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[Research]

An analysis of genetic variation and divergence in Indian tropical polyvoltine silkworm (*Bombyx mori* L.) genotypes

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ABSTRACT

The genetic variation and diversity among fifty-eight polyvoltine silkworm genotypes was estimated by using ten economic traits. The results revealed that the single shell weight showed higher genetic variation such as PCV% (17.20%), GCV% (12.93%), and heritability (56.5%) followed by single cocoon weight, shell ratio and matured larval weight. The D² (Mahalanobis' distance) statistics revealed nine clusters with substantial inter and intra cluster distances. The genotypes included in different clusters varied from 1 to 16. The genotype Pure Mysore was included in isolated cluster indicates its longer adaptation. The genotypes included in cluster VIII and IX showed optimum genetic distance along with higher cluster mean emphasised for utilization in the silkworm breeding.

Key words: Polyvoltine silkworm genotypes, genetic variation, divergence analysis

INTRODUCTION

A great diversity of polyvoltine silkworm *Bombyx mori* L. must exist globally, considering the fact that many number of silkworm breeds are evolved by selection or cross breeding and also some of the tropical countries allowed individual farmers to reproduce silkworm eggs (Anonymous, 1997). This diversity is considered to be the result of adaptation during long generation (Murakami, 1994).

The genetic variation accumulated through adaptation can be used to measure genetic diversity and therefore, be used to monitor and promote efficient conservation and utilization germplasm. In India, Central Sericultural Germplasm Resources (CSGRC), Central Silk Board, Hosur, Tamil Nadu (Latitude: 12°45'N, Longitude: 77°51'E, Altitude: 942 meter above MSL, Average maximum temperature 27-36° C and average minimum temperature 9-14° C with Average annual Rain fall 800-1000 mm, (www.silkgermplasm.com)) is only repository centre exclusively established for conserving the mulberry (*Morus Spp.*) and silkworm genetic resources (*B.mori* L.) during February 1991, which is being maintaining 71 polyvoltine silkworm genetic resources collected from different geographical regions possessing large

amount of genetic variability, which could be exploited well for breeding programme. Moreover, in silkworm when an initial choice of parents has to be made to obtain heterosis, it is important to ascertain the level of parental divergence (Jolly *et al.*, 1989; Chatterjee *et al.*, 1993; Mukherjee *et al.*, 1999; Kumaresan *et al.*, 2003 and 2003^a). The genetic variability included phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %), environmental coefficient of variation (ECV %), heritability (h² in broad sense) and genetic gain (GA) on economically important quantitative traits of silkworm facilitate the choice of characters to be selected for improvement in the breeding programme. The present study was undertaken to ascertain the magnitude of genetic diversity among selected 58 polyvoltine silkworm (*B. mori* L.) genotypes and estimate the genetic variation for 10 economic traits to augment the utilization of silkworm genetic resources for silkworm breeding programmes.

MATERIALS AND METHODS

Fifty-eight polyvoltine silkworm (*B. mori* L.) genotypes (10 geographical races and 48 evolved breeds) were used for the present study. The data collected from the four seasons

viz., January-March, April-June, July-September and October-December from 2003 to 2005 were used. The standard rearing techniques and recommended methodology for maintenance of germplasm was followed (Krishnaswamy, 1978; Kumaresan *et al.*, 2004).

Three replications for each season for each genotype with 300 larvae retained after third moult out were considered for the study. The rearing for maintenance of silkworm germplasm was followed as 'composite population' to avoid inbreeding depression and genetic erosion. Composite laying is defined as collection of known number of eggs from a known number of individual laying sources that represents the whole population. The composite layings are prepared only after the body pigmentation takes place by taking approximately 50 eggs from each layings of 40 dfls are divided into two batches *ie.* 20 dfls in each batch. All the pieces from 20 laying sources are pasted on a slightly thick brown paper with paste and wrapped in white fine tissue paper after drying. Thus, each composite layings consists of 20 layings sources with about 1,000 – 1,500 individual eggs. Ten economically important quantitative traits *viz.*, fecundity (FEC), weight of 10 matured larvae (g) (LWT), total larval duration (hrs) (TLD), fifth age larval duration (hrs) (VLD), cocoon yield (No.)/10,000 larvae (YLN), cocoon yield (Kg)/10,000 larvae (YWT), single cocoon weight (g) (CWT), single shell weight (cg) (SWT), shell ratio (%) (SR) and pupation rate (%) (PR) were considered. To ascertain the genetic diversity through Mahalonobis' distance (D^2); and to estimate the genetic variability (GCV%, PCV%, ECV%, heritability, genetic advancement) among the traits, the computer packages developed by Indostat Service Pvt. Ltd., Hyderabad, India were used.

RESULTS AND DISCUSSION

The mean performance of fifty-eight polyvoltine silkworm genotypes for selected ten quantitative traits is presented in Table 1. The wide variation obtained among the polyvoltine genotypes in respect of fecundity (15.69%) followed by cocoon yield (kg)/10,000 larvae (15.04%) and pupation rate % (11.50%) facilitates to select for breeding on these principal traits will be useful.

The existence of genetic variability in economic characters is obviously a resource for breeding (Frankel & Brown, 1983; Dalton, 1987). Analysis of variance indicated significant variation among the polyvoltine silkworm genotypes as well as their interaction with

different seasons for all the economic traits considered for the study (Table 2).

The components of genetic variation such as variances of environment, genotype and phenotype together with their coefficient of variation, heritability (h^2 in broad sense) and genetic advancement are presented in Table 3.

The single shell weight showed higher PCV% (17.20) and GCV% (12.93) and maximum heritability (56.5%) followed by CWT, SR and LWT. The single cocoon weight showed higher PCV% (12.98%), GCV% (8.85%) and heritability (46.52%) indicated that the trait could be considered for measuring the phenotypic stability of silkworm genotypes in the varying environments as it has correlation with many traits of economic importance (Kumaresan *et al.*, 2005). This suggests that selection based on above characters will be highly effective for improvement (Narasimharaju *et al.*, 1990; Kumaresan *et al.*, 2000).

The characters like YLN and PR have showed less GCV%, PCV% and heritability under study indicating that the environmental effect was more in the expression of those traits. Though the genetic variation obtained in this study is primarily based on the quantitative traits, which are influenced by the environmental factors. Thus the study needs further confirmation through the DNA profile study like ISSR, RFLP *etc.* genetic markers. Chatterjee and Mohandas (2003) confirmed such wide genetic variation in the yield traits of some 20 silkworm stocks.

They studied genetics of productive traits, which are associated with ISSR markers to explore the association between ISSR markers and yield attributes, such markers needs to be tested for their use in marker-assisted breeding programmes for modifying the yield potential of silkworm. On the basis of Mahalonobis' D^2 values (Ward's minimum variance), the fifty-eight polyvoltine silkworm genotypes were classified in to 9 clusters with substantial inter and intra cluster distances. Number of genotypes included in different clusters varied from 1 to 16. Maximum number of genotypes (16) have included in cluster I, among them 5 genotypes are geographical races *viz.*, Sarupat, NK4, Cambodg, Moria and Nistid (Y). Sarupat and Moria are local land races of same eco-geographical region belong to Assam (North-Eastern state of India). The other 11 genotypes included in cluster I are evolved breeds from various conventional breeding programme; and most of them are producing yellow colour cocoons (9 genotypes) except 2 genotypes (MU1 and MY23) that spins greenish yellow.

Table 1. Mean performance of polyvoltine silkworm genotypes for economic traits.

Sl.No.	Race Name	Fecundity	Wt.of 10 matured larvae (g)	Total larval duration (hrs)	Fifth age larval duration (hrs)	Cocoon yield (No.)/10,000 larvae	Cocoon yield (Kg)/10,000 larvae	Single cocoon weight (g)	Single shell weight (cg)	Shell ratio (%)	Pupation rate (%)
1	Pure Mysore	487	20.31	648.33	193.92	7984	8.81	1.13	15.92	14.28	74.19
2	Sarupat	411	25.07	563.29	148.75	8606	10.11	1.17	17.50	15.19	80.64
3	Moria	420	25.37	566.00	145.50	8662	10.45	1.18	17.33	14.79	88.20
4	T.N.White	467	25.82	558.46	144.42	8281	9.83	1.19	17.83	14.95	78.37
5	C.nichi	423	22.43	538.92	122.71	8497	8.71	1.02	12.92	12.84	82.16
6	Hosa Mysore	516	29.65	567.33	149.11	8089	11.45	1.41	22.00	15.62	81.92
7	Mysore Princess	498	27.58	562.75	144.92	7934	10.03	1.28	19.17	15.14	75.56
8	Kolar Gold*	463	27.90	563.42	145.42	8624	11.47	1.33	20.92	15.82	79.76
9	Kollegal Jawan	544	29.05	547.17	133.92	8245	11.17	1.35	20.50	15.30	80.49
10	MY1	520	27.54	571.54	152.50	8536	11.39	1.38	18.92	13.83	83.20
11	P2D1	487	29.43	572.12	156.75	8428	11.39	1.37	21.25	15.55	83.47
12	Rong Daizo	433	29.73	582.54	167.87	8333	11.73	1.44	21.25	14.72	81.60
13	Guangnong Plain	427	24.93	549.92	131.21	8220	9.10	1.16	17.50	15.24	82.37
14	OS-616	447	24.78	567.12	148.83	8478	9.50	1.16	16.33	14.10	84.32
15	Raj	448	23.09	590.46	167.92	7846	8.73	1.12	15.92	14.15	79.31
16	G	457	25.82	560.25	144.62	8248	9.62	1.22	18.50	15.20	85.65
17	Nistari	422	23.41	553.21	141.21	8417	9.49	1.13	15.58	13.71	85.58
18	Nistari(M)	397	21.47	559.46	147.58	8580	9.00	1.04	14.25	13.80	80.98
19	Nistari(P)	407	24.64	546.04	139.71	8759	9.50	1.11	15.00	13.69	84.79
20	ZPN (SL)	362	21.31	566.58	153.42	8136	8.46	1.11	15.33	14.03	85.89
21	CB5	400	25.05	555.25	152.42	8486	9.74	1.23	18.08	14.87	82.00
22	KW2	410	21.85	576.42	151.92	8619	9.40	1.11	15.67	14.43	87.81
23	M2	415	23.59	564.17	149.42	8938	10.03	1.13	16.75	15.06	87.18
24	A23	475	23.62	564.04	147.29	9114	10.28	1.21	17.17	14.32	90.57
25	A25	495	25.50	568.04	156.96	8669	11.20	1.31	20.17	15.58	84.83
26	Oval	420	25.26	557.12	150.79	8731	10.36	1.28	19.33	15.09	85.98
27	O	463	25.27	566.08	149.75	9007	10.68	1.29	20.00	15.62	84.48
28	M83(c)	424	23.95	559.58	146.08	8375	8.95	1.11	16.25	14.77	83.05
29	B	456	22.48	583.67	161.92	8963	9.78	1.15	16.92	14.69	84.45
30	GNM	385	26.36	565.37	145.58	8308	10.08	1.25	20.25	16.40	81.62
31	A14DY	468	28.53	557.25	149.58	8996	11.56	1.35	19.92	14.80	87.53
32	A4E	457	25.99	584.46	158.75	8284	11.31	1.37	21.67	15.95	85.30
33	PA12	498	27.79	589.04	162.50	6293	11.27	1.43	22.92	16.15	79.46
34	AP12	506	28.88	592.08	165.08	8944	12.78	1.45	23.08	16.00	88.84
35	A13	485	29.41	555.53	146.53	8561	11.68	1.36	20.00	14.71	82.99
36	PMX	497	27.23	567.33	149.25	8760	11.30	1.34	20.75	15.56	80.86
37	PMS2	462	26.53	564.14	146.64	8566	9.85	1.21	18.67	15.50	82.69
38	MU-1	448	26.66	548.00	142.42	8721	10.67	1.28	19.00	15.04	84.45
39	MU-11	471	26.51	568.71	150.25	8566	10.92	1.28	19.67	15.47	85.86
40	WAI-1	468	29.04	563.00	144.25	8760	11.19	1.37	20.25	14.98	82.78
41	WAI-4	385	21.54	558.29	135.00	8350	8.96	1.14	16.92	15.07	77.13
42	MY23	456	27.41	558.33	140.75	8859	10.67	1.25	18.58	14.84	85.23
43	MW13	449	27.52	559.00	143.92	8545	11.21	1.36	20.92	15.49	88.31
44	MHMP(W)	426	27.39	567.50	152.08	8196	10.33	1.29	19.25	15.04	81.64
45	MHMP	479	31.37	571.87	154.67	9024	12.55	1.47	22.67	15.60	90.67
46	P4D3	446	26.70	595.50	167.42	8365	11.15	1.41	23.17	16.46	85.02

Table 1. Continued.

47	Nistid (Y)	445	24.57	569.25	148.00	8943	10.36	1.20	16.75	14.15	89.96
48	Nistid (W)	383	20.88	561.14	143.92	8998	9.23	1.04	14.17	13.73	85.90
49	NK4	401	23.69	572.04	160.58	8765	10.85	1.24	17.83	14.45	85.36
50	Cambodg	387	24.81	560.08	151.83	9006	11.17	1.23	18.00	14.83	86.28
51	Daizo	377	19.89	571.87	171.00	8578	8.38	1.01	13.92	13.96	83.94
52	LMP	398	24.63	556.83	147.33	8724	9.83	1.16	17.08	14.77	87.29
53	DMR	391	24.92	548.71	144.67	8842	9.95	1.19	18.42	15.49	81.72
54	LMO	454	25.67	561.50	147.08	8974	10.86	1.22	17.92	14.73	87.32
55	MY1(SL)	492	24.70	572.21	158.92	8589	10.90	1.30	19.92	15.56	84.06
56	PM(SL)	465	26.34	569.83	150.14	8271	10.34	1.31	19.83	15.28	80.99
57	BL23	509	28.91	553.83	146.83	8437	11.51	1.31	22.00	16.84	81.20
58	BL24	457	27.75	552.25	142.33	8868	12.49	1.33	21.33	16.15	87.53
	Mean	447	25.65	566.28	150.24	8567	10.41	1.250	18.600	14.99	83.81
	SE	20.25	0.94	7.62	4.09	232.76	0.45	0.030	0.010	0.27	2.78
	CV%	15.69	12.73	4.66	9.42	9.41	15.04	9.490	11.350	6.38	11.50
	CD at 5%	56.25	2.62	21.17	11.35	646.42	1.25	0.09	0.02	0.77	7.73
	CD at 1%	74.01	3.44	27.86	14.93	850.49	1.65	0.12	0.02	1.01	10.17

Note=* The pure breed Kolar Gold is different from the present popular cross breed in south India, by name it is also called as 'Kolar Gold', where Pure Mysore (Multivoltine female parent) is crossed with CSR2 (Bivoltine male parent). The parentage of Kolar Gold used in the present study is: (PM.NN6D) (Hosho. Shungetsu).

Table 2. Analysis of variance for economic traits and effect of genotype × environment interaction.

Parameters/Source of variation	Race (G)	Season (E)	Race × Season (G × E)	Error
df	57 (g-1)	3 (e-1)	171 ((g-1) (e-1))	462
Fecundity	20603.53**	78996.76**	4028.63**	5264.33
Weight of 10 matured larvae (g)	82.44**	615.38**	8.81**	11.98
Total larval duration (hrs)	3061.79**	139434.69**	917.07**	1226.94
Fifth age larval duration (hrs)	1387.53**	12503.73**	256.33**	233.05
Cocoon yield (No.)/10,000 larvae	1116366.84**	25735345.79**	791841.63**	610074.13
Cocoon yield (Kg)/10,000 larvae	13.42**	100.59**	2.76**	2.62
Cocoon weight (g)	0.160**	1.464**	0.014**	0.017
Shell weight (cg)	0.0074**	0.0185**	0.0005**	0.0005
Shell ratio (%)	7.436**	31.050**	0.996**	0.912
Pupation rate (%)	146.17 ^{NS}	1142.71**	90.70**	121.57

** Significance at 1%; NS= Non significance.

G=Genotype; E=Environment; G×E= Genotype × Environment interaction.

Table 3. Genetic variability in economic traits of polyvoltine silkworm genotypes.

Parameters/Genetic parameters	V _e	V _g	V _p	ECV%	GCV%	PCV%	h ² (in broad sense)	GA at 5%	GA at 1%
Fecundity	4923.49	1306.67	6230.17	15.69	8.08	17.65	20.97	34.100	43.700
Weight of 10 matured larvae (g)	10.67	5.98	16.65	12.73	9.53	15.91	35.93	3.020	3.870
Total larval duration (hrs)	697.47	197.03	894.50	4.66	2.48	5.28	22.03	13.570	17.390
Fifth age larval duration (hrs)	200.51	98.92	299.42	9.42	6.62	11.52	33.04	11.780	15.090
Cocoon yield (No.)/10,000 larvae	650146.94	38851.67	688998.56	9.41	2.30	9.69	5.64	96.420	123.570
Cocoon yield (Kg)/10,000 larvae	2.45	0.91	3.36	15.04	9.19	17.62	27.17	1.030	1.320
Cocoon weight (g)	0.014	0.012	0.026	9.49	8.85	12.98	46.52	0.150	0.190
Shell weight (cg)	0.0004	0.0006	0.0010	11.35	12.93	17.20	56.50	0.040	0.050
Shell ratio (%)	0.91	0.54	1.46	6.38	4.92	8.05	37.31	0.930	1.190
Pupation rate (%)	92.96	4.43	97.39	11.50	2.51	11.76	4.55	0.926	1.186

V_e=Variance environment; V_g= Variance genotypes; V_p=Variance phenotype; ECV%=Environmental coefficient of variation; GCV%=Genotypical coefficient of variation; PCV%=Phenotypical coefficient of variation; h²= Heritability % in broad sense; GA at 5%=Genetic advancement at 5%; GA at 1%=Genetic advancement at 1%.

The results corroborate with earlier findings (Kumaresan and Sinha, 2002). This result further implies that when an initial choice of parents has to be made to obtain heterosis it is important to ascertain the level of parental divergence (Jolly *et al.*, 1989). The genotypes Guangnong Plain (GNP) and GNM (Guangnong Marked) of same origin (China) have included in cluster II indicated their close affinity in the genetic divergence. Cluster IV has showed only one genotype *viz.*, Pure Mysore that indicates its uniqueness for the acquired character of longer adaptation in the tropical zone of sericulture. The genotype Pure Mysore is one of the major local native breeds of Southern India where sericulture is being practised predominantly. This was also used in the breeding for more than three decades as one of the female counterparts in the evolution of new polyvoltine as well as poly × bivoltine hybrids for commercial exploitation.

Arunachalam *et al.*, (1984) emphasised that there is an optimum level of genetic divergence between parents to obtain heterosis in F₁ generation and it may not be logical to advocate the use of extreme diverge parents to obtain heterotic combination.

The cluster V included 5 genotypes *viz.*, Nistari, Nistari (P), Nistari (M), Nistid (W), which produces spindle shaped cocoons; the other one C.nichi is native of Japan producing peanut shaped cocoons. The genotype Nistari

and its sub lines have included in the same cluster suggests that their close affinity due to low genetic distances. The results emphasised that the high genetic variation might not give always a high genetic diversity in the inbreeding population of same species.

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. The cluster VI included 7 genotypes evolved from various conventional breeding programmes.

The cluster VII included 9 genotypes of potential polyvoltine; where two sex limited breeds (PM (SL) and MY1 (SL)) have included in same cluster. The cluster VIII included 6 genotypes among them two genotypes *viz.*, BL23 and BL24 are presently being exploited for their poly × bivoltine hybrid combination in the major tropical sericulture zones. The cluster IX included 6 genotypes comprised of 5 evolved breeds and one geographical race Rong Dazao.

This further confirmed the earlier report that the genetic diversity is not always related with geographical diversity (Ramamohana Rao and Nakada, 1998).

The above results revealed that the inclusion of genotypes of same origin in different clusters clearly indicates presence of considerable genetic diversity among the population used in the study (Table 4).

The genetic distances for inter and intra clusters is presented in Table 5. The results revealed that the cluster IV has isolated with

zero value of intra cluster distance, which has also showed maximum inter cluster distances with almost all the other cluster groups.

Table 4. Cluster information based on Mahalanobis' Euclidean² Distance Ward's minimum variance.

Cluster	No. of Genotypes	List of Genotypes
I	16	Sarupat, M2, M83(c), LMP, CB5, Oval, DMR, NK4, Cambodg, Moria, LMO, MU-1, MY23, A23, Nistid (Y), OS-616.
II	3	Guangnong Plain, Wai-4, GNM.
III	5	Raj, B, ZPN (SL), KW2, Daizo.
IV	1	Pure Mysore.
V	5	Nistari, Nistari (P), Nistari (M), Nistid (W), C.nichi.
VI	7	Tamil Nadu White, Mysore Princess, Kollegal Jawan, A14DY, A13, WAI-1, MY-1.
VII	9	G, PMS2, O, MU-11, PM (SL), MHMP (W), MW13, A25, MY1 (SL).
VIII	6	Hosa Mysore, P2D1, Kolar Gold, PMX, BL23, BL24.
IX	6	PA12, P4D3, A4E, AP12, MHMP (Y), Rong Dazao.

Table 5. Matrix on Euclidean Inter and Intra cluster distances based on Ward's minimum variance.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	1.433	2.133	2.295	4.236	2.484	2.275	1.780	2.641	3.128
II		1.917	3.049	4.758	3.090	2.786	2.191	2.819	3.477
III			2.026	3.539	2.609	3.469	2.882	3.883	4.065
IV				0.000	4.915	4.630	4.165	4.824	4.483
V					1.638	3.497	3.355	4.142	4.751
VI						1.719	1.824	1.995	2.630
VII							1.263	1.795	2.245
VIII								1.482	2.068
IX									1.830

Table 6. Cluster mean for economic traits based on Ward's minimum variance.

Parameter/Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX
Fecundity	424.46	399.01	410.72	487.12	406.52	492.97	464.51	487.91	469.75
Weight of 10 matured larvae (g)	24.94	24.28	21.72	20.31	22.57	28.14	26.18	28.48	28.41
Total larval duration (hrs)	560.71	557.86	577.80	648.33	551.75	559.38	566.20	562.71	585.92
Fifth age larval duration (hrs)	148.23	137.26	161.23	193.92	139.02	145.16	150.36	148.28	162.71
Cocoon yield (No.)/10,000 larvae	8763.94	8292.43	8428.31	7984.07	8650.27	8473.48	8517.44	8534.33	8540.54
Cocoon yield (Kg)/10,000 larvae	10.24	9.38	8.95	8.81	9.19	10.98	10.56	11.60	11.80
Single cocoon weight (g)	1.20	1.18	1.10	1.13	1.07	1.33	1.28	1.35	1.43
Single shell weight (cg)	17.60	18.20	15.50	15.90	14.40	19.50	19.70	21.40	22.50
Shell ratio (%)	14.78	15.57	14.25	14.28	13.55	14.82	15.42	15.92	15.81
Pupation rate (%)	85.60	80.37	84.28	74.19	83.88	81.56	84.28	82.46	85.15

The cluster IV has showed minimum genetic distance (3.539) with cluster III and maximum genetic distance (4.915) with cluster V. The mean performance of each cluster for all the ten economic traits is presented in Table 6.

It could be concluded from the above results that the genotypes included in cluster IV and V are geographical land races, hence genetic enhancement has to be done for these genotypes through pre-breeding with wild relatives or elite silkworm breeds to augment the utilization of local races.

Similarly, the optimum genetic distances obtained between cluster VIII and IX (2.068) along with higher cluster mean values especially for cocoon characters emphasised the utilization of these genotypes in the conventional silkworm breeding programmes for sustainable silk production.

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