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Original research

Genetic diversity of *Cyprinus carpio* from an Albanian fish hatchery based on microsatellite markers

Xhiliola BIXHEKU[1], Anila HODA[2,*], Dhurata BOZO[1]

[1]Quality Assurance Agency for Higher Education, Tirana, Albania

[2]Agricultural University of Tirana, Department of Animal Science, Tirana, Albania

*Corresponding author, e-mail: ahoda@ubt.edu.al

Abstract: The study is focused on the genetic characterization of *Cyprinus carpio* from a fish hatchery in used for the population of ponds of the semi-intensive fish farms in central part of Albania. A total of 30 specimens from this fish hatchery were genotyped for four microsatellite markers. All markers were highly polymorphic, with the number of alleles higher than 14 and PIC values higher than 0.5. Observed and expected heterozygosity values were found as 0.609 and 0.887, respectively, and the mean fixation index was calculated as 0.312. All loci showed significant deviation from Hardy Weinberg equilibrium. Genetic bottleneck analysis did not reveal any recent bottleneck of this population.

Keywords: genetic variation, fixation index, bottleneck

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Introduction

The common carp (*Cyprinus carpio* L.), is one of the most widely distributed and important freshwater fishes in the world. Albania has a long tradition in carp farming, based mainly in rearing of Chinese and common carp (http://www.fao.org/docrep/009/a0141e/A0141E02.htm#ch2.1).

In Albania exists a limited number of hatcheries producing carp fingerlings. One of them is Klosi fish breeding hatchery, located in central part of Albania, which produce fingerlings that are used as restocking material in the ponds of the semi-intensive fish farms and where the study is carried out.

Several phenotypes of *C. carpio* L from "Klosi Fish-Breeding hatchery", Elbasan have been previously studied by Shermadhi et al. (2014). Shermadhi et al. (2013) have studied meristic and morphometric variability among carp populations from Albanian lakes. In order to estimate the genetic diversity of carp reared in this hatchery, we are using high polymorphic microsatellites as markers.

Microsatellites are highly polymorphic simple sequence repeats, distributed throughout the genome, which are co-dominantly inherited in a Mendelian fashion. They display abundant polymorphism and can be easily detected, which make them excellent markers for the estimation of genetic diversity of a specie. Microsatellites also, present high intrapopulation variability as well. Singet al. (2016) demonstrated the use of microsatellite markers as a powerful tool for monitoring the genetic condition of different strains of common carp.

Microsatellites are used for the characterization of genetic diversity of common carp all over the world like in Vietnam (Thaiet al., 2007), Caspian Sea (Ghelichpour et al., 2013), Bangladesh (Mondol et al., 2006), Croatia (Tomljanovic et al., 2013), Poland (Rutkowsi et al., 2017), Korea (Kimet al., 2918), or from native lakes in Albania (Biba et al., 2017).

The aim of this study is to estimate genetic diversity within carp population from a hatchery, by the use of three microsatellite markers. Since, the fingerlings of *C. carpio*

produced in this fish hatchery are used to populate other ponds or farms of Albania, the findings of the study might contribute to the rational planning of sustainable improvement and utilization of this specie, which is of great importance also for the local community.

Material and methods

Sample collection, DNA extraction and microsatellite analysis

Fish fins were collected from 30 random specimens provided from Klosi fish hatchery of Elbasan, central part of Albania and were stored in ultra-pure Ethanol 99%. DNA was extracted from fish fins samples by salt out procedure (Aljanabi and Martinez, 1997). A set of four microsatellite markers (Biba et al., 2014) (Table 1) were amplified by a PCR reaction as described previously (Bixheku et al., 2017). The PCR products were resolved on 6% denaturing polyacrylamide gels using a LICOR 4300 DNA Analyser. Fragment lengths were determined relative to 50-350 IRDye size standard.

Table 1. Microsatellite locus information

Locus	Primer-sequence	T^0 annealing	Mg (mM)
MFW1	F: GTC CAO ATC GTC ATC AGG AG R: GTT TGA GGT GTA CAC TGA GTC AGG	60	1.5
MFW7	F: TAC TTT GCT CAG GAC GGA TGC R: GTT TAT CAC CTG CAC ATC GCC ACT C	60	1.5
MFW6	F: ACC TGA TCA ATC CCT GGC TC R: GTT TGG GAC TTT TAA ATC ACG TTG	60	1.5
MFW18	F: GTCCCTGGTAGTGAGTGAGT R: GTTTGCGTTGACTTGTTTATACTAG	60	1.5

Statistical analysis

Number of alleles per locus, observed and expected heterozygosity, allelic frequencies were calculated using GENEPOP version 3.1 (Raymond and Rousset, 1995). The same software was also used to test for departures from Hardy Weinberg equilibrium. Polymorphic Information Content (PIC) value was calculated according to Botstein et al. (1980) implemented in Cervus 3.0.3 software package (Marshall 1998).

Bottleneck event were tested by sign test, standardized differences test and Wilcoxon sign rank test under three different mutation models: Infinite Allele Model (IAM), Step wise Mutation Model (SMM) and Two Phase Model of mutation (TPM). Also, a qualitative test of mode shift was performed to evaluate the frequency distribution of alleles at different microsatellite loci using BOTTLENECK program (Piry et al., 1999).

Results and Discussion

A total of 80 alleles, across the 4 markers under study, were identified (Table 2); the number of alleles ranges from 14 to 30, showing that the markers are highly polymorphic in our samples. The allele frequency data for all markers are displayed in Fig. 1.

The effective number of alleles varied from 5.57 to 18.23. Mean observed heterozygosity ranged from 0.462 to 0.821, with a mean value of 0.609, which was lower than expected heterozygosity. The mean expected heterozygosity for the population overall loci is 0.887. These values are lower compared to the population of common carp from south eastern part of Caspian Sea (Yousefian, 2011), but are higher than those found in lakes and rivers of China (Liao et al., 2006). Our values are comparable with those found for the common carp of Lake Shkodra (Biba et al., 2015) and Lake Ohrid (Biba et al., 2014). The within population inbreeding index (F_{IS}) is highly positive.

PIC and Shanon information index are also measure of genetic variability. PIC values are higher than 0.5, further supporting that the loci are highly polymorphic.

Genetic bottleneck analysis reveals the mutation drift equilibrium under all tests, for all the three models (Table 3). Also the mode shift indicator (Fig. 2) showed a normal L-shaped curve.

Estimation of the genetic variation showed that all the three markers are highly informative. The markers are highly polymorphic, presenting a number of alleles higher than 14. Considering that, the minimum number of alleles recommended for microsatellite loci is four, the markers used in this study are seen as appropriate for analysis of genetic variation in the population. Allelic diversity and Genetic diversity of the population as whole are also high,

indicating their effective and appropriate use for the breeding programs. The high level of expected heterozygosity can be attributed to the large number of alleles. The high heterozygosity values can be explained with the large population size and the lack of breeding control. These findings and results are of great interest and importance, considering that the fingerlings produced in this fish hatchery are used for the population of ponds of the semi-intensive fish farms of Albania.

Table 2. Number of Alleles (Na), Number of Effective Alleles (Ne), Shanon Information Index (I), Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, and Fixation Index (F)

Locus	Na	Ne	I	Ho	He	uHe	F	PIC
MFW1	14	5.568	2.088	0.522	0.820	0.839	0.364	0.802
MFW7	22	9.941	2.709	0.462	0.899	0.917	0.487	0.893
MFW6	30	18.233	3.170	0.821	0.945	0.962	0.131	0.943
MFW18	15	8.494	2.405	0.632	0.882	0.906	0.284	0.872
Mean	20.250	10.559	2.593	0.609	0.887	0.906	0.316	0.8775
SE	3.705	2.715	0.230	0.079	0.026	0.026	0.075	

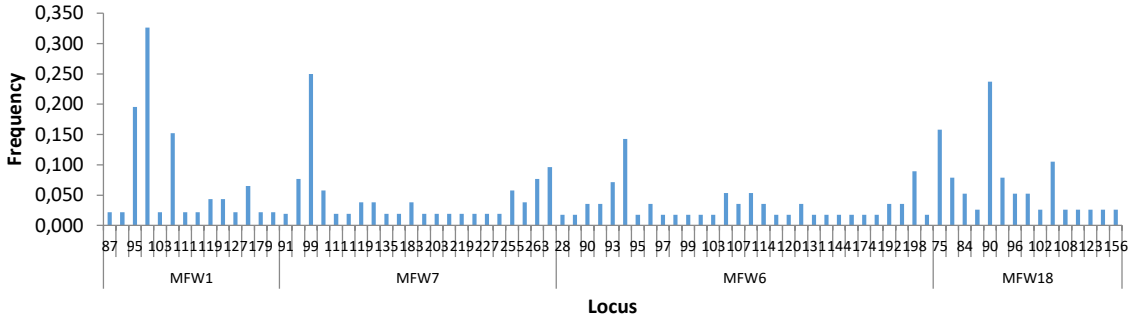

Figure 1. Allele frequencies of *Cyprinus carpio* provided from Klosi fish hatchery with graph over four loci

Table 3. Genetic bottleneck analysis

IAM		TPM		SMM	
Expected	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**
Sign test: Number of Loci with heterozygosity excess					
2.45 (0.164)	1	2.49 (0.0203)	0	2.34 (0.0295)	0
Standard differences test: T2 values (probability)					
-0.884 (0.1882)		-2.789 (0.00264)		-4.966 (0.00009)	
Wiloxon rank test (probability of heterozygosity excess)					
0.968		1.000		1.000	

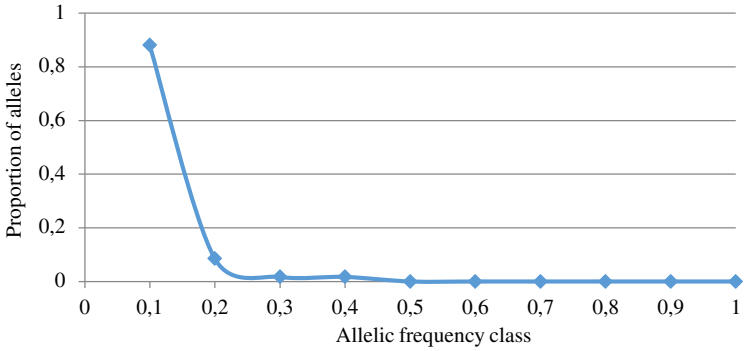

Figure 2. Graphical representation of proportions of alleles and their distribution

The number of effective alleles is much lower than the observed number of alleles. This can be explained with the very low frequency of most of alleles at each locus (Fig. 1). Therefore, a low number of alleles might have contributed to the major part of the allelic frequency at each locus.

All loci displayed heterozygosity deficiency, with F_{IS} values ranging from 0.13 to 0.34. Similar values are found for carp of Lake Vrana and Lake Grudnjak, Croatia (Tomiljanovic et al., 2013).

All loci showed significant deviation from Hardy Weinberg equilibrium. This can be explained by the presence of null alleles, genetic drift and inbreeding.

The high level of polymorphism parameters is explained by David et al. (2003) with the doubling of the genome long history of evolution. Li et al. (2007) considering the doubling of genome as conducive for the enhancement of heterozygosity, as the reason behind the maintenance of higher polymorphism parameters and genetic variation in the modern common carp.

The bottleneck analysis shows that the null hypothesis of mutation drift equilibrium is accepted, showing that the populations hasn't suffered a recent genetic bottleneck.

The carp population from Klosi hatchery contains a high level of genetic diversity. Our results on the genetic variability within population of this fish hatchery, can help for conservation and breeding programs, in order to increase the hybrid vigor of commercial stocks.

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