TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2022) 46: 429-438

10.55730/1300-0128.4191

Determining the genetic diversity of silkworm lines in Turkey

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Received: 11.10.2021 • **Accepted/Published Online:** 08.03.2022 • **Final Version:** 13.06.2022

Abstract: Studies involving molecular genetic characterization of silkworms are quite limited. Therefore, with this study, pure and hybrid silkworm lines raised in Turkey was characterized for the first time by microsatellite DNA markers. Samples were collected from 7 different silkworm lines, of which the pure lines were obtained from the Provincial Directorate of Agriculture and Forestry in Bursa and the hybrid ones from Silkworm Cocoon Association of Turkey (Kozabirlik). DNA samples were extracted from silkworm eggs. Genotyping was done by combining a total of 16 microsatellite markers in a single multiplex. Two hundred and eighty-one alleles were observed for 16 microsatellite loci. The highest allele number was observed for the T01CTA07R locus (30), while the highest effective allele number (17.61) and the highest polymorphic information content (0.94) were observed for the FL0612 locus. The expected heterozygosity values (He) were in the range of 0.72 (K02) to 0.95 (FL0612). The highest and lowest observed heterozygosity values (Ho) ranged between 0.52 and 1.00. When all lines were considered a single population, only three of the sixteen loci were in the HWE. As a result of this study, successful genotyping was performed with 16 microsatellite loci combined in a single multiplex using 3 different fluorescent dyes. The high polymorphism values obtained for the loci used showed that these loci can be used effectively in the genetic identification of silkworm breeds or lines. This study is expected to fill in the gaps in the related literature as well as to contribute to the conservation of silkworm local genetic resources.

Key words: Silkworm, Bombyx Mori, microsatellites, genetic polymorphism, Turkey

1. Introduction

Silkworm (*Bombyx Mori*) is the general name given to the caterpillar of a butterfly for silk [1]. In Turkey, silkworm breeding is carried out as an auxiliary agricultural activity that is carried out on a small scale by families with limited income. The wild silkworm that came to Anatolia by the historical 'Silk Road' route is known as '*Bombyx mandarina*'. The Japanese line of this breed is called "*Japanese Bombyx mandarina*" and the Chinese line is called "*Chinese Bombyx mandarina*". The domestic silkworm is named '*Bombyx Mori*' which has been reported to originate from the Japanese line [2].

Silkworm cultivation and silk embroidery was carried out in 15th and 16th centuries in many cities especially Bilecik, Bursa, and Eskişehir [3,4,5]. In the 16th century, Anatolia reached its golden age in terms of silk and silk products. Some of the raw material of the woven silk was supplied from China and Iran. Thus, the production of silk fabrics in Anatolia reached its peak in the world. In the middle of the 19th century, most of the production was centered around Bursa [5,6].

Over time, many countries formed their own hybrid lines. Shunrei x Shogetsu Japanese hybrid, Vratza 35 x Merefa 2 Bulgarian hybrid, Ukr. 27 x Ukr. 15 known as Ukrainian hybrid are among these hybrid lines [7]. In 1953, box of 5 polyhybrid seeds was brought to Turkey from Italy [5]. These hybrid lines that are used today in Turkey are called N x M and M x N. A hybrid line in Japan has been brought to our country for testing since 1962 with the efforts and contributions of the Cocoon and Silk Insect Breeding Institute. Positive improvements have been observed on this polyhybrid and the domestic silkworm breeding named ''Bursa Beyazı" was terminated and polyhybrid silkworm breeding was started. In 1964, the first polyhybrid seed production was started by the help of Japanese technical personnel [5,8,9]. This hybrid was obtained by crossing "Chinese strain" (M) silkworms with high silk yield and "Japanese strain" (N) silkworms with high resistance to diseases.

These lines are obtained by crossing Chinese female and Japanese male or Chinese male and Japanese female. Then these crossbred lines are distributed free of charge to all manufacturers in Turkey after wintering period.

Microsatellites (SSR or STR) are simple sequence repeats of 1-6 bases that are abundant in the coding and

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noncoding regions of all eukaryotic and some prokaryotic genomes [10]. Microsatellites have been useful markers in many disciplines where fine-scale determination of genetic makeup is required due to the allele hypervariability of these markers, side sequencing and codominant inheritance style, and recent advances in PCR technology [11,12,13]. Numerous microsatellite loci have been identified and definitions have also been made within the silkworm [14,15].

Therefore, in this study, genetic polymorphisms within and differences between silkworm lines farmed in Turkey as pure and hybrid were determined by using microsatellite DNA markers. It was aimed to define the current genetic structure of silkworm lines cultivated in Turkey with microsatellite DNA markers for the first time, to contribute to the relevant literature and to guide the conservation programs for the protection of domestic lines as genetic resources.

2. Materials and methods

2.1. Materials

The animal material of the study consists of 83 samples from a total of 7 silkworm lines; 3 pure lines (Bursa Beyazı, Hatay Sarısı, Bursa Alaca) belonging to Genetic Resources Conservation Program directed by the Bursa Provincial Directorate of Agriculture and Forestry, 2 pure lines (M and N) and two of their hybrids (M x N, N x M) produced by Silkworm Cocoon Association of Turkey (Kozabirlik). A total of 83 silkworm samples were used in genetic characterization (Table 1). DNA samples were obtained from 50 silkworm eggs from each individual.

2.2. Methods

2.2.1. DNA extraction and quantification and quality assessment

DNA extractions from the silkworm eggs were performed with the DNA extraction kit (Qiagen) according to the manufacturer's protocol. The DNA quantity (260 nm) and quality (260 nm/280 nm ratio) was assessed using a NanoDrop ND-2000 UV-Vis Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE).

2.2.2. Target site amplification by polymerase chain reaction (PCR)

Sixteen microsatellite DNA loci selected from the microsatellites reported in the literature were used for genetic analyses. By labeling only one of each primer pairs with one of 3 different fluorescent dyes (D2, D3 and D4), 16 markers were genotyped in a single reaction with the multiplex PCR. Detailed information on the multiplex group formed by the microsatellites and the fluorescent dyes used for labeling are given in Table 2.

The 25-µL PCR reaction mix included: PCR buffer (1X), primers (2.0 mM), dNTP (16.8 mM), MgSO4 (2.0 mM), Taq DNA polymerase, genomic DNA (100 ng) and ddH2O. Touch-Down (TD) PCR conditions given in Table 3 were used in order to perform DNA amplification more efficiently for multiplex that contains primer pairs of all microsatellites used in the study.

2.2.3. Prescreening of PCR products by electrophoresis

PCR products were first exposed to gel electrophoresis using a 2% agarose gel in the presence of an appropriate size ladder, and then photographed with a gel imaging and documentation device. After optimizing the procedure in the existing thermal cycler with the modifications made in the amount of PCR elements and clear visuals in the agarose gel, the stage of genotyping of all samples with fragment analysis was started.

2.2.4. Fragment analysis

Capillary electrophoresis was used for the separation and sizing of the PCR fragments obtained from forward primer labeled with one of the cyanine-based fluorescent dyes by using the Beckman Coulter GeXP genetic analyzer (Beckman Coulter, Inc., USA). GenomeLab™ DNA Size Standard Kit 400 was used for determination of fragment sizes.

Five microliters of PCR product, 0.5 µL of size standard 400 and 34.5 µL SLS (Sample Loading Solution) were put into each well in order to load PCR products to the genetic analyzer. A drop of mineral oil was applied into each well preceding loading the samples to the Beckman Coulter GeXP analyzer in order to prevent the mixture from evaporation when it reaches 90–120 °C during the denaturation phase of the device.

2.2.5. Detection of genotypes and statistical analysis of genetic data

Fragment lengths obtained as a result of fragment analysis were examined and genotype information belonging to individuals was retrieved from the computer environment. Allele number (Na), average allele number (MNA), effective allele number (Ne), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), mean heterozygosity ($\hat{H}$), fit to Hardy–Weinberg equilibrium, Wright's F-statistics (FIT, FIS, FST) [24,25] and null allele frequencies were obtained by using GenAlEx [26,27], POPGENE [28] and CERVUS 3.0.3[1] [29] programs. Dendrogram showing the genetic distances between lines were obtained from Population 1.2.32[2] and

[1] Marshall TC. Cervus, 3.0, Cervus is a computer program for assignment of parents to their offspring using genetic markers. Cervus, a Windows package for parentage analysis using likelihood approach. CERVUS was written by Tristan Marshall (1998/2006) http://www.fieldgenetics.com.Acces date: 09.07.2018; 2006.

[2] Langella O. Populations, 1.2.32. available via http://bioinformatics.org/~tryphon/populations/ Access date: 09.07.2015; 1999.

Table 1. Silkworm lines and sample numbers (n) used in this study.

Lines	n
Bursa Alaca	12
Bursa Beyazı	12
Hatay Sarısı	12
M	12
N	12
M x N	11
N x M	12
Total	83

FigTree 1.4.2.[3] by using Nei's Da distance matrix [30]. Nei's gene diversity (H_T), total genetic diversity distributed among lines (D_{ST}), and coefficient of genetic differentiation (G_{ST}) was calculated using FSTAT 2.9.3 [31] program. Molecular Analysis of Variance (AMOVA), which is a multivariate analysis that determines whether there is a difference between the mean values of the populations specifically for molecular data, was performed using Arlequin 3.5.2.2 [32] program. Factorial correspondence analysis (FCA) was performed using the "AFC sur populations" module in GENETIX v4.05 [33] program in order to reveal possible mixtures between populations. Population structures were visualized in STRUCTURE [34,35,36,37] using the Bayesian approach-based clustering technique. In STRUCTURE analyses using independent allele frequencies and admixture model, the length value was taken as 20,000 and the number of Markov Chain Monte Carlo iterations as 100,000, and the analysis was performed with 20 replications at different K values (K = 2–5). CLUMPAK [38] program was used to create alignment plots from the obtained STRUCTURE results. From the findings obtained as a result of the analysis, the most appropriate cluster - K value is determined by Evanno et al. (2005) [39], using the method ($\Delta K = m \mid L'' (K) \mid / s [L (K)]$) was determined in STRUCTURE HARVESTER [40].

3. Results and discussion

Genetic polymorphism statistics for 16 microsatellite loci used to genotype 83 samples from 7 silkworm lines are presented in Table 4. A total of 281 alleles were identified, the highest number of alleles was observed at the T01CTA07R locus (i.e. 30 alleles), the highest effective allele number (17.61) and the highest polymorphic information content (0.94) were observed at the FL0612 locus. The expected heterozygosity (He) ranged from 0.72 (K02) to 0.95 (FL0612). The highest and lowest observed heterozygosity (Ho) value varied between 0.52 and 1.00.

Observed parameters such as polymorphic information content (0.85), observed heterozygosity (0.83), expected heterozygosity (0.86) were found to be higher than various other studies [14,15,17,41,42,43,44,45,46]. Based on these results, it can be said that the 16 microsatellite loci used in this study revealed a high level of polymorphism. Also, the average allele values obtained in terms of populations for domestic and foreign pure and hybrid lines were lower than the stated values by [41,42].

The averages of the F_{IS}, F_{IT}, and F_{ST} values were calculated as –0.027, 0.037, and 0.062, respectively. Since the average F_{ST} value observed by the loci in our study is low, it is concluded that it has moderate genetic diversity. The F_{IS} values of 10 loci, among the loci used in this study, are lower than those in the relevant literature; the average of the F_{ST} value obtained was lower than that in the study conducted by Chakraborty et al. [47]. The mean diversity between breeds (DST) and the coefficient of gene differentiation (GST) were obtained as 0.014 and 0.017, respectively. The average GST value obtained indicates that 1.7% of the total genetic variation is caused by the difference between populations and 98.3% is due to the difference between individuals.

Obtained results showed that only 2 microsatellite loci (Sat1423, T01CTA07R) had null allele frequencies above 20%. Data for these two loci were not used for genetic diversity analyses due to their null allele frequency above 20%.

The genotype information obtained for each of 16 microsatellite loci were tested for deviation from Hardy–Weinberg equilibrium (HWE) by chi-square ($\chi 2$) test. The number of loci that are not in the Hardy–Weinberg equilibrium ($p < 0.05$) are 2 in Bursa Alaca, 1 in Bursa Beyazı, 3 in Hatay Sarısı population, 5 in M population, 2 in M x N hybrid population, 4 in N, and 2 in the N x M hybrid population. It was determined that the allele distributions of most of the microsatellite markers (13 microsatellites) studied did not conform to this equation. Genetic diversity statistics in terms of silkworm lines are given in Table 5.

The lowest and highest numbers of alleles were observed in silkworms of N x M (6.75) and Bursa Alaca (9.63) lines, respectively. The highest observed heterozygosity values were found in Bursa Beyazı (0.92) line. FIS values, which are of great importance in terms of defining the structure of the populations and determining the loss of heterozygosity, varied between –0.067 (N Line) and –0.292 (N x M hybrid line). When the FIS values defined as inbreeding coefficient are examined, it can be said that there is no loss of heterozygosity in populations.

A total of 85 private alleles were observed in the studied population, and Bursa Alaca line had the highest

[3] Rambout A. FigTree 1.4.2. Available via http://tree.bio.ed.ac.uk/ Acces date: 09.07.2015; 2006.

Table 2. Details for primer pairs of microsatellite markers forming the multiplex.

Dye	Microsatellite	Primer sequence		Allele length	References
D3	Sat 3215	F	AGAACCCAGTCCAAGTAATC	70–118	[16]
		R	ACTAATCAACTCGTGACAGT		
D3	SAT1423	F	CTTTCGATCACCGCGTTCTC	130–176	[17]
		R	CGCTACGAAATACCATTATCTGACA		
D3	D90454	F	TGCGATGTCTACATGGTGG	181–184	[18]
		R	GTCTCTCGATAGCTTCCT		
D3	X05086	F	GTGAAGGCAGAAAAGGAC	191–206	[19]
		R	CGCACCAACTAAGGCACG		
D3	FL0612	F	CAGATTTCGCCAGGACTACACTTT	230–264	[20]
		R	GAGAAGTGCAGAGTGCCCATATT		
D3	FL0657	F	GAGATTAGTCAGAAATAGTGGCAAAGA	300–400	[20]
		R	GTAAATCAAACGGTTGTCTGGAAG		
D4	K02	F	ATTGTAACCGATTTGAGAGA	105–122	not published
		R	ATTCGCACAATAAGTTCAC		
D4	SAT346	F	GAAGACAGAGCGAAGTGGA	139–189	[17]
		R	ATGGATTCCTGCTGGTAGAT		
D4	X17219	F	GCAAGCCACCAGTTAGATATGG	205–223	[21]
		R	CACGTACGTTGCGTTCACCG		
D4	T01CTA07R	F	GTCAGACCAAATAGCGGAGGAA	240–300	[22]
		R	TCGCACGCCTTTTGTTTTG		
D4	FL0555	F	TCCAGGCTCCCTCCTTCTTTA	323–331	[20]
		R	GAAGGCTACAGGAAACGGGG		
D2	Sat 2550	F	GGTCCCTTGAAACTGCGAT	120–160	[16]
		R	CAGAGACCTGCCGGTTGTCGTT		
D2	Bmsat127	F	AGGCTTAGTGACGAGCCGT	180–193	[17]
		R	GGTGCCAATCATTCTTATT		
D2	D49948	F	ACGCAGACGAGAAGCTCAC	200–240	[23]
		R	ACTGCACCGTATATGCGA		
D2	Sat 211	F	GATCGAACTACGCAATTACG	280–300	[16]
		R	CAGCATCCATCCTTATTTAT		
D2	FL0554	F	CAGTGTATCTATGATTTCCATTGTTTCT	338–350	[20]
		R	CGGAGTACGGATCACAACGC		

Table 3. Thermal cycler conditions used for PCR amplification of microsatellite loci.

Steps	Temperature	Time
Initial denaturation	95 °C	5 min
1. Denaturation	94 °C	30 s
2. Annealing (4 cycles per each annealing temperature)	*66–48 °C	30 s
3. Extension	72 °C	60 s
Final extension	72 °C 10 min	
Hold	4 °C ∞	

*Temperatures used for annealing: 66, 65, 63, 62, 60, 59, 56, 55, 51, 49, and 48 °C.

Table 4. Genetic polymorphism statistics for silkworms genotyped for 16 microsatellite loci.

Loci	Na	Ne	PIC	Ho	He	F_{IS}*	F_{IT}*	F_{ST}*	D_{ST}	G_{ST}	HWE	F(Null)
Sat 2550	20	9.16	0.88	0.98	0.90	–0.160	–0.095	0.056	0.021	0.024	**	–0.050
Bmsat127	14	6.77	0.84	0.69	0.86	0.167	0.214	0.057	0.005	0.005	***	0.117
D49948	21	6.08	0.82	0.89	0.84	–0.126	–0.066	0.054	0.017	0.020	***	–0.036
Sat 211	15	9.88	0.89	0.65	0.91	0.189	0.271	0.101	0.013	0.015	***	0.159
FF554	17	11.72	0.91	0.78	0.92	0.104	0.166	0.069	0.010	0.011	ns	0.079
Sat 3215	20	7.00	0.85	0.95	0.86	–0.170	–0.103	0.057	0.021	0.024	***	–0.064
SAT1423	21	6.34	0.83	0.89	0.85	–0.118	–0.059	0.053	0.017	0.020	ns	–0.029
D90454	9	6.57	0.83	0.52	0.85	0.363	0.407	0.069	0.003	0.004	***	0.235
X05086	13	9.51	0.89	0.92	0.90	–0.082	–0.020	0.058	0.020	0.023	***	–0.015
FL0612	22	17.61	0.94	0.80	0.95	0.110	0.180	0.079	0.005	0.005	***	0.082
FL0657	20	12.12	0.91	0.90	0.92	–0.014	0.027	0.040	0.000	0.000	ns	0.011
K02	11	3.47	0.67	0,99	0.72	–0.494	–0.387	0.071	0.037	0.051	***	–0.182
SAT346	20	5.62	0.80	1.00	0.83	–0.294	–0.215	0.061	0.029	0.035	***	–0.108
X17219	14	5.04	0.78	0.92	0.81	–0.217	–0.142	0.062	0.026	0.032	***	–0.078
T01CTA07R	30	8.70	0.88	0.54	0.89	0.351	0.388	0.058	–0.005	–0in.005	***	0.250
FL0555	14	7.46	0.85	0.95	0.87	–0.153	–0.099	0.047	0.012	0.013	***	–0.051
Means	18	8.31	0.85	0.83	0.86	–0.027	0.037	0.062	0.014	0.017		

Na: Number of alleles, Ne: Effective number of alleles, PIC: Polymorphic information content, Ho: observed heterozygosity, He: expected heterozygosity, F_{IT}, F_{IS}, F_{ST}: Wright's F-statistics (* calculated according to Weir and Cockerham, 1984), D_{ST}: Interracial diversity, G_{ST}: coefficient of gene diversity, HWE: Hardy–Weinberg Balance (** $p < 0.01$, *** $p < 0.001$, ns: not significant), F (Null): null allele frequency.

Table 5. The molecular genetic parameters of 7 different silkworm lines.

Lines	MNA	Heterozygosity		F_{IS}	HWE	NPA		
		Ho	He			Freq. ≥5%	Freq. <%5	Total
BA	9.63	0.89	0.84	–0.123	2	11	16	27
BB	8.69	0.92	0.80	–0.278	1	1	6	7
HS	9.13	0.87	0.83	–0.179	3	1	8	9
M	7.50	0.71	0.78	–0.113	5	3	0	3
M x N	8.50	0.85	0.82	–0.184	2	4	10	14
N	8.94	0.78	0.83	–0.067	4	5	11	16
N x M	6.75	0.77	0.76	–0.292	2	5	4	9

BA: Bursa Alaca, BB: Bursa Beyazı, HS: Hatay Sarısı, M: M line, N: N line, MN: M x N Hybrid, NM: N x M Hybrid, MNA: Mean Number of Alleles, Ho: Average observed heterozygosities, He: Average expected heterozygosity, FIS: loss of intraline heterozygosity, HWE: The number of loci that are not in HWE equilibrium ($p < 0.05$), NPA: number of private alleles.

number of unique alleles. Private alleles which have a frequency higher than 5% were also detected in Bursa Alaca line in the highest number (11). Although a total of 85 unique alleles belonging to the 7 breeds are identified, only 30 of them have allele frequencies of 5% or above. The frequency of the other 55 unique alleles is below 5%. Thus, the majority of specific alleles are very poorly capable of identifying populations.

Detection of within and between lines genetic variation has been demonstrated by AMOVA (Table 6).

AMOVA has shown that most of the total genetic variation is shared and dissociated within populations and individuals. Fixation index values give an idea in terms of kinship coefficient and population differences. The FST value obtained from the AMOVA analysis was similar to the GST value. This shows that the existing genetic variation can be explained by genetic differences between individuals. AMOVA showed that a large part of the total genetic variation was shared within lines (96.99%). The difference between individuals is very high as 113.93. These results agree with the value obtained for GST.

Genetic distance dendrogram for the breeds obtained using Nei's Da distance matrix [31] is given in Figure 1. Bursa Alaca line clearly differed from the others. However, genetic distances between other lines are small. The fact that Hatay Sarısı and the M line appear as the closest lines to each other is quite interesting with no concrete explanation.

The graph regarding the factorial correspondence analysis is given in Figure 2. Although 2 clusters were observed in the FCA graph, it was observed that all other populations except Bursa Alaca were relatively close. The graph of the factorial relationship analysis and the dendrogram of genetic distances are quite compatible. In order to make a sound explanation of the similarities between the lines, very detailed information about the formation processes of the lines is required. However, it is not possible to reach this detailed information for now.

According to results of STRUCTURE analysis containing different numbers of clustering, the ΔK value which was taken from 7 studied silkworm lines or their crosses shows that the most suitable group number was 5 (K = 5) due to obtained highest ΔK value as 34.68. The results obtained for the STRUCTURE analysis were similar to the genetic distance dendrogram for the breeds obtained using Nei's Da distance matrix [31], as expected. While individuals belonging to Bursa Alaca population are differentiated from other populations in the FCA graph, there is no clear differentiation between individuals of other populations. Dendrogram of genetic distances and K = 5 value from STRUCTURE analysis shows that the studied populations were partially intertwined.

4. Conclusion

Molecular genetic studies aimed to define inter- and intrapopulation variation in terms of silkworm lines, which have an important place in our country's history and are grown in many regions of our country, are quite scarce. For this reason, this study, which was performed for the first time in Turkey with microsatellite markers with high identification power, in silkworm, is unique and important in terms of both contributing to the literature and contributing to the breeding and genetic resource conservation programs.

In this study, 16 microsatellite DNA loci were combined in one multiplex, optimized and successfully used PCR protocol for genotyping by using 3 different fluorescent dyes. The high polymorphism values obtained for the loci used in our study showed that these loci can be used effectively in the genetic identification of silkworm breeds or lines in our country.

Observing 18 alleles per locus indicates that the allele diversity is quite high. When the FIS values obtained according to microsatellites as a result of our study are examined; it is observed that there is a loss of heterozygosity in terms of microsatellites in 10 working loci (Sat 2550, D49948, Sat 3215, SAT 1423, X05086, FL0657, K02, SAT346, X17219, FL0555).

The resulting GST value (0.017) shows that 98.3% of the total genetic variation is due to the difference between individuals, not the lines. This raises a suspicion on that the breeds/lines could not be bred pure. Apart from Bursa Alaca, the differences between lines are not very clear. This indicates possible historical crosses between lines or that they may have originated from one another. DST, which is the across-line genetic diversity value, was found to be very low. This is an indication that there is not much difference between populations.

As a result of the applied $\chi 2$ test, 13 of 16 microsatellite markers examined were not in Hardy–Weinberg

Table 6. Results for AMOVA.

Variation source	df	SS	VC	PV(%)	FI
Among populations	6	33.05	0.10946Va	3.01	–0.1746
Within populations	76	221.35	–0.61607Vb	–16.93	0.03009
Among individuals	83	344.00	4.14458Vc	113.93	–0.13926
Total	165	598.39	3.638		

df: degrees of freedom, SS: sum of squares, VC: variance components, PV: percentages of variances, FI: fixation index.

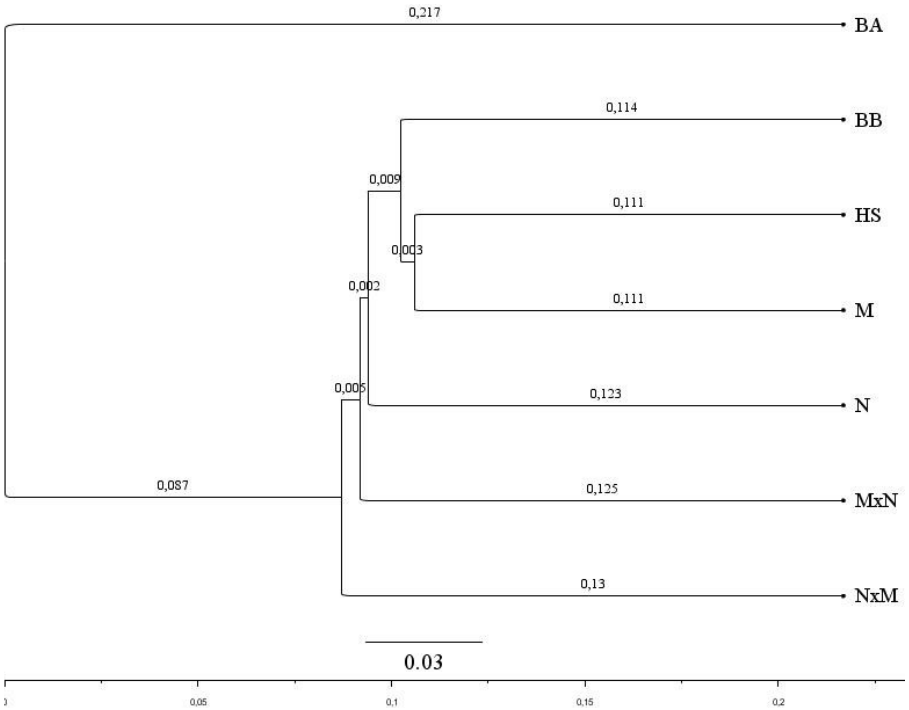

Figure 1. Dendogram of 7 silkworm lines (1000 bootstrap) according to Nei's Da distance matrix.

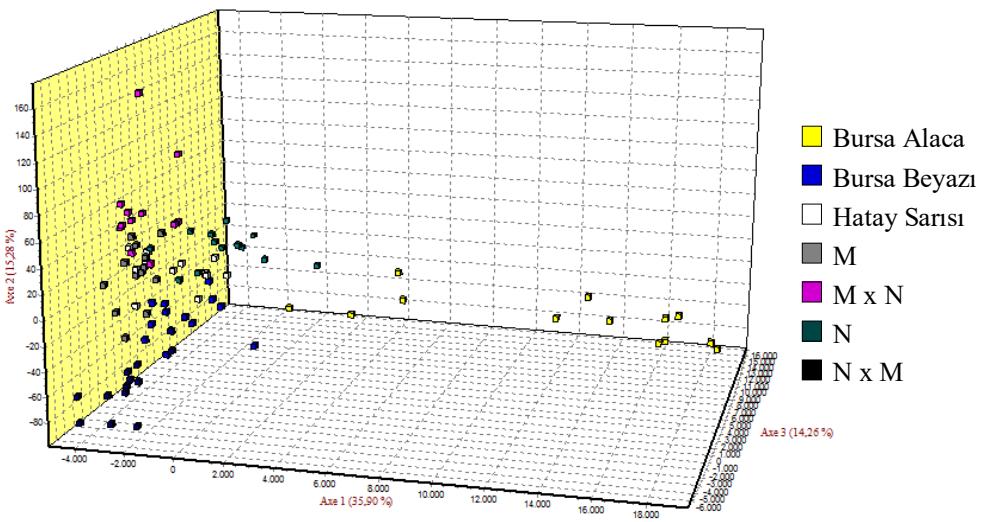

Figure 2. Factorial correspondence analysis of 7 silkworm lines

equilibrium, indicating that breeding studies were carried out in populations or that the other evolutionary forces were in action. Considering the selection studies carried out on silkworm lines, the results were perceived to be normal as expected.

Of the 16 microsatellite loci used in our study, it was revealed that 2 loci with a null allele frequency above 20% cannot be used effectively in defining genetic diversity, while the other 14 microsatellite loci are highly polymorphic and can be used effectively to reveal the genetic diversity of the studied lines.

A high number of unique alleles (85) were detected in the studied sample set. However, the frequency of the vast majority of them is below 5%, indicating that these unique alleles have a weak power to define populations. On the other hand, 30 unique alleles with a frequency of 5% and above can be used in population definitions and line separations.

STRUCTURE analysis results including different clustering numbers (K = 2–7) are presented in Figure 3.

According to the STRUCTURE and dendrogram results of the study, it can be said that the Bursa Alaca line is relatively differentiated, but the other lines are almost in the same group. This situation indicates that the Bursa Alaca line is purer than other lines. As can be understood from the Factorial Relationship Analysis graph, it has been observed that Bursa Alaca line is largely differentiated from other lines similar to the results in the genetic distance dendrogram, but Bursa Beyazı, Hatay Sarısı, M, N, M x N hybrid, N x M hybrid are relatively intertwined and individuals in the studied populations. It has been observed that the diversity between populations is higher than that between populations.

As a result of the study, it has been revealed that the microsatellite markers used are polymorphic and can be used safely in determining genetic diversity in pure and hybrid populations of silkworm. However, in this study material, the lines outside the Bursa Alaca line are not clearly distinguished by genetic analysis, which has led to the need for genetic identification with a higher number of individuals for more efficient identification and orientation. In addition, it is recommended to exert an intense effort in the formation of samples, and to take samples from different locations, if any, for each line. The results obtained will contribute to the registration of the lines or the update of the registration information of the registered lines and will be a guide for the conservation programs of the lines. The results obtained can be considered a preliminary pointing out that the relevant public institutions, nongovernmental organizations, and other relevant groups should show higher sensitivity in terms of protecting the purity of the lines.

Acknowledgments

This study was produced from MSc thesis (Thesis ID: 2019-YL-130) by Ezgi Odabaş and the study was carried out at Aydın Adnan Menderes University, Institute of Science and Technology, Department of Animal Science. First and foremost, we would like to express our deepest gratitude to Associate Professor Onur YILMAZ for his support, guidance, helpful advice, and constructive criticism, and his valuable contribution with the statistical program was very much appreciated. Special thanks go to administrators and staff of Aydın Adnan Menderes University Agricultural Biotechnology and Food Safety Application and Research Center (ADU-TARBİYOMER) for supporting the thesis study. Our sincere appreciation and thanks go to Research Assistant Dr. Nezih ATA for his guidance and help in lab during the thesis. We would also like to express our gratitude to Kozabirlik General Directorate for providing silkworm samples.

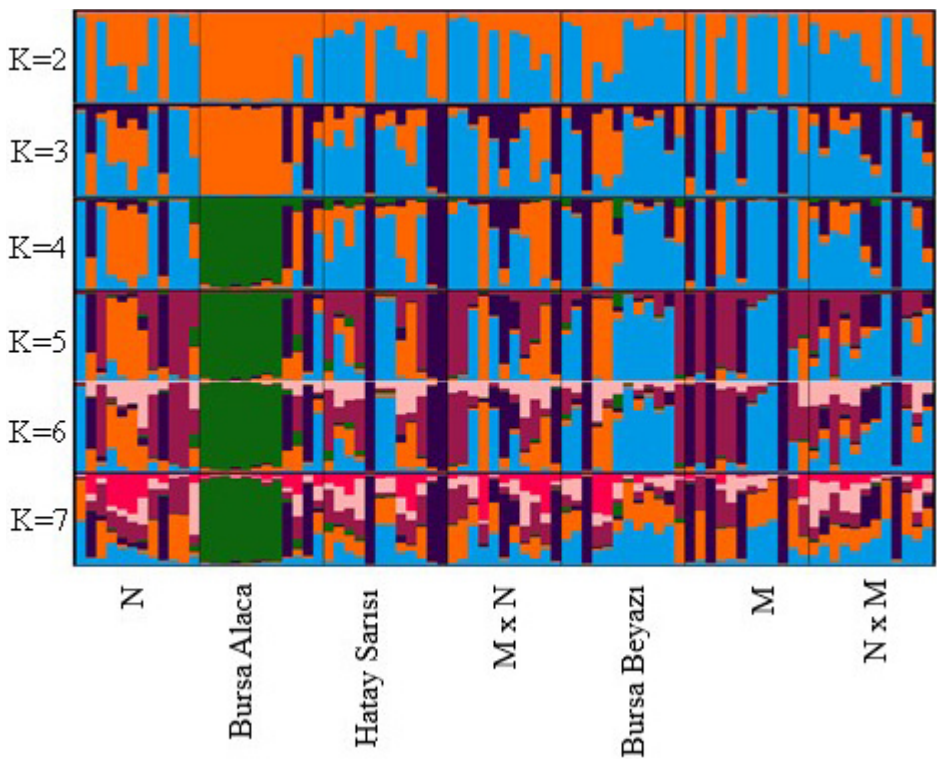

Figure 3. The CLUMPAK results of the populations (K = 2–7).

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