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Study on Productivity and Genotype Structure by Several Enzyme Loci of Silkworm (*Bombyx mori* L.) Parthenoclones Obtained by Thermal and Combined (Low-High Temperature) Method

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The purpose of this study was to establish whether there are differences in the productivity of the same silkworm (*Bombyx mori* L.) parthenoclones, obtained by two different methods – thermal and combined, as well as to study their genotype structure by several enzyme loci. It was established that all individuals of parthenoclones Joana, Joana (↓↑), Pohi and Pohi (↓↑), are homozygous by the studied esterase and phosphoglucomutase loci, which substantiated the clones' genetic stability. By comparative analysis of some biological and technological properties, it was found that parthenoclone Pohi (↓↑) obtained by low-high temperature activation is characterized by higher values of these properties as compared to parthenoclone Pohi obtained by thermal parthenogenesis. Comparing the two methods of inducing ameiotic parthenogenetic development, we would recommend that parthenoclone Joana is sustained by thermal parthenogenesis, and parthenoclone Pohi – by the combined method (low-high temperature).

Key words: *Bombyx mori* L., Parthenogenesis, Isoenzymes

Introduction

The greatest contribution to clarify the nature of the artificial parthenogenesis of silkworm (*Bombyx mori* L.) has been made by the investigations of Vereyskaya (1979), Strunnikov *et al*. (1980a), Klimenko (1980), Sugai *et al*. (1983a), Murakami (1985), Yunqiang *et al*. (2001), Singh *et al*. (2002), who propose the so called thermal method for activating non-fertilized eggs removed from the ovarioles. The method of ameiotic parthenogenesis induction is significantly labour-consuming and a number of authors direct their efforts to developing more efficient methods. Strunnikova and Demyanov (1980) propose another method of inducing parthenogenesis with non-fertilized eggs laid by silkworm, which is based on the combined impact with low (–11°C) temperature and high (46°C) temperature. Vasileva (2006) also works on induced ameiotic parthenogenesis, provoking parthenogenetic development by means of thermal and combined method, to breeds and hybrids from the silkworm genepool in Bulgaria, maintained at the Sericulture Experiment Station – Vratsa.

The aim of this study was to establish whether there are differences in the productivity of the same parthenoclones obtained by two different methods – thermal and combined, as well as to study their genotype structure by some enzyme loci.

Materials and Methods

Obtained of the parthenoclones

The silkworm (*B. mori* L.) parthenoclones included in this study were obtained by two different methods:

1. Thermal method – non-fertilized eggs, isolated from the ovarioles of female silkworm moth, were placed for pre-activation preservation for 12 h at temperature of 25°C and relative air humidity of 80 ~ 90%. The eggs were activated towards ameiotic parthogenetic development by means of dipping into clean water of temperature of 46°C

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for 18 min. and successive cooling for 5 ~ 10 min in water of 18°C. After the treatment the parthenogenetic eggs were kept for 3 days at 15 ~ 20°C and relative air humidity of 85 ~ 90% (Vasileva, 2006).

2. Combined method of low-high temperature (↑↓) – eggs from non-fertilized female silkworm moths were treated at the fourth day after being laid with combined impact of low temperature (–10°C) at exposition of 150 min., successive water bath of high temperature (46°C) for 10 min and sudden cooling for 5 ~ 10 min in water of 18°C (Vasileva, 2006).

The ameiotic parthogenetic clones obtained by the thermal method were designated as Joana and Pohi. The parthenoclones obtained by the combined method of low-high temperature were designated Joana (↑↓) and Pohi (↑↓). All individuals were nourished at a standard regime of silkworm breeding.

Isoenzyme analysis

Twenty individuals from each parthenoclone were analyzed to establish their genotype by several esterase and phosphoglucomutase loci, which showed a breed-specific polymorphism of the silkworm. Larvae in 5th instar were used. The study was carried out by means of Polyacrilamide gel electrophoresis (PAGE). To analyze non-specific esterases individual samples of haemolymph were used and separation in 7.5% gel. To analyze the phosphoglucomutase were used individual extracts of silk glands and separation in 6% gel. Polyacrylamide gel electrophoresis and isolation of haemolymph and silk glands were carried out according to Stoykova *et al.* (2003 and 2004). Methods of Spencer *et al.* (1964) and Shaw and Prasad (1970) were used to visualize the phosphoglucomutase and the esterases respectively.

Results and Discussion

Biological properties

At analyzing the biological properties of the obtained parthenoclones, it was established that the 5th instar period of the parthenoclones, obtained by the thermal method is comparatively shorter, especially for Joana (Table 1). The larval duration of parthenoclone Pohi (↑↓) obtained by low-high temperature is 23 h shorter that the one of Pohi obtained by the thermal method and the difference is significant at degree of probability ***P < 0.001. The pupation rate of the silkworm parthenoclones obtained by low–high temperature is higher than the one of the parthenoclones obtained by thermal parthenogenesis, respectively with 2.66% - for Joana (↑↓) and 3.17% - for Pohi (↑↓) (the difference is significant at degree of probability *P < 0.05). The fresh cocoon yield by one box of eggs for parthenoclone Pohi (↑↓) is 1.71 kg higher than parthenoclone Pohi.

Fresh cocoon technological properties

The data on fresh cocoon technological properties is given in Table 2. It was established that with the studied parthenoclones obtained by low-high temperature activation, the shell weight and shell percentage % values are higher than those of the parthenoclones obtained by thermal method. Parthenoclone Pohi (↑↓) showed higher values for the three properties as compared with parthenoclone Pohi, respectively with: 0.039 g - for the fresh cocoon

Table 1. Biological properties

Parthenoclone	5-th instar duration in h	td	Larval duration in h	td	Pupation rate in %	td	Fresh cocoon yield by one box of eggs (20000 eggs) in kg	td
Joana	213		718		91.00		37.33	
Joana (↑↓)	222***	8.92	719	0.99	93.66	2.64	36.66	0.66
Pohi	213		718		96.33		38.66	
Pohi (↑↓)	214	0.99	695***	22.81	99.50*	3.14	40.37	1.69

* P<0.05, **P<0.01, ***P<0.001

Table 2. Fresh cocoon technological properties

Parthenoclone	Fresh cocoon weight g	td	Shell weight g	td	Shell percentage %	td
Joana	2.159		0.440		20.57	
Joana (↑↓)	2.084***	7.44	0.441	0.99	21.16	0.58
Pohi	2.163		0.438		20.25	
Pohi (↑↓)	2.202*	3.86	0.463*	2.48	21.03	0.77

*P<0.05, **P<0.01, ***P<0.001

Table 3. Filament technological properties

Parthenoclone	Dry cocoon weight in g	td	Filament length in m	td	Filament weight in g	td	Filament size in denier	td	Reelability in %	td	Raw silk percentage (%)	td
Joana	0.882		1129		0.341		2.72		90.21		38.66	
Joana (↑↓)	0.880	0.065	1086	0.63	0.329	0.55	2.72	0	90.88	0.66	37.38	1.26
Pohi	0.869		1027		0.318		2.78		86.64		36.59	
Pohi (↑↓)	1.008	0.69	1292***	4.42	0.396***	4.26	2.76	1.98	91.45**	4.77	39.28*	2.67

*P<0.05, **P<0.01, ***P<0.001

weight property, 0.025 g - for the shell weight property (significant difference at degree of probability ***P<0.05), and 0.78% - for the shell percentage property.

Filament technological properties

The data on the filament technological properties of parthenoclone Pohi (↑↓) showed well distinguished higher values compared to those of parthenoclone Pohi. The differences with the technological properties filament length and weight are significant at degree of probability ***P<0.001, for the reelability percentage - at **P<0.01, and for raw silk percentage - at *P<0.05 (Table 3). The values of the same properties for parthenoclones Joana and Joana (↑↓) do not differ significantly.

Genotype structure of the parthenoclones

The genotype structure of each parthenoclone was analyzed by several loci of non-specific esterases and phosphoglucomutase. In the haemolymph spectrum of all studied parthenoclones the esterases were analyzed of zones BES B, BES D and BES E, described earlier by Stoykova *et al*. (2003). These esterases were determined by three different loci - Bes B, Bes D and Bes E and manifested a breed-specific polymorphism with the breeds grown in Bulgaria, with presence of three codominant alleles in each of them (Stoykova *et al*. 2003; Staykova and Grekov, 2006; Staykova, 2008). For all studied individuals in zone BES B was found only the presence of fraction BES B_1, in zone BES D was only found the presence of fraction BES D_3, and in zone BES E - was not found any esterase activity. The lack of polymorphism by these esterases showed that the individuals of Joana, Pohi, Joana (↑↓) and Pohi (↑↓) are homozygous by the respective esterase genes, and in the genepool of each of the parthenoclones locus Bes B is presented only by allele Bes B_1, locus Bes D - by allele Bes D_3, and locus Bes E - by Bes E_0. In the spectrum of silk glands the expression of phosphoglucomutase was analyzed and monolocus polymorphism was found for the silkworm breeds grown in Bulgaria (Staykova, 2008), with the presence of three codominant alleles (Pgm A_1, A_2 и A_3). For all studied individuals was only found expression of one fraction - PGM A_2, which proved homozygosity by allele Pgm A_2. It showed that in the genepool of the four parthenoclones, locus Pgm A was presented by allele Pgm A_2. The homozygosity established by the analyzed genes proved genetic stability of the parthenoclones.

On the grounds of the above said we can draw the following conclusions:

1. Parthenoclone Pohi (↑↓), obtained by low-high temperature activation has higher values of the studied biological and technological properties as compared to parthenoclone Pohi obtained by thermal parthenogenesis.
2. Comparing the two methods of inducing ameiotic parthenogenic development to the studied parthenoclones, we recommend that parthenoclone Joana is sustained by thermal parthenogenesis, and parthenoclone Pohi – by the combined method (low-high temperature).
3. Parthenoclones Joana, Joana (↑↓), Pohi and Pohi (↑↓), are genetically stable and composed of homozygous individuals. The genepool of the four perthenoclones lack polymorphism by the analyzed enzyme loci.

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