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Examination of Genetic Relationships of Silkworm Stocks in Korea by Additive Isozyme Analysis

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Previously, genetic relationships among 321 silkworm races preserved in the Department of Sericulture and Entomology, NIAST, RDA were studied using six isozymes comprising 20 loci. As a part of additional studies, three additional isozymes with 7 loci [phosphoglucomutase (PGM), glucose phosphate isomerase (GPI), malete dehydrogenase (MDH)] from hemolymphs, midgets, and eggs were employed to reevaluate the previous dendrogram (UPGMA method). Although the current study supported the recognition of the previously identified major groups, some minor changes were apparent among the selected 47 forms. The current study showed that the polymorphic loci of GPI from eggs and MDH from hemolymph appear to be responsible characters for generating a new major group in the genetic relationship. Interpreting the data with current additive isozymes may represent more robust genetic relatioships among 321 silkworm races preserved in Korea, until further evidence is available.

Key words: Isozyme analysis, *Bombyx mori*, Silkworm races, Phylogenetic analysis

Introduction

The silkworm which has been bred in farmhouse is known as the result of natural and artificial selection of primitive silkworm which was derived from wild silkworm. There are many mutant races, except for the commercial races, and the races are classified into Chinese, Japanese, European and tropical races by their geographical origin. NIAST is preserving more than 300 stocks of silkworm. The races will be important genetic resources for development of the insect industry in the future, since they have individual genetic characters.

The most important factor in biological genetic resource conservation regimes is to maintain pure strain of each species and establish accurate genetic relationship among species. Traditional criteria for the classification of silkworm races were morphological and ecological characteristics such as egg color, cocoon color, moltinism and voltinism. However, biochemical and microbiological techniques such as isozyme polymorphism analysis (Eguchi *et al.*, 1988; Kanekatsu *et al.*, 1992; Takeda *et al.*, 1993, Seong, 1997) and DNA polymorphism analysis (Kim *et al.*, 2000; Yoon *et al.*, 2001) are currently used as silkworm species classification criteria.

Seong (1997) made genetic distance dendrogram of silkworm races using the results from electrophoresis isozyme polymorphism analysis of 20 loci such as silkworm blood, midguts, digestive fluid, etc. in 300 preserved silkworm races. And the number of identified loci is increased by addition of new polymorphic isozymes in recent study, hence, it is expected that more accurate comparison of genetic relationship is possible.

In this study, 47 races were randomly selected on the basis of pre-existing genetical distance data, from isozyme analysis of 20 loci in 300 preserving silkworm races, and isozyme polymorphism of these races were analyzed with 7 additional loci. To understand the change of grouping pattern and the cause for the change, the newly obtained dendrogram was compared with previous dendrogram obtained by 20 loci. Based on the result, we tried to suggest more accurate silkworm classification method.

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Table 1. Data matrix of mono- and polymorphic loci summerized for 47 silkworm stocks

No.	Strains	Hemolymph													Midgut						Digestive juice				Egg			
		G_6PDH	*Est_1*	*Est_2*	*Acp_1*	*Acp_2*	*Amy*	*P_1*	*P_2*	*P_3*	*P_4*	*P_5*	*GPI**	*MDH**	*Est_1*	*Est_2*	*Alp_1*	*Alp_2*	*Suc_1*	*Suc_2*	*GP_1**	*Amy_1*	*Amy_2*	*Amy_3*	*GPI**	*MDH_1*	*MDH_2*	*PMG**
1	N74 (J)	11	12	00	00	00	00	0	1	0	1	1	23	11	12	00	33	11	11	11	23	11	00	0	23	11	12	11
40	Heukjam (J)	11	12	00	00	00	00	0	1	1	0	1	22	11	22	00	34	11	11	11	23	00	11	0	33	12	22	11
18	Shansurian (E)	11	12	00	12	00	00	0	1	0	0	1	33	00	12	11	14	11	11	11	33	11	00	0	11	22	12	11
75	Kyunsakjuk (E)	11	12	00	12	00	00	1	0	1	1	1	22	22	13	11	14	11	11	11	12	00	00	0	33	34	12	11
110	C14 (C)	11	12	00	12	00	22	0	1	0	1	1	33	22	34	11	33	11	11	11	13	00	13	0	33	23	12	11
81	Chungchun (C)	11	11	00	12	00	22	0	1	1	0	1	22	22	12	11	33	11	11	11	23	00	13	0	33	22	12	11
184	RHS (J)	11	00	11	00	22	23	1	1	0	0	1	22	11	13	11	14	11	11	11	12	13	13	1	11	12	22	22
94	Suwonjam101(J)	11	12	00	00	00	22	0	1	0	0	1	33	22	13	11	23	11	11	11	33	00	11	0	33	44	12	11
154	Ihenalig (E)	11	12	00	12	00	22	0	1	1	0	1	33	22	22	00	22	11	11	11	33	00	11	0	33	44	22	11
222	LY (C)	11	11	11	00	00	33	0	1	0	0	1	23	22	13	11	14	11	11	11	33	00	13	0	33	33	12	11
212	CHibakran (C)	11	12	00	12	00	22	0	1	1	0	1	33	22	13	11	12	11	11	11	33	13	00	0	33	44	12	11
2	N76 (J)	11	12	00	00	00	11	0	0	1	1	1	33	00	12	11	33	11	11	11	33	11	00	0	22	34	12	11
105	Chinese (E)	11	12	00	00	22	11	0	0	1	1	1	22	11	13	11	14	11	11	11	23	00	11	0	33	24	22	22
269	CH2 (C)	11	12	00	00	22	22	0	1	1	0	1	33	22	13	11	23	11	11	11	33	00	13	0	33	44	12	22
144	DY (J)	11	11	00	00	22	12	0	1	1	0	1	22	12	13	11	14	11	11	00	23	00	13	1	33	24	22	11
207	Hansungbanmun(C)	11	12	00	00	22	22	0	1	0	0	1	33	12	13	00	14	11	11	11	33	00	13	0	33	34	12	11
217	Bukak (J)	11	12	00	00	11	22	0	1	1	0	1	22	22	13	11	12	11	11	00	23	00	13	1	33	22	12	11
219	Soyang (C)	11	12	00	00	22	33	0	1	0	1	1	22	22	22	11	13	11	11	11	22	00	13	0	33	22	12	11
238	C44 (C)	11	00	11	00	23	22	0	1	0	0	1	33	00	13	11	14	11	11	11	33	00	13	0	33	44	12	11
206	E56 (E)	11	11	11	12	22	33	0	1	0	1	1	33	22	13	11	12	11	11	11	33	00	13	1	33	44	12	11
52	Rok1042 (E)	11	12	00	00	22	22	0	0	1	1	1	22	22	22	11	00	11	11	11	22	00	11	0	33	24	12	11
245	IJPE (E)	11	12	00	12	22	33	0	1	0	1	1	11	22	13	00	22	11	11	00	11	00	12	0	33	11	12	11
54	Myun49 (J)	11	12	00	00	22	33	0	1	0	1	1	22	22	12	11	23	11	11	00	22	00	11	0	33	22	12	11
228	SA2 (T)	11	12	00	00	23	22	0	1	1	0	1	33	22	24	11	14	11	11	11	33	13	00	0	33	44	22	11
63	Usungjukei (J)	11	12	00	00	22	22	0	0	1	0	1	22	22	12	11	00	11	11	11	22	13	00	0	33	22	12	11
157	C7 (C)	11	12	00	00	22	00	0	1	1	1	1	22	00	12	00	23	11	11	11	22	00	11	0	11	22	12	11

Table 1. Continued

No.	Strains	Hemolymph												Midgut						Digestive juice				Egg			
		G_6PDH	*Est_1*	*Est_2*	*Acp_1*	*Acp_2*	*Amy*	*P_1*	*P_2*	*P_3*	*P_4*	*P_5*	*GPI**	*MDH**	*Est_1*	*Est_2*	*Alp_1*	*Alp_2*	*Suc_1*	*Suc_2*	*GP_1**	*Amy_1*	*Amy_2*	*Amy_3*	*GPI**	*MDH_1*	*MDH_2*	*PMG**
191	N19 (J)	11	12	00	00	22	22	0	1	0	0	1	11	22	13	11	11	11	11	11	13	13	00	0	23	11	12	11
108	C3 (C)	11	12	00	00	22	00	0	1	1	0	1	22	00	13	11	33	11	11	11	22	13	00	0	11	22	12	11
3	Sammyunjam(K)	11	11	00	00	22	11	0	1	0	0	1	33	22	12	00	33	11	11	11	23	00	13	0	33	44	22	11
260	Sammyunhong(K)	11	12	00	00	22	00	0	1	1	1	1	33	22	13	00	34	11	11	11	33	00	13	1	23	34	12	11
12	Kumkwangju (J)	11	12	00	00	22	00	0	1	0	1	1	33	12	22	11	00	11	11	11	22	00	00	0	33	24	12	11
62	BakanEBK-waingzi.(J)	11	11	00	00	22	11	0	1	0	1	1	22	12	12	00	12	11	11	11	23	00	00	0	33	34	12	11
167	Jam101 (J)	11	12	00	00	11	00	0	1	0	0	1	22	22	13	11	14	11	11	11	23	00	12	1	33	24	12	11
279	AP (E)	11	11	00	00	33	22	1	0	0	0	1	22	22	13	11	14	11	11	11	22	13	12	0	33	24	12	11
30	Pedts (E)	11	12	00	00	33	22	0	1	0	1	1	12	22	34	11	12	11	11	11	11	00	11	0	33	11	12	11
159	GalH (C)	11	12	00	00	22	33	0	0	1	1	1	12	22	34	11	14	11	11	00	33	00	11	1	33	34	12	11
150	Hwangyu (J)	11	12	00	00	33	22	1	0	1	1	1	23	22	34	00	12	11	11	11	23	00	00	0	33	22	12	11
43	Crimson (C)	11	12	00	00	22	22	0	1	0	0	1	22	22	34	11	33	11	11	11	23	00	11	0	33	22	12	11
88	Chunmun (C)	11	12	00	00	22	33	0	1	0	1	1	33	11	34	00	14	11	11	11	33	11	00	0	33	34	12	11
11	Hansammyun (K)	11	00	11	12	33	33	0	0	0	1	1	33	22	23	00	00	11	11	11	33	00	13	0	33	44	12	11
242	E58 (E)	11	00	11	00	11	22	0	0	1	1	2	33	00	24	11	14	11	11	11	33	00	13	0	11	34	12	11
118	Jam103 (J)	11	12	00	00	22	22	0	1	0	1	1	33	12	13	11	14	11	11	11	33	33	00	1	33	44	12	11
134	C27 (C)	11	11	00	00	22	22	0	1	1	0	1	33	22	13	11	12	11	11	11	23	33	11	0	33	23	12	11
93	Woongjinhi (C)	11	12	00	00	22	22	0	1	0	1	1	11	22	24	11	14	11	11	11	11	33	11	0	33	11	22	11
127	I-NOVI (E)	11	12	00	00	12	22	0	1	0	1	1	22	12	13	11	14	11	11	00	23	33	13	0	33	24	12	11
39	7 (C)	11	12	00	00	22	33	1	1	0	1	2	33	22	13	11	11	00	11	11	33	22	11	0	22	44	12	22
15	Bagdad (E)	11	11	00	00	22	22	0	1	0	1	1	22	12	34	11	14	11	11	11	23	33	00	0	33	44	12	11

Aristerisks stand for the newly obtained loci by additive isozyme analysis from the original data (Seong, 1997).

Abbreviations C, E, J, K and T represent Chinese, European, Japanese, Korean and Tropical race of silkworm stocks, respectively.

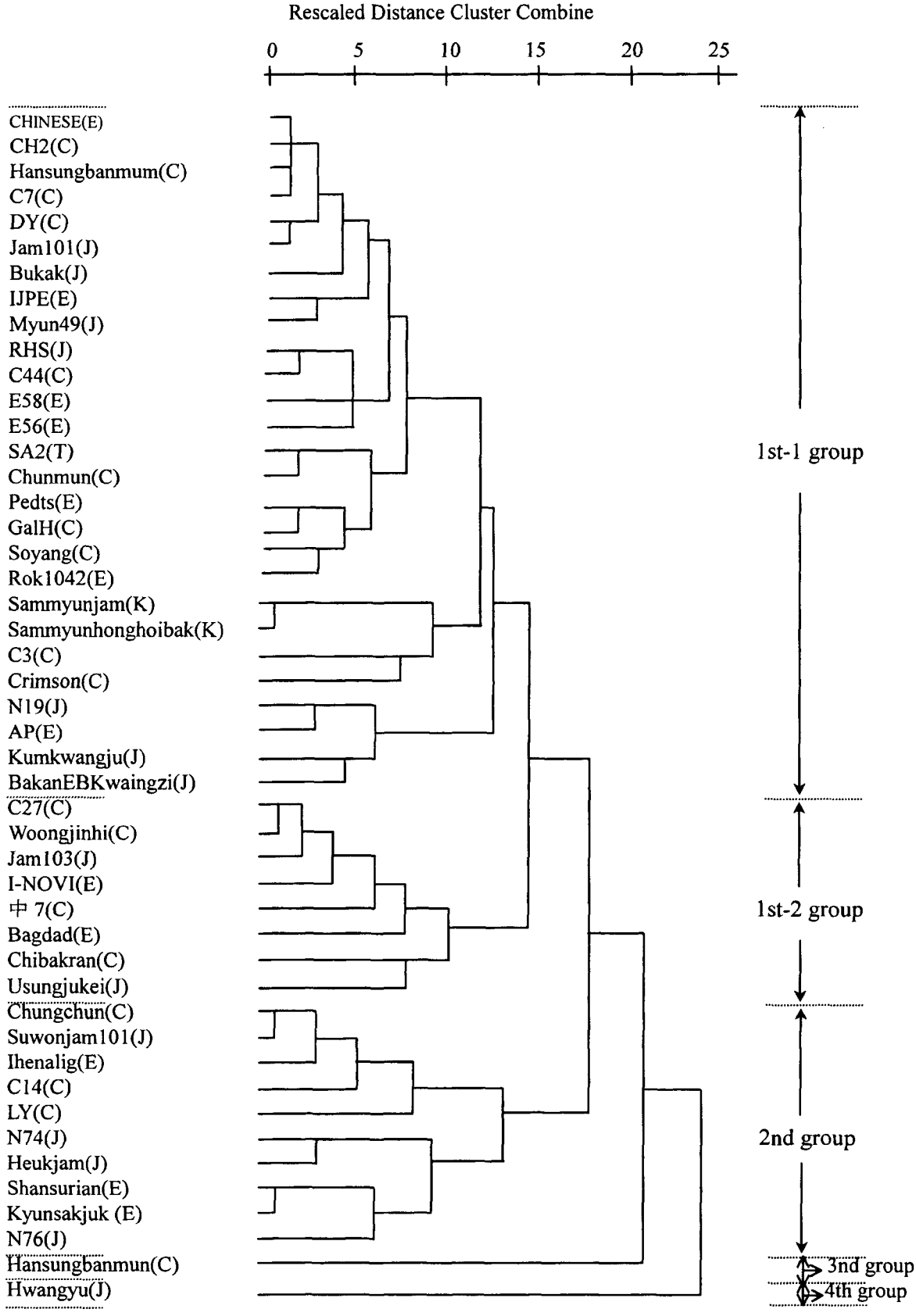

Fig. 1. Genetic similarity dendrogram for 47 silkworm races based on the original data (20 loci, Seong, 1997).

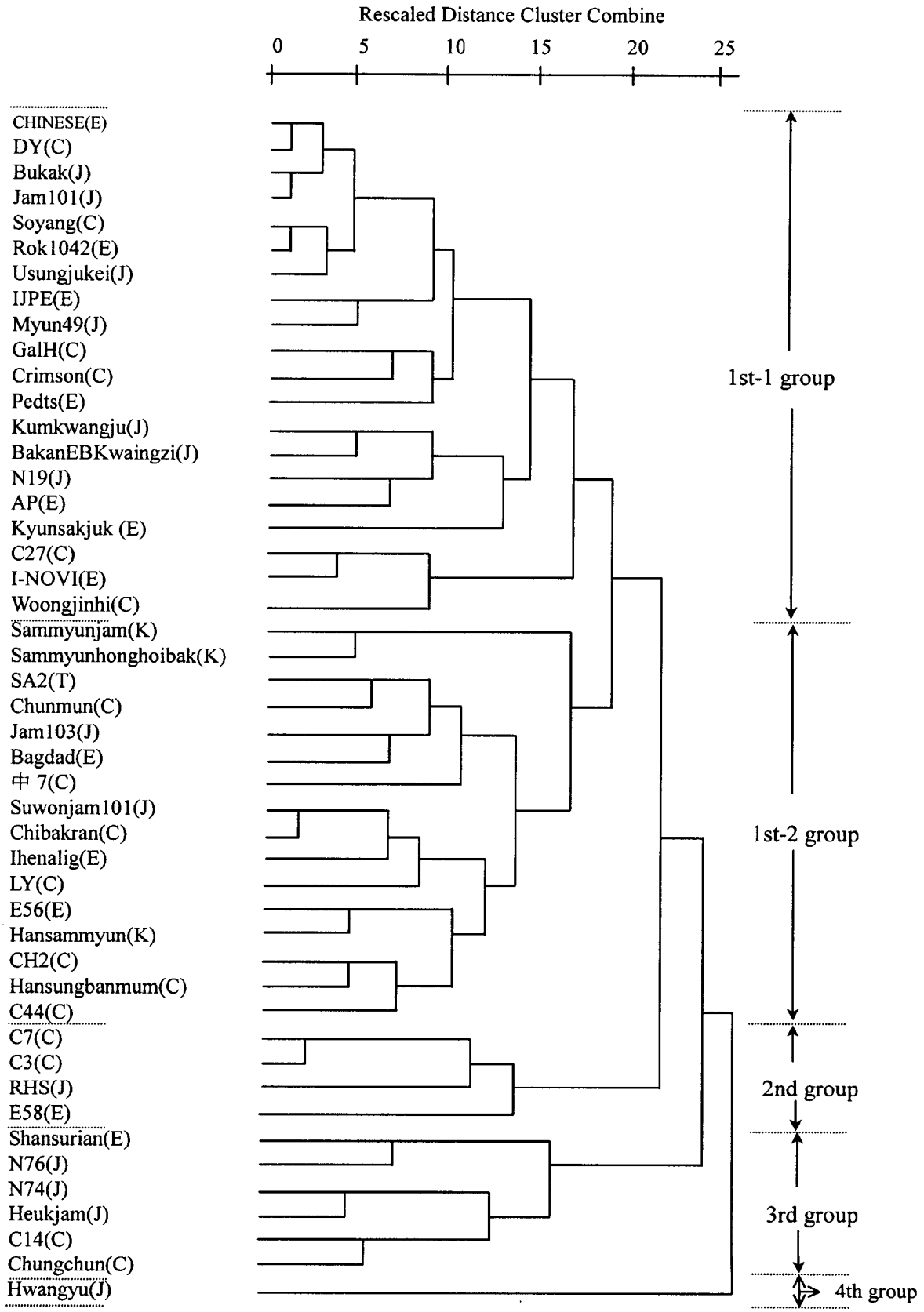

Fig. 2. Revised genetic similarity dendrogram for 47 silkworm races based on the 27 loci.

Materials and Methods

Silkworm races and breeding

Forty-seven silkworm races were randomly selected, considering genetic relationship among races from genetic dendrogram of 20 loci established by Seong (1997). The geographic origins of 47 races were China (16), Japan (15), Europe (12), tropical (1) and Korea (3), respectively. The female larvae of selected races were bred by mulberry leaves, and sampled at 5th day of 5th instar. The quantity of sample obtained from female larvae is larger than from male larvae, so female larvae were used.

Isozyme analysis

Three additional isozymes which exhibit polymorphism in eggs, hemolymps and midguts were detected to use isozyme polymorphism analysis. The added three isozymes were phosphoglucomutase (PGM), glucose phosphate isomerase (GPI), malete dehydrogenase (MDH), and from these isozymes 7 loci which exhibit new polymorphism in eggs, hemolymps and midgut were identified.

Methods for enzyme fluid extraction for isozyme analysis, electrophoresis, enzyme detection and marking of isozyme polymorphism were based on Seong (1997).

Genetic relationship analysis and dendrogram establishiment

Data matrix was made based on isozyme polymorphism of 47 races. Euclidean distance UPGMA (unweighted pair-group method using arithmetic average) method in SPSS program was used to analyze genetic relationship and genetic distance dendrogram was made.

Results and Discussion

Seven new loci were newly identified from three additional isozyme (PGM, GPI, MDH) analysis; two loci in hemolymps, one locus in larvae midgut and three loci in eggs, respectively. Adding polymorphism of 7 new loci to exiting polymorphism of 20 loci, an integrated data matrix was made (Table 1). Then, genetic relationship analysis was conducted based on Table 1, using Euclidean distance UPGMA method in SPSS program (Norusis, 1986). With these results, genetic distance dendrograms of existing 20 loci and new 27 loci were established, respectively (Fig. 1 and 2).

Genetic distance dendrogram (Fig. 1) of 20 loci isozyme polymorphism in 47 races is a simplified version of large scale genetic distance dendrogram in 303 races established by Seong (1997), and consisted of four large clusters. Distribution of races in each group is 35 races in group 1, 10 races in group 2, 1 race in group 3 and 1 race in group 4. Most of races were included in group 1 (74%). Group 1 could be divided into 2 sub-groups, group 1-1 and group 1-2, 27 and 8 races were included in these two sub-groups. Table 2 shows geographical origin of races in each groups.

Dendrogram of 27 loci also shows four large classification groups (Fig. 2). Distributions of races was 36 races in group 1, 2 races in group 2, 6 races in group 3, 1 race in group 4. In this case, group 1 was also largest among the four groups. Table 3 shows the origins of each group

Table 2. Distribution of geographical silkworm races of 47 races from the original data (Seong, 1997)

Group	Origin	Chinses	Japanese	European	Korean	Tropical	Total
1st group	1-1	9	8	7	2	1	27
	1-2	4	2	2	0	0	8
2nd group		3	4	3	0	0	10
3rd group		0	0	0	1	0	1
4th group		0	1	0	0	0	1
Total		16	15	12	3	1	47

Table 3. Distribution of geographical silkworm races of 47 races based on more selected loci (27 loci)

Group	Origin	Chinses	Japanese	European	Korean	Tropical	Total
1st group	1-1	5	8	7	0	0	20
	1-2	7	2	3	3	1	16
2nd group		2	1	1	0	0	4
3rd group		2	3	1	0	0	6
4th group		0	1	0	0	0	1
Total		16	15	12	3	1	47

in Fig. 2.

The main purpose of this study was to identify the effect of loci increment on genetic relationship of races. When the two dendrograms were compared, each dendrogram had four large classification groups. However, the feature of each dendrogram was somewhat different. First, 4 races in group 2 such as Suwonjam, LY, Iherlig, Kyunjuksak grouped together with group 1 and the rest moved into group 2 in the Fig. 1. Second, 4 races (C7, C3, RHS, E58) were separated from group 1, and consist group 2 in Fig. 2. Third, Hansammyun the Korean traditional race which was included in group 3 was grouped together with group 1.

However, the new dendrogram sustained the basic structure of previous dendrogram, except for generating new group (group 2) and movement of some race between groups. This meant that isozyme polymorphism in 7 loci added from isozyme analysis did not cause a significant change in the structure of exiting dendrogram. The results also suggest that previous isozyme analysis of 300 silkworm races was somewhat stable criterion for classification of silkworm races. But it is expected that additional isozyme analysis will be needed to increase the confidence of classification.

Analyzing the causes of races movement, in the case of four races C7, C3, RHS and E58 which was separated from existing group 1 and established new group 2, the major cause of movement was unique isozyme polymorphism of MDH in hemolymp and GPI in eggs. But in case of the other four races such as Suwonjam, LY, Ihenalig and Kyunjuksak which were included in group 2 in previous dendrogram, they moved into group 1 by additional isozyme analysis. It seems that the polymorphism characteristics of these races were changed by additional isozyme analysis and the changed characteristics were similar to those of group 1, so they moved to group 1.

One of the races newly included in group 1 is the Hansammyun, Korean traditional race which was in group 4 in previous dendrogram. In the previous study, it was expected that Hansammyun would be useful material for revealing origin of Korean races and process of differentiation. But this race also was included in group 1 with other Korean traditional races such as Sammyunjam and Sammnyunhonghoibak. The cause of change is that the characteristics of Hansammyun analyized by additional isozyme analysis was similar to those of group 1. However, Hwangyu the Japanese race remained in group 4 separately showing the farthest genetic distance from other races, in spite of addition of new additional isozyme loci. So additional study for analyzing the origin and characteristics of the race is expected.

Increasing loci by additional isozyme analysis made the classification of silkworm more stable through movement of races between groups and establishment of new group, while maintaining the basic structure of previous dendrogram. From the result, it is expected that more reliable criterion for classification of 321 races in Korea is established at isozyme level.

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