# Passport data of six Bulgarian strains of silkworm *Bombyx mori* L. on the base of population genetic parameters

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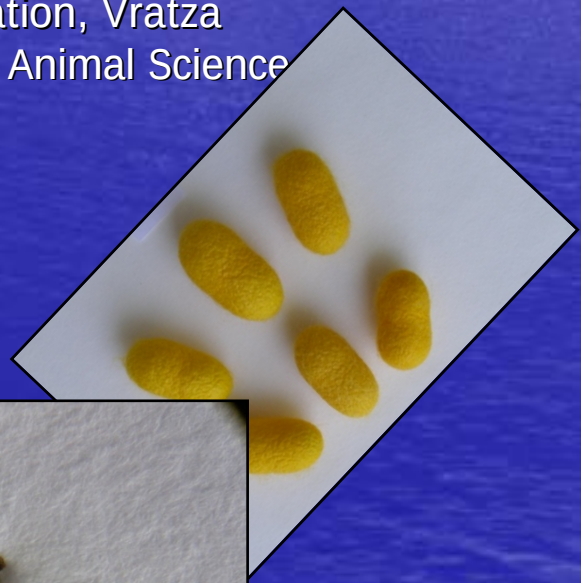


# Introduction

- Isozymes and other molecular markers are very useful to study the genetic diversity of mulberry silkworm *B. mori* L. and to differentiate the particular strains. Assessment of heterozygosity in each silkworm strain is important for efficient management and conservation of genetic resources .

# Introduction

- The choice of parental strains in the silkworm breeding programs in Bulgaria was mainly based on the study of some qualitative and quantitative traits as total larval duration, cocoon shape, cocoon colour, weight of single cocoon, weight of single shell, shell ratio, fecundity and etc.
- The assessment of the parental strains, however, should be complex and should be realized simultaneously by different parameters. One of the main composite parts of such a complex assessment is the passportisation of various strains of isoenzyme specters of key metabolic enzymes.

# Aim

- The aim of this study was to assess the genetic structure of populations of six silkworm Bulgarian strains on the basis of isozyme polymorphism and to describe population genetics passport data of this strains.

# Material and Methods

| Strains | Origin |
|---|---|
| VRATZA 1 | BULGARIA |
| VRATZA 37 | |
| VRATZA 40 | |
| GERGANA 1 | |
| GERGANA 2 | |
| OGOSTA 1 | |

Six silkworm strains created in Bulgaria and maintained by the Sericulture Experiment Station germplasm bank - Vratza, and Agricultural University – Plovdiv were tested with 7.5% polyacrilamide gel electrophoresis (PAGE) (Daevis *et al.*, 1964).

On the fifth day of the fifth instar, 80 - 88 larvae were selected randomly from each strain in order to analyse the following enzyme systems:

- EST (four loci), MDH (one locus) and ACP (one locus) – from hemolymph;
- PGM (one locus), HK (one locus), ADH (one locus) and AST (one locus) - from silk glands.

Several genetic variation parameters as allele frequencies, mean number of alleles per locus, proportion of polymorphic loci at the 99% (P), observed ($H_o$) and expected ($H_e$) heterozygosity... were computed by BIOSYS-1 software. Phylogenetic trees were constructed using Nei's (1972) genetic distance, by UPGMA and Neighbor-joining methods using the PHYLIP software package.

# Results and Discussion

- Among the ten investigated isozyme loci, six loci (Bes B, Bes D, Bes E, Pgm A, Mdh A, Bph A) proved to be polymorphic (Table 1), and four of them (Bes A, Hk A, Adh A and Ast A) were monomorphic. The six polymorphic loci manifested intra- and inter-strain polymorphism. The Mdh A locus was identified as polymorphic only in Ogosta 1. We determined population genetic passport data on studied loci concerning allele frequencies, mean number of alleles per locus, percent of polymorphic loci and heterozygosity for each of the tested strain.

| Locus | Strains | | | | | |
|---|---|---|---|---|---|---|
| | Vratza 1 | Vratza 37 | Vratza 40 | Gergana 1 | Gergana 2 | Ogosta 1 |
| **BesB** | | | | | | |
| $B_1$ | 0.567 | 0.183 | 0 | 0.691 | 0.200 | 0 |
| $B_2$ | 0 | 0.183 | 1 | 0.147 | 0.200 | 0.528 |
| $B_3$ | 0.433 | 0.633 | 0 | 0.162 | 0.600 | 0.472 |
| **BesD** | | | | | | |
| $D_0$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $D_1$ | 0.350 | 0.750 | 1 | 0.824 | 0.833 | 0.556 |
| $D_2$ | 0.350 | 0.250 | 0 | 0 | 0.100 | 0.444 |
| $D_3$ | 0.300 | 0 | 0 | 0.176 | 0.067 | 0 |
| **BesE** | | | | | | |
| $E_0$ | 0.467 | 0.833 | 0.483 | 0.662 | 0.600 | 0 |
| $E_1$ | 0.350 | 0.167 | 0.283 | 0 | 0 | 1 |
| $E_2$ | 0.183 | 0 | 0.233 | 0.338 | 0.400 | 0 |
| **PgmA** | | | | | | |
| $A_1$ | 0 | 0 | 0 | 0.265 | 0 | 0.097 |
| $A_2$ | 0.667 | 0.900 | 0.817 | 0.500 | 1 | 0.236 |
| $A_3$ | 0.333 | 0.100 | 0.183 | 0.235 | 0 | 0.667 |
| **MdhA** | | | | | | |
| $A_1$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $A_2$ | 1 | 1 | 1 | 1 | 1 | 0.972 |
| $A_3$ | 0 | 0 | 0 | 0 | 0 | 0.028 |
| **Bph A** | | | | | | |
| $A_0$ | 0.100 | 0.088 | 0.194 | 0 | 0 | 0 |
| $A_1$ | 0.317 | 0.618 | 0.597 | 0.529 | 0.550 | 0 |
| $A_2$ | 0.400 | 0.206 | 0.048 | 0.471 | 0.450 | 1 |
| $A_3$ | 0.183 | 0.074 | 0.161 | 0 | 0 | 0 |

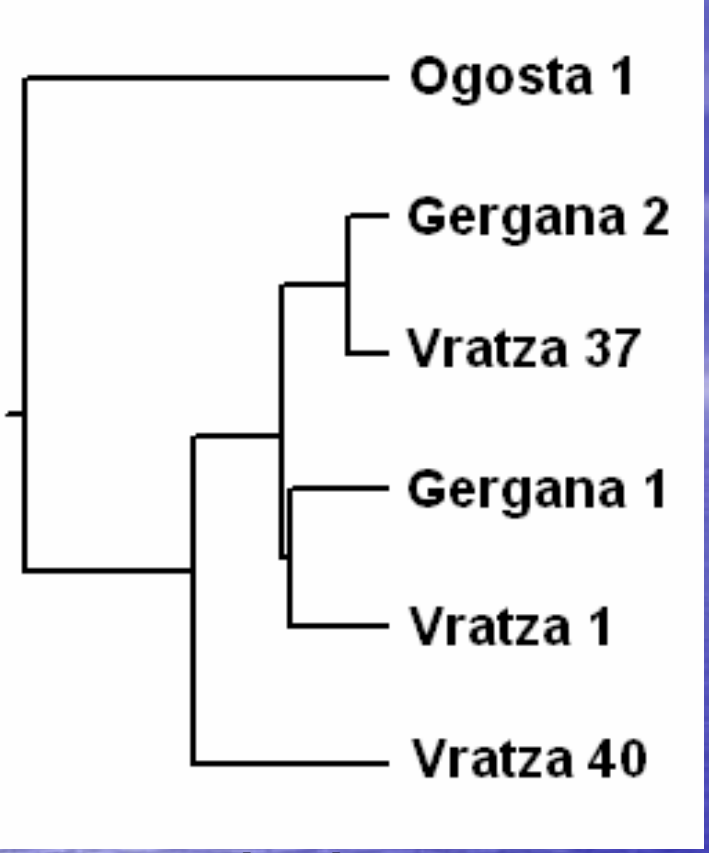
| Locus | Strains | | | | | |
|---|---|---|---|---|---|---|
| | Vratza 1 | Vratza 37 | Vratza 40 | Gergana 1 | Gergana 2 | Ogosta 1 |
| **BesA** | | | | | | |
| $A_0$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $A_1$ | 1 | 1 | 1 | 1 | 1 | 1 |
| $A_2$ | 0 | 0 | 0 | 0 | 0 | 0 |
| **Hk** | | | | | | |
| $A_1$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $A_2$ | 1 | 1 | 1 | 1 | 1 | 1 |
| **Adh** | | | | | | |
| A | 1 | 1 | 1 | 1 | 1 | 1 |
| **Ast A** | | | | | | |
| A | 1 | 1 | 1 | 1 | 1 | 1 |

| Strains | Mean sample size per locus | Mean no. of alleles per locus | Percent polymorphic loci (P=0.99) | $H_o$ | $H_e$ |
|---|---|---|---|---|---|
| **Vratza 1** | 85.0±0.0 | 1.9±0.30 | 50.0 | 0.183±0.070 | 0.297±0.102 |
| **Vratza 37** | 88.0±0.0 | 1.9 ±0.40 | 50.0 | 0.102±0.046 | 0.196±0.074 |
| **Vratza 40** | 86.0±0.0 | 1.6±0.30 | 30.0 | 0.082±0.051 | 0.153±0.083 |
| **Gergana 1** | 84.0±0.0 | 1.7±0.30 | 50.0 | 0.112±0.052 | 0.237±0.083 |
| **Gergana 2** | 80.0±0.0 | 1.6±0.30 | 40.0 | 0.100±0.050 | 0.186±0.079 |
| **Ogosta 1** | 86.0±0.0 | 1.5±0.20 | 40.0 | 0.086±0.042 | 0.156±0.076 |

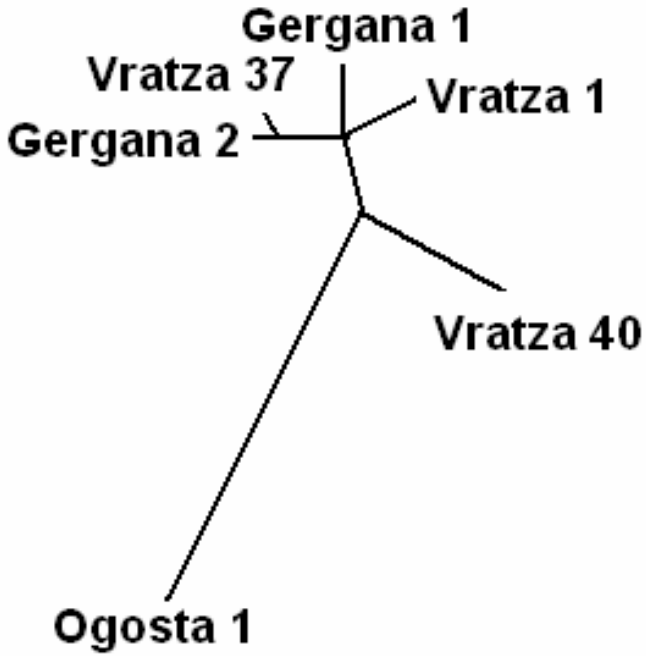
- The results about genotype frequencies showed statistically significant deviations from the Hardy-Weinberg equilibrium for the most loci in six strains. This fact in addition to the deficit of heterozygotes may indicate a high level of inbreeding in the studied populations.
- The $F_{ST}$ values ranged from 0.0228 (Mdh A) to 0.3571 (Bes E). The mean $F_{ST}$ value over all loci was 0.2904, which shows that 29.04% of the overall genetic diversity observed was among strains.

| | Vratza 1 | Vratza 37 | Vratza 40 | Gergana 1 | Gergana 2 | Ogosta 1 |
|---|---|---|---|---|---|---|
| **Vratza 1** | ***** | 0.061 | 0.164 | 0.059 | 0.070 | 0.151 |
| **Vratza 37** | | ***** | 0.093 | 0.072 | **0.023** | 0.233 |
| **Vratza 40** | | | ***** | 0.119 | 0.094 | 0.234 |
| **Gergana 1** | | | | ***** | 0.054 | 0.237 |
| **Gergana 2** | | | | | ***** | **0.238** |
| **Ogosta 1** | | | | | | ***** |

The values of genetic distance (Nei, 1972) were calculated using the allele frequencies and ranged from 0.023 (between the strains Vratza 37 and Gergana 2) to 0.238 (between strains Gergana 2 and Ogosta 1).




**UPGMA dendrogram**




**Neighbor-joining dendrogram**

In UPGMA and Neighbor-joining dendrograms strains studied are grouped in two clades originated from Vratza 40. Gergana 2 and Vratza 37 were clustered in the one clade and Gergana 1 and Vratza 1 were clustered in the second one (Fig.1 and 2). The strain Ogosta 1 is outlying from all others.

# Conclusion

- The specific intra- and inter-strain polymorphism on the isoenzyme loci and some population genetics characteristics as frequencies of alleles, mean number of alleles per locus, proportion of polymorphic loci and heterozygosity could be use for composing passport data of each strain in order to use in the breeding programs of mulberry silkworm *Bombyx mori* L.

# LAB of GENETICS at PLOVDIV UNIVERSITY

Antique theatre

Thank you for your attention!