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PROTHORACICOTROPIC GENE EXPRESSION IN *MORIMUS FUNEREUS* LARVAE.

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Prothoracicotropic hormone (PTTH), a neurosecretory peptide of insect brain, stimulates the prothoracic gland to synthesize and release ecdysone, a hormone regulating insect growth and metamorphosis. Many mutants with a disturbed molting (from non-molting to extranumerary molting mutants) have been described in *Bombyx mori* (D o i r a *et al.* 1992). It is supposed that PTTH could play a main role in this kind of mutations, due to its high position in regulatory cascade of growth and metamorphosis.

The protein product of PTTH gene is a glycoprotein, which plays a key role in larval - larval and larval–pupal ecdysis and regulation of adult development. Molecular mass of this PTTH form in *B. mori* was estimated to be 22 kD (big form) and the sequence length of prepro-PTTH cDNA is about 1.0-1.2 kb (K a w a k a m i *et al.* 1990). Two forms of cDNA were found (A d a c h i-Y a m a d a *et al.* 1994) by RT-PCR amplification of *B. mori* total RNA isolated from brain. First one was fast moving band (225-240 bp) and the other was slowly moving band (440-480 bp).

The cerambycid beetle *Morimus funereus* develops in sapwood of various host plants belonging to *Tiliaceae, Fagaceae, Corylaceae, Salicaceae, Fabaceae* and *Pinaceae* families. Sapwood represents external medium and also a nutritive substrate for *M. funereus* larvae. Larval development of *M. funereus* lasts from 3-4 years in nature, while in larvae reared on artificial diet (R o b e r t s 1986) and constant laboratory conditions development is much shorter (6.5 months) (N e n a d o v i ć *et al.* 1989,). The activation of protocerebral neurosecretory neurons, intensified protein and lipid metabolism, as well as increased in proteolytic activity (N e n a d o v i ć *et al.* 1989; I v a n o v i ć *et al.* 1991, 1992) could explain the decrease of development time in larvae maintained on artificial diet.

The present study was aimed at comparative examinations of PTTH gene expression in *M. funereus* larvae from natural population (NP) and those reared on artificial diet and under constant laboratory conditions (AD).

M. funereus larvae from NP group were collected from oak stumps in Fruška Gora Mt. AD larvae were reared from egg hatching on artificial diet (R o b e r t s 1986) under controlled laboratory conditions: constant temperature of 23°C, relative humidity of 70% and in the dark. After decapitation, the brains were dissected out on ice and homogenized. Total RNA was isolated using RNeasy Kit for isolation of RNA (Quiagen). Afterward a reverse transcription was preformed using 1 μg/mL of total RNA and buffer containing 50 mM KCl, 10 mM TRIS-HCl, pH 8.3, 5 mM $MgCl_2$, 2.5 µM Oligo(dT), 1 U RNase inhibitor, dNTPs 0.5 mM each and 2.5 U of murine leukemia virus reverse transcriptase (MuLV RT) in a final volume of 10 µL. RT reaction was preformed 45 min at 42 °C, 5 min at 95 °C and then cooling to 5°C. The obtained cDNA template submitted to PCR amplification was mixed with PCR buffer containing 2 M $MgCl_2$, 50 mM KCl, 10 mM TRIS-HCl, pH 8.3, 100 µM dNTPs, 0-1 µM of each primer and 2.5 U Taq polymerase (Stoffel fragment) in a total volume of 50 µL. Sense (AGCTATTCCGGATCCACCTTG) and antisense (GTACACAAACACGCCACGCTGAC) primers were designed using the sequence for PTTHs gene (K a w a k a m i *et al.* 1990). Before amplification, the samples were denatured at 94°C for 2 min. Amplification consisted of 30 cycles with following parameters 94°C – 1 min, 50°C – 30 sec, 72°C – 1.5 min followed by 72°C for 8 min. Amplified products were analyzed by electrophoresis in 1.5 % agarose gels (Fig. 1).

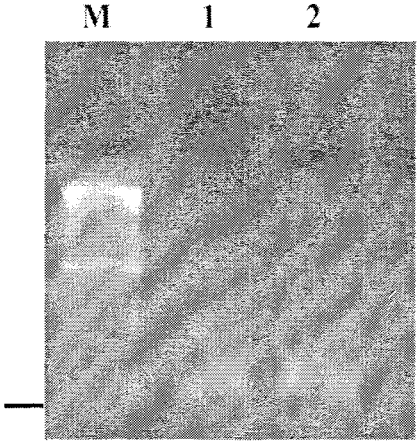

Fig 1. The expression of PTTH cDNA was determined by RT-PCR. RT-PCR products were subjected to electrophoresis in 1.5% agarose gel, stained with ethidium bromide and photographed under UV light. Products of 200-210 bp were amplified from natural and laboratory populations of *M. funereus* larvae. Arrow indicates the 200 bp molecular size.

M – molecular size markers
1 – natural population
2 – laboratory population

Using RT-PCR methods we obtained fragments of 210 bp from total brain RNA of both experimental groups: nature (line 1) and laboratory (line 2). We did not observe differences between natural and laboratory populations at the level of PTTH gene expression. However, changes in brain protein patterns of these larvae were detected in the region of molecular masses for PTTH (A l A r i d 2001).

Many experiments with this species larvae have shown that different environmental factors such as stressful temperature and nutritive substrates of different quality and quantity affect the activity of cerebral neurosecretory cells (I v a n o v i ć *et al.* 1992, L e k o v i ć *et al.* 2001), which are known to be the site of PTTH synthesis (I v a n o v i ć *et al.* 1980).

In conclusion, further studies are necessary to understand in detail biological significance of PTTH gene expression in *M. funereus* larvae, which directly affects insect growth and metamorphosis.

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