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MORPHOMETRIC CHANGES OF *CORPORA ALLATA* IN *MORIMUS FUNEREUS* MÜLS. (CERAMBYCIDAE) LARVAE DURING THERMAL STRESS.

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The *corpora allata* (CA) are a pair of endocrine glands that synthesize and release juvenile hormones (JHs). Together with the *corpora cardiaca*, they also represent a neurohemal organ (Sakakibara and Fugo, 1990). The activity of CA is regulated by numerous hormonal factors, primarily by allatotropin (stimulatory) and allatostatin (inhibitory) neuropeptides. These allatoregulatory neuropeptides may reach the CA via the hemolymph and/or via nervous connections (Stay, 2000; Gäde and Hoffman, 2005). Morphometric and ultrastructural studies demonstrate that fluctuations in JH production always accompany changes in CA gland volume, cell size and quantity of cellular components (Chiang et al., 1998; Pszczolkowski and Chiang, 2000; Chang et al., 2005). The CA undergo cyclic volumetric changes during the period between two larval ecdyses (Nenadović et al., 1993) and during ovarian cycles (Chiang and Schal, 1994).

Juvenile hormones regulate growth, metamorphosis, and reproduction (Tobe and Stay, 1985; Cassier, 1990; Gilbert et al., 2000). Among numerous physiological roles, the JHs are involved in responses to stressful environmental conditions. Temperature is one of the most important environmental factors that determine survival, behavior, development, metamorphosis, reproduction, and population dynamics in insects. It influences all levels of biological organization. Through changes in the cell membranes, temperature differentially affects the activity of neurosecretory neurons in the central nervous system, thereby disturbing hormonal equilibrium. The neuroendocrine system quickly reacts to environmental stress (Ivanović and Janković-Hladni, 1991; Perić-Mataruga et al., 2006); changes in the level and interrelation of hormones affect metabolic pathways and metamorphosis in insects (Borkovec and Gelman, 1986).

Juvenile hormones play a role in insect resistance to both low (Horwath and Duman, 1983; Baust et al., 1985) and high (Rauschenbach et al., 1983) temperatures. Temperature changes cause an increase in the level of JHs and prolong their secretion from the CA, thus enabling insects to survive under stressful conditions.

In the present work, morphometric changes of the left and right CA in response to thermal stress were investigated in *Morimus funereus* larvae (500-700 mg in body weight) collected from a natural population on Mt. Fruška Gora, Serbia during March (when average daily temperatures were in the range of 3-5°C).

The control larvae were sacrificed immediately (natural conditions - NC). Other larvae were divided into five experimental groups. Each larva was kept in a separate flask and reared on natural substrates (crumbled oak subcortical mass). Experimental groups were exposed to a constant temperature of 23°C for 12h, 24h, 48h, 72h, and 30 days, then sacrificed. The insect brains were fixed in Bouin's solution. Serial paraffin sections were stained with Alcian Blue Phloxine and Paraldehyde Thionine Phloxine (Panov, 1980). Four pairs of CA per experimental group were analyzed using a light microscope (Leica, QWIN). Morphometric changes of CA were estimated by monitoring left and right CA volume. Volumes of CA were calculated using the formula $V=1/6\ \pi\ (a \times b^2)$, where "a" represents the larger and "b" the smaller CA diameter (Zhi-Yong Huang et al., 1991). Statistical analyses of results were performed using two-way ANOVA and the LSD multiple range test (Sokal and Rohlf, 1981). Experimental groups (G) and the left/right CA ratio (L/R) were fixed factors.

Our results showed that a temperature of 23°C provoked increase of CA volume after short-term exposure to 23°C (Fig. 1). A statistically significant increase of CA volume was already apparent after 12h. Maximal increase of CA volume was achieved after 48h exposure. After long-term exposure to this temperature, the examined parameter returned to the control level (NC). Two-way ANOVA showed highly significant differences among the groups ($F_{1,31}=10.731$, $P<0.0000$) with respect to CA volume. The present results confirmed those of our previous investigations on the activity of CA in *M. funereus* larvae exposed to different constant temperatures. In those investigation we showed that increase of CA volume is a result of increase in cell number due to mitotic divisions in the CA (Mrdaković et al., 2003).

Previous studies demonstrated that a constant temperature of 23°C is unfavorable for *M. funereus* larvae, especially for young larvae. There are also seasonal differences in the response *of M. funereus* larvae to temperature stress. A temperature of 23°C was stressful for larvae collected in November, but not for those collected in June (Ivanović et al., 1975,

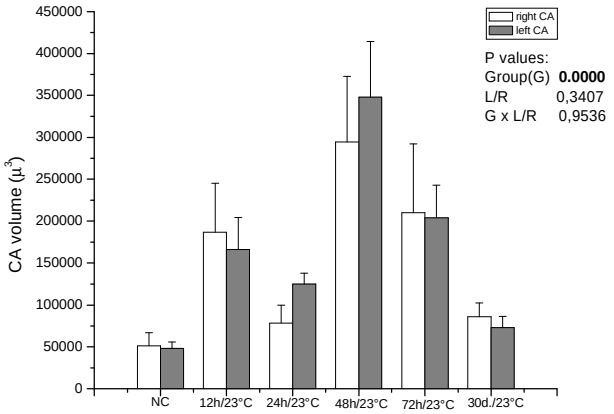

Fig.1. Volumes of left and right CA in *Morimus funereus* larvae exposed to temperature of 23°C. NC - natural control.

1980). Nenadović et al. (1982) showed that a constant temperature of 10°C activated CA cells in winter larvae of *Cerambyx cerdo*.

In their natural habitat, the *M. funereus* larvae used in our experiment lived under the influence of low March temperatures. Short-term exposure to 23°C was stressful for these *M. funereus* larvae owing to the lower temperature in their environment. Increase of CA volume during short-term exposure to 23°C suggests an increase in the titer of JHs, since the biosynthesis and release of JHs are highly correlated and there are no data indicating the possibility of their accumulation in the CA.

The obtained results also showed that there were no significant differences in sensitivity of CA to thermal stress (insignificant G x L/R effect, Fig. 1) and no significant asymmetry between the members within a pair of CA (insignificant L/R effect, Fig. 1). LSD test revealed significant differences between volumes of left and right CA in the control larvae (NC) and left and right CA in larvae exposed to 23°C for 12h (LSD test, $P<0.0043$ for left, $P<0.0054$ for right CA), 24h ($P<0.0367$ for left CA), 48h ($P<0.0000$ for both left and right CA), and 72h ($P<0.0032$ for left, $P<0.0012$ for right CA).

The central nervous system and neurohormones are an important component of the process of adaptation to a changing environment (Ivanović and Janković-Hladni, 1991; Perić-Mataruga et al., 2006). Asymmetry of organs which normally show bilateral symmetry could be expected under various types of environmental stress (Parsons, 1992). However, this was not confirmed by our results.

Similarly, Szibbo and Tobe (1981) showed a high symmetry of CA volume in both members of a pair of CA in relation to the activity cycle in CA of *Diploptera punctata* during ovarial maturation. It has also been shown that CA of *Blattella germanica* adult females exhibited a high degree of bilateral symmetry of gland volume in relation to JH synthesis, with a less than twofold difference between the two glands in CA pairs (Chiang and Schal, 1991). Nervous connection between the left and right *corpus allatum* might be an important part in maintaining symmetry of glands and JH synthesis (Fraser and Pipa, 1977).

The response to temperature changes depends not only on temperatures and time of exposure, but also on the developmental stage (Ivanović et al., 1975), phase of the annual cycle (Ivanović et al., 1982), and nutrition (Ivanović et al., 1989).

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