Int. J. Indust. Entomol. Vol. 9, No. 1, 2004, pp. 59~63

International Journal of
Industrial Entomology

Electrophoretic Analysis of Nonspecific Esterases in Silkworm (*Bombyx mori* L.) Female Genital Organs and Eggs

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(Received 10 May 2004; Accepted 20 July 2004)

By using PAGE, a study was made on the nonspecific esterase spectra of female genital organs and eggs in *Bombyx mori* L. The expression of 11 esterase bands was detected during ontogenesis of races and inter-races hybrids kept in Bulgaria. The gene activity of 9 esterase loci was assumed. Esterases specific for the spectrum of diapausing eggs were observed. In two esterase zones, intra- and inter-breed polymorphism was found. Based on the same breed specific expression, the existence of correspondence between esterase bands from spectra of different silkworm tissues and organs was suggested. Stage-specific expression of esterases in female genital glands, indicative of differentiated gene activity during ontogenesis, was established.

Key words: *Bombyx mori* L., PAGE, Nonspecific esterases

Introduction

The electrophoretic spectrum of nonspecific esterases in the female genital organs and eggs of *Bombyx mori* L. has been studied by many authors, who described a different number of esterase bands. For example, using PAGE, Sankina *et al.* (1975) and Nasirillaev *et al.* (1977) detected from 3 till 7 esterase bands in the spectra of diapausing eggs of different races, while Egorova *et al.* (1977) reported 10 esterase bands. Krishnamurthy *et al.* (1984) detected 11, and Xu and Song (1987) - a total of 12 esterase bands during different stages of embryogenesis. By starch gel electrophoresis, Kai and Hasegawa (1972, 1973) detected 4 esterase bands in the spectra of oviducts with maturing eggs in pupae, and from 2 to 7 esterase bands in the mature eggs of different silkworm races. They proved that the A-esterase occurring on day 3 of the pupal stage was associated with interruption of diapause and restoration of morphogenesis (Kai *et al.*, 1981a, b; Kai *et al.*, 1984a, b). The comparison of results from different studies showed a great variability in the number of esterases detected by different authors. This might be due both to the use of different electrophoretic techniques and to the study of races of different origin. We found no studies on the expression of nonspecific esterases in ovaries during the larval period. In the present paper, data from more detailed investigations on the esterase spectrum in female genital organs and eggs and their stage specificity in different races and hybrids of *B. mori* L. are described and discussed. The breeds reared in Bulgaria were not yet analyzed for studying the esterase spectrum and we are doing the work.

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Materials and Methods

Using polyacrylamide gel electrophoresis (PAGE) 365 specimens of sixteen races 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-race hybrids P14 × P15, P15 × P14, M1 × M2, M2 × M1, UK17 × UK19, UK19 × UK17, UK18 × UK20, and UK20 × UK18 have studied. The race B517 is polyvoltine, while all others are monovoltine. Specimens from different families (different ovipositions) of each race have chosen for investigation. The esterase spectra of female genital organs were analyzed during ontogenesis,

i.e.,

- In the 2nd, 3rd, 4th and 5th larval instars, in the spinning period and in the 0 – 1 day-old pupae we tested the esterase spectra of ovaries.
- In the 1 – 2, 5 – 6, and 8 – 10 day-old pupae and in the imago stage we analyzed the spectra of oviducts with maturing eggs.

In the 2nd, 3rd, 4th and 5th larval instars we tested individual samples (ovaries isolated from one specimen) as well as mixed samples (ovaries isolated from 10 specimens per race or hybrid and squashed together). During the subsequent ontogenetic stages, we analyzed individual samples only. Using PAGE, we studied also the individual esterase spectra of oviducts in 20 adults specimens of the tested races and hybrids, as well as those of diapausing eggs (60 samples).

The female genital organs (ovaries, oviducts and oviducts with maturing eggs) were 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 hrs at 4°C. Thirty of eggs per female were collected for each of the diapausing egg sample, squashed with quartz sand in 0.8 M tris-phosphate buffer at pH 6.7 and left for extraction for 18 hrs at 4°C, also. Then the samples were centrifuged for 45 min at 5,000 rpm. The electrophoretic separation involved using 7.5% polyacrylamide vertical gel (pH 8.9) at 4.5 mA/cm for 3 hrs, together with 3.3% concentrating gel (pH 6.7) and 0.05 M tris-0.2 M glycine electrode buffer at pH 8.3 (Stoykova, 2001).

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 hrs and stored in 7% acetic acid.

Results

In the female genital organs and eggs, we detected a total of 11 esterase bands, marked with letters (A, B, C, D, E, F, G1, G2, G3, H and I1) in order of their decreasing mobility towards the anode (Fig. 1). In the spectra of ovaries and oviducts with maturing eggs during the silkworm

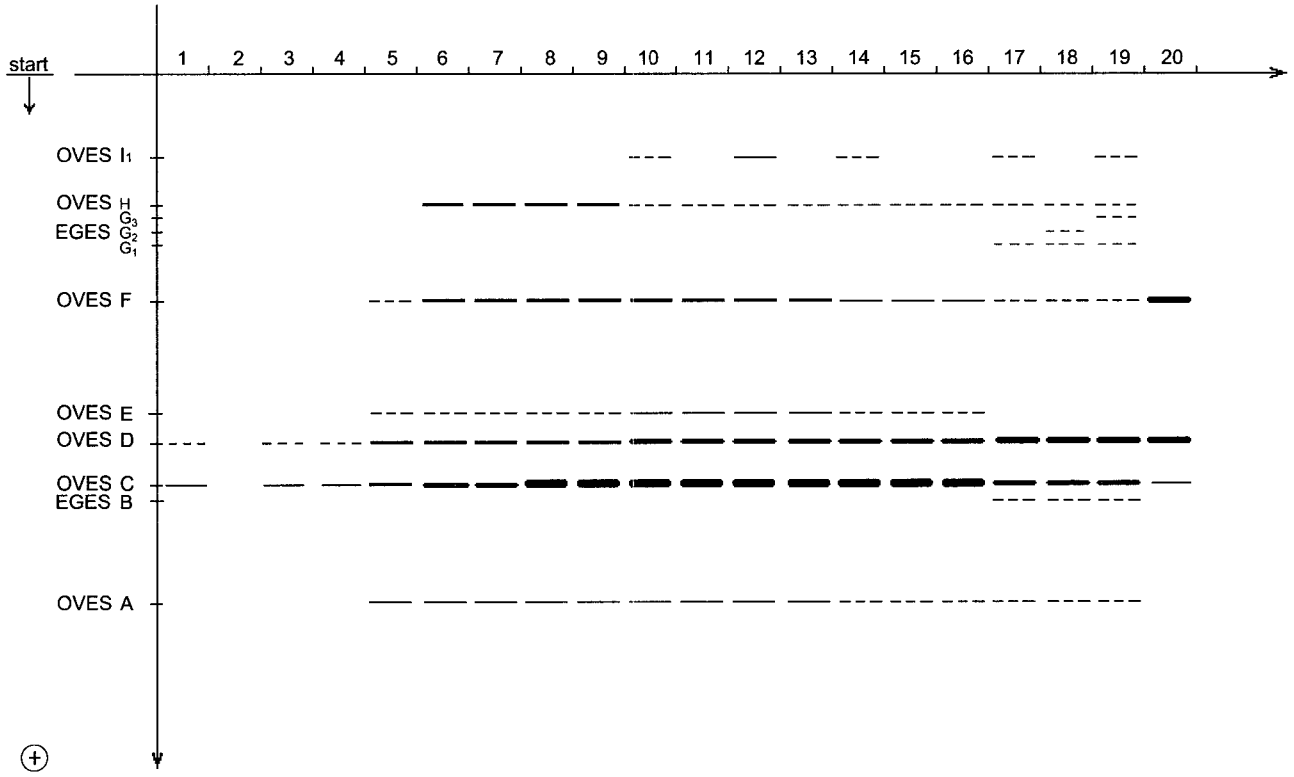

Fig. 1. Scheme of the esterase spectra of ovaries (lanes 1 – 9), oviducts with maturing eggs (lanes 10 – 16), diapausing eggs (lanes 17 – 19) and oviducts (lane 20) of *B. mori* L. in different stages of ontogenesis - 7.5% PAGE: 2nd larval instar (1 and 2); 3rd larval instar (3 and 4); 4th larval instar (5); 5th larval instar (6 and 7); spinning period (8); pu pae 0– 1st day (9); pupae 1st – 2nd day (10 and 11); pupae 5th – 6th day (12 and 13); pupae 8th – 10th day (14 and 15); imago (16). Mixed probes of ovaries (1 and 3), individual probes of ovaries and oviducts with maturing eggs (2, 4, 16 and 20), mixed probes of diapausing eggs per female (17 – 19).

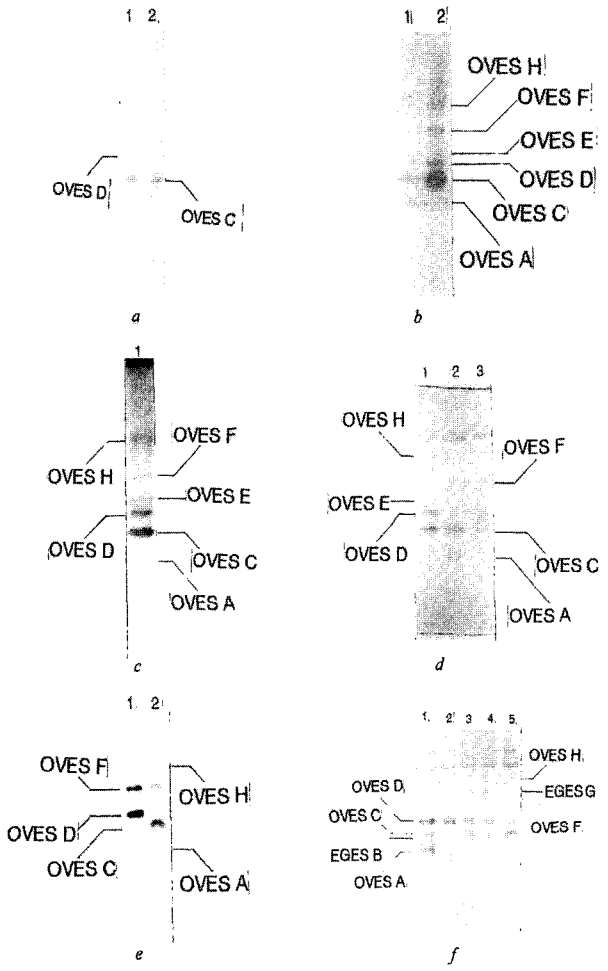

Fig. 2. Esterase spectra of silkworm female genital organs and eggs in 7.5% PAGE: a. ovaries of 2nd larval instar, races M1 (1) and T108 (2) - mixed probes; b. ovaries of 5th larval instar, race P15 (1) - individual probe and hybrid P15 × P14 (2) - mixed probe; c. ovaries of pupa 0 – 1st day (1), race P14; d. oviducts with maturing eggs of pupa 5th – 6th day (1), race P15; oviducts with maturing eggs of pupa 8th – 10th day (2), race P15; oviducts with maturing eggs of imago (3), hybrid P14 × P15; e. oviducts of imago (1), race Tashkent 16 and oviducts with maturing eggs of pupa 8th – 10th day (2), race Tashkent 16; f. diapausing eggs, races UK18 (1) and UK20 (2 – 5).

individual development, we found 7 of the esterase bands (Ovarium esterases - OVES), which were named as OVES A, OVES C, OVES D, OVES E, OVES F, OVES H and OVES I1. The expression of bands EGES B, EGES G1, G2 and G3 was detected only in egg spectra (Egg esterases - EGES).

No esterase activity was found in individual extracts from ovaries of 2nd instar larvae. In mixed samples from that period, as well as in mixed and individual samples from ovaries of 3rd instar larvae, we detected the bands OVES C and OVES D (Fig. 1 and 2a). In the spectrum of 4th larval instar, we detected the occurrence of OVES A, OVES E and OVES F both in mixed and individual probes (Fig. 1). In the spectrum of the 5th larval instar (mixed and individual samples), besides the bands already described, we found also OVES H. In individual extracts, all esterase bands showed a weaker expression (Fig. 2b). The most intensive and distinct in that stage were OVES C, OVES D and OVES F (Fig. 1 and 2b). In individual ovarian probes of spinning larvae and 0 1 day-old pupae, we observed an increased expression of band OVES C (Fig. 1 and 2c).

In extracts from oviducts with maturing eggs of pupae (1 – 2, 5 – 6 and 8 – 10 day-old) and adults of all races and hybrids, bands OVES A, OVES C, OVES D, OVES E, OVES F and OVES H were detected (Fig. 1 and 2d). OVES H was with weak expression in these stages (Fig. 2d). During the first half of the pupal period, the intensity of OVES E was high and then decreased.

In individual extracts from adult oviducts, we detected the presence of bands OVES C weak, and OVES D and OVES F very intensive (Fig. 1 and 2e).

In the spectra of oviducts with maturing eggs in pupae and adults of races 19, M1, M2, Tashkent 15 and F1 hybrids M1 × M2 and M2 × M1, we determined band OVES I1, having different intensity in the different individuals (Fig. 1). This esterase band was not found either in the spectra of other individuals from the same races or in those of all individuals from the other races tested and their hybrids (Table 1). No other interbred differences in the esterase spectra of female genital organs were detected.

In the spectra of diapausing eggs, we found the occurrence of a weak band - EGES B, as well as the expression of OVES A, OVES C, OVES D, OVES F, OVES H and OVES I1 (Fig. 1 and 2f). The most distinct were OVES D and OVES C. In the egg spectrum we determined also some very weak bands in zone EGES G (G1, G2 and G3), which were expressed either separately or in combinations by pairs in the different races and their hybrids (Table 1). Race specificity was also detected in the expression of band OVES I1, which confirmed that found in the electrophoretic spectra of oviducts with maturing eggs.

Discussion

Based on the PAGE results, we assumed that the nonspecific esterases in the *B. mori* L. female genital organs were probably products of seven loci. The expression of OVES I1 justified the assumption for the presence of polymorphism with a null allele at the specific locus in races 19, M1, M2, Tashkent 15 and hybrids M1 × M2 and M2 ×

Table 1. Race specificity esterase expression of EGES G (G1, G2, G3) and OVES I1 bands: (+) - expression, (−) - absent expression

Races	Esterasebands			
	OVES I1	EGES G		
		EGES G 1	EGES G 2	EGES G 3
P14	-	-	+	-
P15	-	+	-	-
19	+	+	+	-
20	-	+	+	-
B517	-	+	-	+
T106	-	+	+	+
T108	-	+	-	+
M1	+	+	+	+
M2	+	+	+	-
UK17	-	+	+	+
UK18	-	-	+	-
UK19	-	+	+	+
UK20	-	+	-	-
Tashkent 12	-	+	+	-
Tashkent 15	+	+	+	-
Tashkent 16	-	+	+	+
Hybrids				
P14 × P15	-	+	+	-
P15 × P14	-	+	+	-
M1 × M2	+	+	+	-
M2 × M1	+	+	+	-
UK17 × UK19	-	+	+	+
UK19 × UK17	-	+	+	+
UK18 × UK20	-	+	+	-
UK20 × UK18	-	+	+	-

M1. In the parallel study of nonspecific esterases found in different tissues (oviducts with maturing eggs, fat body and haemolymph), isolated from the same individuals, we detected that the presence and absence of band OVES I1 in the spectra of the female genitals correlated with the presence and absence of specific esterase bands from the spectra of fat body and haemolymph described by us earlier as FBES J1 and BES E1 (Stoykova, 2001; Staykova *et al.*, 2003). In our opinion, this fact confirmed the assumption that the lack of this isozyme in the spectra of different tissues was genetically determined and associated with the presence of a null allele. The race specificity of the OVES I1 expression confirmed that found of the FBES J1 (from the fat body spectrum) and BES E1 (from the haemolymph spectrum).

In the egg spectrum we detected some specific esterases - in zones EGES B and EGES G. Assumption was made that the presence of these egg-specific esterases was associated with the expression of two different esterase loci. The expression of esterases from zone EGES G was probably due to polymorphism with three codominated alleles. Using electrophoretic analysis on gels of different concentrations (6% and 7.5%) we found that the three esterase bands in zone EGES G (G1, G2 and G3) from the egg spectrum were expressed in the zone previously described by us as BES D (D1, D2 and D3) bands from the haemolymph spectrum (Staykova *et al.*, 2003). The race specificity detected in the expression of EGES G esterases, confirmed that detected in the expression of BES D esterases. In the spectrum of eggs however, the respective bands showed a very weak expression.

Eguchi and Yoshitake (1967), Kai *et al.* (1982) and Eremina (1985) reported identity between esterases from different *B. mori* L. tissues based on their equal gel position. The identical race specificity and correlation in the expression allowed our assumption that band OVES I1 corresponded both to FBES J1 and BES E1, and the bands in zone EGES G corresponded to those in zone BES D. We assumed that the respective esterases from the spectra of different tissues were probably controlled by the same genes. The results obtained by us confirmed the existence of intra- and interbreed polymorphism in the esterase spectra of silkworm female genital organs and eggs, reported previously by other authors (Sankina *et al.*, 1975; Egorova *et al.*, 1977; Nasirillaev *et al.*, 1977; Naletova *et al.*, 1978; Xu and Song, 1987).

Our results demonstrated clearly the stage-specific expression of esterases in female genital glands, assumed to occur in result of a differentiated gene activity during silkworm ontogenesis. This supported the idea of Krishnamurthy *et al.* (1984), Xu and Song (1987), Raju and Krishnamurthy (1995). The stage specificity observed was probably associated with the processes of gland formation, growth and functioning.

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