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The Effect of Temperature on Midgut and Brain Protein Profiles in *Morimus funereus* Larvae (Coleoptera: Cerambycidae)

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The 7-days shift of *M. funereus* larvae, from nature to a constant temperature of 23°C led to changes in midgut and brain protein quality and quantity. The changes in midgut protein profiles are characterized by an intensified protein band Mr of 29 kD, the absence of protein Mr of 22 kD and less intense bands Mr of 8.5-2.5 kD. Electrophoretic patterns of brain proteins showed less intense Mr of 66-2.5 kD protein bands.

Key words: *Morimus funereus*, temperature, brain and midgut proteins.

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Morimus funereus inhabits deciduous and coniferous trees in the forests of southeastern Europe. Temperature is a limiting factor for their spreading to the north (STANIĆ *et al.* 1989). The larvae are phloem feeders. Due to a long time of larval development they are a suitable model system for studying biochemical and physiological changes during acclimation and acclimatization (IVANOVIĆ *et al.* 1987). The authors' goal was to determine whether change in temperature regime caused modification in midgut and brain protein profiles indicating induction and/or repression of digestive enzymes and/or neurohormones.

Material and Methods

Larvae were collected from oak trees (Fruška Gora Mt) during November and divided into two groups i.e.: NC-natural conditions and WM23-larvae reared on wood mass under a constant temperature of 23°C for 7 days. Their midguts and brains were homogenized in RIPA buffer (50mM Tris pH 7.4, 150mM NaCl, 1% Nonidet P-40, 0.5% Triton - X100, 0.1 % SDS; 1:5 wet wt/vol.) and centrifuged at 20 000 g for 5 min. Crude extracts were subjected to 13.5% and 16% SDS-PAGE (LAEMMLI 1970). The gels were stained with 0.1% $AgNO_3$. The authors used Sigma Mr standards 66-14.2 and 16.95-2.51 kD.

Results and Discussion

The stimulatory effect of constant temperature of 23°C on body mass, fat body amount, midgut and brain total protein concentration (AL ARID 2001), and fat body glycogen content in *M. funereus* larvae (DORDEVIĆ 1995) has previously been shown indicating increased protein synthesis and accumulation of energy resources. In the present experiment, the detected changes in electrophoretic patterns of midgut proteins (Fig. 1A) were mostly located in the region of insect digestive enzymes. Molecular masses of some insect proteases are within this range (20-30 kD) (APPLEBAUM 1985; CAMPOS *et al.* 1989; WIEMAN & NIELSEN 1988). In addition to trypsin-like enzymes (DURDEVIĆ *et al.* 1997), chymotrypsin and chymotrypsin-like enzymes were found in the midgut of *M. funereus* larvae (VUJIČIĆ *et al.* 1998) and recent data indicates the possible presence of cysteine proteases, as well (AL ARID 2001).

Midgut protein patterns within the range of Mr 8.5-2.5 kD of the two groups are similar (Fig. 1B). In *M. funereus* larvae, collected from oak logs during the hibernation period (November), compensatory reactions at the level of protease activity have been detected in response to increased temperature (23°C) (IVANOVIĆ *et al.* 1987). The present authors' results show a switch of protein bands with

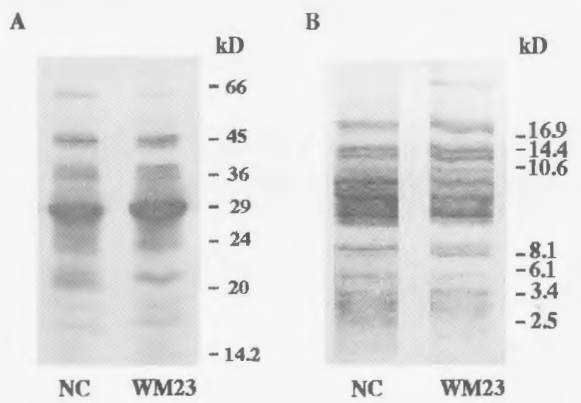

Fig.1. Electrophoretic patterns of midgut homogenates of *Morimus funereus* larvae obtained by 13.5% (A) and 16% (B) SDS-PAGE; NC - natural control; WM23 - larvae exposed to constant temperature of 23°C.

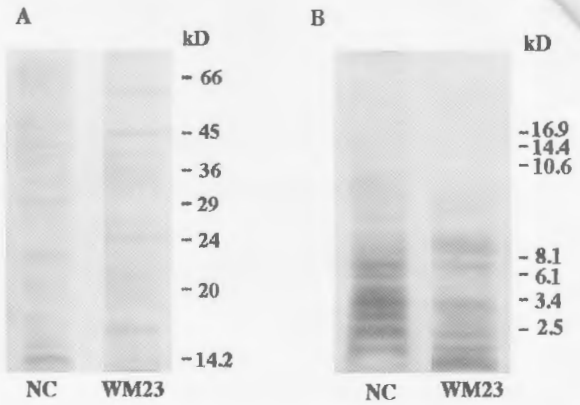

Fig. 2. Electrophoretic patterns of brain homogenates of *Morimus funereus* larvae obtained by 13.5% (A) and 16% (B) SDS-PAGE; NC - natural control; WM23 - larvae exposed to constant temperature of 23°C.

close molecular masses in some regions (Fig. 1B); i.e. there is a possibility of isoenzyme induction.

Changes in *M. funereus* brain protein patterns (Figs 2A & B) are mainly detected in the Mr region of 20-60 kD and 3-9 kD. In *Manduca sexta* and *Bombyx mori* the large prothoracicotropic hormone (PTTH) forming (28 and 30 kD, respectively) is synthesized in lateral neurosecretory cells (LNSC), while small PTTH form (7 and 5 kD, respectively) in medial neurosecretory cells (MNSC) (ISHIZAKI *et al.* 1992; MUEHLEISEN *et al.* 1993). KIM *et al.* (1997) have isolated and characterized a 45 kD pure form and 60 kD native form of this hormone in *Drosophila melanogaster* larvae. In *M. funereus* larvae A1 MNSC seem to be the site of PTTH synthesis on the basis of indirect proof that the 7-day exposure to constant temperature of 23°C leads to their activation (IVANOVIĆ *et al.* 1985) and concomitant increase in titer of ecdysteroids in the haemolyph (IVANOVIĆ *et al.* 1980). A significant correlation between the activity of MNSC and activity of digestive enzymes was observed in earlier studies on *M. funereus* larvae after temperature shift (LEKOVIĆ *et al.* 2001). This could be achieved through the synthesis of different neurohormones in response to stressful temperature.

M. funereus is a polyphagous insect and is exposed to significant seasonal changes in temperature during development. Plasticity of midgut enzymes and brain neurohormones could be considered as an adaptive mechanism that enables adequate matching of environmental changes, as well as the efficient utilization of nutritionally poor food.

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References

AL ARID L. 2001. Effect of food Quality on Midgut and Brain Proteins in *Morimus funereus* Larvae (Coleoptera; Cerambycidae). M. A. Thesis, University of Belgrade, Belgrade.

APPLEBAUM S. W. 1985. Biochemistry of digestion. (In: Comprehensive Insect Physiology, vol. 4. Kerkut G. A. & Gilbert L. I. eds. Pergamon Press, New York): 279-311.

CAMPOS F. A. D., XAVIER-FILHO J., SILVA C. D., ANDARY M. B. 1989. Resolution and partial characterization of proteinases and l-amylase from midguts of larvae of the bruchid beetle *Callosobruchus maculatus* (F.). Comp. Biochem. Physiol. **92B**: 51-57.

DORDEVIĆ S. 1995. The role of neurohormones- regulators of carbohydrate and lipid metabolism in *Morimus funereus* (Coleoptera) in stress. Ph. D. Thesis, University of Belgrade, Belgrade.

DURDEVIĆ A., VUJIČIĆ Z., JANKOV R., NENADOVIĆ V., IVANOVIĆ J. 1997. Trypsin-like enzymes from the midgut of *Morimus funereus* larvae (Coleoptera, Cerambycidae). Arch. Biol. Sci. **49**: 19-20.

ISHIZAKI H., SUZUKI A. 1992. Brain secretory peptides of the silkmoth *Bombyx mori*: Prothoracicotropic hormone and bombyxin. (In: Progress in Brain Research, vol. 92. Joosse J., Buijs R. M. & Tilders F. J. H. eds. Elsevier Science Publishers B.V.): 1-14.

IVANOVIĆ J., JANKOVIĆ-HLADNI M., STANIĆ V., MILANOVIĆ M. 1980. The role of the cerebral neurosecretory system of *Morimus funereus* larvae (Insecta) in thermal stress. Bull. Acad. Serbe Sci. Arts **20**: 91-97.

IVANOVIĆ J., JANKOVIĆ-HLADNI M., STANIĆ V., KALAFATIĆ D. 1985. Differences in the sensitivity of protocerebral neurosecretory cells arising from the effect of different factors in *Morimus funereus* larvae. Comp. Biochem. Physiol. **80A**: 107-113.

IVANOVIĆ J., JANKOVIĆ-HLADNI M., SPASIĆ V., FRUŠIĆ M. 1987. Compensatory reactions at the level of digestive en-

zymes in relation to acclimatization in *Morimus funereus* larvae. Comp. Biochem. Physiol. **86A**: 217-224.

LAEMMLI U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**: 680-685.

LEKOVIĆ S., LAZAREVIĆ J., NENADOVIĆ V., IVANOVIĆ J. 2001. The effect of heat stress on the activity of A1 and A2 neurosecretory neurons of *Morimus funereus* (Coleoptera: Cerambycidae). Eur. J. Entomol. **98**: 13-18.

KIM A. J., CHA G. H., KIM K., GILBERT L. I. LEE C. C. 1997. Purification and characterization of the prothoracicotropic hormone of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **94**: 1130-1135.

MUEHLEISEN D. P., GRAY R. S., KATAHIRA E. J., THOMAS M. K., BOLLENBACHER W. E. 1993. Immunoaffinity purification of the neuropeptide prothoracicotropic hormone from *Manduca sexta*. Peptides **14**: 531-541.

STANIĆ V., JANKOVIĆ-HLADNI M., IVANOVIĆ J., NENADOVĆ V. 1989. Joint effects of temperature, food quality and season on development of cerambycid *Morimus funereus* under laboratory conditions. Entom. exp. appl. **51**: 261-267.

VUJIČIĆ Z., MOKRANJAC D., JANKOV R., NENADOVIĆ V., IVANOVIĆ J. 1998. Comparative analysis of proteolytic enzymes in *Morimus funereus* larvae from natural conditions and reared in the laboratory. 4th Yug. Congr. Plant Protection, Vrnjačka Banja, p.8

WIEMAN K. F., NIELSEN S. S. 1988. Isolation and partial characterization of major gut proteinase from larvae *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae). Comp. Biochem. Physiol. **89B**: 419-426.