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Inter- and intra-population genetic variability of introduced silkworm (*Bombyx mori* L.) strains raised in Bulgaria

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ABSTRACT

The genetic variability of four populations belonging to two introduced silkworm strains (*Bombyx mori* L.) of various origins has been studied using isoenzymic analysis of six enzyme systems. Nonspecific esterases, phosphoglucomutase, malate dehydrogenase, acid phosphatase, alkaline phosphatase and hexokinase from different tissue of larvae 5th instar have been analysed using PAGE. Polymorphism in six from a total of nine loci has been found. Inter- and intra-population differences have been ascertained expressed in different allele composition of the gene pool and different frequencies of alleles. A higher degree of inter-population variability has been reported on the acid phosphatase and a lower one – on the phosphoglucomutase.

Key words: *Bombyx mori* L., *genetic variability, isoenzymes.*

Introduction

The choice of parental strains in the selection of mulberry silkworm (*Bombyx mori* L.) was mainly based on the study of their biological and technological properties and their genealogical data. The assessment of the parental strains, however, should be complex and should be carried out simultaneously by different indexes. Finding suitable markers to study the genetic heterogeneity is important for the selection of this type with regard to increasing the adaptive potential and their productivity (Mirhoseini & Gholami, 2002). Using isoenzymes as markers makes it possible to study the genetic diversity of the mulberry silkworm as well as to differentiate the strains (Eguchi, 1995; Goldsmith, 1995; Etebari et al., 2005). The established genetic variability could be used also to study the gene flow and origin of the separate strains (Gamo & Othsuka, 1980).

The purpose of this study was to determine the degree of genetic variability of various populations of the two introduced strains of mulberry silkworm raised in Bulgaria using isoenzyme markers.

Materials and Methods

Four populations from two strains of mulberry silkworm (*Bombyx mori* L.) with different origin were studied:

Line 22 – uni-bivoltine strain introduced from Uzbekistan. The egg serosa color is gray, chorion color is white and eggs are sticky. The larvae are white in color whit markings. The cocoons are white in color and oval-elongated in shape.

Mziuri 1 – uni-bivoltine strain introduced from Georgia. The egg serosa color is gray, chorion color is white and eggs are sticky. The larvae are white in color whit high marking. The cocoons are white, elongated with low constriction (Petkov et al., 2006).

All individuals were nourished at a standard regime of silkworm breeding in Sericultural Experiment Station Vratza and Agricultural University - Plovdiv. According to locations of rearing, populations were marked as Line 22 Vr and Mziuri 1 Vr (from Vratza) and Line 22 Pv and Mziuri 1 Pv (from Plovdiv). On the fifth day of the fifth instar 50 - 81 larvae were selected randomly from each population.

The spectrum of 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 hemolymph and the spectra of phosphoglucomutase (PGM, EC 5.4.2.2) and hexokinase (HK, EC 2.7.1.1) from silk glands as well as the spectrum of alkaline phosphatase (ALP, EC 3.1.3.1) from gut of larvae were studied by means of 7.5% PAGE (Daevis, 1964). Isolation of the tissues and preparation of the samples was made according to Stoykova et al. (2003) and Staykova

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et al. (2004). Method of Shaw & Prasad (1970) was used to visualize the nonspecific esterases and malate dehydrogenase. Methods of Spencer et al. (1964) and Eaton et al. (1966) were used to visualize the phosphoglucomutase and hexokinase, respectively. Acid phosphatase isoenzymes were visualized according to Staykova et al. (2010) and alkaline phosphatase according to Boyer (1961).

Allele frequencies, mean number of alleles per locus, proportion of polymorphic loci at the 99% level, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium and Wright's fixation index (F_{ST}) (Wright, 1965), were calculated using BIOSYS-1 (Swofford & Selander, 1981).

Results and Discussion

Four of the studied enzyme systems – nonspecific esterases, phosphoglucomutase, malate dehydrogenase and acid phosphatase showed polymorphism (Table 1). Alkaline phosphatase and hexokinase were monomorphic across all individuals of the studied populations.

The nonspecific esterases in the mulberry silkworm (*Bombyx mori* L.) heamolymph are coded by different genes (Bes A, B, D and E), showing polymorphism (Egorova et al., 1985; He, 1995; Stoykova et al., 2003; Staykova, 2008). In the gene pool of all populations included in this study, we found a three-allele polymorphism in Bes B locus, in the presence of Bes B_1, B_2 and B_3 alleles (Table 1). Bes D locus was also polymorphic and presented with three alleles (Bes D_1, D_2 and D_3) in the gene pool of Line 22 Vr, Line 22 Pv and Mziuri 1 Pv. Only in the gene pool of Mziuri 1 Vr we established a fourth allele (Bes D_o). We observed also a three-allele polymorphism in the Line 22 Pv population by Bes E locus (Bes E_1, E_2 and E_o). In the gene pool of Line 22 Vr and Mziuri 1 Pv, this gene was presented by two alleles (Bes E_1 and E_o). In all populations, where we found polymorphism in Bes E locus, Bes E_o allele showed the highest frequency, which was fixed in the gene pool of Mziuri 1 Vr. In all studied populations of the two strains, Bes A locus was monomorphic.

Staykova (2006, 2008) describes polymorphism at the phosphoglucomutase locus (Pgm A) with the strains of mulberry silkworm raised in Bulgaria. In the gene pool of the studied populations of Line 22 and Mziuri 1, we ascertained the presence of three alleles (Pgm A_1, A_2 and A_3). In three out of four populations, the highest frequency was the one of Pgm A_2 (Line 22 Pv, Mziuri 1 Vr and Mziuri 1 Pv).

We found three-allele polymorphism by Mdh A locus in Line 22 Pv (Mdh A_1, A_2 and A_3). In the gene pool of the rest populations, Mdh A_2 allele was fixed. Marcato et al. (1990) also describe monomorphism by this locus. Egorova & Nasirillaev (1993) reported the presence of polymorphism of malate dehydrogenase in hemolymph, which coincides with our findings in Line 22 Pv.

Polymorphism in codominant alleles at the locus, coding the acid phosphatase of *Bombyx mori* L. hemolymph (blood phosphatase - Bph) was reported for the first time by Yoshitake & Akiyama (1964). Later on, it was also described by some other authors (Eguchi et al., 1988; Staykova et al., 2010). The Bph locus was mapped in 23rd chromosome of the mulberry silkworm (Fujimori et al., 1984). In the present study we also ascertained polymorphism in Bph gene, which was presented with three alleles in the gene pool of Mziuri 1 Vr (Bph A, B and 0) and with two alleles in the gene pool of Line 22 Vr (Bph B and 0) and of Mziuri 1 Pv (Bph A and B). Bph B allele was fixed in the gene pool of Line 22 Pv (Table 1).

In the gene pool of all studied populations, we established monomorphism in the loci controlling the alkaline phosphatase (Alp A) and hexokinase (Hk A). Staykova & Ivanova (2012) also reported lack of inter-breed polymorphism in hexokinase locus.

In the present study we ascertained differences in the composition of the gene pools of the pairs of populations of the same strain. These differences were related to the different frequencies of ranging of separate alleles in polymorphic loci, as well as to the lack or fixation of some alleles – only with one of the populations in the pair (Table 1). For example, Bes E_2 allele rarely found in the gene pool of Line 22 Pv (frequency 0.067), was eliminated by the gene pool of Line 22 Vr. Mdh A_2 allele, frequently found in the gene pool of Line 22 Pv (frequency 0.683), was fixed in the gene pool of Line 22 Vr. Bes D_o allele, which was found in Mziuri 1 Vr with no high frequency (0.218), was eliminated by the gene pool of Mziuri 1 Pv. Bes E_o allele, which was frequently found in Mziuri 1 Pv (frequency 0.703) , was fixed in the gene pool of Mziuri 1 Vr. The elimination of rare alleles and the fixation of frequently found alleles in the gene pools of the populations of the same strain is probably a result of the effect of genetic drift. The fixation of Bph B allele in the gene pool of Line 22 Pv and the lack of Bph 0 allele of the gene pool of Mziuri 1 Pv, could also be a result of genetic drift, related with the so called “founder effect”.

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Table 1. *Allele frequencies in populations tested.*

Locus	Populations			
	Line 22 Vr	**Line 22 Pv**	**Mziuri 1 Vr**	**Mziuri 1 Pv**
Bes A				
A_1	1.0	1.0	1.0	1.0
Bes B				
B_1	0.250	0.683	0.462	0.516
B_2	0.208	0.117	0.500	0.281
B_3	0.542	0.200	0.038	0.203
Bes D				
D_1	0.181	0.533	0.141	0.516
D_2	0.681	0.350	0.141	0.438
D_3	0.139	0.117	0.500	0.047
D_0	0	0	0.218	0
Bes E				
E_1	0.181	0.400	0	0.297
E_2	0	0.067	0	0
E_0	0.819	0.533	1.0	0.703
PgmA				
A_1	0.167	0.267	0.013	0.063
A_2	0.389	0.467	0.577	0.547
A_3	0.444	0.267	0.410	0.391
MdhA				
A_1	0	0.200	0	0
A_2	1.0	0.683	1.0	1.0
A_3	0	0.117	0	0
Bph				
A	0	0	0.205	0.625
B	0.389	1.0	0.205	0.375
C	0	0	0	0
D	0	0	0	0
0	0.611	0	0.590	0
Alp A				
A_1	1.0	1.0	1.0	1.0
Hk A				
A_1	0	0	0	0
A_2	1.0	1.0	1.0	1.0

The mean number of alleles per locus varied from 1.9 (for Line 22 Vr and Mziuri 1 Pv) to 2.1 (for Line 22 Pv) (Table 2). The estimated percentage of polymorphic loci was lowest in Mziuri 1 Vr (44.4%). The observed heterozygosity (H_o) varied from 0.119 (for Line 22 Pv) to 0.164 (for Line 22 Vr). With all analyzed populations the expected heterozygosity (H_e) by polymorphic loci was higher than the observed one. There were significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in most populations ($P<0.05$). Chi-Square (df: 1-6) tests showed that the deviations were generally in favour of homozygotes. The lower degree of observed heterozygosity and the higher degree of homozygotes proved the inbreeding effect.

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

Populations	Mean sample size per locus	Mean No. of alleles per locus	Percent polymorphic loci (P=0.99)	H_o	H_e
Line 22 Vr	50.0±0.0	1.9±0.30	55.6	0.164±0.059	0.279±0.094
Line 22 Pv	81.0±0.0	2.1 ±0.40	55.6	0.119±0.045	0. 308±0.099
Mziuri 1 Vr	50.0±0.0	2.0±0.40	44.4	0.157±0.067	0. 255±0.102
Mziuri 1 Pv	80.0±0.0	1.9±0.30	55.6	0.142±0.059	0.292±0.094

The estimated mean F_{ST} value was 0.1752 which shows that 17.52% of the overall genetic diversity observed was among populations. The highest value of intra-population genetic variability was calculated by Bph locus (0.3734) and the lowest one was calculated by Pgm locus (0.0324).

The results obtained in this study showed that in the studied populations of the two introduced strains Line 22 and Mziuri 1, there is an inter- and intra-population polymorphism at six enzyme loci (Bes B, Bes D, Bes E, Pgm, Mdh, Bph) and monomorphism at three loci (Bes A, Alp and Hk). The acid phosphatases from the hemolymph of the mulberry silkworm are very suitable markers to analyze the intra-population polymorphism. Apart from this group of isoenzymes, malate dehydrogenase and nonspecific esterases could also be used for the same purpose. Phosphoglucomutase is more suitable to study the inter-population polymorphism and determination of the degree of population genetic variability. The genetic variability determined in the course of this study is important for the analysis of separate populations and strains in order to use them in selection programs.

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