IDENTIFICATION OF ALLELIC VARIANTS OF MICROSATELLITE DNA IN THE GENETIC POPULATION OF BESTER (*ACIPENSER NIKOLJUKINI*)

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It was investigated genetic polymorphism of microsatellite DNA markers of hybrid beluga and sterlet - bester (Acipenser Nikoljukini). From DNA markers studied LS-19, LS-68, LS-39, Aox-27, LS-54 and Aox-45 it was identified 56 allelic variants. It was found that the LS-68 locus was the most polymorphic, and the least polymorphic - Aox-27. Based on this data and calculations we have shown a trend of substitutions of allelic variants of observed population in the homozygous state of bester, indicating the negative impact of its artificial reproduction.

***Allele variations, microsatellite DNA markers, bester, locus, polymorphism*.**

Due to increased illegal harvesting, deterioration of the environment places of natural foraging and spawning fish sturgeon family (*Acipenseridae*) almost on the verge of extinction [2]. Now, the only one alternative to increasing the production of sturgeon products in Ukraine is the development of artificial reproduction and breeding herds and domestication of the sturgeon species [4].

According to the analysis of process parameters and biological indicators of fish-breeding one of the most convenient and suitable facilities and modern product of sturgeon breeding is bester (*Acipenser Nikoljukini*) - hybrid of beluga (*Huso huso*) and sterlet (*Acipenser ruthenus*) first obtained in 1952 by Nikolyukin [1, 10]. Bester is one of the most productive members of the family of sturgeon, which combines valuable economic properties of both parental forms: fast growth

rate - from beluga and relatively early puberty and property to live in fresh water - from sterlet. This interspecific hybrids was able to playback enough industrially manageable and malleable to different growing conditions. Therefore, bester is a valuable and desirable object of sturgeon breeding which commercial value and industrial cultivation is increasing every year [11, 13].

Successful work on artificial reproduction largely depends on the knowledge and understanding of the genetic processes that occur in populations. At present definition of the genetic diversity and population structure is carried out using molecular genetics methods [3, 5]. Along with the use of analysis of nucleotide sequences of mitochondrial DNA, restriction fragments length polymorphism (RFLP), nuclear DNA loci analysis using random primers by PCR (RAPD), more popular becomes DNA analysis using microsatellite DNA markers (DNA fingerprinting) [12, 14, 17]. Microsatellite markers are characterized by high speed of mutation and codominant mode of inheritance, which allows for species and population differentiation individuals with a high level of allelic variation. Using microsatellite markers allows to monitoring of genetic processes in natural and artificial populations and ensure compliance with the conservation of genetic diversity of threatened and endangered species [16, 22].

In the Ukrainian Laboratory of Quality and Safety of Agricultural Products in recent years carried out researches on the identification and study of the genetic structure of sturgeon fish species, among which are studied species such as Russian sturgeon, sterlet and stellate sturgeon [6, 7, 9]. Next for molecular genetic studies we have selected new one - bester, who among the various types and forms of hybrid sturgeon is one of the best species which combine commercial cultivation and effective production of food caviar [10, 13].

Purpose of research: identification of allelic variants of Bester population and studying its genetic structure by DNA microsatellite markers analysis.

Materials and methods of research. Study material was sample of 34 individuals bester held at the farm "Biosyla». Sampling for the study was carried out in vivo by cutting off slice thoracic or caudal fin. The selected biological

material fixed with 96% ethanol in specially labeled sterile tubes. DNA isolation was performed using the set of "DNA-sorb" ("Amplisens", Russia) according to the manufacturer's instructions.

For molecular genetic studies we have used six microsatellite DNA markers: LS-19, LS-68, LS-39, LS-54, Aox-27 and Aox-45 which nucleotide sequences are deposited in the international genetic database GenBank [19, 20].

PCR was performed under conditions optimized at the molecular diagnostic department Ukrainian Laboratory of Quality and Safety of Agricultural Products [8]. Products amplification was denatured by HiDi formamide (Sigma, USA) and separated by capillary electrophoresis using genetic analyzer "ABI Prism 3130" Genetic Analyser (Applied Biosystems, USA). Sizes of alleles were determined using the "Gene Mapper 3.7" software (Applied Biosystems, USA) and a size standard S-450 (Syntol, Russia). Determination of the number and frequency of identified alleles was performed by counting and analysis of the genotypes studied individuals. Calculations of the observed (Ho) and expected heterozygosity (He), polymorphism index content (PIC) and probability of exclusion coincidence alleles (PE) was performed using the next free distributed software Cervus 3.0.3. and Power Stats V12 (Promega) [18, 21].

Results of research: As a result we have identified 56 alleles in the bester population (Figure 1). To facilitate alleles counting, we have developed own nomenclature by using the Latin alphabet. This nomenclature by allele coding for each of the studied microsatellite DNA markers and features in the perception and operation data described earlier [6, 7].

For locus LS-19 was detected 7 allelic variants, including most often allelic variant K (38.2%), another allels H, J and L were less often found (1.5%). Locus LS-68 was the most polymorphic and contained 19 allele variants. More often found allelic variant P with frequency 19.1%, and less - E, K, O, W, Z3, Z5 and Z6 with the same frequency of 1.5%. Locus Aox-27 was the least polymorphic among the studied markers and consisted only five allelic variants. More often met allelic variant I (58.8%), and least likely - allelic variant K (1.5%). For locus LS-54 there

were detected 10 allelic variants, including variant G met more often (33.7%), whereas allelic variants J, K, M, V, W met least equally (2.9%).

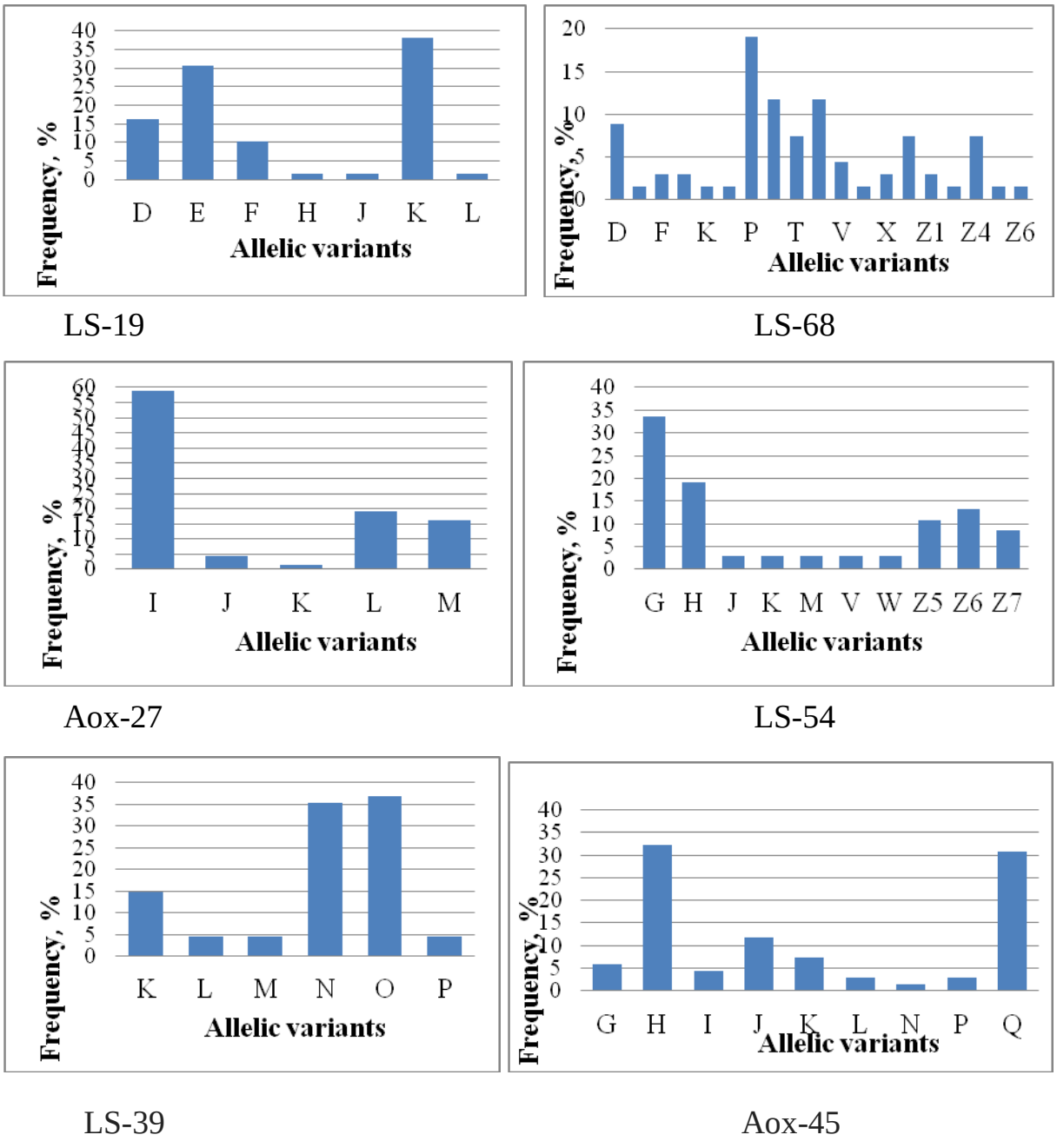

Figure 1 – Distribution of bester allelic variants identified by microsatellites DNA analysis.

For locus LS-39 was detected 6 allelic variants, including variant O which met with the highest frequency of 36.8%, whereas variants L, M and P met with

the same lowest rate of 4.4%. For locus Aox-45 was found 9 allelic variants, including the most commonly encountered variant H (32.4%), and least - variant N (1,5%).

According to the estimates of parameters of heterozygosity we have found that the actual level of heterozygosity (Ho) ranged from 0,235 to locus LS-54 to 0.912 for locus Aox-45 (table 1).

As shown in the following table is theoretically expected heterozygosity (He) ranged from 0.598 to 0.918 for loci Aox-27 and LS-68, respectively. Average actual heterozygosity was at 0.564, while the average value theoretically expected heterozygosity was higher and amounted up to 0,775 (He> Ho), indicating a lack of heterozygous genotypes in the observed bester population.

1. Indicators of genetic polymorphism of microsatellite loci in the bester population

Locus	Number alleles	Ho	He	PIC	PE
LS-68	19	0,412	0,918	0,898	0,121
LS-39	6	0,294	0,723	0,665	0,061
Aox-27	5	0,735	0,598	0,542	0,485
LS-54	10	0,235	0,821	0,787	0,040
Aox-45	10	0,912	0,859	0,827	0,820
Mean	9,5	0,564	0,775	0,732	0,353
				CPE	0,970

Index of polymorphism (PIC) for bester ranged from 0.542 for locus Aox-27 to 0.898 for locus LS-68, indicating that the locus Aox-27 is the least polymorphic, and locus LS-68 most polymorphic. The average value of the index for the studied polymorphism loci DNA equal to 0.732, which indicates a rather high level of polymorphism of selected markers for this type of fish (PIC> 0,500).

Index exclusion probability of accidental coincidence alleles (PE) averaged 0.353 and ranged from 0.040 to 0.820 for loci LS-54 and Aox-45 respectively. The value of the combined probability of exclusion coincidence alleles (CPE) was equal to 0.970, indicating a fairly high level of informativeness selected panel of

DNA microsatellite markers. Using this panel of markers allows us to genotyping bester population with probability at least as 97%.

Conclusions

Our studies on DNA microsatellite markers show the efficiency of microsatellite loci to identify allelic variants and population-genetic analysis bester. For a selected panel of 6 microsatellite markers were identified 56 alleles. The most polymorphic locus was LS-68 (19 allelic variants), the locus of Aox-27 was the least polymorphic (5 allelic variants).

On the basis of population-genetic calculations, it was found that the genetic structure bester has tendency to go allele in homozygous condition, which in turn is a result as the negative impact on these fish allele fund the present conditions of artificial breeding and reproduction.

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