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BREED SPECIFIC EXPRESSION OF GUT SILKWORM *(Bombyx mori L.)* NONSPECIFIC ESTERASES

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ABSTRACT. Nonspecific esterases of silkworm (*Bombyx mori* L.) gut were investigated by means of polyacrilamide gel electrophoresis (PAGE). Stage-specific expression of eleven esterase bands was detected during larval development of breeds and inter-breed hybrids kept in Bulgaria. In two esterase zones, intra- and inter-breed polymorphism was found. The polymorphism in fraction GES I_1 specific for the gut may be used for testing of the breeds raised in our country with reference to determining the degree of genetic heterogeneity. The specific expression in zone GES L_1 observed at present study confirmed gene determinate polymorphism in zone BES E_1, observed earlier.

KEY WORDS. *Bombyx mori* L., PAGE, gut esterases, polymorphism.

INTRODUCTION

The study of polymorphous enzyme proteins of *Bombyx mori* L. is significantly important for the selection of this species. The efforts of the selection research workers are directed to creating highly productive breeds and hybrids of mulberry silkworm. Besides, some negative tendencies can be observed resulting from the decrease of the overall adaptive potential, which is related to decreasing the degree of genetic changeability. The establishment of suitable biochemical markers for analyzing the degree of genetic heterogeneity may be used for a preliminary evaluation and selection of the different breeds and families when making optimal variants for cross-breeding with reference to improving the most important economic

signs and increasing the effectiveness of heterozygous selection. With this regard different polymorphous enzyme groups have been studied (Marcato et al., 1990; Takeda et al., 1993; Kanekatsu et al., 1993; Eguchi, 1995; Asano et al., 1997). When studying different breeds raised mainly in Japan, China, Korea, India and the former Soviet Union, in the group of nonspecific esterases from different tissues, a genetically determined polymorphism has been ascertained (Egorova et al., 1985; He, 1995; Krishnamurthy et al., 1997). The breeds of mulberry silkworms and their hybrids raised in Bulgaria have not been studied for analyzing the nonspecific gut esterases and finding out an eventual polymorphism. This was the motivation to provide the present study.

MATERIAL AND METHODS

Using polyacrylamide gel electrophoresis (PAGE) 850 specimens from different ovipositions of sixteen breeds designated as 19, 20, B517, T106, T108, M1, M2, UK17, UK18, UK19, UK20, P14, P15, Tashkent 12, Tashkent 15, Tashkent 16 as well as the F1 inter-breed hybrids P14xP15, P15xP14, M1xM2, M2xM1, UK17xUK19, UK19xUK17, UK18xUK20, and UK20xUK18 have studied. The esterase spectra were estimated at different ontogenetic stages: 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} larval instars. Individual samples as well as mixed ones from equalized number of specimens per race or hybrid were used for 1^{st}, 2^{nd} and 3^{rd} larval instars. Only individual samples were used during later developmental stages.

The gut was isolated through dissection, rinsed with distilled water, squashed with quartz sand in 0.8 M tris–phosphate buffer at pH 6.7 and left for extraction for 18 hours at 4°C. Then the samples were centrifuged for 45 minutes at 5000 rpm before PAGE. The electrophoretic separation involved using polyacrylamide vertical gels (6%; 6.5%; 7.5%) at pH 8.9 at 4.5 mA/cm for 3 hours, together with concentrating gels (3.3%) at pH 6.7, and 0.05 M tris–0.2 M glycine electrode buffer at pH 8.3.

The nonspecific esterases were visualized in 1 M phosphate buffer at pH 7.0. α- and β-naphthylacetate were used as substrates and Fast blue BB salt as a dye. The stained plates were fixed in a 14% trichloroacetic acid for 2 hours and stored in 7% acetic acid.

RESULTS AND DISCUSSION

In the course of larvae development in the electrophoretic spectrum of the alimentary tract we visualized total eleven esterase fractions (gut esterase - GES), which we marked in the sequence of their decreasing electrophoretic mobility (GES A ÷ GES L_1, Fig.1).

In mixed extracts from larvae gut 1^{st} instar we visualized fractions GES A, B, C, D, E, F, G, H, I_1 and J, as in the individuals from the breeds B517, T106, T108 and Tashkent 16, there missed GES I_1. The bands GES D and GES E form a common spot, where two separate fractions have been differentiating only in a greater rarefaction. In the case of a higher concentration of the samples, fraction GES F is masked (disappears) in this zone, too. The bands GES A and GES C are with a weak

expression in most of the samples. GES A is missing from the individual spectrum, and all the rest of the bands are weaker probably as a result of a low concentration of the respective products. Due to the same reason the bands GES D and GES E have been well differentiated as two separate fractions in the individual extracts (Fig.1, Fig.2).

In mixed extracts of larvae gut 2nd instar GES A is intensive. The same band is visualized also in the individual spectrum but as a weak one. Fractions GES B, F and J are more distinct in the individual spectrum of 2nd larval instar compared to the 1st instar (Fig.1, Fig.3).

In 3rd larval instar we ascertained GES A, B, C, D, E, F, G, H, I_1 and J, as in mixed, as well as in individual samples. We visualized these esterase bands also in the spectrum of 4th and 5th larval instars (Fig.1). Because of the great intensity fractions GES D and GES E have not been differentiated as separate zones, but form a common spot, where sometimes fraction GES F has been masked also at 7.5% PAGE. By means of a comparative study of gut extracts of one and the same individuals in gels with different concentration (6%÷7.5%), as well as by means of a greater rarefaction of some of the samples, prepared for 7.5% PAGE, we proved the presence of the fraction GES F in the spectrum (Fig.4).

In the course of the study we ascertained inter-breed differences in the expression of fractions GES I_1 and GES L_1 as follows (Table 1):

- We ascertained esterase GES I_1 with all analyzed individuals of the breeds P14, P15, 19, 20, M1, M2, UK19 and Tashkent 15, and the hybrids P14xP15, P15xP14, M1xM2, M2xM1, UK17xUK19, and UK19xUK17. It is not present in the spectra of B517, T106, T108 and Tashkent 16. We observed GES I_1 with different intensity only with some individuals of the breeds UK17, UK18, UK20 and Tashkent 12 and F_1 hybrids UK18xUK20 and UK20xUK18 (Fig.2, Fig.3, Fig.4).
- We visualized esterase GES L_1 in the spectra of some of the larvae studied of 19, M1, M2, Tashkent 15, M1xM2 and M2xM1 – with different intensity (Fig.1). The fraction is missing from the spectra of other individuals of these breeds and hybrids. We observed this band in an electrophoretic position, corresponding to fraction BES E_1 from the haemolymph spectrum (Stoykova et al., 2003). The expression of GES L_1 manifests an inner-breed and inter-breed polymorphism, which is analogical to the one described under BES E_1. The similar breed specificity in the expression and the same electrophoretic mobility of these two fraction (BES E_1 и GES L_1) ascertained by us give us grounds to consider that they are one and the same isoenzymes, which are present in the haemolymph and gut tissue.

By means of a comparative analysis of the nonspecific esterases from front, middle and back part of the gut of one and the same individuals we ascertained, that all esterase bands, visualized in the spectrum of the whole gut, are present also in the spectra of the parts though with a different intensity. The expression of the esterases from the middle part is stronger. In the spectrum of the front and back part fractions GES A, GES B, GES C, GES H and GES I_1 are very weak. In the electrophoretic spectrum of the front part the band GES G is with a higher intensity.

By means of a comparative study of gut extracts after freezing at -18°C we ascertained, that some esterase bands are not expressed or are with a very weak intensity (GES F, GES J).

Various authors determine different number of esterase fractions in the gut spectrum of different breeds of *B. mori* L. (Eguchi and Sugimoto, 1965 - six; Eguchi and Iwamoto, 1975 - two; Egorova et al., 1977 - seven; Eremina, 1985 - six). In the course of ontogenesis, from 1st larval instar to the end of the larval period, we ascertained a greater number of esterase fractions. We reckon that the differences between the results we have obtained and the results of other authors are related to the use of different electrophoretic techniques (electrophoresis in an agaroze and in a polyacrylamide gels with different concentration), the study of breeds with different origin, and the analyzing of gut spectrum in different stages of the ontogenesis. Egorova et al. (1977) report about an inner-breed and inter-breed polymorphism with regard to the number and intensity of the esterases of gut spectrum. The results we have obtained confirm the presence of a breed-specific polymorphism in the expression of the gut esterases. We admit that the expression of fraction GES I_1 in the spectra of some individuals of the breeds UK17, UK18, UK20 and Tashkent 12, and the absence of the fraction from the spectra of other individuals of the same breeds is a result of the polymorphism with a null allele present, which homozygous state is connected with the absence of an esterase fraction in the zone of GES I. The polymorphism we observe with the studied F_1 hybrids UK18xUK20 and UK20xUK18 is a result of the presence of polymorphism in the initial parents' breeds. We admit that the presence of esterase GES L_1 in the spectra of some individuals is also a result of a biallele control with the presence of a null allele. The similar breed specificity in the expression and the similar electrophoretic mobility of fractions GES L_1 (of the gut spectrum) and BES E_1 (of the haemolymph spectrum) give us grounds to consider that they are one and the same isoenzymes, products of a polymorphous locus.

Our results indicate also a stage-specific expression of the esterases of the gut spectrum, related to a change in the number and intensity of esterase fractions in the different larval instars. The determined stage specificity is a result of the differentiated regulation of the gene activity in the process of ontogenesis.

In the comparative analysis of the non-specific esterases from different tissues and organs of the mulberry silkworm – silk glands, testes, fat body, haemolymph, ovaries, which we have studied earlier (Stoykova et al., 1998; Stoykova et al., 1999; Stoykova, 2001; Stoykova et al., 2003; Staykova et al., 2004) and having compared them to those of a gut, we ascertained that the gut spectrum comprises the greatest number of fractions, thus confirming the data of other authors. Eguchi and Yoshitake, 1967; Minina and Phillippovich, 1974). In the spectrum of the alimentary tract we visualized also the fastest mobile esterase bands (GES A and GES B).

The ascertained polymorphism in fraction GES I_1 specific for the gut may be used for testing of the breeds raised in our country with reference to determining the degree of genetic heterogeneity.

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Table 1. Breed specificity esterase expression of GES I_1 and GES L_1 bands: (+) – expression in all specimens, (–) - absent expression from all specimens, (+/–) - expression in some specimens.

Breed	Esterase bands	
	GES I_1	GES L_1
P14	+	–
P15	+	–
19	+	+/–
20	+	–
B517	–	–
T106	–	–
T108	–	–
M1	+	+/–
M2	+	+/–
UK17	+/–	–
UK18	+/–	–
UK19	+	–
UK20	+/–	–
Tashkent 12	+/–	–
Tashkent 15	+	+/–
Tashkent 16	–	–
Hybrid		
P14xP15	+	–
P15xP14	+	–
M1xM2	+	+/–
M2xM1	+	+/–
UK17xUK19	+	–
UK19xUK17	+	–
UK18xUK20	+/–	–
UK20xUK18	+/–	–

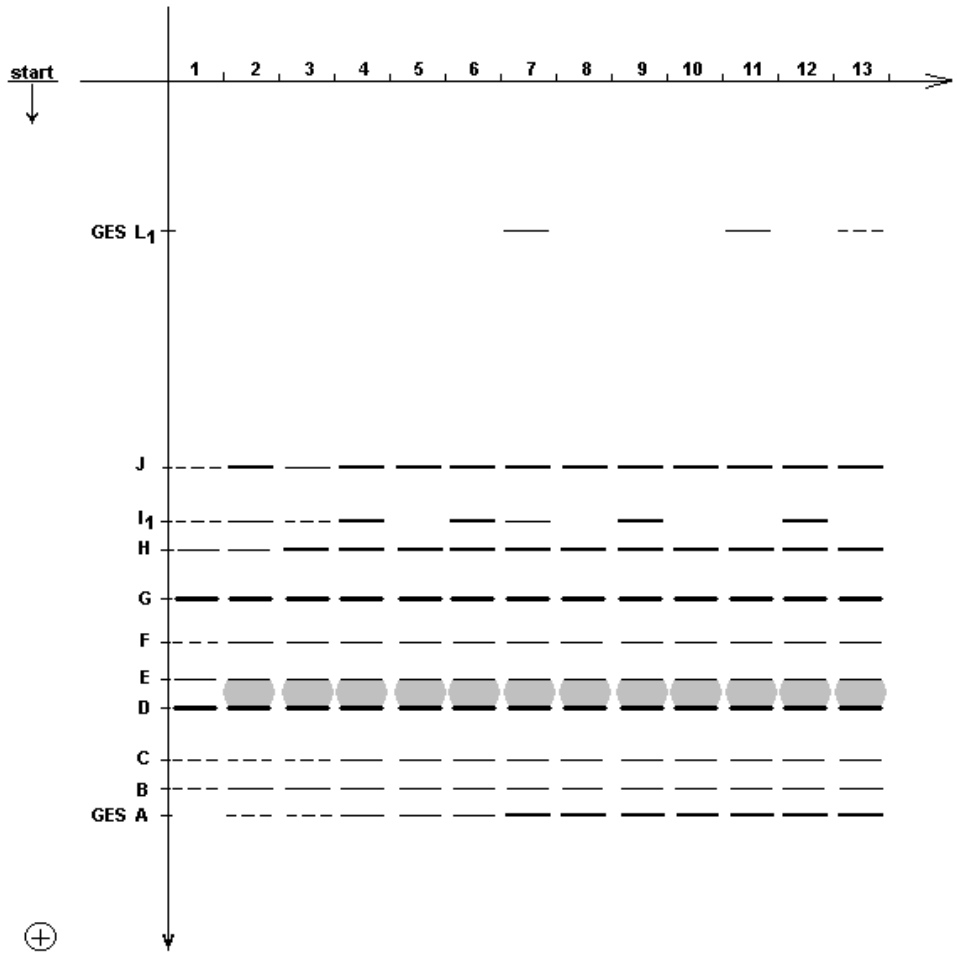

Fig. 1. Scheme of the esterase spectra of silkworm gut in PAGE: 1, 2 – 1st larval instar; 3, 4 – 2nd larval instar; 5, 6 – 3rd larval instar; 7÷9 - 4th larval instar; 10÷13 – 5th larval instar; 2, 4, 6 – mixed probes; 1, 3, 5, 7÷13 – individual probes.

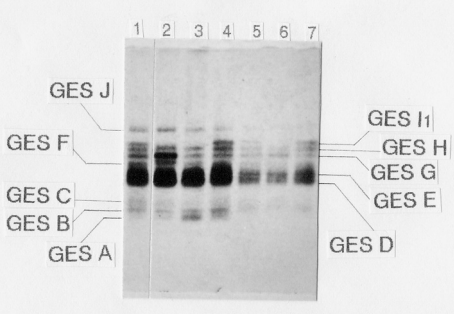

Fig. 2. Esterase spectra of silkworm gut in 7.5% PAGE, 1st larval instar: 1 – breed P15; 2 - breed T108; 3 - breed T106; 4, 7 - breed M2; 5 - hybrid P15xP14; 6 - breed B517; 1÷4 - mixed probes, 5÷7 - individual probes.

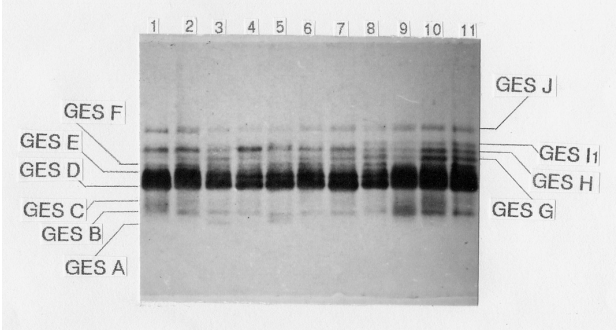

Fig. 3. Esterase spectra of silkworm gut in 7.5% PAGE, 2nd larval instar: 1, 2 - breed UK20; 3÷7 - breed Tashkent 16; 8÷11 - breed M2; individual probes only.

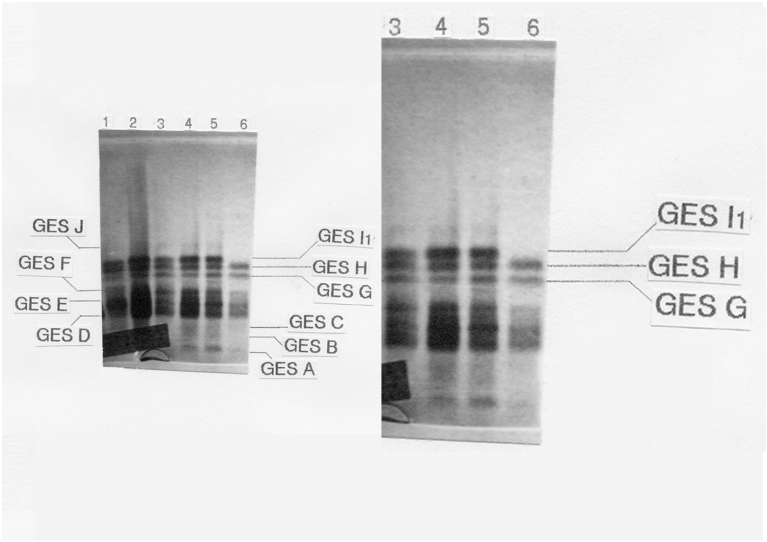

Fig. 4. Esterase spectra of silkworm gut in 6.5% PAGE, 4th larval instar (6) and 5th larval instar (1÷5): 1 - breed P15; 2, 3 - breed M1; 4, 5 - breed M2; 6 - breed T106.