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ELECTROPHORETIC STUDIES ON THE NAD-DEPENDENT MALATEDEHYDROGENASE DURING THE LARVAE STAGE FROM THE ONTOGENESIS OF *APIS MELLIFERA L. (HYMENOPTERA; APIDAE)*

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Abstract

Electrophoretic analysis in polyacrylamide gel was made on 230 unsealed and sealed larvae of apian families from three culture populations, inhabiting Central Sredna Gora, the West Rhodopes and Yambol region.

It was found out that the synthesis of the NAD-dependent MDH during the larvae stage is controlled by two (2) linked gene loci. No report has been made of sexually related, sexually restricted and sexdependent differences on the NAD-dependent MDH-loci in the specimens studied.

Key words: *Apis mellifera*, electrophoresis, izoenzymes.

Introduction: The NAD-dependent MDH have already been the subject of study for a number of authors (Cornuet 1979; Garstide 1980; Nunamaker et al 1984; Li Shao Wen et al 1988; Page et al 1988).

Nunamaker and Wilson (1982) study the coenzymes formation in honey bees during the period of morphogenesis in larvae and they report availability of eight coenzymes in the haemolymph, among them MDH. The authors notice general reduction of the enzymatic activity in later stages from the development of the larvae.

The aim of the present study is, on the basis of electrophoresis in polyacrylamide gel, to reveal the peculiarities of gene control over the NAD-dependent MDH in honey bees during the larvae stage, by means of a comparative analysis of the genous activity in unsealed and sealed larvae of both sexes.

Materials and methods: We used electrophoresis in polyacrylamide gel according to Maurer's first system (1968). An analysis was made on 230 larvae of different sex from 14 families of 3 cultural populations of bees, inhabiting Central Sredna

Gora, the West Rhodopes and Yambol region.

Results and discussion: In the gel samples, from unsealed larvae, we found presence of three fractions having electrophoretic mobility (EPhM), as follows: 0,45; 0,40; 0,35. The three fractions are expressed in a part of the specimens studied, the most intensive of them being the medial. Either the fastest only or the slowest only are found in the other specimens (Fig. 1).

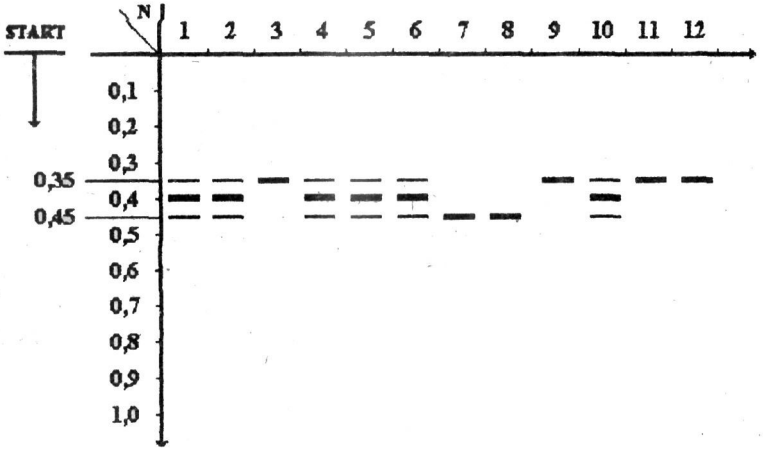

Fig. 1. Electrophoregram of NAD-dependent MDH in Polyacrylamid gel (unsealed larvae).

One more fraction of EPhM 0,50 was reported for the sealed larvae and the expression rate of the separate fractions is in different variants for the different specimens (Fig. 2).

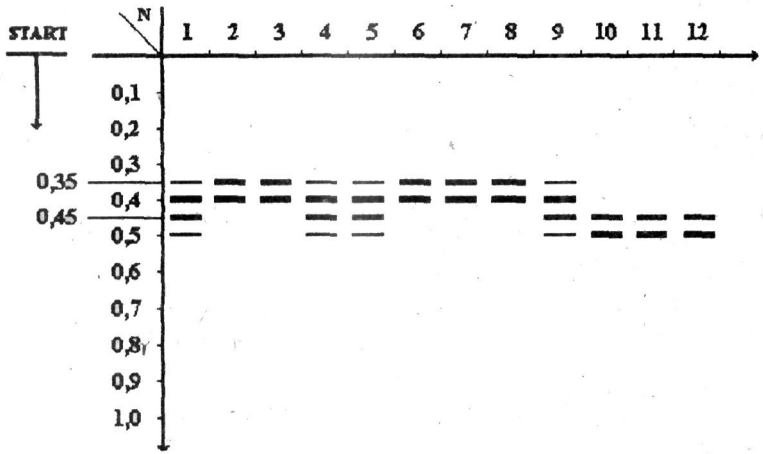

Fig. 2. Electrophoregram of NAD-dependent MDH in Polyacrylamid gel (sealed larvae).

The expression pattern of the fractions in polyacrylamide gel provids us to accept the action of two gene loci, designated as Mdh A and Mdh B. Both loci are polymorphous. The Mdh B locus products are expressed in all larvae forms. The phoregrams give information for the availability of two allele of this locus, designated as Mdh B_1 and

Mdh B_2. Both types of homozygotes are found in the bee families studied, as well as heterozygotic forms (Mdh B_1/B_1; Mdh B_2/B_2; Mdh B_1/B_2).

The action of the Mdh A locus is quite interesting, whose gene products can be found only in sealed larvae. The expression pattern of this locus fractions indicates the presence of twoallele system of determination, with report for the following genetic types: Mdh A_1/A_1; Mdh A_2/A_2; Mdh A_1/A_2.

Considering the male sealed larvae, in result of the action of both gene loci (Mdh A and Mdh B), both faster or both slower fractions are expressed. The homozygotness expressed is a result of the partenogenesis, characteristic of male specimens.

Typical heterozygotes on both gene loci are expressed in the female straight larvae, besides the homozygotic combinations; a result of which is the expression of 4 fractions in the phoregrams (Fig. 2).

Impressive is the fact that the homozygotes on the Mdh A_1 allele are always homozygotes also on the Mdh B_1 allele; the heterozygotes on the Mdh A locus are compulsary heterozygotes also on the Mdh B locus. This fact provids evidence to suppose that the two loci are closely linked.

In the course of the experiments carried out, we found increased genous activity of both loci befor the basic organformation processes of the sealed larvae. In our opinion, this fact is related to the active processes of morphogenesis. This statement of ours is different from the opinion of Nunamaker et al. (1982) for reduced enzymatics activity during later stages from the morphogenesis in larvae of honey bees.

CONCLUSIONS

1. The NAD-dependent MDH in bees during the larvae stage from their ontogenesis are controlled by two (2) gene loci (Mdh A and Mdh B).
2. The Mdh A and Mdh B loci are closely linked.
3. Polymorphism is presence on the two gene loci.
4. Sexually related, sexually restricted or sexdependent differences on the MDH loci in the specimens studied were not investigated.

REFERENCES

Cornuet, Y. M. The MDH system in honey bees of Guadoloupe., J. Hered., 70, 3, 223-224, 1979.

Garstide, D. F. Similar alloztme polymorphism in honey bee *A. mellifera* from different continents., Experientia, 36, 6, 649-650, 1980.

Li Shao Wen, Meng Jo-Pin, Chang J. T., Li Ju-Huai. Studies on the isozymes composition of 4 enzymes in two species of honey bees — *Apis mellifera* and Apis cerana, Acta entomol. sin. 31, 1, 15-19, 1988.

Maurer, G. Disk Electrophose, Heidelberg Walter de Gruyter & Co, Berlim, 1968.

Nunamaker, R. A., Wilson, W. T., Ahmad, R. Malate dehydrogenase and non-specific esterase isoenzymes of *Apis mellifera*, J. Kans Entomol. Soc., 57, 4, 591-595, 1984.

Nunamaker, R. A., Wilson, W. T. Isozyme changes in the honey bee Apis mellifera during larvae morphogenesis., Insect Biochem., 12, 1, 99-104. 1982.

Page, R. A population estimate of MDH allozyme frequencies for the honey bees, A. mellifera., Pan. Pacif. Entomol., 64, 3, 285-289, 1988.

Sheppard, W. S., Berlosher, S. H. Enzyme polymorphism in *Apis mellifera* from Norway., J. Apicult., Res., 23, 2, 64-69; 1984.

ЕЛЕКТРОФОРЕТИЧНИ ИЗСЛЕДВАНИЯ НА НАД — ЗАВИСИМИ МДХ ПО ВРЕМЕ НА ЛАРВНИЯ СТАДИЙ ОТ ОНТОГЕНЕЗАТА НА *APIS MELLIFERA (HYMENOPTERA: APIDAE)*

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(Резюме)

На електрофоретичен анализ в полиакриламиден гел са подложени 230 незапечатани и запечатани ларви на 14 семейства от 3 културни популации пчели, населяващи Централна Средна гора, Западни Родопи и Ямболски район.

Установява се, че синтезата на НАД-зависимите МДХ по време на ларвния стадии се контролира от два (2) генни локуса, които са тясно скачени. Не са открити половосвързвани, половоограничени и ползависещи различия по НАД-зависимите МДХ-локуси при изследваните индивиди.