Appraisal of Eighteen Silkworm (*Bombyx mori* L.) Genotypes Using Pre and Post Cocoon Characters

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ABSTRACT

This study was done on simultaneously reared silkworm genotypes over four consecutive years (2004-2007) and at different seasons. Eighteen silkworm genotypes were tested in the study from bivoltine, multivoltine and mutants from the Germplasm Bank of DOS in Sericulture Science, University of Mysore, Mysore. Analysis of variance was done for nine quantitative pre and post cocoon characters in eighteen silkworm genotypes, results clearly showed that all nine economic traits exhibited highly significant difference. The highest record for mean hatching percentage was from the G_{111} genotype at 98.51% and the MU_1 race revealed the least mean value of 98.02%. Genotypes MG_{414} (3.79 g) and PM (661 h) exhibited maximum evaluations for larval weight and larval duration, respectively. Results of this investigation provide information on morphological dissimilarity among silkworm strains calculated from the generalized distance, which is an extension of the Euclidian distance. The dendrogram UPGMA, produced from the cluster analysis, demonstrated two distinct clusters, divided in terms of having genetic distances 0.45 to 3.27 from the respective clusters and the genetic architecture of traits is used as a key for evolutionary geneticists.

Key word: *Bombyx mori*, quantitative characters, clustering

INTRODUCTION

The silkworm is not only a commercially important insect but identification of its genetic characters is also an important laboratory tool for scientific breeding. This type of genetic analysis has been applied during the last few decades to enable animal and plant breeders to increase the productivity of commercial plants and animals. It has been estimated that, more than 3000 silkworm stains are available worldwide due to various ongoing breeding programs[1,2]. These univoltine and bivoltine silkworm varieties are qualitatively and quantitatively superior races, whereas multivoltine are relatively inferior in terms of traits but superior in terms of survival rate and hardiness. However, in tropical countries like India, multivoltine silkworm strains are used for silk production, as they are well adapted to tropical climates.

During the process of breeding, the basic principles of genetics are applied to understand patterns of inheritance that determine many of qualitative and quantitative characters, successfully applied to increase livestock production. It is imperative to know that in the silkworm *Bombyx mori*, quality and quantity of the silk produced are determined by the interaction between the genetic potential of silkworm races and the specific environment to which they are exposed. An extensive study is needed to improve existing strains for commercial purposes and to develop new strains through breeding programmes aimed at improving silk productivity, adaptability to a local environment and disease resistance and/or tolerance[3,4].

The primary goal of silkworm breeding programmes is the simultaneous genetic improvement of multiple traits in general and the superiority of silkworm varieties is judged according to manifestations of traits in any selection programme. Normally breeders deal with more than one character, either directly or indirectly but a common index could be used to evaluate multiple traits in order to judge the superiority of a variety impartially. In order to maintain these genetic resources for heterosis, classification and characterization of these strains is very important. In this study, eighteen *Bombyx mori* strains were evaluated for nine quantitatively important characters and for feasibility of using these strains in breeding programs.

MATERIALS AND METHODS

This study was done on the simultaneous rearing, over about four years (2004-2007) in different seasons of the year, using eighteen different genotypes trains of bivoltine, multivoltine and mutant varieties drawn from

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the Germplasm Bank of DOS in Sericulture Science, University of Mysore, Mysore. In this study, 18 silkworm strains were evaluated for nine quantitatively important traits to determine their feasibility for use in breeding programmes providing data that will be useful in genetic and breeding programmes for the production of superior silkworm strains and hybrids. After incubation of eggs at $25 \pm 10°C$ and relative humidity of $80 \pm 5\%$, the block box was taken out on the 8th day to achieve uniformity in hatching. Rearing was conducted in three seasons viz., pre-monsoon, monsoon and post-monsoon and larvae hatched from each laying were reared separately under uniform laboratory conditions as described by Yokoyama[5] and Krishnaswami[6].

The variance components were estimated using SAS and Minitab software[7]. The statistical models for estimates of each trait were:

$$Y_{ijk} = \mu + G_i + Y_j + (GY)_{ij} + e_{ijk}$$

Where:

μ	=	Population mean
G_i	=	ith Genotype effect
Y_j	=	jth year effect
$(GY)_{ij}$	=	ith Genotype and jth year instruction effect
e_{ijk}	=	ijkth Error

RESULTS AND DISCUSSION

Table 1 shows the analysis of variance (mean square values) for nine quantitative pre and post cocoon characters in eighteen silkworm genotypes. The Table clearly demonstrates that all the nine economic traits exhibited significant difference at 0.01 level of probability. The interaction data of ANOVA shows that none of the traits revealed significant difference except for that of larval weight at 5.02, exhibiting highly significant difference.

Furthermore, characteristics of nine traits in eighteen different voltine groups including indigenous and exotic genotypes were analyzed in this study to determine levels of fecundity in terms of hatching percentage, larval weight, larval duration, yield by number/10,000 larvae brushed (YBN), yield by weight/10,000 larvae brushed (YBW), cocoon weight, shell weight and shell percentage: characteristics that have an important role in the selection of specialized

strains and are related to silkworm cocoon and silk yarn production. During the larval stage, the silkworm accumulates energy reserves for cocoon production and for the last stage of its life cycle. The mean values of nine pre and post cocoon traits of eighteen selected genotypes (Table 2) show different quantitative traits' variations in selected races among which, the MG_{414} and *C. nichi* races exhibited mean values for maximum and minimum fecundity at 571.47 and 437.42, respectively. Whereas in the case of hatching percentage, the mean value of G_{111} genotype at 98.51% was the highest and the MU_1 race revealed the least mean value of 98.02%. However, only the strains MG_{414} (3.79 g) and PM (661 h) exhibited maximum levels of larval weight and larval duration, respectively. The *C. nichi* race recorded the lowest amount for traits of larval weight and larval duration. However, these parameters were indicated as correlates to predict silkworm silk production capacity[8].

Furthermore, this Table demonstrates that by extending the parameters of YBN and YBW the highest evaluations of 9387.70 No. and 13.25 kg in *C. nichi* and MU_{406}, respectively in the same traits the least being recorded by Knobbed (8603.50 No.) and Ursa (7.62 g) so far. However, it is very important to glance over the *C. nichi* race and Ursa mutant that exhibited the lowest of three post cocoon parameters (CW, SW and SR) at records of 0.90, 0.10 and 11.37 g), respectively, whereas, the highest records for CW and SW traits were scored by MU_{405} and MU_{406} at constant 1.49 and 0.27 g in both races, respectively but the maximum SR was recorded in MU_{406} race at 18.33%. The most important traits to correlate with silk production performance of the silkworm are those of cocoon weight, shell weight and shell percentage. An initial analysis of all strains showed that the mean value of cocoon weight, shell weight and shell percentage of the MU_{405}, MU_{406} and MG_{414} indigenous races were superior and that the lowest values were recorded for *C. nichi* Chinese races and Ursa Japanese mutants are from exotic genotypes. Therefore, the shell ratio measure is an estimate that involves cocoon weight and shell weight traits simultaneously with other factors and defines the cocoon's final value. Furthermore, in general other races/mutants, although small differences among them were observed, they were not significant.

Shell weight and silk gland weight are traits that determine the potential for silk production because the energy for silk formation is contained within this gland[8].

Table 1: Analysis of nine quantitative characters of eighteen silkworm genotypes in different years

Source of variation	df	FC	HP	LW	LD	YBN	YBW	CW	SW	SR
Genotype	17	26461.73**	0.35	747.93**	14081.77**	19216.80**	49.39**	0.59**	0.05**	65.55**
Year	3	19935.96**	3.45**	219.09**	54001.18**	582317.85**	13.25**	0.17**	0.01**	6.31**
Interaction	51	0.77	0.31	5.02**	177.45	88327.17	1.57	0.02	0.0007	1.09
Error	268	1456.75	0.24	2.75	1995.06	72578.38	2.65	0.03	0.0008	1.15

**Significant at 0.01 level of probability, FC: Fecundity, HP: Hatching, LW: Larval weight, LD: Larval duration, YBN: Yield/10000 larval brush, YBW: Yield/10000 larval weight, CW: Cocoon weight, SW: Shell weight, SR: Shell ratio

Table 2: Analysis of pre and post-cocoon traits of eighteen silkworm genotypes

Genotypes	FC	HP	LW	LD	YBN	YBW	CW	SW	SR
C. nichi	437.42±7.55^e	98.16±0.15^ab	1.79±0.36^g	530.10±10.90^f	9387.70±19.60^a	8.07±0.26^gh	0.90±0.03^e	0.10±0.00^f	11.37±0.17^g
G₁₁₁	500.20±12.20^bc	98.51±0.09^a	3.34±0.61^b	560.37±9.87^bc	8468.00±120.00^f	9.73±0.28^def	1.21±0.04^bc	0.21±0.01^b	16.94±0.17^b
G₁₁₂	503.70±10.60^b	98.20±0.13^ab	3.31±0.53^b	569.47±9.67^b	8423.00±135.00^de	10.22±0.44^cde	1.26±0.05^b	0.21±0.01^b	16.42±0.22^bc
GNP	479.40±10.30^bcd	98.26±0.13^ab	2.93±0.40^d	551.00±10.30^bc	9203.50±30.90^ab	10.19±0.68^cde	1.22±0.05^b	0.20±0.01^b	16.25±0.52^bcd
Hosa mysore	488.42±9.63^bcd	98.33±0.13^ab	2.92±0.53^d	556.30±10.20^bc	9189.50±38.30^ab	11.58±0.39^b	1.29±0.05^b	0.21±0.01^b	16.17±0.32^bcd
Kolar gold	470.05±9.91^d	98.22±0.13^ab	3.02±0.53^cd	543.70±11.20^bc	9250.90±28.00^ab	10.75±0.42^bcd	1.24±0.05^b	0.20±0.01^b	16.36±0.35^bc
Knobbed	507.90±10.50^b	98.26±0.11^ab	2.96±0.56^d	552.50±10.40^bc	8603.50±97.40^de	9.91±0.35^cdef	1.21±0.04^bc	0.20±0.01^b	16.37±0.16^bc
MG₄₁₄	571.47±4.17^a	98.50±0.08^a	3.79±0.70^a	568.21±9.78^b	9291.20±40.90^ab	12.73±0.26^a	1.44±0.03^a	0.26±0.01^a	18.11±0.20^a
MU₁	484.26±9.18^bcd	98.02±0.15^b	3.01±0.43^cd	547.70±10.60^bc	9208.80±54.80^ab	10.60±0.36^bcd	1.20±0.04^bc	0.19±0.01^b	16.22±0.15^bcd
MU₄₀₅	557.89±6.09^a	98.46±0.09^a	3.68±0.57^a	563.90±10.10^bc	9268.40±54.60^ab	13.11±0.46^a	1.49±0.04^a	0.27±0.01^a	18.05±0.15^a
MU₄₀₆	567.37±4.43^a	98.50±0.08^a	3.77±0.73^a	563.40±10.10^bc	9286.00±46.30^ab	13.25±0.40^a	1.49±0.04^a	0.27±0.01^a	18.33±0.20^a
Nistari	462.06±7.37^de	98.32±0.14^ab	2.30±0.47^e	554.80±12.00^bc	9243.10±31.10^ab	9.82±0.21^cdef	1.10±0.03^cd	0.16±0.00^cd	14.27±0.21^e
Pure Mysore	462.47±6.88^de	98.35±0.12^ab	1.81±0.28^g	661.90±14.50^a	9319.30±40.50^ab	8.99±0.27^fg	1.02±0.03^d	0.14±0.01^e	13.35±0.34^f
Tamil Nadu white	473.00±11.30^cd	98.26±0.12^ab	3.09±0.60^c	544.90±11.00^bc	9228.10±25.90^ab	11.55±0.34^b	1.32±0.04^b	0.21±0.01^b	16.21±0.13^bcd
Ursa	469.95±8.34^d	98.34±0.12^ab	2.06±0.66^f	547.30±10.70^bc	8831.60±41.00^c	7.62±0.24^h	0.90±0.03^e	0.12±0.00^f	12.95±0.24^f
White eyes mutant	468.10±10.60^d	98.15±0.13^ab	2.34±0.37^e	547.90±11.40^bc	9131.60±60.70^b	9.35±0.35^ef	1.07±0.04^d	0.17±0.01^c	15.52±0.23^d
Yellow mutant	484.05±8.52^bcd	98.26±0.14^ab	2.27±0.33^e	557.10±10.40^bc	8629.80±85.50^d	8.75±0.30^fg	1.04±0.04^d	0.15±0.00^de	14.06±0.18^e
Zebra	502.60±11.30^b	98.18±0.11^ab	3.04±0.43^cd	562.10±10.10^bc	8859.60±60.90^c	10.98±0.34^bc	1.29±0.04^b	0.20±0.01^b	15.64±0.24^cd

Values (Mean±SD) followed by different superscript letters in the same column are different by the Duncan test (p < 0.01), FC: Fecundity, HP: Hatching, LW: Larval weight, LD: Larval duration, YBN: Yield/10000 larval brush, YBW: Yield/10000 larval weight, CW: Cocoon weight, SW: Shell weight, SR: Shell ratio

Table 3: Analysis of silkworm nine important larval traits in four different years

Year	FC	HP	LW	LD	YBN	YBW	CW	SW	SR
2004	497.54±5.08^ab	98.43±0.04^a	30.14±0.72^a	592.09±6.83^a	9151.50±24.20^a	10.67±0.26^a	1.22±0.03^a	0.19±0.006^b	15.34±0.21^b
2005	470.31±6.26^c	98.31±0.06^a	28.76±0.75^b	545.17±3.79^c	8977.80±51.20^bc	10.11±0.25^b	1.16±0.03^b	0.19±0.006^b	15.83±0.22^a
2006	506.99±4.66^a	98.39±0.05^a	28.73±0.63^b	561.50±4.46^b	9052.20±41.20^b	10.70±0.21^a	1.25±0.02^a	0.20±0.004^a	15.94±0.20^a
2007	494.24±7.04^b	97.96±0.08^b	26.26±0.67^c	532.61±4.71^c	8964.30±62.50^c	9.92±0.22^b	1.16±0.02^b	0.18±0.006^b	15.70±0.29^a

Values (Mean±SD) followed by different superscript letters in the same column are different by the Duncan test (p < 0.01), FC: Fecundity, HP: Hatching, LW: Larval weight, LD: Larval duration, YBN: Yield/10000 larval brush, YBW: Yield/10000 larval weight, CW: Cocoon weight, SW: Shell weight, SR: Shell ratio

Evaluations for MU₄₀₆ MU₄₀₅ showed cocoon weights 1.49, 1.49 shell weights 0.27, 0.27 and shell percentages of 18.33 and 18.05, respectively; mean values obtained for all eighteen strains were higher than those recorded in other studies in the available literature[8,9,10]. Evaluations for cocoon weight, shell weight and shell percentage are very important silkworm parameters in terms of economic viability, they are highly inheritable, affected by the additive gene action and they allow a better response to the selection process[11,12].

However, mean values of nine economic traits in four different years (Table 3) show that, the fecundity trait was highest (506) in the year 2006 and much less in 2005 (470) followed by rates of 487 and 494 in the years 2004 and 2007, respectively. But in these years the trait of hatching percentage exhibited 98.43 and 97.96 maximum and minimum percentages in the years 2004 and 2007, respectively and moderate traits recorded for the remaining two years were 2005 (98.31) and (98.39). Furthermore, in this Table, evaluations for larval weight, larval duration and YBN recorded the highest levels of 30.14 g, 592.09 h and 9151.50 No. in the same year (2004) and in 2007 all three traits showed the least mean values of 26.26 g, 532.61 h and 8964 No. and the remaining two years showed moderate mean values, respectively. Evaluations for YBW were highest in 2006 (10.70) and

least in 2007 (992) followed by moderate in the other two years. However, evaluations for CW, SW and SR were very high 1.25, 0.20 and 15.94 in the same year 2006 but records for the year 2007 were CW (1.16) and SW (0.18) except that SR exhibited in 2004 was 15.34.

Figure 1 clearly showed the comparisons analysis between nine quantitative traits of all eighteen races. In this study, information on morphological dissimilarity among the silkworm strains of the Germplasm Bank was calculated from the generalized distance of Mahalanobis[13] which is an extension of the Euclidian distance. However, the dendrogram produced is that of an unweighted pair group method with arithmetic means (UPGMA) and through cluster analysis (Fig. 2); results demonstrate a clear division into two huge clusters (groups) on the basis of genetic distance of 0.45 to 3.27 from the respective two clusters and the genetic architecture of these traits is thus a key concern to evolutionary geneticists. These grouping methods allowed for the subdivision of an original group of observations into several subgroups in such a way that homogeneity was obtained inside the subgroups and heterogeneity among the subgroups. The method employed for grouping in terms of UPGMA uses the average distance among all the equal genotypes for the formation of each group[14,15].
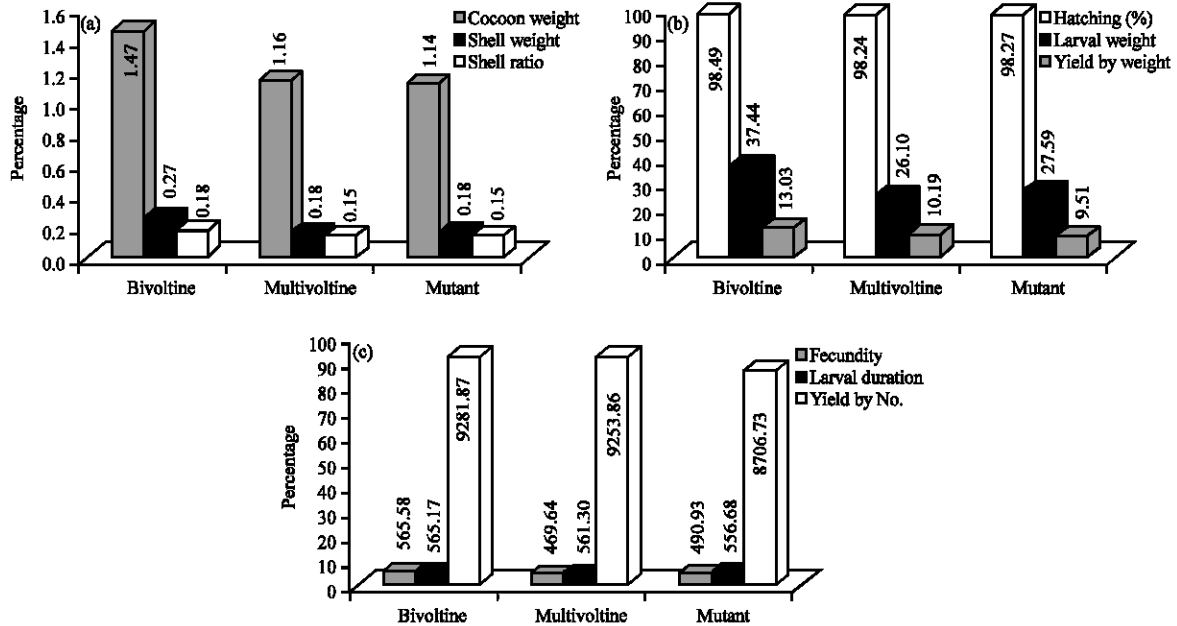
Fig. 1 (a-c): Comparison between nine quantitative traits of Bivoltine, Multivoltine and Mutant *Bombyx mori* L.
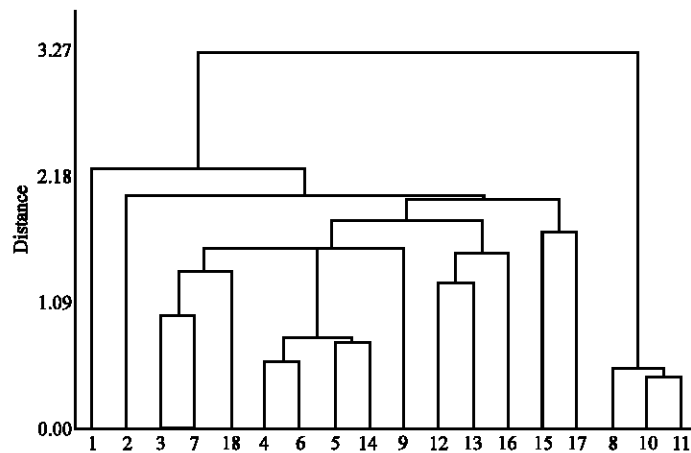

Fig. 2: Dendrogram showing the relationships among eighteen silkworm genotypes, 1: *C. nichi*, 2: G_{111}, 3: G_{112}, 4: GNP, 5: Hosa Mysore, 6: Kolar gold, 7: Knobbed, 8: MG_{414}, 9: MU_1, 10: MU_{405}, 11: MU_{406}, 12: Nistari, 13: Pure Mysore, 14: Tamil Nadu white, 15: Ursa, 16: White eyes mutant, 17: Yellow mutant and 18: Zebra

However, strains such as Ursa, Zebra, Knobbed, Yellow Mutant, G_{111} and G_{112} were clustered in one group and the remaining genotypes were grouped together and formed another cluster. Furthermore, these two clusters are divided into several sub-groups (sub-clusters), whereby Ursa and Zebra mutants fall in one sub-group and another Knobbed, Yellow Mutant, G_{111} and G_{112} fall together in another sub-group.

However, in the case of other cluster races such as GNP, MU_1, HM, TNW, Kolar Gold, Nistari and White Eyes Mutant, they fall in to one sub-group and MG_{414}, MU_{406} and MU_{405} races fall together to form another sub-group and ultimately PM and *C. nichi* races were interlinked together to the previous sub-groups, respectively (Table 4).

Figure 3 indicates different distances between all genotypes. Results of this research confirm that optimum difference between genotypes might be used for prediction and even to obtain heterosis in a silkworm-breeding programme[15,16]. Arunachalam[16]

Table 4: Standardized variables amalgamation steps, Euclidean distance and single linkage of eighteen silkworm genotypes

| Step	No. of clusters	Similarity level	Distance level	Clusters joined		New cluster	No. of obs. in new cluster
1	17	94.84	0.45	MU_{405}	MU_{406}	10	2
2	16	94.21	0.51	MG_{414}	MU_{405}	8	3
3	15	93.44	0.58	GNP	Kolar gold	4	2
4	14	91.53	0.74	Hosa Mysore	Tamil Nadu white	5	2
5	13	91.05	0.78	GNP	Hosa Mysore	4	4
6	12	88.60	1.00	G_{112}	Knobbed	3	2
7	11	85.52	1.27	Nistari	Pure Mysore	12	2
8	10	84.55	1.35	G_{112}	Zebra	3	3
9	9	82.56	1.53	Nistari	White eyes mutant	12	3
10	8	82.18	1.56	G_{112}	GNP	3	7
11	7	82.13	1.57	G_{112}	MU_1	3	8
12	6	80.61	1.70	Ursa	Yellow mutant	15	2
13	5	79.46	1.80	G_{112}	Nistari	3	11
14	4	77.29	1.99	G_{112}	Ursa	3	13
15	3	76.76	2.04	G_{111}	G_{112}	2	14
16	2	74.22	2.26	*C. nichi*	G_{111}	1	15
17	1	62.67	3.27	*C. nichi*	MG_{414}	1	18
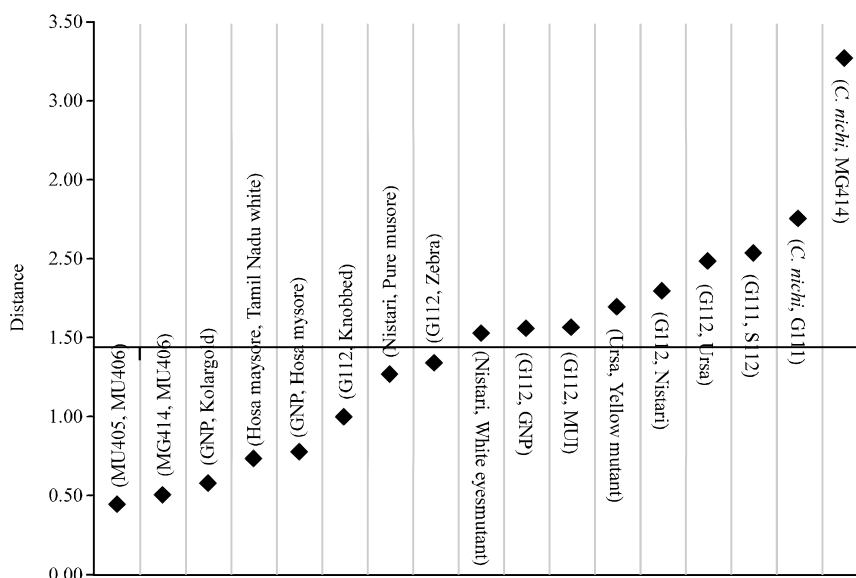
Fig. 3: Two-dimensional display of distance between eighteen silkworm genotypes based on nine quantitative traits

reported that there is an optimum level of genetic divergence between parents to obtain heterosis in the F_1 generation and that they reported that it may not be logical to advocate the use of extremely divergent parents to obtain heterotic combinations. In this investigation, the genotypes Nistari and White Eyes Mutant, G_{112} and GNP, G_{112} and MU_1 all showed moderate distance and it is envisaged that they would have significant positive heterosis in cross breeding programmes (Table 4). Evaluations of traits through this cluster method indicates that these genotypes may be suitable for future crossings, maintenance of parental strains and hybridizations to maximize heterosis and to avoid inbreeding depressions.

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