Proc. IVth Intern.Confer.Ephemeroptera
V.Landa et al.(eds.),225-226,ČSAV,1984

AN ELECTROPHORESIS OF PROTEINS: POSSIBILITIES IN MAYFLY BIOSYSTEMATICS

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Summary. If we suppose each protein to be a direct phenotypic manifestation of a certain gene than a set of some tissues proteins can be understood as an expression of a group of genes /operon/. Apparently it is possible to carry out either genetical analysis or to use this characteristics as one of the objective criteria for the taxon determination by means of a set of individual proteins.

The proteins can be analysed only provided that they are genetically polymorphic and synthetised morphogenetically in corresponding tissues at the same ontogenetic period. Ways of an identification and classification of proteins from various individuals are of the first-rate importance for taxon specificity determination. Immunological and electrophoretical methods become widely used at present but they were utilized rather rarely in the study of mayflies and aquatic insects in general /see e. g. Gysels 1975 and others/.

In the present contribution we deal with some possibilities of an electrophoresis application in the biosystematics of mayflies. In order to know the usefulness of these methods we aimed at comparison the hemolymph proteins of both male and female specimens in several species of different families and egg proteins in some species. An influence of parasitism on the protein patterns and comparison of affinity of some protein fractions were studied as well.

Proteins were devided by the polyacrylamide gel electrophoresis according to Davis /1964/. Mostly body homogenates and hemolymph mixed with 40 % sucrose in distilled water were used as the source of proteins in almost all the cased. An attempt to separate the proteins of some internal organs was made, too /e. g. ovaries, testes, fat body and others/. Protein zones were visualised with the Coomassie Brilliant Blue G-250 staining according to Holbrook and Leaver /1976/.

Immunoelectrophoresis was performed in 0.8 % agarose gel in veronal-acetate buffer of the pH 8.6. A rabbit

was immunised intraveneously with 12 doses of 7 % antigen for preparation of antibodies. An antigen was prepared from the whole-body homogenate of 100 specimens /sex ration 1 : 1/ of the last instar nymphs of Ephemera danica the body appendages /legs, gills, cerci/ of which were removed. Homogenate was filtered, freeze-dried and resuspended in saline before use. Antibody concentration was tested by means of an oncoming diffusion in the electrical field. Interspecies comparison of protein identity was performed with the help of the Ochterloni's precipitation method.

The relatively very simple situation can be found in the mayfly eggs where only 3 or 4 protein fractions occur in homogenates and interspecific differences can be easily evaluated.

When working on nymphal proteins it seems to be more precise to use nymphs of the same age and sex. Due to intrapopulation polymorphism the differences among individual specimens are manifested by slightly different position of several zones and the protein pattern changes are more pronounced during the nymphal development and growth. Differences between male and female nymphs are apparent as well. Interspecific differences are considerable especially those of species from different families. They were not further studied on account of the lack of data on intrapopulation polymorphism and a relatively small number of specimens investigated. On the other hand, these differences can be undoubtedly utilized in regional taxonomy.

A simple hemolymph electrophoresis can be successfully applied when studying some physiological problems im mayflies, e. g. effects of parasitism of chironomid Symbiocladius rhithrogenae on host nymphs. In nymphs infested with the earlier instar parasite larva there are almost no visible changes in the hemolymph proteins in comparisom with those of controls. In nymphs infested with the 3th or 4th instar parasite larva the changes concerning the mayfly hemolymph proteins are very considerable. Most of fractions disappear and on electropherogram only a single broad zone can be observed.

The immunoelectrophoretic methods enable to compare the species on the basis of antigene similarities of their proteins. Identical proteins show common precipitates which, according to our opinion, would manifest apparent phylogenetic relationships, very pronounced at the familial and generic levels and very useful also in species or species-group separation.