UDC 575.17:639.371.13(477) https://doi.org/10.15407/biotech14.02.037

ASSESSMENT OF GENETIC STRUCTURE VARIABILITY OF RAINBOW TROUT *Oncorhynchus mykiss* (Walbaum, 1792) OF UKRAINIAN LOCAL STOCKS USING POLYMORPHIC BLOOD PLASMA PROTEINS

O. Yu. Bielikova
S. I. Tarasjuk
A. I. Mruk
T. A. Nahorniuk
L. P. Buchatskyi

Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, Kyiv

E-mail: belikova.e.y@gmail.com

Received 03.02.2021
Revised 16.04.2021
Accepted 30.04.2021

Background. Rainbow trout is a valuable species of aquaculture, which is characterized by a high level of variability.

Aim. The goal was to study the peculiarities of genetic variability of rainbow trout based on polymorphism of transferrin (TF), posttransferrin (PTF), esterase (EST) (EC 3.1.1.1.) and albumin (ALB) loci.

Methods. Electrophoretic separation of plasma proteins of rainbow trout from three local stocks (Chernivtsi, Kharkiv and Transcarpathian) was carried out in the native polyacrylamide gel.

Results. Peculiarities of the distribution and relative electrophoretic mobility of allelic variants of the studied loci of rainbow trout of Ukrainian local stocks were established. The results demonstrated the effectiveness of the analysis using methods of biochemical genetics to establish the characteristics of the genetic structure, the level of heterozygosity of local stocks and to carry out their differentiation.

Conclusion. The use of selected markers would allow monitoring the dynamics of changes in the state of local stocks under the established conditions of cultivation in the future.

Key words: rainbow trout, polymorphic blood plasma proteins, transferrin, posttransferrin, esterase, albumin.

Fish show a high level of variability because they are more closely connected with the environment due to poikilothermicity. Phenotypic variations emerge as a result of adaptive responses to the effect of the environment (short-term phenotypic corrections) in combination with genetically determined changes over generations (long-term adaptations) [1, 2]. For a long time, the study of variability in fish was carried out using polymorphic proteins. Electrophoretic separation of proteins revolutionized fish population genetics [3]. Isozymes and non-enzymatic proteins with polymorphism have been long proved to be an effective type of markers for studying gene activity at different stages of ontogenesis and in various tissues, as well as for studying peculiarities of gene expression [4]. The biochemical genetics allow studying the role of isoenzymes in morphogenetic processes, cell differentiation with the determination of genetic mechanisms of isozyme expression regulation and the level of their use, analyzing the role of isozymes in the regulation of metabolic processes [5]. Such markers can be used to study not only population diversity and structure but also to analyze processes of adaptation of the organism to changes in conditions during fish breeding at the genetic level. This is possible since allozyme markers allow studying changes in proteins with known biochemical functions at the metabolic level [6–8]. Systematic studies using polymorphic proteins make it possible to track stability and variability parameters in fish under the effect of various environmental factors and conditions of fish kept in aquaculture at different stages of their life cycle [9].

Salmonids are characterized by a high multiplicity of protein and isozyme systems due to their tetraploid origin [10]. Taking into account the fact that rainbow trout is a valuable species of aquaculture and a convenient object for biological studies, the amount of studied polymorphic proteins becomes quite significant. Analysis of the literature indicates continuing interest and importance of allozyme analysis of rainbow trout in different countries [11, 12] to study the genetic diversity of natural populations, the variability of local aquaculture stocks for effective management and improvement of the quality of the genetic structure of fish stocks, and also for the conservation of valuable species to maintain the ecological balance [13]. Biochemical genetics methods have long been actively used to determine the level of population heterogeneity, which is extremely necessary for fish farmers. The analysis of protein polymorphism in salmonids was carried out by Altuhov in his work "Population genetics of salmonids" [10], which presented a generalization of the studied mono- and polymorphic protein in rainbow trout muscular tissues, heart, liver, and eyes. A number of works was devoted to the study of allozyme (enzyme) loci in tissues; however, this is unacceptable for monitoring works with species cultivated in aquaculture. Therefore, to study the variability of the genetic structure of rainbow trout cultivated in trout farms, it is advisable to analyze the polymorphism of plasma proteins. The use of allozyme markers is very convenient and informative for assessing the variability, since blood plasma is the main liquid participating in protein transport, quickly responds to the effects of external factors and allows determining the state of the body and the passage of biochemical processes in it [14–15]. A very important advantage of the method used is the ability to carry out *in vivo* sampling of fish blood and the ability to analyze the selected loci on one gel plate does not involve expenses for DNA isolation, which significantly accelerates monitoring works.

Therefore, our work was aimed at studying the peculiarities of the genetic variability of rainbow trout by polymorphism of plasma proteins.

Materials and Methods

All manipulations with rainbow trout were carried out following the provisions recommended by the European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes (Strasbourg, 1986) ETS No.123.

The assessment of genetic variability using polymorphic proteins was carried out on rainbow trout of three local stocks (LS): Chernivtsi (Berehomet, n = 42), Kharkiv (Novaya Vodolaga, n = 60) and Transcarpathian region (Turya Polyana, n = 55) in 2018–2019. Fish before taking blood samples were kept in a solution of the anesthetic clove oil at a concentration of 0.033 ml/L according to the recommendations [16] to avoid fish traumatizing.

Blood from the caudal vein was collected with a sterile syringe with heparin (25 IU/ml). Blood was centrifuged (15 min at 3.5 thousand rpm), plasma and erythrocyte mass were separated into two different clean Eppendorf microtubes for further work. Samples were transported at 4 °C and stored at –20 °C in the laboratory until research.

The study of the genetic structure of rainbow trout was carried out at the loci of transferrin (TF), posttransferin (PTF), esterase (EST) (EC 3.1.1.1.), and albumin (ALB). The loci were designated according to the nomenclature [17]. Electrophoresis was carried out using a "Helicon" camera VE-20 (Russian Federation) with platinum electrodes with a power supply unit "Elf-4" (Ukraine). Plasma proteins were separated by electrophoresis in 9% native polyacrylamide gel (PAG) using Tris-glycine electrode buffer (pH 8.3) [18, 19]. Reagents of the following companies were used in the study: Sigma (USA), "Sfera sim" (Ukraine), CarlROTH, Appli Chem Gmbn (Germany). Blood plasma (3.5 µl) was mixed with 2.5 µl of the dye for loading into the gel (1 part of TBE buffer, 1 part of glycerol and bromophenol blue 0.02%). Electrophoresis was carried out at a temperature of 4 °C at a voltage of 140 V and 20 mA for 30 min to the exit from the concentrating gel, then 160–180 V 30 mA 30 min and at a voltage of 250 V 45 mA until the leading dye passes 1.5 camera lengths.

Staining of the analyzed proteins on gels was carried out following generally accepted methods [18–21].First, a solution containing α-naphthyl acetate (5 mg), β-naphthyl acetate (5 mg), Fast Blue RR salt (10 mg) and 0.1 M phosphate buffer pH = 7.4 (200 ml) was used to detect esterase bands. The gel plate was incubated in this solution at 37 °C for 20 min in a dark place. Then the gel was stained with a 0.04% solution of Coomassie Brilliant Blue G-250 in 7% acetic acid to detect allelic variants of albumin, transferrin, and posttransferrin.

Washing of the gel plate was performed in a solution consisting of H_2O (100 ml), alcohol (50 ml) and acetic acid (20 ml).

Relative electrophoretic mobility (R_f) was determined in accordance with the recommendations [22]. The electrophoresis was terminated when the lead dye reached 2 cm to the end of the gel. First, the absolute mobility of the leading dye (solution of bromophenol blue in 50 % glycerol) in cm and the mobility of each component after dyeing were measured. These values of the length of the path passed by each component were divided by the path length of the leading dye [23].

Statistical analysis of the frequency distribution of alleles and genotypes, calculation of the heterozygosity level and Wright's F-statistics were performed using Biosys-1 [24]. To assess the stability of the local stock of rainbow trout according to the Hardy-Weinberg equilibrium, the deviation of the actual frequencies of genotypes from the theoretically expected ones at the studied loci was calculated using the Pearson test with a 5% level of error significance.

Results and Discussion

The population genetic analysis of rainbow trout from three local stocks showed that the studied loci (TF, PTF, EST, ALB) of blood plasma were polymorphic. Albumin (ALB), posttransferin (PTF), and esterase (EST) were biallelic systems represented by fast and slow-migrating alleles. TF locus was represented by a triallelic system, which was typified as alleles A, B and C, among which allele A had the highest electrophoretic mobility. The presence of multiple molecular forms may be due to the addition of different amounts of sialic acid residues to transferrin [25].

The presence/absence of allelic variants at certain loci of allozymes and the level of their activity allow tracing the effect of abiotic factors on the body since the allelic products of some loci are usually associated with certain physiological functions that ensure the stability of the conditions of a certain habitat of fish [26].

Transferrin is very polymorphic (the number of alleles varies within 2–13, although most species usually have 3–4 alleles) and is easily determined by electrophoresis [25]. Polymorphism at transferrin locus in rainbow trout was also studied by a Chilean group of scientists [11], who found that Tf was polymorphic in muscular tissues and liver in some of the studied groups and was represented by two alleles, but it was monomorphic in some populations.

The selected protein systems are quite popular as tools for studying variability at the population level. Among the many allozymes, transferrin and esterase are among the most variable [27]. Fractions of albumin and beta globulins (which include transferrin) of blood plasma are recommended for individual labelling for population genetic analysis of rainbow trout from both natural populations and domesticated stocks [28].

The study of serum esterase fractions attracts attention by the fact that that fish are characterized by a linear correlation of the frequency distribution with geographical latitude. In cases where representatives of the same latitude are studied, there is a relationship with the height of the location of water bodies, where the studied fish are cultivated, above sea level [29]. Fish esterase is characterized not only by genetic variability but also by the presence of ontogenetic variations [10, 29]. Transferrin and albumin, which have no enzymatic activity, are often represented in fish as isoforms [25].

The relative electrophoretic mobility of the investigated loci was found to be specific for the studied local stocks of rainbow trout (Fig. 1).

The mobility of the leading dye (Fig. 1, *A*) was taken as a unit when calculating the electrophoretic mobility of the alleles of the studied loci. At esterase locus, the relative electrophoretic mobility of fast-migrating F allele was 0.293 ± 0.001, and that of slow-migrating S allele was 0.269 ± 0.007 (Fig. 1, *B*). The electrophoretic mobility at albumin locus was greater than the mobility of the transferrin fraction (Fig. 1, *C*). The fast allele Alb A had a relative R_f equal to 0.394 ± 0.004, and the slow allele Alb B to 0.369 ± 0.003. At transferrin locus, the electrophoretic mobility was: 0.305 ± 0.002 for allele A, 0.274 ± 0.005 for allele B, and 0.261 ± 0.003 for allele C. The lowest values of the relative electrophoretic mobility were observed at the posttransferin locus: 0.239 ± 0.002 allele F and 0.199 ± 0.001 for allele S.

Determination of the electrophoretic mobility of protein isoforms in their native state by their total charge, molecular weight and spatial configuration of the polypeptide chain [23] gives quantitative characteristics to the study, allows characterizing the species-specific and intraspecific constant of polymorphic proteins, can be used for species identification [22] and interspecies comparison [30]. The relative electrophoretic mobility provides a comparative characteristic

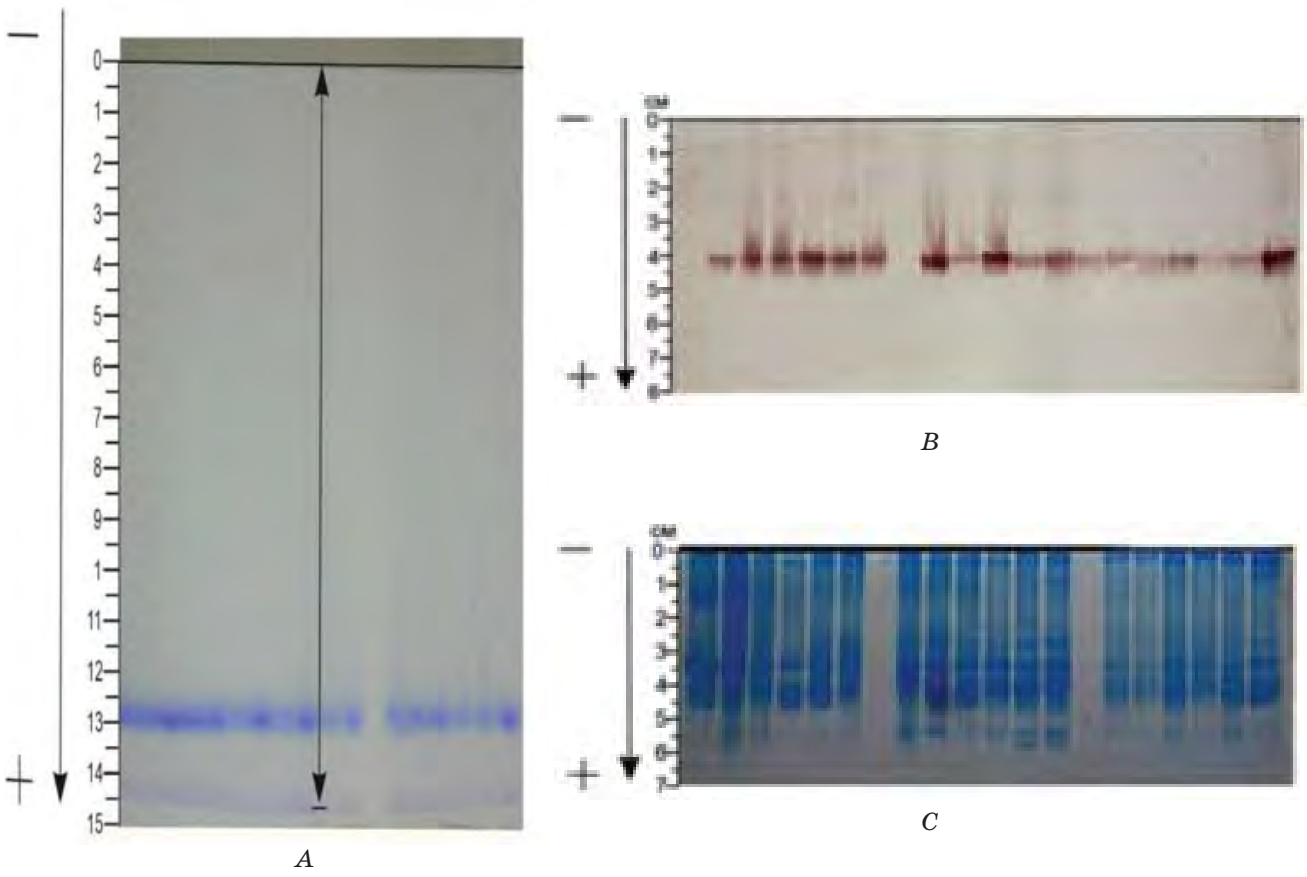

Fig. 1. **Electropherograms demonstrating the mobility of the leading dye (*A*) and allozyme loci: esterase (*B*) and non-enzymatic proteins (albumin, transferrin and posttransferin) (*C*)**

of the distribution of multiple molecular forms of acid phosphatase in various animals, including rainbow trout [30]. The distribution of heteroforms of this enzyme had tissue species specificity in freshwater fish species and less expressive species features.

Allelic variability of loci underlies biochemical polymorphism, which is widespread in nature, and the correlation between the geographical area or conditions of fish cultivation and changes in the frequency distribution of allelic variants is often observed [25].

An analysis of the frequencies of allelic variants of the studied loci allows establishing the peculiarities of their distribution in rainbow trout from Ukrainian local stocks (Fig. 2). According to esterase locus, the fast-migrating allele (F) was found to occur with a lower frequency (0.383 ± 0.013) than the slow-migrating allele (S) (0.617 ± 0.013).There were no differences in the distribution of allelic frequencies at EST locus among the three studied local stocks. According to albumin locus, the mean frequency of the fast allele (A) was 0.515 ± 0.082, and that of the slow allele (B*)* was 0.485 ± 0.082. However, the Kharkiv local stock was characterized by a predominance of the allele Alb B in contrast to other studied stocks.

At transferrin locus, which is the most variable among proteins in fish, three identified alleles occurred with the same frequency (in equal proportions) and were: 0.318 ± 0.024 for allele A, 0.35 ± 0.008 for allele B, and 0.331 ± 0.023 for allele C. No differences in allele frequencies were found among three studied local stocks. The frequency of the slow-migrating allele (S) at the posttransferin locus was 0.575 ± 0.076, and that of the fast-migrating allele was 0.425 ± 0.076. The frequency of allele S at PTF locus in the Transcarpathian local stock was significantly different from its frequency in the other two stocks. The study of rainbow trout in Ukraine showed the relative uniformity of allelic frequencies among the studied groups was clearly expressed at all loci, except for esterase.

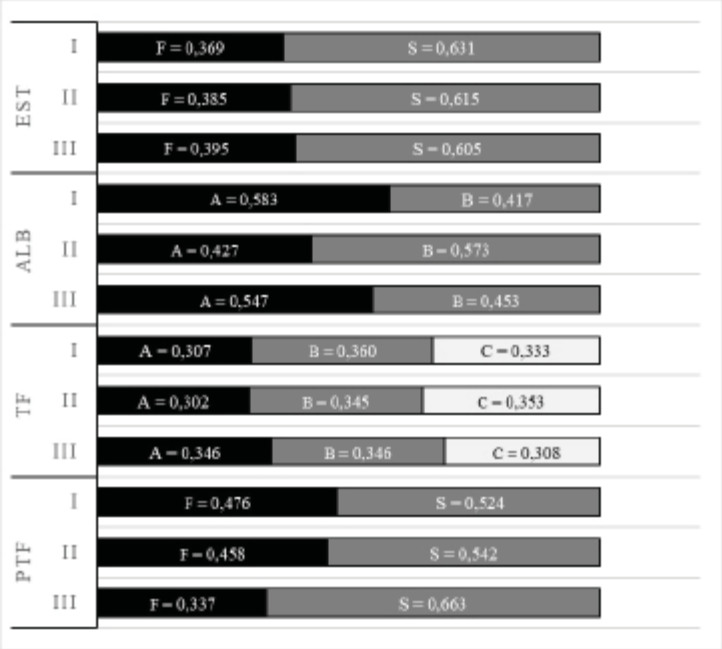

Fig. 2. **Allele frequencies of allozyme loci in rainbow trout among three local stocks:** *I* — Chernivtsi; *II* — Kharkiv; *III* — Transcarpathian

Analysis of the distribution of genotypes of studied loci (EST, ALB, PTF) in accordance with the Hardy-Weinberg law showed that a equilibrium distribution of genotypes was observed only in the Chernivtsi local stock at the loci of EST ($P < 0.05$) and PTF ($P < 0.05$), except for ALB ($P < 0.025$) (Fig. 3). The imbalance in the distribution at esterase locus was the largest in the Transcarpathian LS, FF genotype was absent in this local stock. In the Kharkiv stock, the deviation from the expected distribution of FF genotype was 12.5%. In the Kharkiv and Transcarpathian local stocks, FS genotypes were predominant at esterase locus.

At albumin locus, all studied stocks were characterized by a predominance of the heterozygous AB genotype; deviations from the expected number of individuals with this genotype were 15.1% in Chernivtsi, 23.5% in Kharkiv and 17.3% in Transcarpathian stocks. At posttransferin locus in the Kharkiv local stock, a 29.0% excess of the expected amount was observed for heterozygous FS genotypes, and that in the Transcarpathian stock was 22.2%. The latter stock was characterized by the absence of FF genotype at PTF locus.

Analysis of the distribution of genotypes at transferrin locus showed that differences in the distribution of ABC, AA, and AC genotypes frequencies were observed in rainbow trout from the Chernivtsi local stock in comparison with other stocks (Fig. 4). In the Transcarpathian stock, there was a significant predominance of individuals with AB genotype, in contrast to the Chernivtsi and Kharkiv stocks, by 9 and 10%, respectively. In turn, individuals of the Kharkiv local stock were characterized by the presence of CC genotype, which was not recorded in other stocks. ABC genotype was found at a higher frequency compared to other genotypes in all three local stocks.

Studies of protein polymorphism in rainbow trout were aimed at establishing the presence of a genetically determined relationship between fish-breeding biological characteristics and polymorphic proteins [31]. No connection was found between the maturation time of females and the presence of AA and AB genotypes of albumin locus, and no differences in size and weight parameters were observed between these genotypes when studying the polymorphism of albumin and transferrin in blood plasma of rainbow trout from the Ropsha experimental station [31]. However, Yablokov et al. [31] reported the presence of differences in trout weight with different transferrin genotypes (TF B and TF AB) and showed that heterozygous individuals with AB genotype of transferrin locus had a 20-day shorter spawning period than homozygous individuals [31].

Heterozygosity level is a very important parameter in population genetics that allow

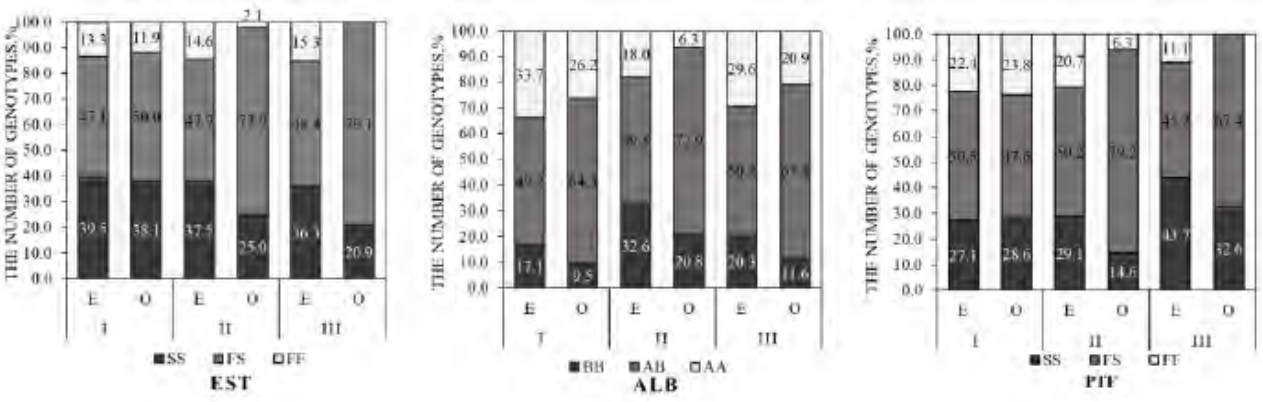

Fig. 3. **The number of genotypes (%) at esterase, posttransferin and albumin loci:**
E — expected; *O* — observed

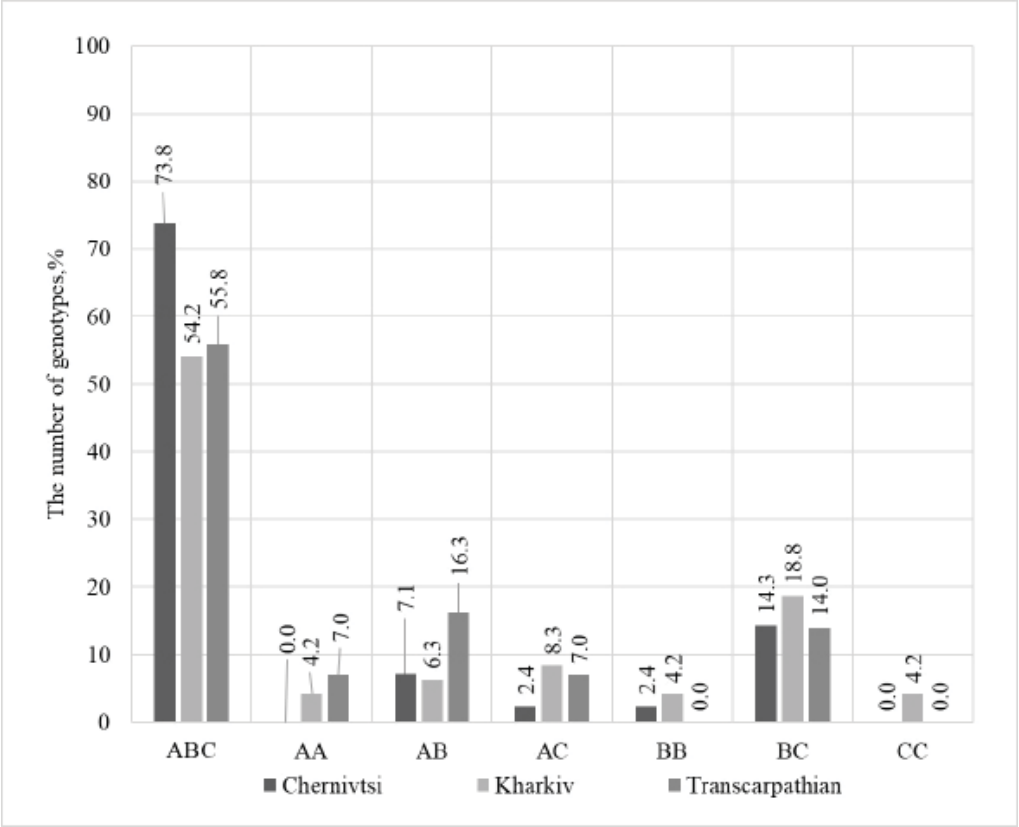

Fig. 4. **Distribution of genotypes (%) at transferrin locus**

assessing changes caused by human activity in breeding work [32]. Heterozygosity affects fitness (survival, fertility, quality of offspring) and thus determines many aspects of their relationship with the environment [32]. Analysis of the heterozygosity level was carried out using the fixation index, the average values of the observed and expected heterozygosity (Fig. 5). The highest values of the expected (H_e) and observed (H_o) heterozygosity at three loci (EST, ALB, and PTF) were found in the Kharkiv local stock and were 0.492 ± 0.007 and 0.750 ± 0.021, respectively. In the Chernivtsi stock, H_e was recorded at the level of 0.489 ± 0.01, and H_o −0.54 ± 0.052. In the Transcarpathian local stock, the expected heterozygosity was 0.479 ± 0.015, and the observed heterozygosity was 0.713 ± 0.039. The fixation index (F_{is}) calculated from the values for three locihad the lowest value in the Chernivtsi local stock (−0.117 ± 0.108), which significantly differed from F_{is} value in the Kharkiv and Transcarpathian stocks (−0.531 ± 0.039 and −0.508 ± 0.085, respectively). If we consider the differences in the values of heterozygosity and fixation indices (Fig. 5), it seems that the Chernivtsi local stock differed most at esterase and posttransferin loci (F_{is} was −0.074 at EST locus and 0.045 at PTF locus).

An increase in the heterozygosity index can be expected in the case of an increased adaptation of fish to a particular environment. A decrease in heterozygosity, as well as its excessive increase, is unfavorable for the normal functioning of population [32]. It is known that a decrease in the genetic variability of fish during inbreeding may be accompanied by deterioration in such important biological parameters as the survival of embryos and larvae, growth rate, the efficiency of food assimilation, body shape, etc [33].

To assess the degree of differentiation of the genetic variability of the studied groups, Wright's F-statistics were calculated, reflecting the individual (F_{is}), subpopulation (F_{st}), and general population (F_{it}) levels of genetic variability [34]. F_{st} value, which is a measure of genetic differentiation between subpopulations, is highly dependent on the distribution of allele frequencies in the compared subpopulations, while F_{is} and F_{it}, which are a measure of deviations from the Hardy-Weinberg ratio within a subpopulation and throughout the population, will, on the contrary, depend on deviations in the distribution of genotypes in subpopulations [34]. The analysis of variability at the individual level of individuals relative to the sample showed that the average excess of heterozygotes per individual in the studied local stocks was 38.7%; in total, the predominance of heterozygotes was at esterase locus (42.5%). As for albumin locus, the predominance of heterozygous genotypes was F_{is} = 39.1%,

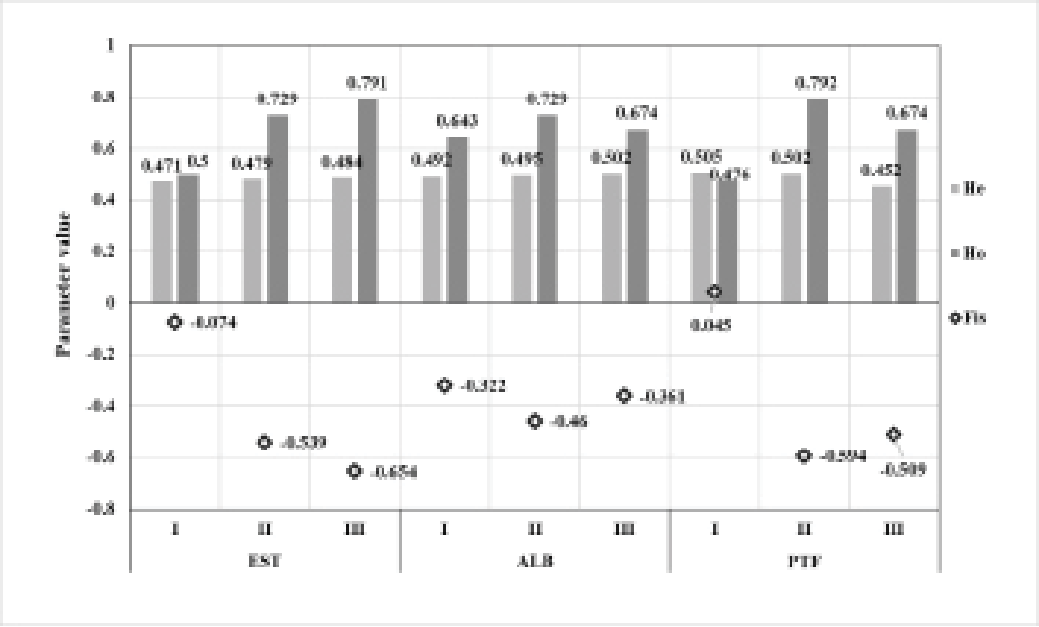

Fig. 5. **Fixation index, observed and expected heterozygosity among three local stocks:** *I* — Chernivtsi; *II* — Kharkiv; *III* — Transcarpathian

and for the posttransferin locus the lowest value was 34.7%. Analysis of interpopulation variability for F_{st}, which reflected the degree of differentiation between local stocks, showed that the share of interpopulation variability by the studied polymorphic loci was 1.1%. The stocks differed most of all by albumin and posttransferin loci, F_{st} was 0.018 and 0.016, which indicates a high level of differentiation in these systems. No genetic differentiation was observed at esterase locus. According to the identity index (Nei (1978) unbiased genetic identity), the Chernivtsi and Transcarpathian local stocks (0.997) were the most closely related by polymorphism of plasma proteins, while the identity index of the Kharkiv stock with the aforementioned stocks was 0.993.

The results obtained demonstrate the efficiency and convenience of using blood plasma proteins to determine the level of population heterogeneity, comparative characteristics of different populations by polymorphism of the same enzymes. The developed methodological basis for the biological interpretation of the results obtained using polymorphic proteins allows tracking the dynamics of changes in the genetic structure of populations when the physiological state of rainbow trout changes [20, 27, 28]. The parameters of variability were determined, which made it possible to give recommendations to farms for the scientifically grounded implementation of some biotechnological measures to maintain genetic biodiversity and rational economic management of the trout farm.

The research was performed within the research programs of the National Academy of Agrarian Sciences of Ukraine: "To study the mechanisms of adaptation of certain valuable fish species using methods of population genetics" (DR №0116U001227) and "To investigate the genetic variability of rare and endangered species of salmon and sturgeon fish in the water bodies of the Carpathian region and to develop methods for their reproduction" (DR № 0119U100578). The authors declare no conflict of interests.

REFERENCES

1. *Pavlov D.A.* Morphological variability in the early ontogenesis of teleost fishes. *Moskva: GEOS*. 2007, 264 p. Available at: https://www.researchgate.net/publication/311570706_Morphological_Variation_in_the_Early_Ontogeny_of_Teleost_Fishes (In Russian).
2. *Pulcini D., Wheeler P.A., Cataudella S., Russo T., Thorgaard G. H.* Domestication shapes morphology in rainbow trout *Oncorhynchus mykiss*. *J. Fish Biol.* 2013, 82 (2), 390–407. https://doi.org/10.1111/jfb.12002
3. *Gomelsky B.* Fish Genetics: Theory and Practice Publisher: VDM Verlag Dr. Müller. 2011, 200 p.
4. *Kartavcev Yu. F.* Molecular Evolution and Population Genetics: A Study Guide, 2-e izd. *Vladivostok: Izd-vo Dal'nevostochnogo gos. un-ta.* 2008, 562 p. (In Russian).
5. *Tarasiuk S. I., Bielikova O. Yu., Kolisnyk S. O.* Relevance of molecular genetic research in aquaculture. *Problemy ekolohichnoi biotekhnolohii.* 2018, No 1. Available at: http://ecobio.nau.edu.ua/index.php/ecobiotech/article/view/12877/17714. (In Ukrainian).
6. *Kovalchuk N. A., Vishchur O. I., Kras S. I., Tarasiuk S. I.* Analysis of the genetic structure of populations of horse breeds by individual genetic and biochemical markers. *Veterynarna biotekhnolohiia.* 2013, No 23, P. 187–194. Available at: http://nbuv.gov.ua/UJRN/vbtb_2013_23_32. (In Ukrainian).
7. *Artamonova V., Mahrov A.* Genetic methods in salmon and trout breeding: from traditional breeding to nanobiotechnology. *Moskva: Tovarishchestvo nauchnykh izdaniy KMK*. 2015, 128 p. (In Russian).
8. *Das P., Meena D. K., Singh S. K., Akhtar M. S., Gupta S. K., Alexander C., Mandal S. C.* Molecular markers (protein and DNA origin): Application in fisheries and aquaculture. *Reprinted with perission from World Aquaculture*. 2010, 41 (4), 11–14. Available from: https://www.researchgate.net/publication/236961450_Molecular_markers_protein_and_DNA_origin_Application_in_fisheries_and_aquaculture
9. *Shishanova E. I., Ryabova G. D., Labenec A. V.* On the expediency of using protein markers in assessing the genetic status of fish populations (populations). *Akvakultura Centralnoy Vostochnoy Evropy: nastoyashchee i budushchee. Sb. II Mezhdunar. Sezd NACEE. Kyshynev*: 2011, P. 285–287. (In Russian).
10. *Altuhov Yu. P., Salmenkova E. A., Omel'chenko V. T.* Population genetics of salmonids. *Moskva: Nauka.* 1997, 287 p. (In Russian).
11. *Cárcamo C. B., Díaz N. F. Winkler F. M.* Genetic diversity in Chilean populations of rainbow trout *Oncorhynchus mykiss*. *Lat. Am. J. Aquat. Res*. 2015, 43 (1), 59–70. https://doi.org/10.3856/vol43-issue1-fulltext-6

12. *Pavlov S. D., Semenova A. V.* Allozyme variation in Mykiss *Parasalmo (O.) mykiss* from the Kol River. *Biological Sciences*. 2011, 436 (1), 59–61. https://doi.org/10.1134/s0012496611010194
13. *Nafath-ul-Arab I., Ahmad T. H., Shah O. A., Asimi Z., Yousuf A., Iqbal S., Baba H., Fatima A., Razak N.* Protein polymorphism in fishes. *J. Entomol. Zool. Studies.* 2020, 8 (2), 62–66. Available from: https://www.entomoljournal.com/archives/2020/vol8issue2/PartB/8-1-281-193.pdf
14. *Morro B., Doherty M. K., Balseiro P., Handeland S. O., MacKenzie S., Sveier H., Albalat A.* Plasma proteome profiling of freshwater and seawater life stages of rainbow trout (*Oncorhynchus mykiss*). *PLoS ONE*. 2020, 15 (1), e0227003. https://doi.org/10.1371/journal.pone.0227003
15. *Mikodina E. V., Lapteva T. I., Sedova M. A., Ganzha E. V., Pavlov E. D.* Functional markers of fish. Directory. *Moskva: VNIRO.* 2016, 118 p. (In Russian).
16. *Hamackova J., Kouril J., Kozak P., Stupka Z.* Clove Oil as an Anaesthetic for Different Freshwater Fish Species. *Bulgar. J. Agricul. Sci*. 2006, 12 (2), 185–194. https://www.agrojournal.org/12/02-04.htm
17. *Shaklee J. B., Allendorf F. W., Morizot D. C., Whitt G. S.* Gene Nomenclature for Protein-Coding Loci in Fish. *Transactions of the American Fisheries Society.* 1990, 119 (1), 2–15. https://doi.org/10.1577/1548-8659(1990)119<0002:gnfpli>2.3.co;2
18. *Gahne B., Juneja R. K., Grolmus J.* Horizontal polyacrylamide gradient gel elelectrophoresis for the simultaneous phenotyping of transferring, post-transferrin, albumin and post-albuminin the blood plasma of cattle. *Anim. Blood Group Biochem. Genet*. 1977, 8 (3), 127–137. https://doi.org/10.1111/j.1365-2052.1977.tb01637.x
19. *Tarasiuk S. I., Mruk A. I., Zaloilo O. V., Mariutsa A. E., Hlushko Yu. M., Ustych V. I., Kucheruk A. I., Bielikova O. Yu., Sukhoverska L.* Genetic and morphometric assessment of individual populations of rare and endangered fish species of Ukraine: guidelines. *Kyiv: Instytut rybnoho hospodarstva NAAN*. 2018, 73 p. (In Ukrainian).
20. *Glazko V. I., Sozinov I. A.* Genetics of animal and plant isoenzymes. Ed. I. A. Sozinov. *Kyiv: Urozhai.* 1993, 528 p. (In Russian).
21. *Korochkin L. I., Serov O. L., Pudovnik A. I..* Genetics of isoenzymes. *Moskva: Nauka.* 1977, 275 p. (In Russian).
22. *Karnauhov G. I., Chebanov M. S., Vasiliadi V. D.* Methodical recommendations for determining the species of sturgeon fish by electrophoreticmethod. *Krasnodar: KrasNIIRH*. 2003, 16 p. (In Russian).
23. *Struchkova I. V., Kal'yasova E. A* Theoretical and practical bases for carrying out electrophoresis of proteins in polyacrylamide gel: Electronic teaching aid. *Nizhniy Novgorod: Nizhegorodskiy gos. un-t*. 2012, 60 p. (In Russian).
24. *Swofford D. L., Selander R. B.* BIOSYS-1: a Fortain programm for the comprehensive analysis of electroforetic data in population genetics and systematics. *J. Heredity*. 1981, 72, 281–283.
25. *Kirpichnikov V. S.* Functional differences between isozymes (isoforms) and between allelic forms of proteins in fish. *Biokhimicheskaya i populyacionnaya genetika ryb: sbornik nauchnykh trudov. Leningrad.* 1979, P. 5–9. (In Russian).
26. *Karabanov D. P.* Resistance to abiotic factors of some bony fish isoenzymes. *Water: Chemistry and Ecology*. 2012, V. 7, P. 50–58. Available from: https://www.researchgate.net/publication/269100956_Funkcionalnye_razlicia_v_ustojcivosti_k_abioticeskim_faktoram_nekotoryh_izofermentov_kostistyh_ryb.
27. *Andriyasheva M.A.* Genetic aspects of whitefish breeding. *Sankt-Peterburg: Izd-vo FGNU "GosNIORH"*. 2011, 640 p. (In Russian).
28. *Varzegova S. A.* Technique for individual marking of rainbow trout using blood plasma proteins. *Sbornik nauchnykh trudov GosNIORH*. 1987, No 259, P. 40–45. (In Russian).
29. *Salmenkova E.A.* Genetics of fish isoenzymes. *Uspekhi sovremennoy biologii*. 1973, No 75, P. 217–235. (In Russian).
30. *Ruokolajnen T. R., Sidorov B. C., Bolotnikov I. A.* Distribution of multiple forms of acid phosphatase in the liver of different freshwater fish species. *Biokhimiya molodi presnovodnykh ryb.* 1985, 72–76. (In Russian).
31. *Yablokov A. G., Varzegova S. A.* Evaluation of six-year-old rainbow trout breeders for selected serum proteins. *Biologicheskie osnovy industrialnogo rybovodstva: Sbornik nauchnykh trudov. Redaktor Levitan A. I. Lenigrad*. 1982, No 188, P. 39–52. (In Russian).
32. *Altuhov Yu. P.* Fish population genetics. *Moskva: Pishch. prom-st.* 1974, 245 p. (In Russian).
33. *Hrynzhevskyi M. V., Sherman I. M., Hrytsyniak I. I., Vasylets S. V., Tretiak O. M., Tomilenko V. H., Oleksiienko O. O., Mruk A. I.* Organization of selection and breeding work in fish farming. Ed. Hrynzhevskoho M. V., Shermana I. M. *Kyiv: Rybka moya.* 2006, 352 p. (In Ukrainian).
34. *Hedrik F.* Population genetics. *Moskva: Tekhnosfera*. 2003, 592 p. (In Russian).

ОЦІНЮВАННЯ МІНЛИВОСТІ ГЕНЕТИЧНОЇ СТРУКТУРИ РАЙДУЖНОЇ ФОРЕЛІ *Oncorhynchus mykiss* (Walbaum, 1792) УКРАЇНСЬКИХ ЛОКАЛЬНИХ СТАД ІЗ ВИКОРИСТАННЯМ ПОЛІМОРФНИХ ПРОТЕЇНІВ ПЛАЗМИ КРОВІ

О. Ю. Бєлікова
С. І. Тарасюк,
А. І. Мрук
Т. А. Нагорнюк
Л. П. Бучацький

Інститут рибного господарства
НААН України, Київ

E-mail: belikova.e.y@gmail.com

Вступ. Райдужна форель є цінним видом аквакультури, який має високий рівень мінливості.

Мета. Вивчити особливості генетичної мінливості райдужної форелі за поліморфізмом локусів: трансферину (TF), посттрансферину (PTF), естерази (EST) (КФ 3.1.1.1.) та альбуміну (ALB).

Методи. Розділення протеїнів плазми крові райдужної форелі трьох локальних стад (Чернівецького, Харківського та Закарпатського) проводили за допомогою нативного електрофорезу в поліакриламідному гелі.

Результати. Встановлено особливості розподілу та відносну електрофоретичну рухливість алельних варіантів досліджених локусів у райдужної форелі українських локальних стад. Результати демонструють ефективність обраного методу аналізу з використанням методів біохімічної генетики для встановлення особливостей генетичної структури, рівня гетерозиготності локальних стад та проведення їхньої диференціації.

Висновки. Застосування обраних маркерів дасть змогу надалі здійснювати моніторинг динаміки змін стану локальних стад за встановлених умов культивування.

Ключові слова: райдужна форель, поліморфні протеїни плазми крові, трансферин, посттрансферин, естераза, альбумін.

ОЦЕНКА ИЗМЕНЧИВОСТИ ГЕНЕТИЧЕСКОЙ СТРУКТУРЫ РАДУЖНОЙ ФОРЕЛИ *Oncorhynchus mykiss* (Walbaum, 1792) УКРАИНСКИХ ЛОКАЛЬНЫХ СТАД С ИСПОЛЬЗОВАНИЕМ ПОЛИМОРФНЫХ ПРОТЕИНОВ ПЛАЗМЫ КРОВИ

Е. Ю. Беликова
С. И. Тарасюк,
А. И. Мрук
Т. А. Нагорнюк
Л. П. Бучацкий

Институт рыбного хозяйства
НААН Украины, Киев

E-mail: belikova.e.y@gmail.com

Вступление. Радужная форель является ценным видом аквакультуры, который имеет высокий уровень изменчивости.

Цель. Изучить особенности генетической изменчивости радужной форели по полиморфизму локусов: трансферрина (TF), посттрансферрина (PTF), эстеразы (EST) (КФ 3.1.1.1.) и альбумина (ALB).

Методы. Разделение протеинов плазмы крови радужной форели трех локальных стад (Черновицкого, Харьковского и Закарпатского) проводили с помощью нативного электрофореза в полиакриламидном геле.

Результаты. Установлены особенности распределения и относительная электрофоретическая подвижность аллельных вариантов исследованных локусов у радужной форели украинских локальных стад. Результаты демонстрируют эффективность выбранного метода анализа с использованием методов биохимической генетики для установления особенностей генетической структуры, уровня гетерозиготности локальных стад и проведения их дифференциации.

Выводы. Использование выбранных маркеров позволит в дальнейшем проводить мониторинг динамики изменений состояния локальных стад в установленных условиях культивирования.

Ключевые слова: радужная форель, полиморфные протеины плазмы крови, трансферрин, посттрансферрин, эстераза, альбумин.