18}, CSR_2, CSR_4 and NB_4D_2 fell in Cluster 1 and one NJ_3 formed a separate cluster. The three silkworm genotypes (Sannish, Sheiki II and Belkokona II) originating from Russia and two silkworm genotypes (Jam $_{21}$, Jam $_{18}$) evolved in Jammu also fell in Cluster 1. Out of two races (SRC and JBEL) evolved in West Bengal one, SRC fell in Cluster 1 and JBEL formed a single separate cluster. Cluster 1 also included CSGRC-5 evolved at CSGRC, Hosur, JD_6 and YS_3 evolved in Dehradun and A, originated in Poland. However, SH_6, a genotype evolved in Dehradun formed a separate solitary cluster.

The clustering pattern indicated a mixed trend.

Table 3. Average inter- and intra-cluster distances.

Cluster	1	2	3	4	5
1	(4546.91)	8062.63	7424.25	8281.53	14675.27
2		(0.00)	17109.14	13484.62	19605.60
3			(0.00)	14343.42	16227.71
4				(0.00)	6142.45
5					(0.00)

Figures in parenthesis indicate intra-cluster distance.

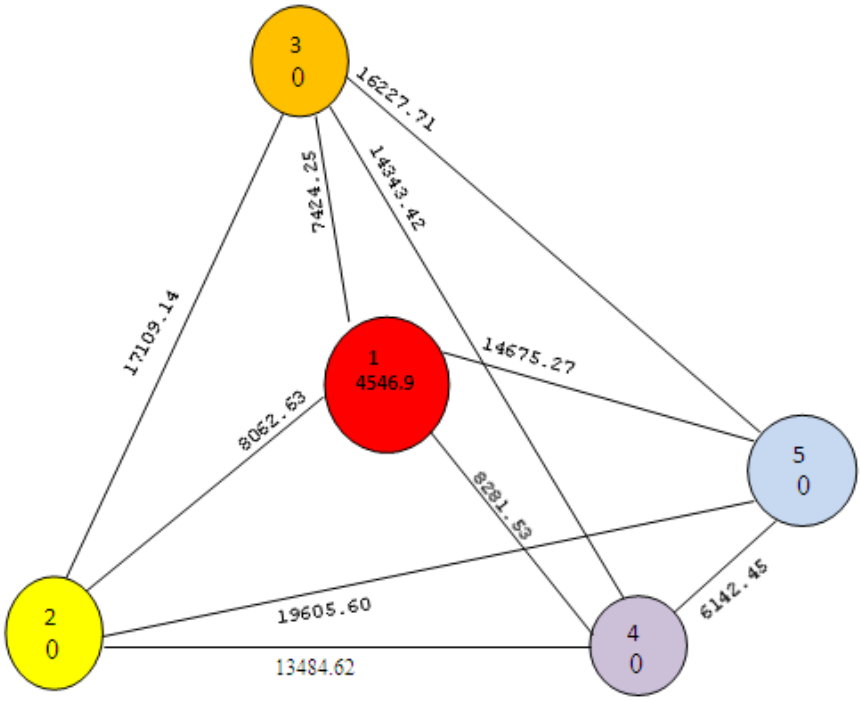

Figure 1. Mahalanobis Euclidean Distances (Not to the scale).

The silkworm genotypes originating/evolved from different geographical regions viz., Japan, Russia, Mysore, Hosur, Jammu, Dehradun, Poland, Pampore and West Bengal, fell in the same cluster. It has been reported by Farooq et al., 2005, that silkworm genotypes originating from different geographical regions fell in one cluster, while those originating from a single geographic region fell in different clusters. Ahmad and Borah, (1999) reported that the relative contribution of different genotypes into different clusters at times reveals no parallelism between genetic diversity and geographical origin. The populations overtime differentiate due to human selection and genetic drift and get adapted to specific agroclimatic environments leading to divergence. Zanatta et al., (2009) also reported that the silkworm strains of same origin did not group together demonstrating they can have different biological and developmental performance. Nehzad et al., (2010) also revealed the inclusion of genotypes of the same origin in different clusters.

The results reveal that while identifying parents for hybridization programme, genetic distances and not the geographic diversity are to be considered. The inter-cluster distances in the present investigation are high showing considerable degree of divergence among various clusters. So the parents for hybridization and future breeding programme can be selected from among the divergent groups. Among the fifteen quantitative characters studied, six characters viz., 5^{th} age larval duration, total larval duration, weight of mature silk gland, single cocoon weight, cocoon yield/10,000 larvae by number and denier contributed about 99.40% towards the total genetic divergence. The results also indicate that the choice of the characters is also important as pointed out

Table 4. Contribution of each character towards genetic divergence.

Character	5th age larval duration (hr.)	Total larval duration (hr.)	Weight of mature larvae (g. larva^{-10})	Weight of silk gland (g.)	Single cocoon weight (g.)	Single shell weight (g.)	Shell ratio (%)	Cocoon yield/ 10,000 larvae by no.	Cocoon yield/ 10,000 larvae by wt. (kg.)	Pupation rate (%)	Filament length (m.)	Denier	Raw silk (%)	Fecundity	Hatching (%)	Total
No. of times appearing first in ranking	97	79	1	83	56	0	0	113	0	1	1	65	0	0	0	496
Percentage contribution	19.56	15.93	0.20	16.73	11.29	0.00	0.00	22.78	0.00	0.20	0.20	13.11	0.00	0.00	0.00	100

by Farooq et al. (2004). It could be conceived that these yield contributing characters must be taken into consideration while planning the cross breeding programmes.

Conflict of Interest

The authors have not declared any conflict of interest.

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