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Elektroforetyczny rozdział białek surowicy krwi i esteraz kiełbi (*Gobio gobio L.*) żyjących w różnych rzekach

Electrophoretic separation of proteins of blood serum and esterases in gudgeon (*Gobio gobio L.*) living in various rivers

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A b s t r a c t — The electrophoretic separation of esterases on starch gel was carried out in four local populations of gudgeon (*Gobio gobio* L.) living in rivers greatly differing and lying in various geographical regions.

It was found that the esterases of the liver and kidney of the investigated fish showed different electrophoretic patterns. They suggest variability in the structure of proteins and thus the occurrence of a system which has an influence on the physiological adaptation of individual populations to the conditions of the environment in which they live.

Material and method

The gudgeon (*Gobio gobio* L.) is a bottom fish living in montane streams, in lowland rivers, and even in lakes. It occurs almost throughout Europe, with the exception of Spain, Italy, Greece, and northern Sweden, thus in various geographical regions to which individual populations must be suitably adapted. The adaptation of a population to certain environmental conditions usually occurs by way of natural selection, which finally results in a genetic differentiation in consequence of some gene falling out of the gene pool. Therefore the question arose as to the degree in which the local populations of gudgeon differ from each other.

In order to obtain an answer to this question electrophoretic separation of proteins was carried out on starch gel since the structure of pro-

tein, differentiated by the genes which governed its structure, was manifested in the number and rate of the migration of lines of proteins in the electric field.

The investigation was conducted on sexually mature representatives of four gudgeon populations from rivers differing considerably: the fish were caught in the period from 21st May — 8th June 1974. The first group originated from the upper course of the River Raba, between the towns of Rabka and Mszana Dolna. In this sector the river is of submontane character, with a stony bottom and cold transparent water, the mean gradient being 8‰. The second group of fish came from the River Mierzawa in the neighbourhood of Michałowa, where the mean gradient was 1.3‰, the bottom was covered with slimy stones, and the water slightly turbid. The third group originated from the typical lowland stream Gzel, with a mean gradient of 0.75‰ and slimy-sandy bottom. The fourth group also came from a typical lowland river, the Nida, at Motkowice. Its bottom was gravelly-sandy and slimy, with a 0.47‰ gradient and turbid water.

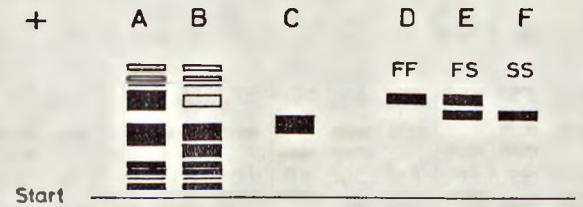
Of the fish caught in the places described above and transported to the laboratory at Mszana Dolna blood samples were taken from the anal vein as well as the samples of liver and kidney tissue. After the centrifugation of morphotic elements, the blood serum as well as liver and kidney samples were placed in tightly closed containers and stored in a frozen state. 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 in general and of enzymes of the ester hydrolases group was carried out on horizontally laid 10.55% starch gel, placed on trays prepared according to the method given by Tsuyuki (1963). The Ashton and Bradon set of buffers (1961) was used in starch gel and electrode basins, only the molarity of the solution being changed from 0.181 to 0.3 M. The electrophoresis took 1 hr and 50 min. at 6.5 V per 1 cm of gel. The stains described by Nyman (1970) were used for staining proteins and enzymes.

Results and discussion

The proteins of the blood serum

All the four gudgeon populations discussed have two patterns of the electrophoretic separation of blood serum (fig. 1 A B). The percentage frequency of the occurrence of these patterns (A and B) in the investigated fish is as follows: in the River Raba pattern A amounts to 50% and pattern B also to 50%. In the River Mierzawa pattern A was found with 83% of fish and pattern B with 17%. In the Gzel stream 75% of A patterns



Ryc. 1. Wzory elektroforetycznego rozdziału: białka surowicy krwi — A, B; esteraz surowicy krwi — C; esteraz nerki — D, E, F, czterech badanych populacji kiełbi. Oznaczenia według Nymana: FF, FS, SS (fast, slow)

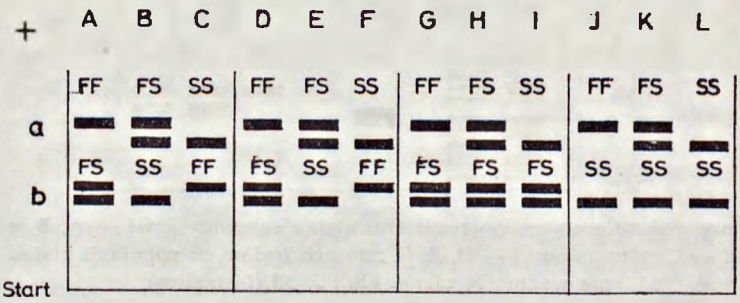
Fig. 1. Patterns of the electrophoretic separation: of the protein of the blood serum — A, B; of the esterases of the blood serum — C; of the esterases of the kidney — D, E, F, of the four gudgeon populations investigated. Symbols according to Nyman: FF, FS, SS (fast, slow)

and 25% of B patterns were found in 72 fish investigated. In the River Nida the ratio of A and B patterns was 14 to 86%.

Esterases

The electrophoretic separation of enzymes of the ester hydrolases group in the blood serum, liver, and kidney shows polymorphism with the exception of the blood serum (fig. 1 C), which has a monomorphic pattern common for all the investigated fish. It is probably effected by two alleles of the co-dominant genes which in consequence of random crossing form 2 homozygous patterns composed of a rapidly migrating FF line and a slower SS line as well as of two heterozygous FS lines, thus similarly as was described by N y m a n (1965, 1967, 1972).

The patterns of esterases of gudgeon liver are divided into two parts: the upper (fig. 2 a) and the lower (fig. 2 b). In the four investigated population the upper part is composed of 3 polymorphic patterns. The lower part is also polymorphic in the fish from the Raba and Mierzawa (fig. 2 b, A B C, D E F) while in the fish from the Nida the lower part (fig. 2 b. G H I) has one pattern composed of 2 lines which correspond with the heterozygous lines (FS) from the Rivers Raba and Mierzawa. The fish from the Gzel stream have also one pattern, this being a single line (fig. 2 b J K L) lying at the same distance from the starting point as the more slowly migrating SS band in the fish from the Raba and the Mierzawa. The polymorphism of the upper and lower parts of the patterns is independent and probably effected by the alleles of other genes since individual fish sometimes have with the heterozygous FS pattern an upper FF, FS, or SS pattern. The frequencies observed in the occurrence of the upper and lower pattern of the liver (FF, FS, and SS) of the gudgeon from the Mierzawa and the Raba do not show any significant deviations from those expected. It suggests that these populations are in a state of relative



Ryc. 2. Wzory elektroforetycznego rozdziału esterazy wątroby: A, B, C — kiełbi z rzeki Mierzawy; D, E, F — kiełbi z rzeki Raby; G, H, I — kiełbi z rzeki Nidy; J, K, L — kiełbi z potoku Gzel. (a — wzór górny, b — wzór dolny)

Fig. 2. Patterns of the electrophoretic separation of liver esterases: A, B, C — gudgeon from the River Mierzawa; D, E, F — gudgeon from the River Raba; G, H, I — gudgeon from the River Nida; J, K, L — gudgeon from the Gzel stream. (a — upper pattern, b — lower pattern)

Tabela I. Wartości obserwowane i przewidywane, frekwencja esteraz F oraz chi-kwadrat polimorficznych wzorów esteraz wątroby badanych populacji kiełbi

Table I. The observed and expected values, frequency of F esterases, and chi-square of polymorphic patterns of liver esterases of the investigated gudgeon populations

Miejsce występowania Place of occurrence		FF	FS	SS	Razem Total	fF	χ^2	Przybliżone prawdopodobieństwo Approximate probability
Mierzawa	wzór pattern a	13.0 11.3	21.0 23.5	13.0 11.7	47	0.50	0.57	0.70-0.80
	wzór pattern b	4.0 1.4	8.0 12.2	35.0 32.4	47	0.16	2.09	0.10-0.20
Raba	wzór pattern a	14.0 13.9	24.0 23.0	10.0 9.6	48	0.54	0,66	0.70-0.80
	wzór pattern b	8.0 10.1	28.0 24.0	12.0 13.4	48	0.46	2.56	0.20-0.30
Nida	wzór pattern a	10.0 3.4	6.0 9.5	34.0 27.5	50	0.26	15.64	< 0.001
Gzel	wzór pattern a	9.0 15.1	48.0 36.0	15.0 20.9	72	0.46	16.06	< 0.001

equilibrium, contrary to those from the Nida nad the Gzel which show great deviations of the values observed from the expected ones (Table I). Similarly as the liver esterases, the esterases of the kidney form three polymorphic patterns (fig. 1 D E F), differing only in the frequency of alleles in individual populations. In the fish from the Mierzawa, Raba, and Nida they show a state of relative equilibrium, while in the individuals from the Gzel stream the values observed depart considerably from those expected (Table II).

The discrepancy between the expected and observed frequencies of patterns of liver esterases in gudgeon from the Nida and the Gzel as

Tabela II. Wartości obserwowane i przewidywane, frekfencja esteraz F oraz chikwadrat polimorficznych wzorów esteraz nerki badanych populacji kiełbi

Table II. The observed and expected values, frequency of F esterases, and chishare of the polymorphic patterns of kidney esterases of the investigated gudgeon populations

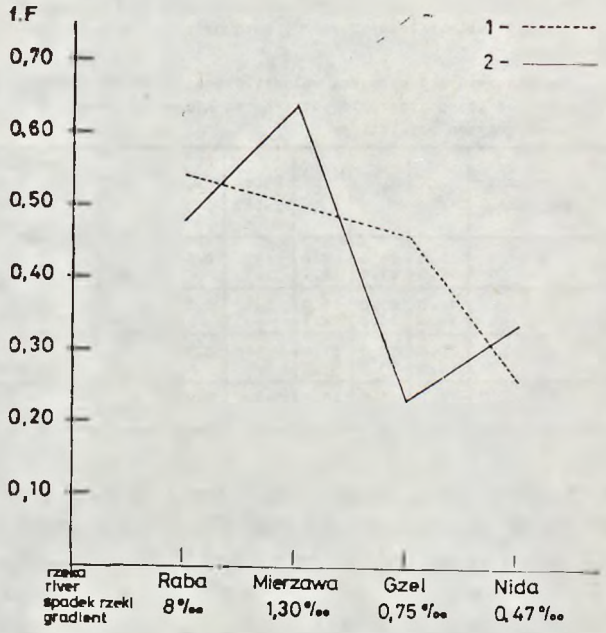
Miejsce występowania Place of occurrence	FF	FS	SS	Razem Total	fF	χ^2	Przybliżone prawdopodobieństwo Approximate probability
Mierzawa	20.0 19.3	20.0 21.2	7.0 6.1	47.	0.64	0.26	0.80-0.90
Raba	10.0 11.0	26.0 24.0	12.0 12.9	48	0.48	0.34	0.80-0.90
Nida	10.0 8.0	19.0 23.0	21.0 19.0	50	0.39	1.40	0.30-0.50
Gzel	9.0 3.8	15.0 25.2	48.0 42.5	72	0.23	11.90	0.01-0.001

well as of kidney esterases in the fish from the Gzel is a phenomenon similar to that found in the populations of loach *(Nemachilus barbatulus* L.) from the Gzel stream (S t a r m a c h 1975). Similarly as gudgeon, loach occur in both montane and lowland rivers. Thus, some environmental factors probably play a role in limiting the state of genetic equilibrium in a population in consequence of the various adaptability of each of the three genotypes occurring according to Hardy-Weinberg law ($p^2 : 2pq : q^2$).

The frequency of esterases (F) of the upper patterns of the liver and kidney of the four panminctic populations investigated, plotted on the diagram (fig. 3), decreases with a decrease in the unit gradient of the river, thus the decreased frequency in the occurrence of F esterases of the liver and kidney is probably connected with changes occurring in the environment in consequence o the slower flow of water, with which, as is known, the water temperature, deposition of silt, oxygen content, changes in the flora and fauna, and hence, in the kind of food of the fish, are connected.

The geographical variability and the coincidence of genetic changes with the environmental ones, particularly of polymorphic populations, has already been described by many authors (F o r d 1964, M a y r 1963, N y m a n 1967, 1972, S i c k 1961, 1965 a, W i l k i n s 1972).

The pattern of the electrophoretic separation of esterases depends on their molecular form (N y m a n, S h a w 1971), which is differentiated by genes governing the structure of protein according to the genetic code. Each gene shows to a greater or lesser degree a pleiotrophic action, i. e. an action conditioning various properties, therefore certain specifities in the electrophoretic patterns of the four investigated populations of gudgeon may suggest a physiological differentiation of their organisms. This may be supported by the investigations of N y m a n (1972), who



Ryc. 3. Frekwencja esteraz F czterech badanych populacji. 1 — fF wątroby; 2 — fF nerki
Fig. 3. Frequency of esterases F of the four populations investigated. 1 — fF of the liver; 2 — fF of the kidney

found a dominance of S esterases in the populations of *Salvelinus alpinus* in northern waters of low temperatures as compared with more southern populations, as well as by the investigations on the local populations of loach from montane and lowland rivers (S t a r m a c h 1973, 1975) in which a correlation was found between the specifficity of the electrophoretic patterns of enzymes and the oxygen demand and the ability of increasing the number of erythrocytes in the pheripheral blood under difficult oxygen conditions.

Thus the differences in the electrophoretic separation of liver and kidney esterases of gudgeon from four rivers suggest changes in the structure of protein and, therefore, a system which has an influence on the physiological adaptation of individual local populations to the conditions of the environment in which they live.

STRESZCZENIE

Przeprowadzono elektroforetyczny rozdział enzymu esterazy wątroby i nerki czterech lokalnych populacji kiełbi (*Gobio gobio* L.) żyjących w rzekach znacznie różniących się od siebie i położonych w odmiennych obszarach geograficznych.

Stwierdzono, że frekwencja esteraz F polimorficznych wzorów wątroby oraz nerki wszystkich badanych populacji zmniejsza się ze zmniejszeniem spadku jednostkowego

rzeki. Obniżona częstotliwość występowania esteraz F jest więc prawdopodobnie związana ze zmianami zachodzącymi w środowisku na skutek wolniejszego przepływu wody.

Rozdział esteraz jest uzależniony od ich formy molekularnej. Jest ona różnicowana przez geny rządzące budową białka. Ponieważ każdy gen w mniejszym lub większym stopniu ma działanie plejotropowe, odrębności we wzorach elektroforetycznych badanych populacji dowodzą zmian w strukturze białka, a tym samym układu mającego wpływ na fizjologiczne przystosowanie się poszczególnych populacji do środowisk w których żyją.

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