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Research Article

Population Genetic Studies of Silkworm (*Bombyx mori* L.) Reared in Bulgaria and Phylogenetic Relationships

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Abstract

The mulberry silkworm (*Bombyx mori* L.) is a species of great economic importance. Currently, there is a great variety of breeds, which is related to their different origins, as well as the selective activities carried out in different breeding centers around the world. Various methods have been used to assess genetic diversity in this species. One of them is the method of polyacrylamide gel electrophoresis (PAGE), which is used in the present work.

This study aimed to evaluate the degree of genetic variability and phylogenetic relationships between thirteen breeds of mulberry silkworm (*Bombyx mori* L.) from genetic resources of Bulgaria through isozyme polymorphism. PAGE was used. Among nine studied isoenzyme loci, by eight loci (Bes A, Bes B, Bes D, Bes E, Pgm A, Mdh A, Bph and Alp A) was found intra-breed and inter-breed polymorphism. At the Hk locus, we found inter-breed polymorphism only. The number of alleles per polymorphic locus ranged from one to two. The degree of polymorphism ranged from 0% to 77.80%. Low levels of observed heterozygosity in comparison with the expected one have been calculated in all of breeds. The combined F_{IS} value over all polymorphic loci was 0.3205, which reflects a substantial deficit of heterozygotes. The value of F_{ST} showed that 49.21% of the overall genetic diversity observed was among breeds. The dendrogram constructed manifested that the two breeds of Japanese origin (Daizo and Japanese 106) were genetically most distant from other breeds. The data for isoenzyme polymorphism and genetic structure of the tested breeds can be used for genetic improvement and to develop new hybrids for silk production.

***Keywords**: Bombyx mori; Isoenzyme Polymorphism; Phylogenetic Relationships*

Introduction

Until the end of the 80s of the last century, Bulgaria was one of the best producers of cocoons in Europe. For various reasons, mostly economic, this industry is now in decline. The favorable climate and the existing rich national traditions are prerequisites for its restoration and further development of sericulture in Bulgaria, because the demand for silk and its place in everyday life will continue to be highly valued due to its hygienic qualities and finesse. Bulgaria maintains a rich genetic resources of more than 250 breeds of different origins, which is also a prerequisite for the recovering of sericulture.

The selection process of the mulberry silkworm is related to the solution of some basic issues as selection of individuals with the highest productivity, reproductive ability, viability and resistance to diseases, as well as analyzing and evaluating the capabilities of the breed gene pool. The creation of new highly productive breeds requires evaluation of the promising features for selection, creation of lines with desired qualities and analysis of their gene pool, development of methods for creation of synthetic lines and evaluation of their genotypic and phenotypic features.

A basic principle for improving breeds is the presence of genetic diversity. Genetic variability can be analyzed by different methods and markers. The method of electrophoresis provides an opportunity to analyze the genetic heterogeneity in populations by studying genetically determined protein polymorphism [1-6]. The established variability in isozyme markers can be used to characterize the genetic heterogeneity and degree of polymorphism of breeds, to study the intensity of gene flow and the origin of individual breeds [7]. Application of isoenzymes and other molecular markers helps to estimate genetic diversity much more accurately than that of morphological traits [8]. Isoenzyme analysis is useful for the study of intra- and inter-breed polymorphism of mulberry silkworm and determining the level of genetic variability and genetic relationships [4,6,9-11]. Isoenzymes like esterase, acid phosphatase, alkaline phosphatase, malate dehydrogenase, phosphoglucomutase have been used by various researchers to study diversity in silkworm genotypes [8,12].

Studies for detection of polymorphic enzymatic and non-enzymatic protein systems in the breeds of *B. mori* kept in Bulgaria were made [4,6,10,11,13-15]. Data on the level of polymorphism and heterozygosity in more than 50 breeds with different origin have been established. The level of polymorphism varied between 0 and 77.8%, and the degree of heterozygosity between 0 and 0.280 in different breeds. Phylogenetic relationships between breeds have been identified on the basis of the established genetic distance and similarity. All these data are important for the maintenance of biodiversity in the silkworm, as well as for the selection of this species. However, there is still no information about other breeds, as the genetic resources of the silkworm maintained in Bulgaria are rich and include a large number of breeds. All this motivates the present investigation which aim was to study the degree of genetic variability and phylogenetic relationships between thirteen breeds of silkworms, introduced and created in Bulgaria, on the basis of isozyme polymorphism.

Material and Methods

The silkworm resources used in the present investigation include a total of thirteen breeds with different geographical origin and phenotype characteristic. They were obtained from the Scientific Center of Sericulture in Vratsa at the Agricultural Academy in Bulgaria. Breed Vratza 16 was created in Bulgaria. All others have been introduced as follow: breeds AES-1 wh and AES-1 zb originated from Spain, Tg - from Italia, Japanese 106 and Daizo originated from Japan, Mir 5 - from Egypt, Mziuri 1 - from Georgia, Tahvon 106 - from Notrh Korea, Ukrainian 19 - from Ukraine, Sh 4 - from China, Line 22 - from Uzbekistan, MNB - from Madagascar. Daizo is polyvoltine while all other breeds are mono-bivoltine. Breeds Tg, Daizo, AES-1 wh and AES-1 zb have color cocoons. All other breeds have white cocoons. All individuals were nourished at a standard regime of silkworm breeding.

Totally 493 larvae on the fifth day of the fifth instar were studied. Larvae were selected randomly from each breed and were submitted to electrophoretic analysis of hemolymph, silk glands and midgut tissues.

The tissue extracts were prepared according to the procedure described earlier [11,14,16,17]. The individual samples were studied by 7.5% polyacrylamide gel electrophoresis (PAGE) [18] for nonspecific esterases (EST, EC 3.1.1), malate dehydrogenase (MDH, EC 1.1.1.37) and acid phosphatase (ACP, EC 3.1.3.2) - from the hemolymph; hexokinase (HK, EC 2.7.1.1) - from the silk glands and alkaline phosphatase (ALP, 3.1.3.1) - from the midgut. The 6% PAGE was used to analyse phosphoglucomutase (PGM, EC 5.4.2.2) from the silk glands. The staining mixtures for the enzymatic activities tested were pointed previously [19].

The phenotypes of the discovered loci were recorded after the revelation of the isozyme activity regions. Allele frequencies, mean number of alleles per locus, proportion of polymorphic loci, observed (H_0) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium, Nei's genetic distance (D) [20], and Wright's fixation index, F_{ST} [21] were calculated using BIOSYS-1 [22]. Dendrogram was constructed using Nei's [20] genetic distance, by UPGMA [23] method using the PHYLIP [24] software package.

Results

Isoenzyme and allozyme polymorphism of nonspecific esterases and allozyme polymorphism of phosphoglucomutase, malate dehydrogenase, acid phosphatase, alkaline phosphatase and hexokinase were detected by polyacrylamide gel electrophoresis (Figure 1).

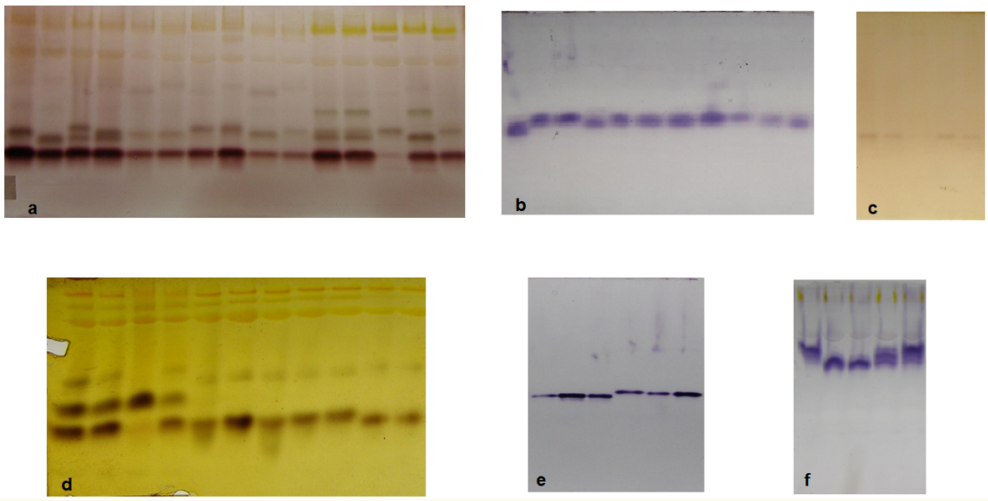

Figure 1: *PAGE spectra of: a. Nonspecific esterases of hemolymph; b. Phosphoglucomutase of silk glands; c. Alkaline phosphatase of midgut; d. Acid phosphatase of hemolymph; e. Hexokinase of silk glands; f. Malate dehydrogenase from hemolymph of Bombyx mori L.*

The tested enzymes recorded a total of nine polymorphic loci with 26 alleles (Table 1). Breed specificity of gene pools with respect to allele content and allele frequencies was established.

In the gene pool of AES 1 zb and AES 1 wh breeds (Table 1) we found two alleles polymorphism with "null" allele of the blood esterase A locus - Bes A_0 and A_1. In all rest breeds Bes A_1 allele was fixed. Bes A_2 allele, which was described earlier [6] in some Egyptian breeds was not detected in the current sample of breeds. For the Bes B locus three alleles were recorded (Bes B_1, B_2 and B_3) in the gene pools of seven breeds - Mir 5, MNB, Mziuri 1, Line 22, Sh 4, Ukrainian 19 and Vratza 16. Polymorphism with two alleles (Bes B_2 and B_3) was established in AES 1 zb, AES 1 wh, Tg and Tahvon 106. Bes B_2 allele was fixed in the gene pool of Daizo and Japanese 106. Among the breeds with polymorphism on Bes B locus, the allele Bes B_1 showed the highest frequency in Vratza 16, Bes B_2 - in AES 1 wh and Bes B_3 - in Sh 4. Polymorphism with four alleles (Bes D_1, D_2, D_3 and D_0) was found on the Bes D locus. Bes D_1 allele was fixed in the gene pool of Ukrainian 19 and Vratza 16, Bes D_2 - in Japanese 106, Bes D_0 - in Daizo. All four alleles were presented in the gene pool of the breeds Mziuri 1 and Sh4. Bes D_1, D_2 and D_3 alleles were presented in the gene pool of Line 22, Bes D_1 and D_2 - in all the rest tested breeds. We obtained the highest frequency of the allele Bes D_1 in Tahvon 106, of Bes D_2 - in AES 1wh, of Bes D_3 - in Sh 4 (except for breeds with fixed Bes D alleles). Mziury 1 and Sh 4 have similar frequencies of the allele Bes D_0. Polymorphism with three alleles was found in Bes E locus (Bes E_1, E_2 and

E_0) in AES 1 zb, AES 1 wh, Tg, and Tahvon 106 breeds. Two of these alleles we obtained in Line 22 (Bes E_1 and E_o), Mir 5 and Sh 4 (Bes E_2 and E_o). Bes E_1 allele was fixed in Japanese 106 and MNB gene pool, Bes E_2 - in Daizo and Bes E_0 - in Mziuri 1, Ukrainian 19 and Vratza 16. The "null" allele Bes E_0 demonstrated the highest frequency in all polymorphic breeds tested (Table 1).

Locus (alleles)	Breeds												
	AES1 zb	AES1 wh	Tg	Mir5	Japanese 106	MNB	Tahvon 106	Mziuri 1	Daizo	Line 22	Sh 4	Ukrainian 19	Vratza 16
Bes A													
A_1	0.622	0.697	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
A_0	0.378	0.303	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bes B													
B_1	0.000	0.000	0.000	0.424	0.000	0.371	0.000	0.462	0.000	0.250	0.140	0.750	0.891
B_2	0.514	0.645	0.500	0.288	1.000	0.429	0.346	0.500	1.000	0.208	0.135	0.212	0.076
B_3	0.486	0.355	0.500	0.288	0.000	0.200	0.654	0.038	0.000	0.542	0.716	0.038	0.033
Bes D													
D_1	0.581	0.158	0.538	0.470	0.000	0.771	0.808	0.141	0.000	0.181	0.108	1.000	1.000
D_2	0.419	0.842	0.462	0.530	1.000	0.229	0.192	0.141	0.000	0.680	0.149	0.000	0.000
D_3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.139	0.527	0.000	0.000
D_0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.218	1.000	0.000	0.216	0.000	0.000
Bes E													
E_1	0.135	0.092	0.200	0.000	1.000	1.000	0.077	0.000	0.000	0.181	0.000	0.000	0.000
E_2	0.041	0.092	0.062	0.182	0.000	0.000	0.179	0.000	1.000	0.000	0.027	0.000	0.000
E_0	0.824	0.816	0.738	0.818	0.000	0.000	0.744	1.000	0.000	0.819	0.973	1.000	1.000
Pgm A													
A_1	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.013	1.000	0.167	0.000	0.000	0.000
A_2	0.338	0.474	0.550	0.697	1.000	1.000	1.000	0.577	0.000	0.389	0.473	0.700	1.000
A_3	0.662	0.526	0.450	0.288	0.000	0.000	0.000	0.417	0.000	0.444	0.527	0.300	0.000
Mdh A													
A_2	0.946	1.000	1.000	1.000	1.000	1.000	0.143	1.000	1.000	1.000	1.000	0.925	1.000
A_3	0.054	0.000	0.000	0.000	0.000	0.000	0.857	0.000	0.000	0.000	0.000	0.075	0.000
Bph A													
A	0.176	0.197	0.000	0.364	1.000	0.100	0.103	0.205	1.000	0.000	0.162	0.188	0.250
B	0.027	0.039	0.000	0.636	0.000	0.500	0.000	0.205	0.000	0.389	0.000	0.000	0.304
C	0.000	0.000	0.000	0.000	0.000	0.271	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0	0.797	0.763	1.000	0.000	0.000	0.000	0.897	0.590	0.000	0.611	0.838	0.813	0.446
Alp A													
A_1	1.000	0.566	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
A_0	0.000	0.434	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hk A													
A_1	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A_2	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 1: *Allele frequencies in breeds tested.*

Among the studied breeds we found polymorphism at the phosphoglucomutase (Pgm) locus with three alleles - Pgm A_1, A_2 and A_3 in Mir 5, Mziuri 1 and Line 22 breeds. Pgm A_2 and A_3 were presented in the gene pool of AES 1 zb, AES 1 wh, Tg, Sh 4 and Ukrainian 19 (Table 1). Monomorphism of phosphoglucomutase demonstrated Daizo (with fixed Pgm A_1 allele), Japanese 106, MNB, Tahvon 106 and Vratza 16 (with fixed Pgm A_2 allele). Pgm A_1 allele had the highest frequency in Line 22, Pgm A_2 - in Ukrainian 19 and Pgm A_3 - in AES 1 zb.

We found two alleles at the malatedehydrogenase locus (Mdh) in breeds AES 1 zb, Tahvon 106 and Ukrainian 19 (Mdh A_2 and A_3). Among these three breeds Mdh A_2 was the most common allele in AES 1 zb and Ukrainian 19, while Mdh A_3 was the most common in Tahvon 106. Mdh A_2 allele was fixed in the gene pools of the rest ten breeds (Table 1). Mdh A_1 allele, which was described earlier [6,12] in other breeds was not detected in the current sample of breeds.

Total of five alleles of the acid phosphatase locus (Bph) were found in tested breeds (Bph A, B, C, D and the "null" allele Bph O) (Table 1). Four of them were presented in the gene pool of MNB breed (Bph A, B, C, D), three - in AES 1 zb, AES 1 wh, Mziuri 1 and Vratza 16 (Bph A, B and 0) and two - in Mir 5 (Bph A and B), Tahvon 106, Sh 4 and Ukrainian 19 (Bph A and O) and Line 22 (Bph B and O). Bph A allele was fixed in Japanese 106 and Daizo breeds, whereas Bph O allele was fixed in Tg. The "null" allele was the most common in eight of the tested breeds. Bph B was the most expressed allele in Mir 5 and MNB.

Two alleles at the alkaline phosphatase locus (Alph A_1 and A_0) with a higher allele frequency of Alph A_1 were recorded in the breed AES 1wh, only (Table 1). Alph A_1 allele was fixed in the gene pool of the rest twelve breeds.

We found inter-breed polymorphism with two alleles on the hexokinase (Hk A) locus. The Hk A_1 allele was presented only in the gene pool of Japanese 106 breed, whereas Hk A_2 allele was fixed in the gene pools of all the rest twelve breeds.

The number of alleles per locus calculated with BIOSYS-1 software package in the silkworm breeds analysed using nine enzyme loci ranged from 1.0 (Japanese 106 and Daizo) to 2.0 (Mziuri 1, AES 1 zb and AES 1wh) (Table 2). The degree of polymorphism (according to the criterion 0.99) was the highest for the two Spanish breeds AES 1 zb and AES 1 wh (77.80%), and the lowest - for the two Japanese breeds Japanese 106 and Daizo (0%). The observed heterozygosity (H_o) by polymorphic loci varied from 0.000 (for Japanese 106 and Daizo) to 0.222 (for AES 1 zb). The expected heterozygosity (H_e) was higher than the observed one (H_o) in all breeds with polymorphism. Significant differences ($P < 0.05$) in genotype frequencies were seen at the most loci in breeds studied. Chi-Square test (DF = 1÷3) showed that the deviations from the Hardy-Weinberg equilibrium were in result of excess of homozygotes and deficiency of heterozygotes.

Breeds	Mean sample size per locus	Mean number of alleles per locus	Percent of polymorphic loci (P=0.99)	H_o	H_e
Vratza 16	46,0 ± 0,0	1,4 ± 0,3	22,2	0,085 ± 0,074	0,095 ± 0,073
Ukrainian 19	40,0 ± 0,0	1,6 ± 0,2	44,4	0,125 ± 0,059	0,141 ± 0,062
Sh 4	37,0 ± 0,0	1,9 ± 0,4	44,4	0,144 ± 0,057	0,215 ± 0,087
Line 22	36,0 ± 0,0	1,9 ± 0,3	55,6	0,164 ± 0,059	0,279 ± 0,094
Mziuri 1	39,0 ± 0,0	2,0 ± 0,4	44,4	0,157 ± 0,067	0,255 ± 0,102
Tahvon 106	39,0 ± 0,0	1,6 ± 0,2	44,4	0,097 ± 0,042	0,153 ± 0,065
MNB	35,0 ± 0,0	1,7 ± 0,4	33,3	0,156 ± 0,085	0,185 ± 0,097
Japanese 106	36,0 ± 0,0	1,0 ± 0,0	0,0	0,000 ± 0,000	0,000 ± 0,000
Mir 5	33,0 ± 0,0	1,8 ± 0,3	55,6	0,189 ± 0,074	0,264 ± 0,089
Tg	40,0 ± 0,0	1,6 ± 0,2	44,4	0,119 ± 0,063	0,214 ± 0,085
Daizo	37,0 ± 0,0	1,0 ± 0,0	0,0	0,000 ± 0,000	0,000 ± 0,000
AES 1 wh	38,0 ± 0,0	2,0 ± 0,2	77,8	0,164 ± 0,048	0,319 ± 0,066
AES 1 zb	37,0 ± 0,0	2,0 ± 0,2	77,8	0,222 ± 0,058	0,297 ± 0,070

Table 2: *Mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected heterozygosity (H_e).*

The mean F_{ST} value over all loci, which is associated with the level of inter-breed differentiation, was 0.4921 and shows that 49.21% of the overall genetic diversity observed was among breeds (Table 3). 50.79% of genetic variations were within the breeds. The highest level of genetic diversity among breeds we found for the Hk A locus (1.0000) and the lowest one - for the Mdh A locus (0.0571). For two loci (Bes D and Bes E) we established a level of inter-breed differentiation over than 50%. The heterozygosity in total populations F_{IT} averaged to 0.6549 and shows that there was a deficit of heterozygotes in the tested breeds and correlates with the obtained lower level of heterozygosity observed compared to the expected one and with deviations from the Hardy-Weinberg equilibrium as well. The combined F_{IS} value for all polymorphic loci was 0.3205, which also reflects a significant deficiency of heterozygotes.

Locus	F_{IS}	F_{ST}	F_{IT}
Bes A	0.6711	0.3084	0.7725
Bes B	0.1455	0.3701	0.4618
Bes D	0.4742	0.5151	0.7450
Bes E	0.5643	0.6692	0.8559
Pgm A	0.5023	0.4191	0.7109
Mdh A	-0.0714	0.0571	-0.0102
Bph A	0.0618	0.4592	0.4925
Alp A	0.3037	0.4146	0.5924
Hk A	0.0000	1.0000	1.0000
Mean	0.3205	0.4921	0.6549

Table 3: *F-statistics for all polymorphic loci studied.*

The values of genetic distance [20] were calculated using the allele frequencies and ranged from 0.029 (between the breeds Vratza 16 and Ukrainian 19) to 0.730 (between Japanese 106 and Ukrainian 19).

Analysis of the results obtained from genetic distances and UPGMA dendrogram (Figure 2) revealed that, all the 13 breeds were grouped into two major clusters. The first cluster included Japanese breeds Daizo and Japanese 106, while the second included the rest 11 breeds, which was distributed in two subgroups. The first of them included MNB breed. The second one included all others. This subgroup was distributed in two - four breeds (Mir 5, Tahvon 106, Ukrainian 19 and Vratza 16) were grouped to form one subgroup and six breeds (Sh4, Mziuri 1, AES 1 wh, Line 22, Tg and AES 1 zb) were grouped to form another subgroup.

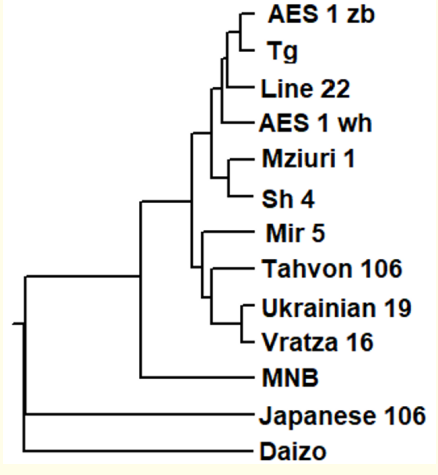

Figure 2: *UPGMA dendrogram.*

Discussion

The study of polymorphic enzymatic and non-enzymatic proteins in mulberry silkworm is important for the selection of this species. They could serve as a kind of "passport" of the parent breeds, on the basis of which it is possible to compile optimal variants of crossbreeding and predict the effect of heterosis. Studies on proteins and enzymes in the silkworm (*Bombyx mori* L.) have long since begun. Breeds of different origins and geographical distribution, bred in different countries as Japan, China, Korea, India, Russia and others, have been studied. Different methods of analysis have been used. A wide range of results has been obtained, which reflects not only the application of different research methods, but also the existing huge variety of breeds that are used in various breeding centers around the world. Significant and positive correlation has been established between some isoenzymes and some yield parameters [25,26]. Successes in this area of research could significantly facilitate the production of desired phenotypes and introduce elements of rigorous planning in the selection process.

In this study we indicated a total of twelve alleles of four esterase loci. Three of them were "null" alleles. In earlier studies [6] have reported an allele Bes A_2. We did not find this allele among the tested thirteen breeds. "Null" alleles of the Bes A, D and E loci were described in other breeds from Bulgarian germplasm resources of silkworm [3,6,12,19,27]. Polymorphism with five alleles was determined of the acid phosphatase. One of them was found as "null" type. Allozyme polymorphism with codominant alleles of this enzyme was reported earlier [1,6,10,12,28]. We found intra-breed polymorphism with three or two alleles of the phosphoglucomutase, malate dehydrogenase and alkaline phosphatase, as well as inter-breed polymorphism with two alleles of the hexokinase. Some of the alleles of the polymorphic loci demonstrated breed specificity. For instance Bes A_0 allele was presented only in AES 1 zb and AES 1 wh. Alp A_0 allele was presented in the gene pool of AES 1 wh and Hk A_1 was presented in Japanese 106, only.

The results based on population genetic analyses showed a certain degree of differentiation between the tested breeds. 50.79% of isoenzyme diversity is observed between breeds and 49.21% is maintained within breeds, which is in line with the diversity based of AFLP markers found in some Iranian breeds [29]. Larger proportion of genetic variations among *B. mori* strains (84.08%) and a relatively smaller within strains (15.92%) have been established by RAPID analysis [30]. The UPGMA dendrogram resolved thirteen breeds into two main clusters. The two breeds of Japanese origin (Daizo and Japanese 106) form one cluster and all the others the other cluster. This grouping of silkworm breeds is probably related to their adaptation to the specific geographical conditions of the environment from which they originate, as well as to the founder effect in the introduced breeds. Japanese 106 and Ukrainian 19 were the most distant breeds whereas Vratza 16 and Ukrainian 19 were the closest breeds.

Low level of heterozygosity among tested breeds have observed in this study. Heterozygote deficiencies probably results from low effective number of reproductive individuals, selection process and inbreeding effect. Some authors [8] pointed that reduction in genetic diversity in silkworm, might be mainly due to domestication, breeding systems, selection, genetic drift and inbreeding. The effects of inbreeding can accumulate over many generations [31,32]. Breeders use artificial selection for target characteristics which also leads to a reduction in genetic variations in the population. The import of breeds of different origins and their use in breeding programs would help maintain a higher level of genetic diversity, which is very important for selection of suitable parents required for successful development of improved breeds and hybrids of silkworm that have high adaptive potential [8]. In view of the differences found in the genetic structure of the studied breeds with different origins, the results obtained here would be useful for breeders in planning crossbreeding strategies to produce new hybrids and in the conservation programs of silkworm *Bombyx mori*.

Conclusion

Our results complement the knowledge of the genetic variations among the silkworm breeds bred in Bulgaria. They confirmed that nonspecific esterases, acid phosphatase and malate dehydrogenase from hemolymph, phosphoglucomutase and hexokinase from the silk

glands and alkaline phosphatase from the midgut are applicable to the study of genetic structure and phylogenetic relationships between breeds. The number of alleles, allelic and genotypic frequencies at polymorphic loci show breed specificity. It is important to perform continuous evaluate the polymorphism degree of the breeds, to avoid a marked increase of the homozygosity. This would result in the expression of deleterious genes that can cause high mortality or other adverse effects. The results obtained in the present study could help breeders in selecting parental pairs for crossbreeding and in determining the quality of parental forms in the early stages of development. The analysis of genetic structure on the basis of isoenzyme markers showed that the tested breeds of silkworm are genetically differentiated. Most of them have high degree of polymorphism and can be used for genetic improvement and to develop new hybrids for silk production.

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Conflicts of Interest

The author declare no conflict of interest.

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