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Full Length Research Paper

Genetic diversity and classification of 51 strains of silkworm *Bombyx mori* (*Lepidoptera: Bombycidae*) germplasm based on larval phenotypic data using Ward's and UPGMA methods

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The aim of this experiment was to study and classify all 51 pure lines of Iran silkworm germplasm based on larval traits and identification of pure lines relationships. 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. The grouping methods allowed the study to subdivide the observations into several subgroups in such a way that homogeneity was obtained inside the subgroups and heterogeneity among the subgroups. Various methods generated similar dendograms. On the basis of these dendrograms, analyzed strains were divided into 2 distinct groups. Frequent divisions were also observed in major groups. The first group was divided into two sub groups including three and eight strains, respectively. The second major group was divided into two sub groups which include 4 and 36 strains, respectively.

Key words: Silkworm, unweighted pair-group method using arithmetic average (UPGMA), ward, cluster, gene bank, Iran.

INTRODUCTION

The Islamic Republic of Iran has a few thousand years history of silkworm rearing and sericulture. Iran collected and maintained different silkworm pure lines with different genetic potential in silkworm gene bank by means of genetic and breeding activities in the two past decades till now. It is necessary for silkworm breeders to use these resources in order to generate genetic potential supply and high potential silkworm-eggs production for farmers.

Sericulture is dependent on silkworm hybrid-egg for cocoon production commercially. We need appropriate pure lines for crosses and hybridization in order to increase the production of hybrid-eggs. One of the most important goals in silkworm breeding is larval properties improvement and reduction of larval development time, especially feeding and molting time. Reduction of larval rearing duration, first, will reduce labor costs and secondly will reduce time of silkworm exposure against pathogens and reduce larvae infection to common diseases.

As Mohammadi and Prasanna (2003) stated, genetic distance-similarity between some genotypes, populations or individuals may be calculated by various statistical measures depending on the data set (Felsenstein, 1984; Nei, 1987; Weir, 1990, 1996; Beaumont et al., 1998). Classification of pure lines of germplasm based on larval durations and determination of relationships amount of these pure lines are necessary in order to carry out successfully any silkworm breeding project. There are many

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Abbreviations: UPGMA, unweighted pair-group method using arithmetic average) methods; **ISRC,** Iran Silkworm Research Center.

pure lines in the Iran silkworm gene bank and therefore, the selection of the best parents is difficult without future scientific investigation. As a result, we must reduce the number of potential parents. Hence, classification can lead us to detect and select those pure lines that are good candidates for future usages and which can have an important role in subsequent breeding programs. To date, researches at Iran did not grouped and classified peanut cocoon strains of Iran germplasm based on larval development and characters. These classifications can improve the efficiency of parental selection as scientific. On the other hand, a number of parental pure lines have similar characteristics from the point of important larval traits and as a result, we can merge these characteristics and improve special breeding projects. These findings will achieve a future breeding research and have an effective role in silkworm rearing management. Previously, Gupta et al. (1992) analyzed 50 silkworm pure lines and classified them into 5 clusters, so that the third cluster had 34 pure lines. Meanwhile, Kumar et al. (1995) studied 46 silkworm germplasm pure lines and grouped them into 5 clusters for future breeding programs.

Iran Silkworm Research Center (ISRC) is responsible for research activities about silkworm breeding and rearing and could organize Iran silkworm germplasm. This gene bank constitutes 51 various peanut cocoon strains until 2009. At previous years, there was no research regarding larval duration and development characters, as well as classification and grouping of these pure lines based on larval traits.

The aim of this experiment was to study and classify all 51 pure lines of Iran silkworm germplasm based on larval traits and identification of pure lines relationships.

MATERIALS AND METHODS

This study was conducted in ISRC and Islamic Azad University, Ghaemshahr Branch, Iran. All insect rearing and experiments were done under the following laboratory condition at ISRC during 2008 - 2009. Eggs of the silkworm strains were obtained from ISRC and incubated at a breeding laboratory. Rearing was done in Iran Silkworm Research Center following the standard procedure described by ESCAP (1993). Each strain was reared under three replications, and after the third ecdysis, larvae were counted in 250 larvae for each replication.

Fifty one silkworm strains were used in the present study. These strains included: (1) 107-K, (2) 119-K, (3) 113-K, (4) 105, (5) 31, (6) 51, (7) 103, (8) BH-2, (9) B2-09, (10) 1003-4, (11) 1003-5, (12) 1005, (13) M2-6-22-2, (14) M2-6-18(109), (15) M-1-2(5), (16) M2-6-22(107), (17) M2-6-18.3, (18) 307-300-2, (19) 202A-204B, (20) I 20, (21) 101433-9-5, (22) 101433-1-4, (23) 101433-6-6, (24) 1126 (111), (25) 113 (2029), (26) 151 (103×M-1-1), (27) Xihang 2.3, (28) Xihang 3.3, (29) 153 (Xihang-1), (30) 5118×10133-2-2, (31) 5118×10133-3-3, (32) Black-White, (33) 101×F6, (34) F6×101, (35) Kinshu, (36) M-1-1×31, (37) 31×M-1-1, (38) M-1-1×103, (39) 103 Poly Marking, (40) Shaki, (41) 101, (42) T1-J, (43) T5-M, (44) 236, (45) 1524, (46) 1433-15, (47) 1433-9, (48) 7409, (49) N19, (50) White Larvae-Yellow Cocoon, and (51) Black Larvae-White Cocoon. Studied quantitative characteristics included larval duration (h), feeding larval duration (h), molting larval duration (h), 1-3 instars larval duration (h), 1-3 instars feeding larval duration (h), 1-3 instars molting larval duration (h), 4-5 instars larval duration (h), 4-5 instars feeding larval duration (h), 4-5 instars molting larval duration (h), 5 instar feeding larval duration (h) and cocoon spinning duration (h).

The grouping methods allowed the study to subdivide observations into several subgroups in such a way that homogeneity was obtained inside the subgroups and heterogeneity among the subgroups. Hierarchical agglomerative clustering was done by using NTSYS-pc, version 2.02 e (Rohlf, 1998) based on complete, single, UPGMA (unweighted pair-group method using arithmetic average), UPGMC and FLEXI approaches and SAS-pc (SAS, 1997) based on WARD and average approaches. However, the method of average linkage between groups (Romesburg, 1984) under UPGMA was considered as the major and final protocol for data conclusion (Sneath and Sokal, 1973) and the resulting clusters were expressed as dendrograms. This method that was employed for grouping, UPGMA, uses the average distance among all the equal genotypes for the formation of each group (Cruz and Regazzi, 2001; Zanatta et al., 2009). 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 the studied parameters together.

RESULTS AND DISCUSSION

Figures 1 - 7 obtained from the hierarchical analysis of these strains represent the studied parameters. Various methods generated similar dendograms. On the basis of these dendrograms, analyzed strains were divided into 2 distinct groups. Frequent divisions were also observed in major groups. The first group was divided into two sub groups including three and eight strains, respectively, while the second major group was divided into two sub groups including four and 36 strains, respectively.

As indicated earlier, the study's final analysis and conclusion have been done on the basis of the average linkage between groups or UPGMA, since other researchers have shown (Peters and Martinelli, 1989; Chatterjee and Data, 1992) that UPGMA yields more accurate results for classification purposes than other hierarchical methods. Thus, the present paper also presents the result of other clustering approaches.

As Mohammadis and Prasanna (2003) stated, more comprehensive definition of genetic distance is "any quantitative measure of genetic difference, which may be at the sequence level or the allele frequency level that is calculated between individuals, populations or species" (Beaumont et al., 1998; Mohammadis and Prasanna, 2003). This kind of classification for determining the best candidates can produce the maximum amount of heterosis with the most distant genetics and is very important (Etebari et al., 2005).

In the present study, we developed quantitative approaches and analyzed 51 silkworm strains with different geographical distribution. From obtained results, the silkworm strains could be clustered into different groups according to the geographic areas that were initially observed. As a result of the low effective population size

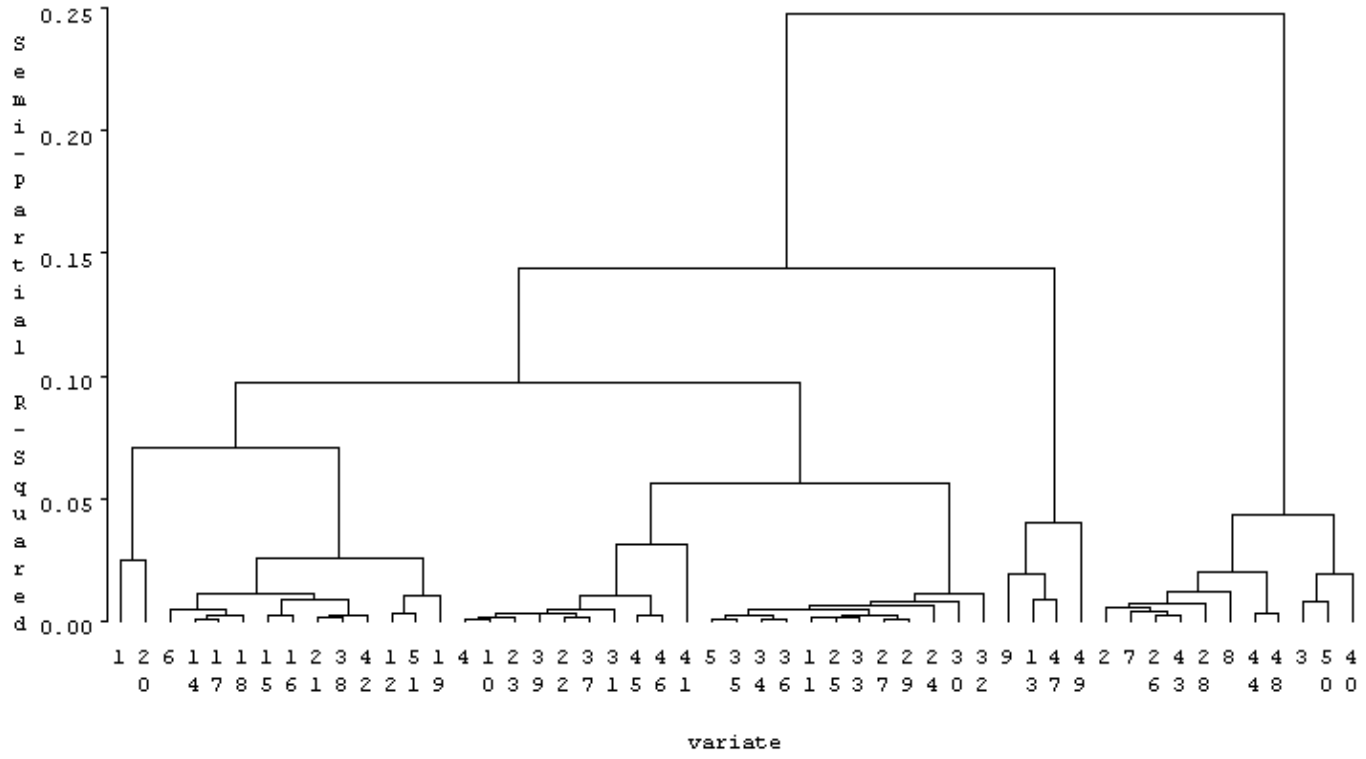

Figure 1. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from WARD method using SAS.

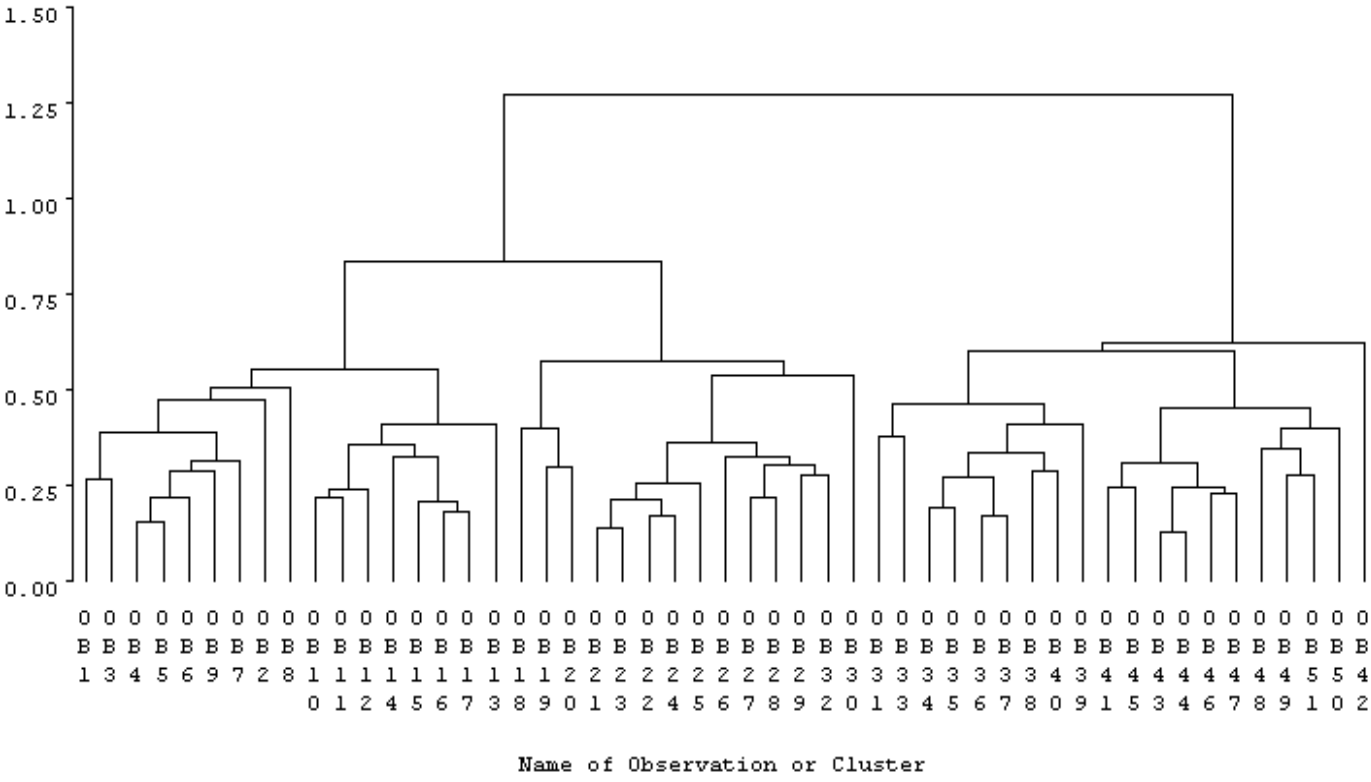

Figure 2. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from average method using SAS.

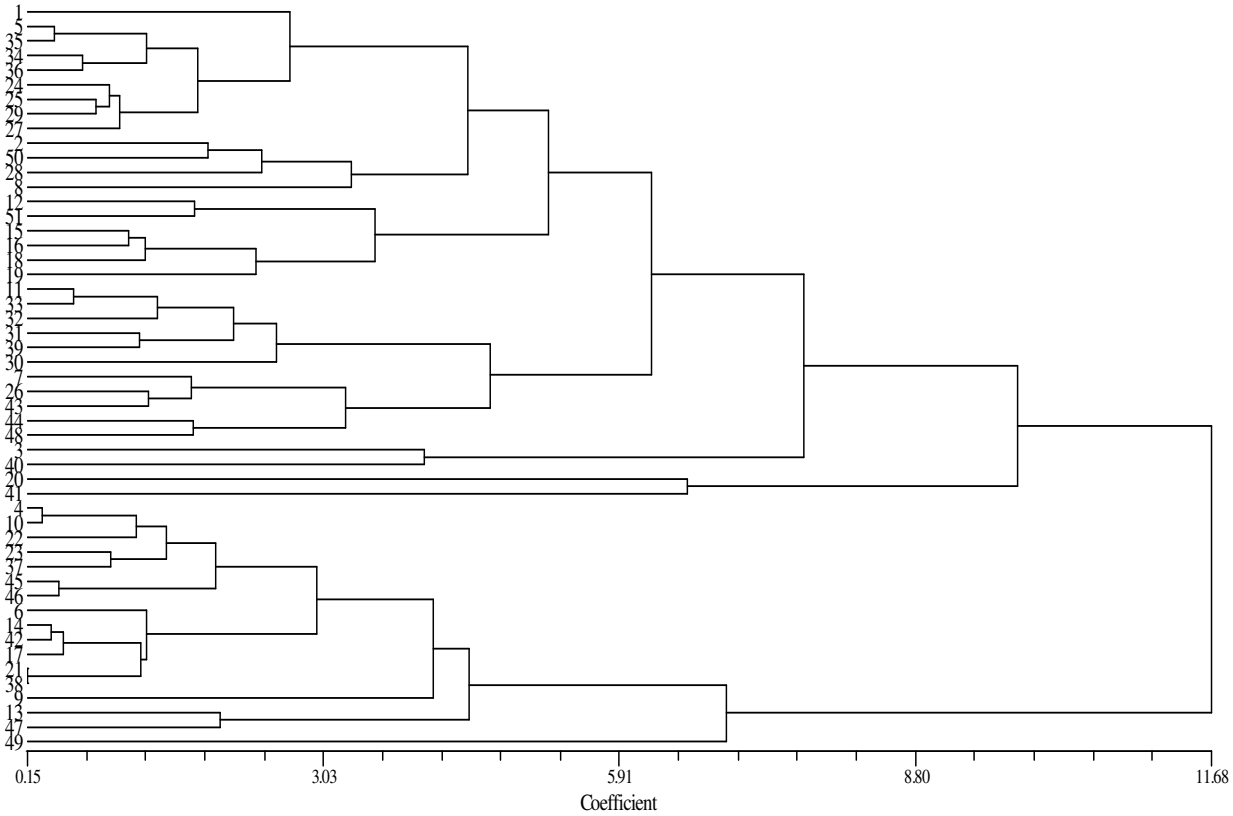

Figure 3. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from complete method using NTSYS.

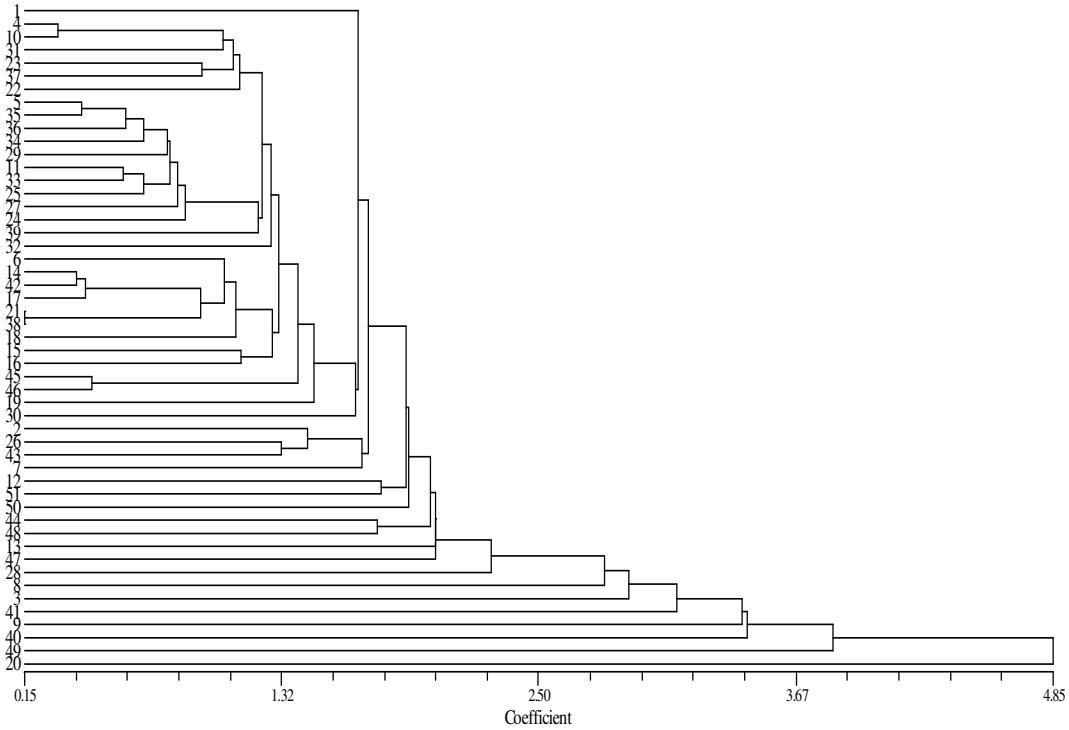

Figure 4. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from single method using NTSYS.

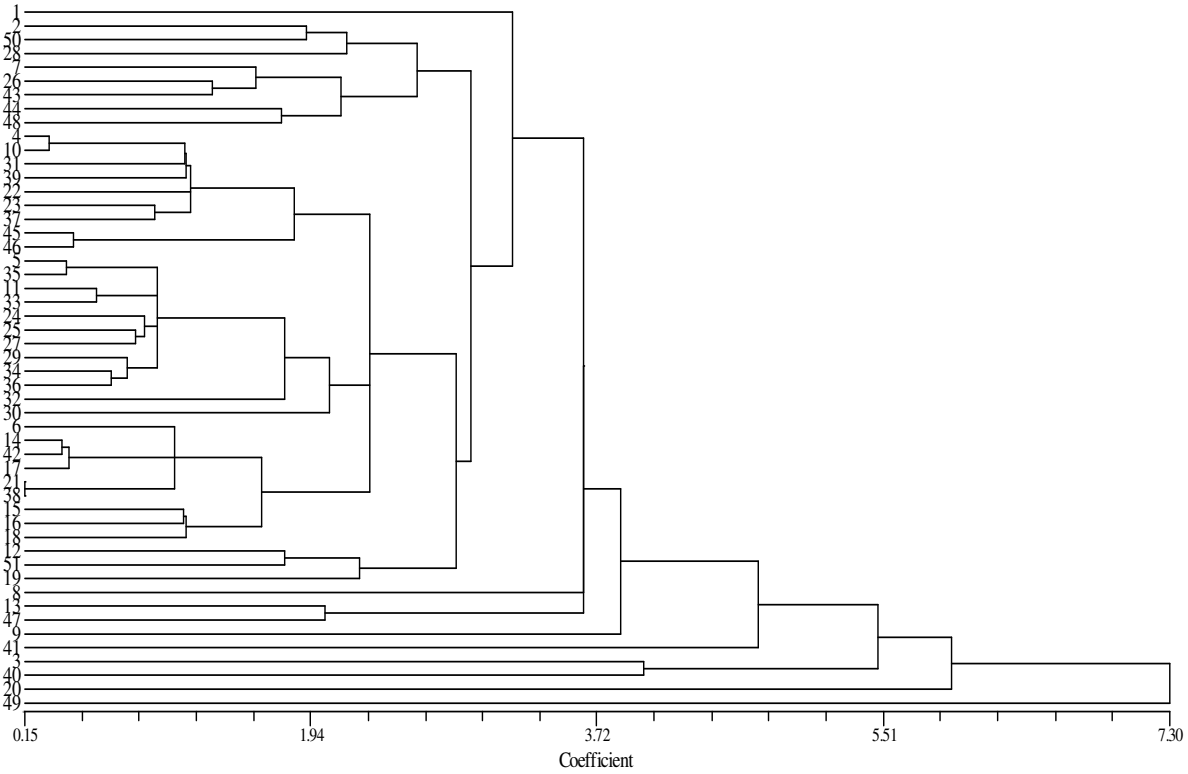

Figure 5. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from UPGMC method using NTSYS.

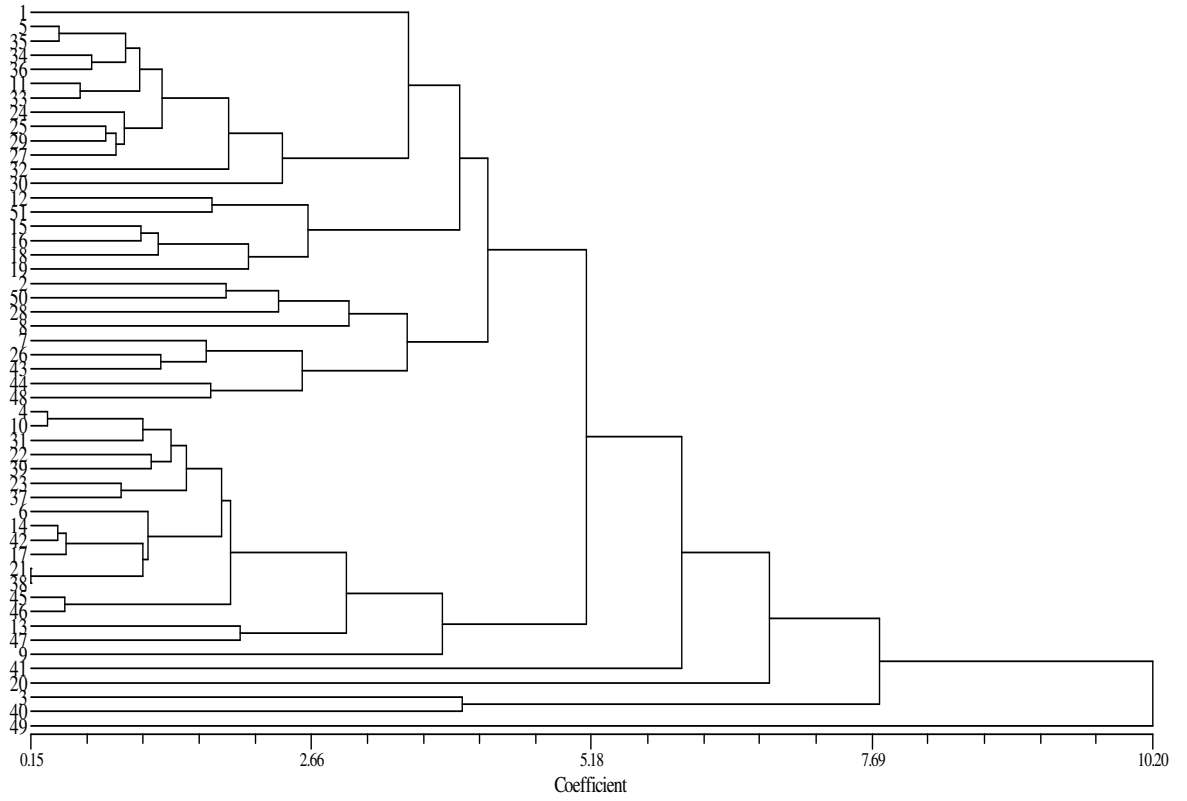

Figure 6. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from FLEXI method using NTSYS.

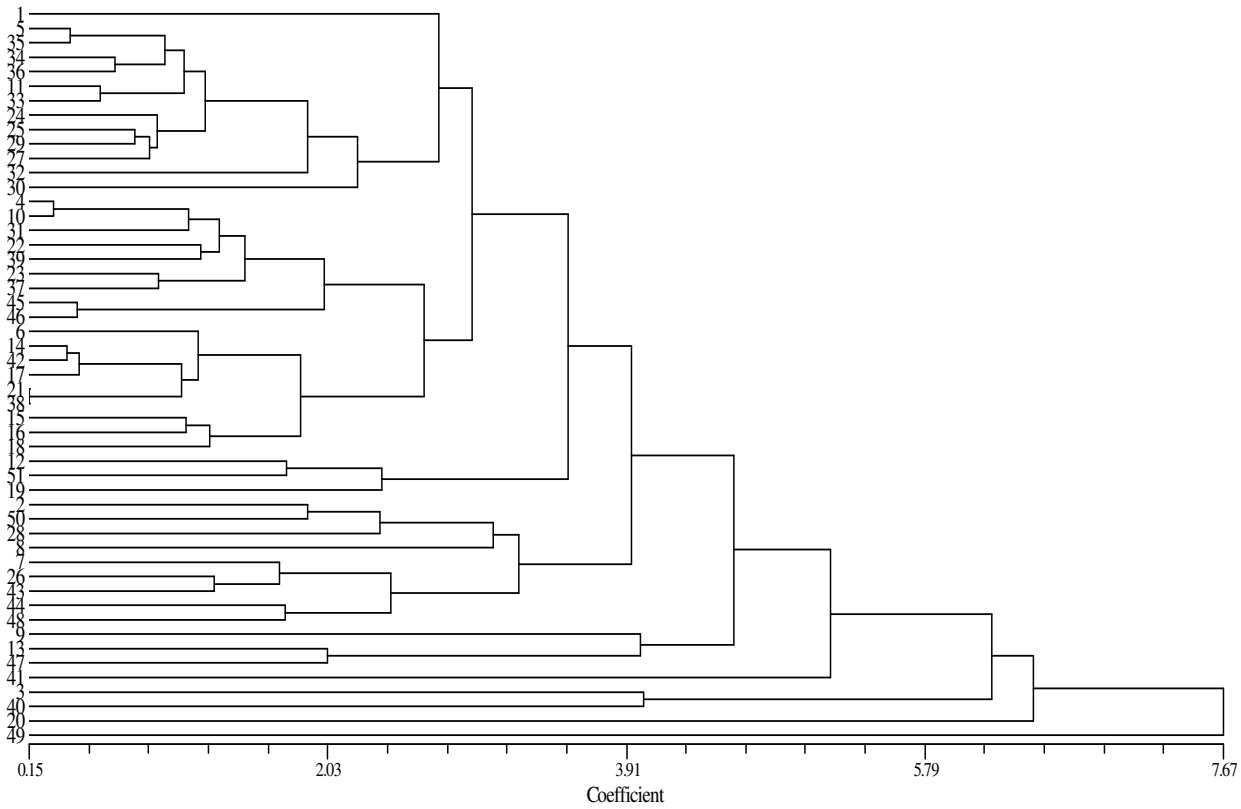

Figure 7. Cluster analysis based on all 11 studied larval phenotypic data and larval duration traits for 51 silkworm strains according to the grouping from UPGMA (unweighted pair group method average) method using NTSYS.

and each female mating only with one male (thus, all her offspring are full-sibs), the inbreeding rate is very high. However, this could cause more differentiation among these strains (Falconer, 1989; Mirhosseini et al., 2007).

As Chatterjee and Data (1992) presented, domesticcation has played a major role in genetic diversification of silkworm (Gamo 1983; Chatterjee and Data, 1992). As sericultural regions of the world have different climatic conditions, physiological diversification has also been influenced by agro-climatic factors. Thus, given geographic isolation and limited cultural exchange, some strains may have acquired similar genotypes due to similar selection pressures. On the other hand, there is still a possibility that there were exchanges of genetic material between the two countries at an earlier time (Chatterjee and Data, 1992). Comparable examples are also available in the present analysis.

The above results revealed that the inclusion of genotypes of the same origin in different clusters clearly indicate the presence of considerable genetic diversity among the population used in this study (Kumaresan et al., 2007).

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