Phylogenetic differentiation of silkworm (*Bombyx mori* L.) strains with different origin raised in Bulgaria

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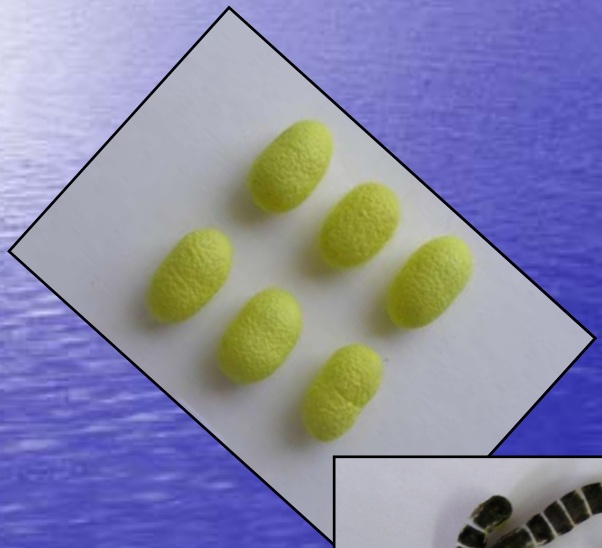
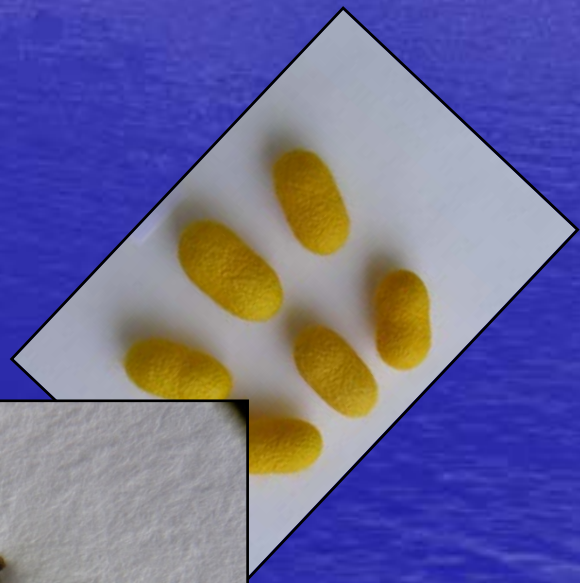




Introduction

The mulberry silkworm, *Bombyx mori* L., has long been used as a model system for basic studies. The economic and scientific significance of silkworm have made this species the subject of intensive genetic studies since the beginning of the last century, and thus, the most important insect genetic model after *Drosophila melanogaster*.

The mulberry silkworm has a large number of geographical strains and inbred lines which show substantial variation for a large number of quantitative traits. The traditional breeding activities, involving hybridization between members of elite groups, are adding new breeds every year. At present, in the silkworm, traits such as cocoon shape, cocoon colour, silk fibre length, larval duration, together with many other ethological traits, are used to differentiate varieties. The selection of parental strains for a breeding programme is based on these characteristics. But the silkworm varieties, particularly those which have been bred from crosses involving many varieties, cannot be distinguished by the use of conventional characteristics. The use of molecular markers could provide a solution to the problem, by providing specific DNA and isozyme profiles (Reddy et al., 1999).

The isozyme profiles of the strains would be useful in producing reliable estimates of genetic diversity, for the selection of parents for the development of elite hybrids.

The aim

- to determine the degree of diversity and the existing relationships between ten silkworm strains with different origin belonging to the silkworm germplasm bank of Bulgaria, using isoenzyme markers.

Material and Methods

Strains	**Origin**
Vratza 1	BULGARIA
Belopol 1/18	BULGARIA
Belopol 2/21	BULGARIA
Gergana 1	BULGARIA
Gergana 2	BULGARIA
Ogosta 1	BULGARIA
Alb Cislau 29	ROMANIA
E 29	EGYPT
Ukrainian 20	UKRAINE
Syria 1	Syria

- From 30 to 40 larvae were selected randomly from each strain on the fifth day of the fifth instar and were used in the study. The larval haemolymph was taken with a transactional cut through one of the prolegs. The spectrum of nonspecific esterases (EST) (EC 3.1.1) from hemolymph was studied by means of 7.5% PAGE (Stoykova et al., 2003). 10μl of each sample was applied into the gel. Method of Shaw and Prasad (1970) was used to visualize the nonspecific esterases.

- Allele and genotype frequencies, observed (Ho) and expected (He) heterozygosity, deviation from the Hardy-Weinberg equilibrium, Nei's genetic distance (D), and Wright's fixation index (FST) were calculated using BIOSYS-1.

- The UPGMA dendrogram was constructed using the PHYLIP software package.

Results and Discussion

- The nonspecific esterases from the *B. mori* haemolymph were under polygene control and for four of the esterase genes (Bes A, Bes B, Bes D and Bes E) was described polymorphism (Egorova et al., 1985; He, 1995; Stoykova et.al., 2003; Staykova, 2008), which was also confirmed in this study. Genepool of the studied ten races regarding their allele composition and the frequencies of different alleles was analyzed on the basis of this polymorphism. Race specificity was ascertained (Table 1).

Table 1. Allele frequencies in strains tested

Strains	Locus												
	Bes A			Bes B			Bes D				Bes E		
	A_0	A_1	A_2	B_1	B_2	B_3	D_0	D_1	D_2	D_3	E_0	E_1	E_2
Vratza 1	0	1	0	0.567	0	0.433	0	0.350	0.350	0.300	0.467	0.350	0.183
Belopol 1/18	0	1	0	0.600	0.325	0.075	0	0.138	0.500	0.363	0.775	0.112	0.112
Belopol 2/21	0	1	0	0.256	0.423	0.321	0	0.115	0.654	0.231	0.692	0.167	0.141
Gergana 1	0	1	0	0.691	0.147	0.162	0	0.824	0	0.176	0.662	0	0.338
Gergana 2	0	1	0	0.200	0.200	0.600	0	0.833	0.100	0.067	0.600	0	0.400
Ogosta 1	0	1	0	0	0.528	0.472	0	0.556	0.444	0	0	1	0
Alb Cislau 29	0.300	0.700	0	0.517	0.050	0.433	0	0.667	0	0.333	0.750	0.250	0
E 29	0	0.500	0.500	0	0.317	0.683	0.533	0.467	0	0	0.633	0	0.367
Ukrainian 20	0	1	0	1	0	0	0	0.551	0.449	0	1	0	0
Syria 1	0	1	0	0.053	0	0.947	0	0.487	0	0.513	0.566	0.303	0.132

- In this study we found polymorphism on the Bes A locus in the races Alb Cislau 29 and E 29. This locus was monomorphic in all other tested strains and presented with Bes A_1 allele.
- The Bes B_1 frequency was highest for the Gergana 1 strain, the Bes B_2 allele – for Ogosta 1, and Bes B_3 – for Syria 1.
- We established the highest frequency of the Bes D_1 allele for the strain of Gergana 2, of Bes D_2 – for Belopol 2/21, and of Bes D_3 – for Syria 1. The Bes D_0 allele was presented in the genepool of the strain E 29, only.
- The Bes E locus was polymorphic and presented by three alleles. For Ukrainian 20, there was fixed the Bes E_0 allele, while for Ogosta 1 – the Bes E_1 allele. Among all strains where we found polymorphism by locus Bes E the highest frequency was demonstrated by the Bes E_0 allele.

The average number of alleles per locus calculated with BIOSYS-1 varied from 1.3 (for Ukrainian 20) to 2.5 (for Belopol 1/18 and Belopol 2/21).
The degree of polymorphism was highest for strains Alb Cislau 29 and E 29 (100%), and lowest – for Ukrainian 20 (25%).
The observed heterozygosity (Ho) calculated with BIOSYS-1varied from 0.392 (for Alb Cislau 29) to 0.083 (for Ukrainian 20). The expected heterozygosity (He) was higher than the observed one in all

tested strains.

Strains	Mean sample size per locus	Mean no. of alleles per locus	Percent Polymorphic loci (P=0.95)	H_o	H_e
Vratza 1	30.0±0.0	2.3±0.50	75.0	0.283±0.097	0.453±0.156
Belopol 1/18	40.0±0.0	2.5 ±0.50	75.0	0.262±0.107	0.380±0.135
Belopol 2/21	39.0±0.0	2.5±0.50	75.0	0.192±0.107	0.413±0.143
Gergana 1	34.0±0.0	2.0±0.40	75.0	0.096±0.058	0.308±0.111
Gergana 2	30.0±0.0	2.3±0.50	75.0	0.142±0.067	0.338±0.127
Ogosta 1	36.0±0.0	1.5±0.30	50.0	0.153±0.089	0.252±0.145
Alb Cislau 29	30.0±0.0	2.3 ±0.30	100	0.392±0.044	0.453±0.036
E 29	30.0±0.0	2.0±0.00	100	0.275±0.086	0.482±0.016
Ukrainian 20	39.0±0.0	1.3±0.30	25.0	0.083±0.083	0.125±0.125
Syria 1	38.0±0.0	2.0±0.40	75.0	0.125±0.054	0.296±0.144

Results and Discussion

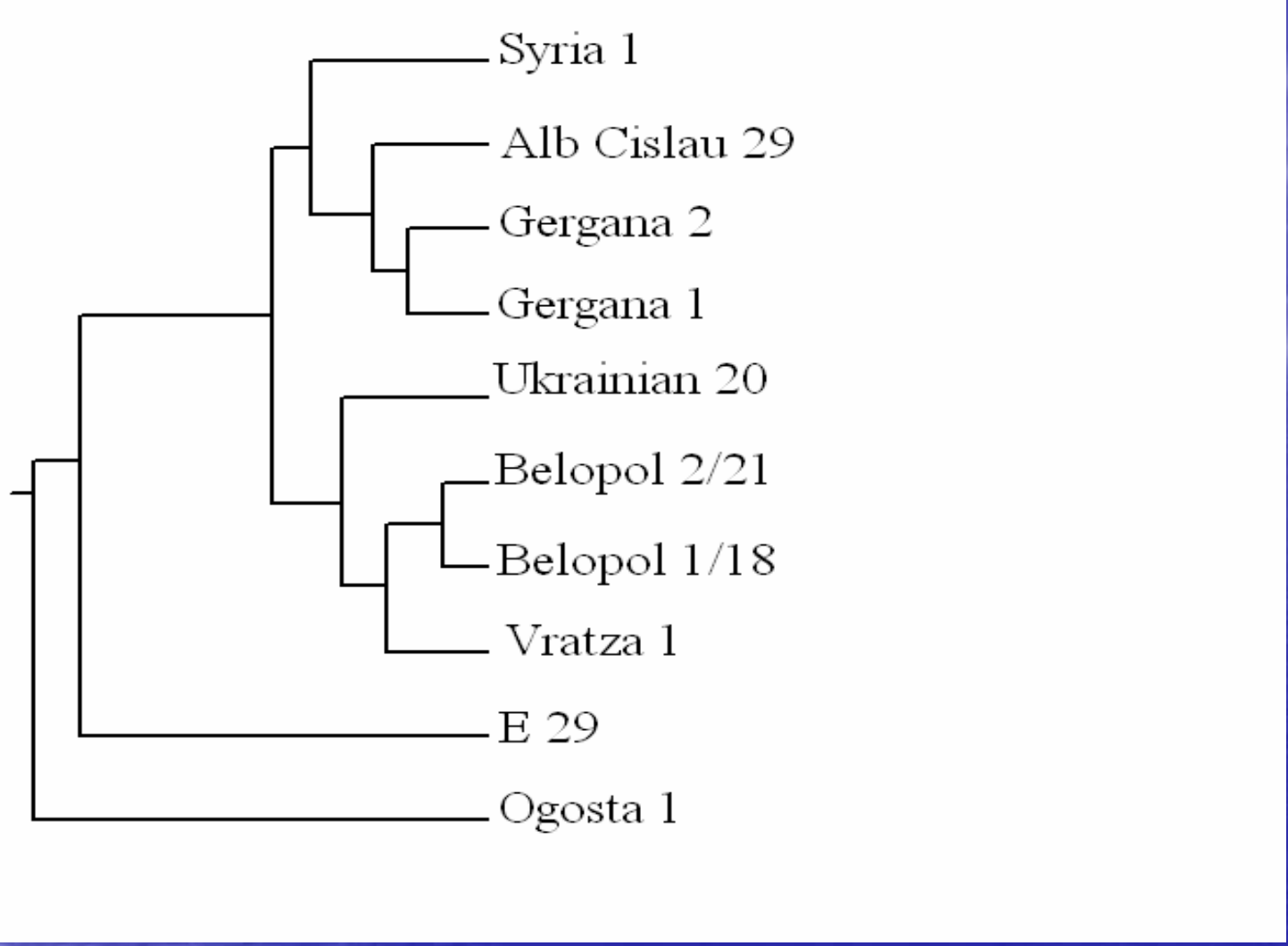
- The test for conformance to Hardy-Weinberg equilibrium manifested significant differences between the obtained and expected genotype frequencies for the most of the strains (except Alb Cislau 29) resulting from the selection.

- The mean value of FST (0.3116) calculated on the base of the established polymorphism, showed that 31.16% of the genetic variability was observed between the different strains, which corresponds to the level of the inter-strain genetic differentiation.

Table 3. Nei's (1972) genetic distance (above diagonal) based on isoenzymes

	Vratza 1	Belopo 1 1/18	Belopo 1 2/21	Gergana 1	Gergana 2	Ogosta 1	Alb Cislau 29	E 29	Ukrainian 20	Syria 1
Vratza 1	*****	0.102	0.121	0.134	0.160	0.305	0.128	0.491	0.157	0.149
Belopol 1/18		*****	0.050	0.180	0.292	0.527	0.224	0.606	0.101	0.366
Belopol 2/21			*****	0.285	0.240	0.342	0.294	0.469	0.223	0.282
Gergana 1				*****	0.089	0.586	0.105	0.424	0.108	0.291
Gergana 2					*****	0.402	0.147	0.227	0.283	0.131
Ogosta 1						*****	0.510	0.700	0.768	0.367
Alb Cislau 29							*****	0.400	0.178	0.161
E 29								*****	0.670	0.315
Ukrainian 20									*****	0.512
Syria 1										*****

- On the grounds of the allele frequencies was also calculated a genetic distance by Nei, which varied from 0.050 (between the strains Belopol1/18 and Belopol 2/21) to 0.768 (between strains Ogosta 1 and Ukrainian 20).



The phylogenetic tree constructed by the UPGMA method consisted of two major subgroups from E 29 strain. The first one contained strains Syria 1, Alb Cislau 29, Gergana 1 and Gergana 2, and the second one included Ukrainian 20, Belopol 1/18, Belopol2/21 and Vratza 1.

The data obtained in this study for the inter- and intra-strain diversity and strain differentiation, could be used for breeding purposes of the mulberry silkworm *Bombyx mori* L. and marker-assisted selection.

Acknowledgments

This work has been financed by "Fund for Scientific Researches" at Ministry of Education and Science – contract DO 02–63/11.12.2008.

Thank you !