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Analysis of Genetic Divergence for Classification of Morphological and Larval Gain Characteristics of Peanut Cocoon Silkworm (*Bombyx mori* L.) Germplasm

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Abstract: A hierarchical agglomerative clustering analysis was undertaken for grouping the 51 lines silkworm, *Bombyx mori* L., based on larval gains parameters in the clustering process. The analysis was based on data from one rearing seasons with all 51 peanut cocoon strains of silkworm and varying morphological development potentials. The results indicate that two clusters can be realized based on larval development parameters. Further sub-grouping under these groups highlights genetically differences associated with the differentiation of various groups of lines as well as their significance for silkworm breeding. Estimates of all variables were further subjected to quick clustering and the results showed that both clusters constituted by three sub groups.

Key words: Silkworm · Hierarchical clustering · Larva · Gain · Germplasm

INTRODUCTION

Silkworm is one of the most economically important insects because of the importance of the silk industry in many countries around the world. In the long history of domestication, several thousand silkworm strains have been developed and maintained. Among them, some different strains are similar in morphological characters although they were collected from different parts of the world [1].

Sericultural industry is facing against a range of challenges and problems despite high economic potential. Thus it is inevitable us organization of appropriate strategies in this section. Any decision in this industry requires having sufficient information in order to data analysis and appropriate outputs presentation [2,3].

Silkworm genetic resources are rearing under small population sizes in silkworm gene bank usually and are used for breeding programs [3]. Knowledge and recognition of genetic distances of these groups have effective role in decision making for effective breeding programs and careful genetic selection based on their genetic characteristics and gene structure. Breeding programs are requiring information regarding similarities and differences of relationship and genotypes in various pure lines and the breeders have need of close and not-close genotypes for crossing and hybridization programs. Thus, it must be specified relationships between different pure lines [5].

Analysis of genetic relationships in animal and plant species is an important component of silkworm larval rearing improvement programs, as it serves to provide information about genetic diversity and is a platform for stratified sampling of breeding populations [6]. In modern genetics study, researchers aim to construct classifications and phylogenetic relationships studies. To do this, many characters need to be observed, recorded and analyzed for all the varieties included in an investigation. Chatterjee and Datta [7] analyzed efficiency of the clustering method for grouping of the 54 silkworm pure line from different geographic origins based on biochemical parameters. They report two main clusters based on biochemical parameters. Meanwhile, Govindan *et al*., [8] grouped 16 silkworm pure lines based on six traits and stated pure lines from common ancestors can classified into different clusters.

There are 51 various races in silkworm gene bank of Islamic Republic of Iran at Iran Silkworm Research Center

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(ISRC) and these races conserved under small population size and inbreeding crosses during twenty previous years. Larval characters have significant correlation with silkworm economical performance and farmers benefits. To date, there is not any research regarding larval gains and phylogenetics relationships between silkworm varieties in Iranian gene bank. Thus, the present paper addresses the relationships of these 51 races of the *Bombyx mori* based on larval gains in an attempt to achieve a classification in agreement with phylogeny.

MATERIALS AND METHODS

This study was conducted in Iran Silkworm Research Center (ISRC) and Islamic Azad University, Ghaemshahr Branch, Iran during 2008-2009. For the present study, 51 germplasm strains with genetic diversity in their characters were selected.

The strains were reared under standard rearing conditions and all rearing stages including larval rearing, feeding, cocoon production, silkworm egg preparation and conservation, hatching and related ancillary activities such as pebrin microscopic experiment, investigation on fetal development, recording and collecting data was conducted in the Iran Silkworm Research Center under standard conditions [5].

The 1st-3rd instars of the young larvae were reared at 27 to 28°C with 85-90% relative humidity and the late age larvae (4th and 5th instars) were maintained at 24-26°C with a relative humidity of 70-80%. Each strain was reared in three replications. At the beginning of 4th instar, 250 larvae were counted from each strain and retained for further studies. Rearing was carried out under hygienic conditions.

Fifty one silkworm strains were used in the present study and they presented in Table (1).

The three quantitative characters studied included larval weight at 1st day of 5th instar (g), larval weight at 3rd day of 5th instar (g) and larval weight at last day of 5 th instar (g).

The grouping methods were allowed to subdivide observations into several subgroups in such a way that we obtained homogeneity inside the subgroups and heterogeneity among the subgroups. Hierarchical agglomerative clustering was done by using NTSYS-pc, version 2.02e [9] based on complet, single, UPGMA, UPGMC, FLEXI approaches and SAS-pc [10] based on WARD and average approaches. However, method of average linkage between groups [11] under UPGMA (Unweighted Pair-Group Method using Arithmetic average) was considered as major and final protocol for data conclusion [12] and the resulting clusters were expressed as dendrograms. This method employed for grouping, UPGMA, uses the average distance among all the equal genotypes for the formation of each group [13,14]. The clustering was based on the squared Euclidean distance. The average linkage between two groups is considered as the average of distance between all pairs of cases with one number from each group. Hierarchical clustering analysis was carried out by considering all studied parameters together.

Table 1: The 51 silkworm strains used in the present study

No.	Strain Name	No.	Strain Name	No.	Strain Name	No.	Strain Name
1	107-K	15	M-1-2(5)	29	153 (Xihang-1)	43	T5-M
2	119-K	16	M2-6-22(107)	30	5118×10133-2-2	44	236
3	113-K	17	M2-6-18.3	31	5118×10133-3-3	45	1524
4	105	18	307-300-2	32	Black-White	46	1433-15
5	31	19	202A-204B	33	101×F6	47	1433-9
6	51	20	I 20	34	F6×101	48	7409
7	103	21	101433-9-5	35	Kinshu	49	N19
8	BH-2	22	101433-1-4	36	M-1-1×31	50	White Larvae-Yellow Cocoon
9	B2-09	23	101433-6-6	37	31×M-1-1	51	Black Larvae-White Cocoon
10	1003-4	24	1126(111)	38	M-1-1×103		
11	1003-5	25	113(2029)	39	103 Poly Marking		
12	1005	26	151(103×M-1-1)	40	Shaki		
13	M2-6-22-2	27	Xihang 2.3	41	101		
14	M2-6-18(109)	28	Xihang 3.3	42	T1-J		

RESULTS AND DISCUSSION

From the obtained results, it was clear that different strains of silkworm *Bombyx mori* showed different performance based on larval growth potential. The analysis of variance regarding to studied traits, showed that different strains have significant different for traits ($P<0.01$).

From obtained results, it is showed the larval weight at 1st day of 5th instar of the Black-White (1.076 gr), 105 (1.020 gr), I 20 (1.009 gr), 119-K (0.997 gr) and 1003-5 (0.989 gr) strains remained significantly at upper level than other strains respectively (Table 2).

The larval weight at 3rd day of 5th instar in BH-2 (3.282 gr), 119-K (3.202 gr), 202A-204B (3.148 gr), 153 [Xihang-1] (3.147 gr) and M-1-2[5] (3.064 gr) strains increased significantly in comparison with other strains (Table 2).

The larval weight at last day of 5th instar remained significantly at upper level in the 119-K (3.814 gr), 307-300-2 (3.734 gr), 51 (3.728 gr), 1003-5 (3.690 gr) and 5118×10133-2-2 (3.681 gr) increased significantly in comparison with other strains (Table 2).

The cluster analyses divided the 51 strains into two groups as shown in (Fig 1 to 7). However, the strains of the same origin did not grouped together, demonstrating they can have different biological and development performance. First and second groups divided into three sub-group separately. Three sub-group of first group included 8, 15 and 1 strains respectively. Other strains were grouped together and far from other silkworm strains, indicating they might be suitable for future crossings, maintenance of parental strains and hybridizations with oval cocoon strains so as to maximize heterosis and to avoid depression inbreeding.

Table 2: Mean (± standard deviation) performance of morphological traits in studied silkworm pure lines of gene bank

Traits Pure Lines	Larval Weight at 1st Day of 5th Instar (gr)	Larval Weight at 3rd Day of 5th Instar (gr)	Larval Weight at Last Day of 5th Instar (gr)
107-K	0.729^{klm}±0.05	2.480^{c-h}±0.18	3.183^{a-g}±0.36
119-K	0.997^{abc}±0.06	3.202^{ab}±0.37	3.814^{a}±0.33
113-K	0.697^{l-m}±0.00	2.297^{f-h}±0.10	2.98^{efg}±0.19
105	1.020^{a-b}±0.07	2.733^{a-h}±0.05	3.409^{a-e}±0.09
31	0.899^{c-h}±0.05	2.704^{a-h}±0.22	3.353^{a-e}±0.31
51	0.912^{b-g}±0.07	2.997^{abc}±0.12	3.728^{ab}±0.11
103	0.846^{e-j}±0.05	2.659^{a-h}±0.33	3.240^{a-f}±0.06
BH-2	0.893^{c-i}±0.18	3.282^{a}±0.49	3.199^{a-g}±0.02
B2-09	0.892^{c-i}±0.03	3.064^{a-d}±0.16	3.639^{a-d}± 0.06
1003-4	0.924^{b-g}±0.06	2.908^{abc}±0.07	3.420^{a-e}±0.17
1003-5	0.989^{a-d}±0.06	2.880^{abc}±0.07	3.690^{abc}±0.43
1005	0.854^{e-j}±0.07	2.607^{a-h}±0.10	3.013^{d-f}±0.35
M2-6-22-2	0.924^{b-g}±0.06	$2.72\,6^{a-h}$±0.14	3.299^{a-f}±0.27
M2-6-18(109)	0.814^{fgh}±0.04	2.377^{a-h}±0.14	3.268^{a-f}±0.09
M-1-2(5)	0.919^{c-h}±0.03	3.002^{abc}±0.06	3.653^{abc}±0.27
M2-6-22(107)	0.912^{b-g}±0.09	2.961^{abc}±0.29	3.464^{a-e}± 0.26
M2-6-18.3	0.901^{b-g}±0.03	2.736^{a-h}±0.06	3.533^{a-e}±0.02
307-300-2	0.989^{a-d}±0.03	2.656^{a-h}±0.20	3.734^{a-b}±0.13
202A-204B	0.944^{b-e}±0.09	3.148^{abc}±0.20	3.615^{a-d}±0.31
I 20	1.009^{abc}±0.03	2.932^{abc}±0.08	3.495^{a-e}±0.16
101433-9-5	0.898^{c-h}±0.06	2.792^{a-g}±0.10	3.473^{a-e}±0.04
101433-1-4	0.844^{e-j}±0.04	2.840^{abc}±0.11	3.628^{a-d}±0.10
101433-6-6	0.876^{d-i}±0.01	2.706^{a-h}±0.65	3.236^{a-f}±0.13
1126 (111)	0.920^{b-g}±0.01	1.541^{i}±1.06	3.511^{a-e}±0.07
113 (2029)	0.917^{b-g}±0.02	2.847^{a-g}±0.21	3.544^{a-e}±0.13
151 (103×M-1-1)	0.902^{c-h}±0.02	2.893^{abc}±0.21	3.638^{a-d}±1.47
Xihang 2.3	0.823^{fgh}±0.09	2.604^{h}±0.45	3.175^{d-g}±0.03
Xihang 3.3	0.877^{b-g}±0.07	2.679^{h}±1.01	2.680^{g-f}±0.33
153 (Xihang-1)	0.900^{c-h}±0.01	3.116^{ab}±0.02	3.401^{a-e}±0.15
5118×10133-2-2	0.927^{b-f}±0.06	2.771^{a-h}±0.19	3.681^{abc}±0.12
5118×10133-3-3	0.877^{d-i}±0.01	2.523^{c-h}±0.08	3.176^{d-g}±0.08
Black-White	1.076^{a}±0.07	2.737^{a-h}±0.19	3.593^{a-e}±0.17

Table 2: Continued

101×F6	0.880^{d-i}±0.02	2.699^{a-h}±0.21	3.362^{a-e}±0.11
F6×101	0.902^{c-h}±0.02	2.832^{a-g}±0.11	3.217^{a-g}±0.53
Kinshu	0.808^{h-g}±0.03	2.522^{c-h}±0.14	3.083^{c-f}±0.08
M-1-1×31	0.877^{d-i}±0.03	2.790^{a-g}±0.34	3.377^{g-f}±0.11
31×M-1-1	0.847^{e-i}±0.05	2.650^{a-h}±0.16	3.343^{g-f}±0.10
M-1-1×103	0.787^{h-l}±0.03	2.338^{e-h}±0.15	3.017^{d-f}±0.25
103 Poly Ma	0.726^{klm}±0.01	2.420^{d}±0.06	3.215^{a-g}±0.28
Shaki	0.747^{j-m}±0.10	2.590^{a-h}±0.31	3.109^{d-g}±0.08
101	0.867^{e-i}±0.03	2.523^{c-h}±0.13	3.386^{a-e}±0.22
T1-J	0.849^{e-i}±0.04	2.705^{a-h}±0.04	3.207^{a-g}±0.32
T5-M	0.949^{b-e}±0.08	2.371^{e-h}±1.13	3.165^{d-g}±0.21
236	0.920^{b-g}±0.04	2.873^{abc}±0.21	3.494^{a-e}±0.06
1524	0.846^{e-i}±0.00	2.460^{e-h}±0.14	3.407^{d}±0.09
1433-15	0.841^{e-i}±0.10	2.741^{a-h}±0.08	3.478^{a-e}±0.48
1433-9	0.851^{e-i}±0.04	2.625^{h}±0.18	3.301^{a-f}±0.09
7409	0.750^{i-m}±0.01	2.651^{a-h}±0.07	2.613^{g-f}±0.48
N19	0.715^{klm}±0.01	2.194^{h-g}±0.04	2.979^{efg}±0.07
White Larvae- Yellow Cocoon	0.779^{i-l}±0.01	2.699^{a-h}±0.07	3.333^{a-e}±0.11
Black Larvae-White Cocoon	0.669^{h}±0.02	2.449^{e-h}±0.08	2.964^{efg}±0.17

Means in each column followed by the same letters are not significantly different at α=0.01

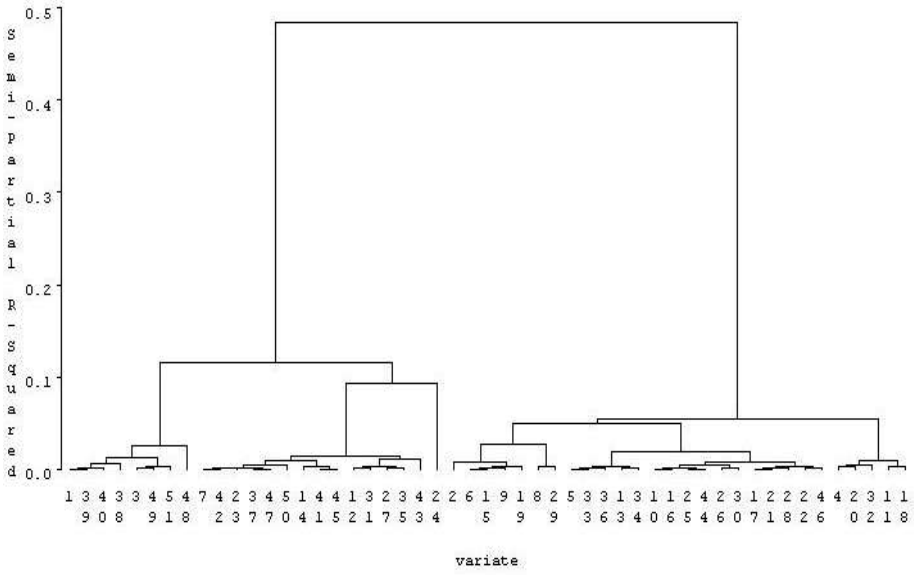

Fig. 1: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from WARD method using SAS

As indicated earlier, our final analysis and conclusion has been done on the basis of the average linkage between groups or UPGMA, since as others researchers have shown [15,7] UPGMA yields more accurate results for classification purposes than other hierarchical methods.

Systematic studies of resource material are very important for the classification and characterization of varieties and also for 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 the future [14].

The cluster analysis provides scope for adopting a recombinational breeding program using distant cluster members. Thus, the subgrouping of high-yielding bivoltine strains offers an opportunity to exploit the genetically differences between high-yielding strains. The clustering also indicates the possibility for

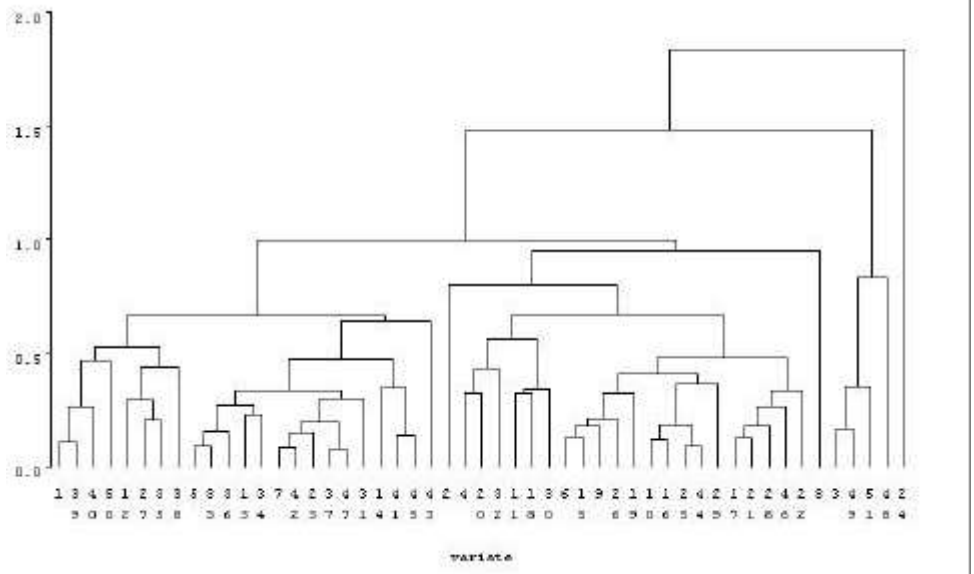

Fig. 2: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from average method using SAS

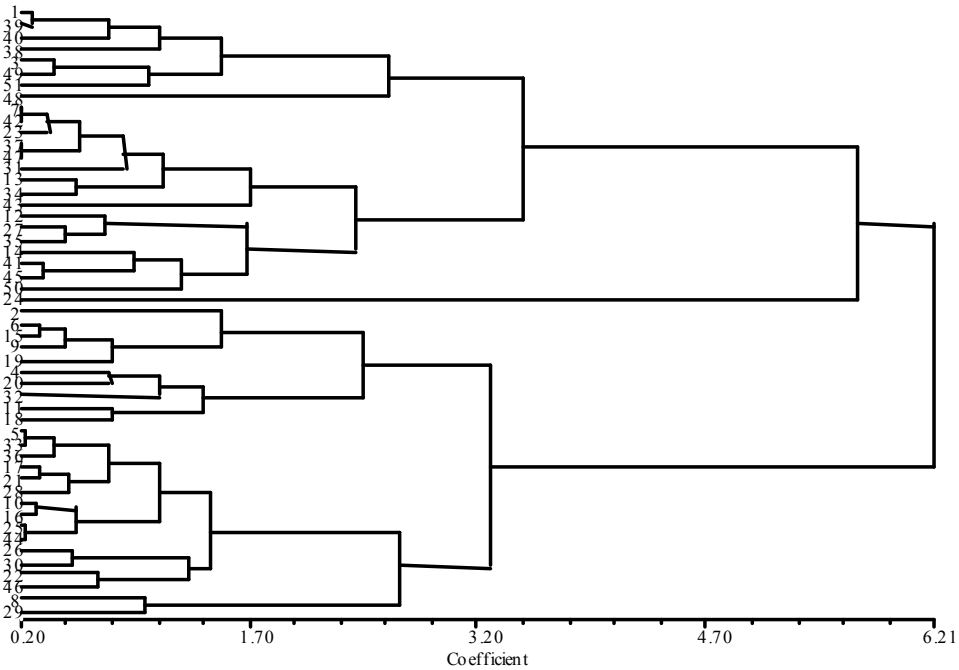

Fig. 3: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from complet method using NTSYS

recombining low and high-yielding members from genetically distant clusters. The results presented here establish its usefulness in realizing a better projection of the genetical difference between silkworm strains of different yield potentials [7].

As Mohammadis and Prasanna [6] stated cluster analysis refers to "a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster" [16]. The resulting clusters of individuals should then exhibit high internal (within cluster) homogeneity and high external (between cluster) heterogeneity. Thus, if the classification

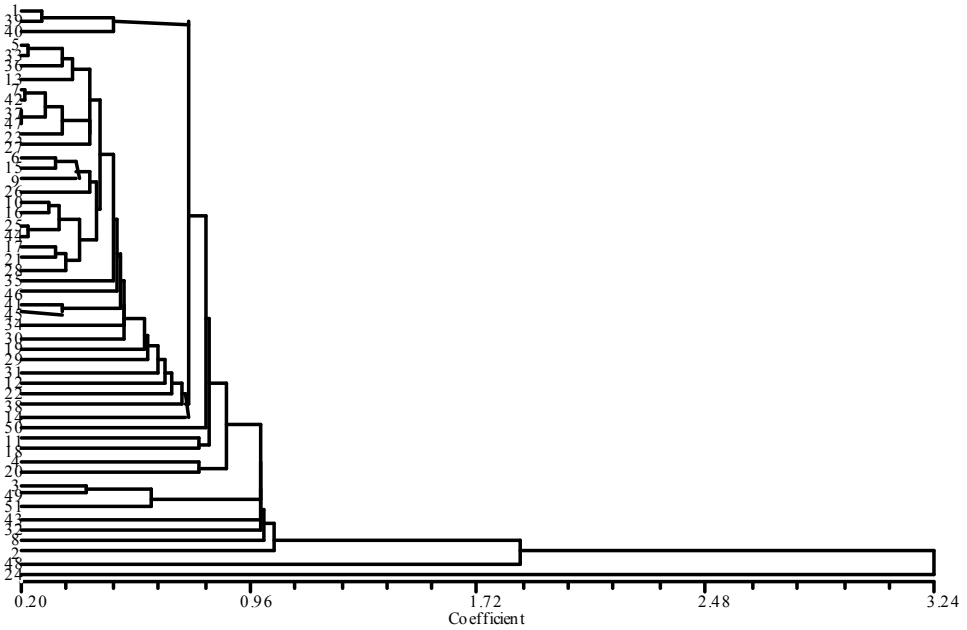

Fig. 4: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from single method using NTSYS

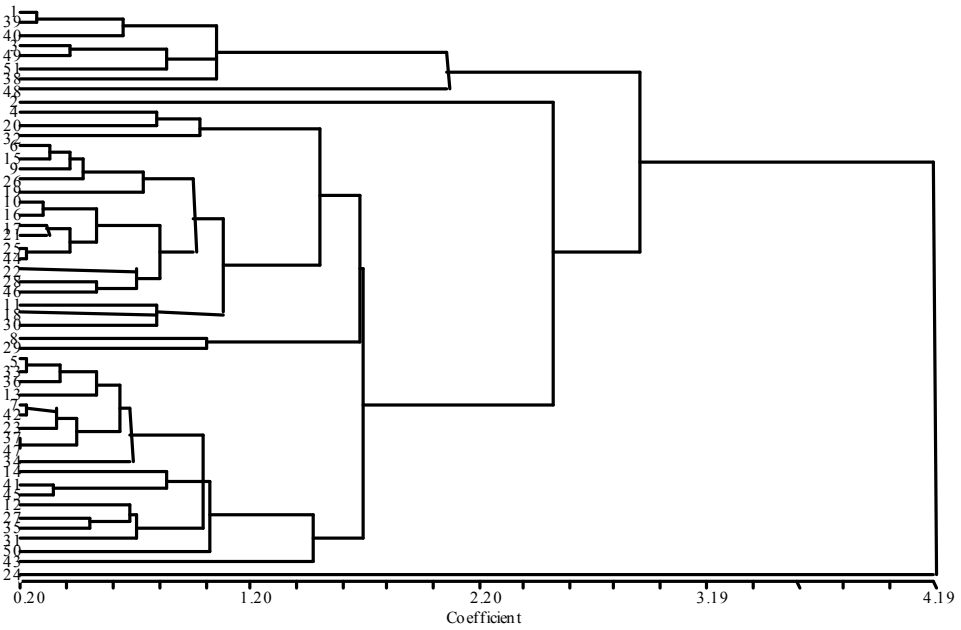

Fig. 5: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from UPGMC method using NTSYS

is successful, individuals within a cluster shall be closer when plotted geometrically and different clusters shall be farther apart [16].

Constant efforts are being made to develop productive polyvoltine silkworm hybrids suitable for sericulture, since more than ninety percent of the raw silk is still coming from polyvoltine silkworm hybrids only. Therefore, maintenance of polyvoltine resource material and their effective utilization has become very important [17].

Researchers emphasized that the high genetic variation might not give always a high genetic diversity in

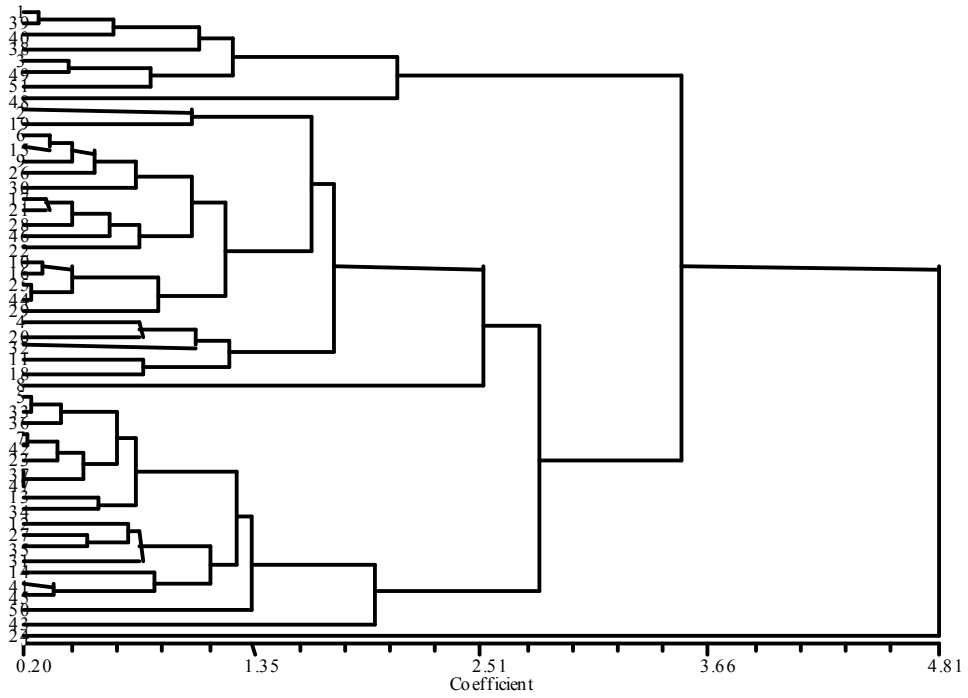

Fig. 6: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from FLEXI method using NTSYS

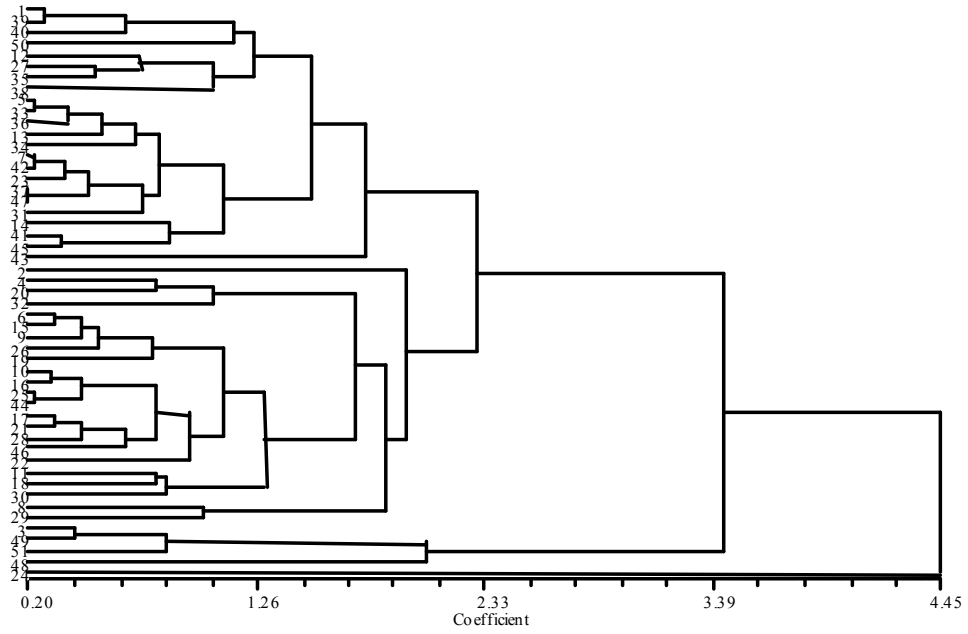

Fig. 7: Cluster analysis based on all 3 studied larval weight gain traits for 51 silkworm strains according to the grouping from UPGMA (Unweighted Pair Group Method Average) method using NTSYS

the inbreeding population of same species. This further confirmed the earlier report that the genetic diversity is not always related with geographical diversity [18]. 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 [19].

The obtained data showed that there are highly significant differences among the genotypes for all the studied characters. Varietal differences for studied traits

in *Bombyx mori* have been reported by Ahsan and Rahman [20]. Similar results on varietal diversity have also been substantiated by the findings of Reza and Rahman [21], Ahsan *et al*., [22] and Ahsan and Rahman [23].

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