| ACTA HYDROBIOL. | 17 | 2 | 141—148 | KRAKÓW 1975 |

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Różnice pomiędzy dwoma populacjami ślizów *(Nemachilus barbatulus* L.*)* na tle badań elektroforetycznego rozdziału białek

Differences between two populations of loach *(Nemachilus barbatulus* L.*)* against the background of investigations on the electrophoretic separation of proteins

Wpłynęło 29 maja 1974 r.

Abstract — Electrophoretic separation of proteins on starch gel of two loach populations (*Nemachilus barbatulus* L.) from a river of sub-montane character and from a typical lowland stream was carried out. The differences in the patterns of the electrophoretic separation of esterases and peroxydase enzymes, presented in figures and photographic tables, indicate a certain specificity of the structure of proteins of the investigated fish populations. This fact suggests that in spite of the lack of any biometric or meristic differences fish living in the two different environments show separate genetical characters.

The loach (*Nemachilus barbatulus* L.) is widely distributed in the waters of almost the whole of Europe, both in mountain and in lowland running and stagnant waters, in cold and warm waters, in clean waters and in those polluted with organic wastes (Smyly 1955). Thus it occurs in waters different distinctly in respect of physical, chemical, and biotic factors and this may have a great influence on the formation of various adaptable characters which allow the growth and propagation of individual populations of the same species in various environments.

It was found in previous investigations on the adaptability of the loach to life in mountain and lowland rivers (Starmach 1973) that if they enter water of low oxygen content they began to differ in their oxygen demand and in their ability to increase the number of erythrocytes in the peripheral blood. However, they do not differ in their biometric or meristic traits.

Hence the question arose whether the previously observed internal traits, and probably also some others, which accommodated the investigated populations to

different environmental conditions and did not disappear after the acclimatization of the fish, are conditioned genetically. In the investigated populations the influence of the environment and the territorial isolation are factors which by way of natural selection of the best adapted genotypes, according to the principles of population genetics, may bring about the formation of separate closed pools of genes, finally leading to a certain genetical separateness.

In order to obtain an answer to the question the electrophoretic separation on starch gel of the proteins of loach from the River Raba and the Gzel stream was proposed since it was known that the structures of proteins, composed of nucleic acids, are differentiated by genes which governe their structure according to the genetic code.

The differences in the protein structure of separate species and even of individual populations of the same species are manifested in the number and rate of the migration of lines of proteins in the electric field. On account of great — not genetical but phenotypical — variability of the fish, which occurs under the influence of local conditions of the environment (Mayr 1963, Sick et al. 1962), these differences often give a more substantial basis for taxonomic investigations of the fish than the biometric and meristic methods used hitherto. This is suggested by numerous works on the electrophoretic separation of fish proteins on starch gel, reported for the first time by Smithies (1955) and included in the biochemical taxonomy (Thompson 1960, Tsuyuki et al. 1965, Tsuyuki, Roberts 1966, Nyman 1965, 1966, 1967, 1971, 1972, Nyman, Westin 1968, 1969, Jonson et al. 1972, and many other authors).

Material and method

The investigated material were fish from 2 loach populations. One part was caught in the Raba above the mouth of the Mszanka stream, a river of sub-montane character in this sector, with a 6 pro mille gradient and a bottom lined with stones covered with algae. The other part of the material originated from a typical lowland Gzel stream, an affluent of the Ruda, whose unit gradient was 0.75 pro mille and the bottom sandy and muddy.

From these two streams differing in their environmental conditions, 30 loach, 115—126 mm in length, were caught in the first half of July, thus already after the spawning-season (Starmach 1966). The fish were transported to the laboratory and placed in aquaria where they spent three weeks being fed with the same food. The similar length of the investigated fish, time of catching, and period of acclimatization was chosen with the purpose of avoiding possible changes in the formulas of analysed proteins, which could have been brought about by age differences, physiological changes connected with the maturation of gonads, the kind of food, and physico-chemical conditions of the water.

The blood for the investigations was taken from the anal vein. After the centrifugation of morphotic elements, the blood serum was stored in heparinized capillaries

in a state of deep freezing. Immediately after removal from the fish, the liver, spleen, brain, and kidney were placed in tightly closed containers, frozen, and stored at —20°C. The preparation of protein extract of the above-mentioned tissues was carried out according to the method reported by Tsuyuki et al. (1962).

The electrophoretic separation of proteins and of enzymes, esterases and peroxydase, was performed on horizontally laid 10.55 per cent starch gel, placed on trays prepared according to the method given by Tsuyuki (1963). A combined set of buffers (according to Ashton, Braden 1961) was used in all the analyses carried out. The electrophoresis took 1 hr and 50 min. at 6.5 V on 1 cm of gel. The stains described by Nyman (1970) were used for staining the proteins of blood serum, the enzymes which belonged to ester hydrolases, and peroxydase.

Results of investigations

The results of the electrophoretic separation of proteins of the blood serum and of enzymes, esterases and peroxydase, of the blood serum, liver, spleen, brain, and kidney are presented in photographic tables and in figures 1—6.

The protein of the blood serum

In the investigated populations of loach from the River Raba and the Gzel stream (figs 4, A, B) the patterns of the electrophoretic separation of proteins of the blood serum do not show differences either in the number of lines or in the rate of their migration towards the anode.

Esterases

The esterases in the blood serum of both investigated populations show polymorphism. This is visible in 3 patterns of the electrophoretic separation (figs 1, 5 A, B, C; D, E, F) and is probably effected by two alleles of the co-operating genes which is consequence of random crossing form two homozygous (AA and BB) and one heterozygous (AB) pattern. A similar observation was made by Nyman in *Salvelinus alpinus* Nyman (1967, 1972). The presented formulas of esterases of the loach from the River Raba and the Gzel stream are similar and differ only in the frequency of occurrence which is AA = 16, AB = 12, BB = 2 with the loach from the Raba, and AA = 22, AB = 5, and BB = 3 with those from the Gzel.

In the loach from the Raba the observed frequencies of occurrence of individual formulas, calculated according to the law of binominal distribution of genotypes (Table I), may indicate a certain genetical equilibrium of this population, while in the loach from the Gzel the ratio of the observed and calculated frequencies is different, particularly in fishes with the genotypes AB and BB. The specificity of the observed and calculated frequencies of formulas of the esterases in the blood

Tabela I. Częstość występowania poszczególnych wzorów esteraz surowicy krwi

Table I. Frequency of occurrence of individual formulas of esterases of the blood serum

Wzór Formula	A A	A B	B B	Miejsce występowania Locality of the occurrence
Częstość obserwowana Frequency observed	16	12	2	Raba
Częstość obliczona wg Hardy-Weinberg Frequency calculated according to Hardy-Weinberg	15,99	11,83	2,12	
Częstość obserwowana Frequency observed	22	5	3	Gzel
Częstość obliczona wg Hardy-Weinberg Frequency calculated according to Hardy-Weinberg	20,17	8,86	0,97	

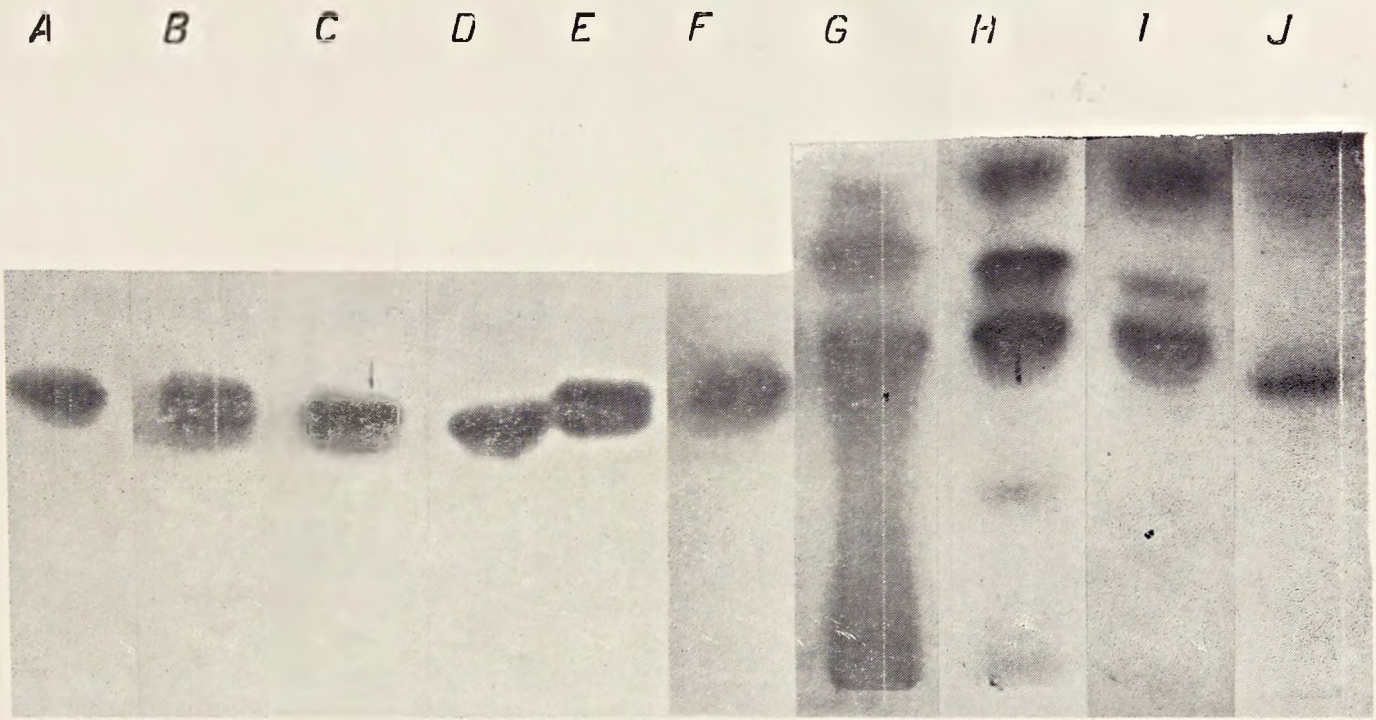
serum of the loach from the Gzel is probably controlled by some factors limiting the state of equilibrium of genotypes in the population ($p^2:2pq:q^2$) presented in Hardy-Weinberg law. This occurs in consequence of various levels of the adaptability of each of the three genotypes.

Contrary to the blood serum, the separation of liver esterases is different with the discussed populations (figs 1, 5 G, H), since they are arranged in three bands with the loach from the River Raba and in four bands with those from the Gzel stream. A slowly migrating line visible in the c zone is characteristic for the fish from the Gzel. Besides, these patterns differ in the rate of migration of individual lines.

In the electrograms of brain esterases of the loach from the Raba and Gzel distinct differences were also observed (figs 1, 5 I, J). The pattern occurring with the fish from the Raba has a single line placed in the c zone. This line does not occur with the fish from the Gzel, a pattern composed of only two lines being observed there. Another trait distinguishing the esterases from the Raba and those from the Gzel is the intensity of the coloration of upper lines in the a zone.

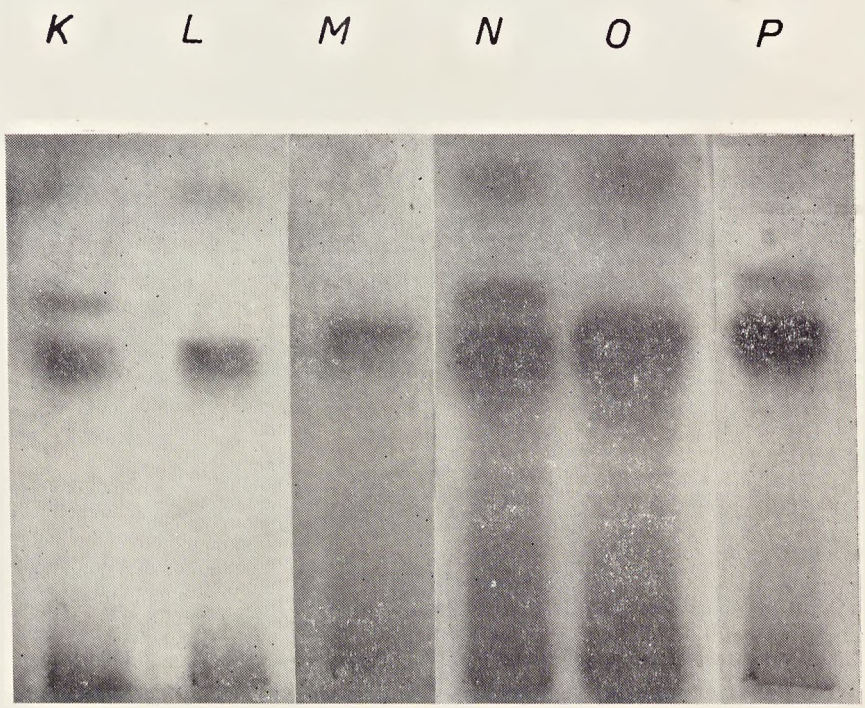
Spleen esterases of the investigated populations (figs 2, 5 K, L, M) are also different. The loach from the Raba have two patterns occurring in the 8K : 22L ratio. They differ in the absence of one line in the L pattern. The loach from the Gzel have a different pattern: this is composed of three lines, one single in the b zone and one double, weakly coloured, in the a zone.

Esterases of the kidney of the fish from the Raba separate on the starch gel similarly as those of the spleen, forming two patterns occurring in the ratio 26N:4 0 (figs. 2, 5 N, O). Besides a slight difference in the rate of migration towards the anode, both these patterns are similar to those of spleen esterases. The pattern of kidney esterases of the loach from the Gzel (figs 2, 5 P) is composed of 3 lines migrating slower than those of the fish from the Raba and grouped in the b zone.



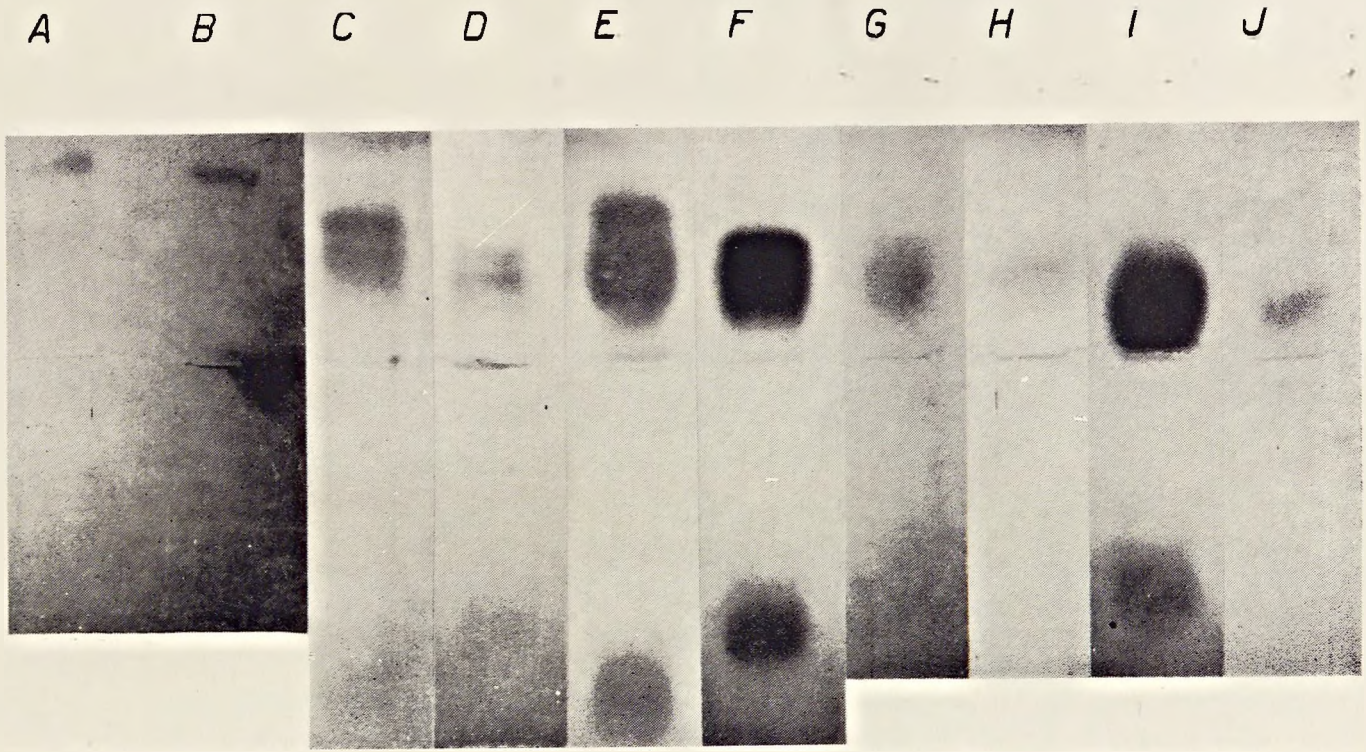
Ryc. 1. Wzory rozdziału esteraz: A, B, C — surowica krwi ryb z Raby; D, E, F — surowica krwi ryb z Gzelu; G — wątroba ryb z Raby; H — wątroba ryb z Gzelu; I — mózg ryb z Raby; J — mózg ryb z Gzelu

Fig. 1. Separation patterns of esterases: A, B, C — blood serum of fish from the River Raba; D, E, F — blood serum of fish from the Gzel stream; G — liver of fish from the Raba; H — liver of fish from the Gzel; I — brain of fish from the Raba; J — brain of fish from the Gzel



Ryc. 2. Wzory rozdziału esteraz; K, L — śledziona ryb z Raby; M — śledziona ryb z Gzelu; N, O — nerka ryb z Raby; P — nerka ryb z Gzelu

Fig. 2. Separation patterns of esterases: K, L — spleen of fish from the Raba; M — spleen of fish from the Gzel; N, O — kidney of fish from the Raba; P — kidney of fish from the Gzel



Ryc. 3. Wzory rozdziału peroksydazy: A — surowica krwi ryb z Raby; B — surowica krwi ryb z Gzelu; C — wątroba ryb z Raby; D — wątroba ryb z Gzelu; E — śledziona ryb z Raby; F — śledziona ryb z Gzelu; G — mózg ryb z Raby; H — mózg ryb z Gzelu; I — nerka ryb z Raby; J — nerka ryb z Gzelu

Fig. 3. Separation patterns of peroxydase: A — blood serum of fish from the Raba; B — blood serum of fish from the Gzel; C — liver of fish from the Raba; D — liver of fish from the Gzel; E — spleen of fish from the Raba; F — spleen of fish from the Gzel; G — brain of fish from the Raba; H — brain of fish from the Gzel; I — kidney of fish from the Raba; J — kidney of fish from the Gzel

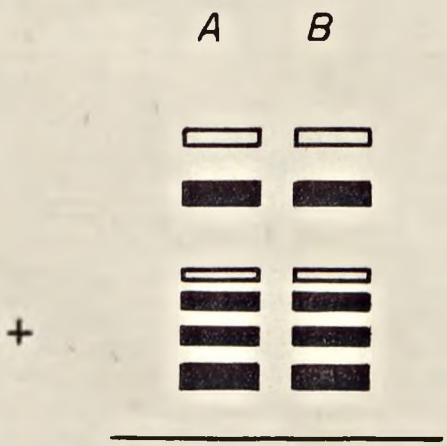
Peroxydase

The electrophoretic separation of peroxydase enzymes — with the exception of blood serum — gives various patterns distinguishing the investigated populations of loach (fig. 6). These patterns are characterized by the rate of migration in the electric field towards the anode and cathode (liver, spleen) and in the fish from the Gzel (brain and kidney figs. 3, 6) in the absence of a line migrating towards the cathode, thus with a positive electric charge, which is visible in the loach from the Raba (figs. 3, 6 G, I).

Recapitulation

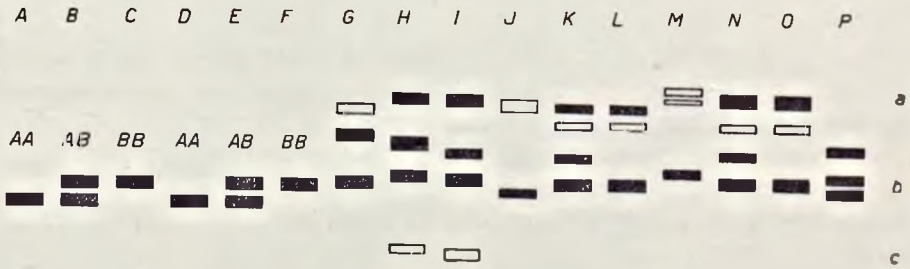
In the case of electrophoretic investigations carried out with the aim of finding the specificity of the structure of proteins in the investigated loach populations, the enzymes of the group of esterases and peroxydase of the liver, spleen, brain, and kidney tissue were found particularly useful. According to Nyman (1971), esterases are also an excellent material for taxonomic studies in other osseous fish, owing to rapid migration, high tolerance for co-operation with other protein molecules, and resistance to storage in a state of refrigeration. Peroxydase which, as Nyman and Westin (1969) claimed, was not a good indicator of the variability between species and within a species, showed differences between the discussed population of loach, particularly in the brain and kidney tissue. Thus it served as an indicator of the taxonomic differences within a species.

The presented differences in the patterns of the electrophoretic separation of esterases and peroxydase enzymes show a certain specificity of the structure of



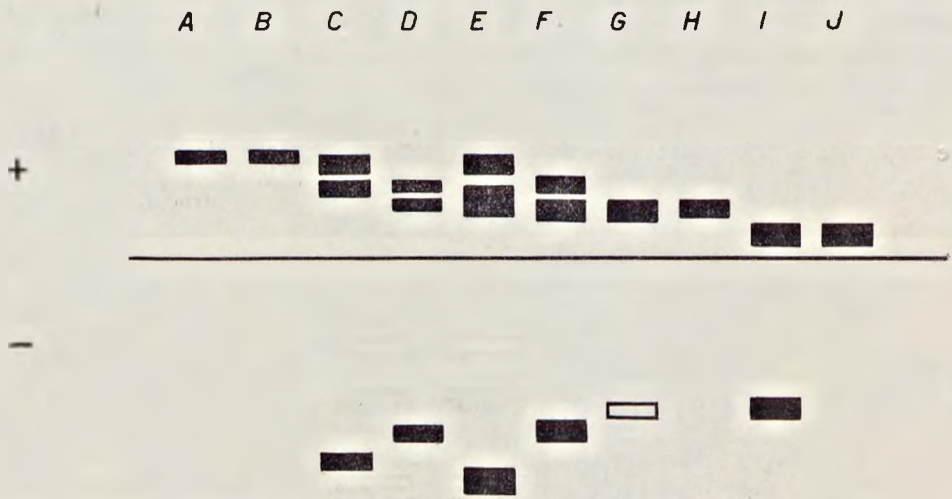
Ryc. 4. Wzory rozdziału białka surowicy ryb: A — z rzeki Raby; B — z potoku Gzel

Fig. 4. Separation patterns of blood serum proteins of fish: A — from the River Raba; B — from the Gzel stream



Ryc. 5. Wzory rozdziału esteraz: A, B, C — surowica krwi ryb z Raby; D, E, F — surowica ryb z Gzelu; G — wątroba ryb z Raby; H — wątroba ryb z Gzelu; I — mózg ryb z Raby; J — mózg ryb z Gzelu; K, L — śledziona ryb z Raby; M — śledziona ryb z Gzelu; N, O — nerka ryb z Raby; P — nerka ryb z Gzelu

Fig. 5. Separation patterns of esterases: A, B, C — blood serum of fish from the Raba; D, E, F — blood serum of fish from the Gzel; G — liver of fish from the Raba; H — liver of fish from the Gzel; I — brain of fish from the Raba; J — brain of fish from the Gzel; K, L — spleen of fish from the Raba; M — spleen of fish from the Gzel; N, O — kidney of fish from the Raba; P — kidney of fish from the Gzel



Ryc. 6. Wzory rozdziału peroksydazy: A — surowica krwi ryb z Raby; B — surowica krwi ryb z Gzelu; C — wątroba ryb z Raby; D — wątroba ryb z Gzelu; E — śledziona ryb z Raby; F — śledziona ryb z Gzelu; G — mózg ryb z Raby; H — mózg ryb z Gzelu; I — nerka ryb z Raby; J — nerka ryb z Gzelu

Fig. 6. Separation patterns of peroxydase: A — blood serum of fish from the Raba; B — blood serum of fish from the Gzel; C — liver of fish from the Raba; D — liver of fish from the Gzel; E — spleen of fish from the Raba; F — spleen of fish from the Gzel; G — brain of fish from the Raba; H — brain of fish from the Gzel; I — kidney of fish from the Raba; J — kidney of fish from the Gzel

proteins in the investigated populations of fish. It seems to suggest that in spite of the absence of biometric and meristic differences the fish living in two different river environments, in the Raba and the Gzel, show certain genetic specificities.

STRESZCZENIE

Przeprowadzono elektroforetyczny rozdział białek w żelu skrobiowym dwóch populacji ślizów (*Nemachilus barbatulus* L.), pochodzących z rzeki o charakterze podgórskim oraz typowego nizinnego potoku.

Wyniki obserwacji rozdziału białek surowicy krwi oraz enzymów z grupy hydrolaz estrowych i peroksydazy, surowicy krwi, wątroby, śledziony, mózgu i nerki zilustrowano na rysunkach od 1 do 6.

Przedstawione różnice we wzorach elektroforetycznego rozdziału, świadczą o pewnej odrębności struktury białek badanych populacji ryb. Wskazuje to, że ryby żyjące w dwóch odmiennych środowiskach rzecznych pomimo braku różnic biometrycznych i merystycznych wykazują pewne odrębności genetyczne.

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