World Journal of Zoology 6 (1): 56-60, 2011
ISSN 1817-3098

Genetic Variations of Common Carp *(Cyprinus carpio* L.) In South-Eastern Part of Caspian Sea Using Five Microsatellite Loci

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Abstract: Five highly variable microsatellite loci were used to investigate genetic variations and population structure of common carp of Caspian Sea. A total of 54 fish were genotyped giving 35 alleles over all loci. The mean number of alleles, per locus in population ranges from 5.5 to 13.0 and the mean observed heterozygosity at the five loci was 0.84. An analysis of genetic variations indicated the Ho within each groups varied between 0.412 to 1 which the lowest was in female at Loc5 loci and highest at Syp4 loci. The range of He was 0.696-0.856 that the lowest was in Lid II in male. Highly significant deviation from Hardy–Weinberg, mostly due to deficits of heterozygote except for Ca3/4 and Z9/10 loci in male, based on χ2-test, showed no deviation form HWE.

Key words: Genetic variation · Cyprinus carpio · Microsatellite

INTRODUCTION

Common carp (*Cyprinus carpio* L.) is an important economic species of fresh water fish, widely distributed as culture fish In Iran. The wild common carp also are present in Caspian Sea which people prefer the wild ones.

This make the wild carps more expensive than the culture ones, but due to over caught, the wild carp are reduced in resources. For restocking millions of carp fingerling are released to Caspian Sea by fishery organization (Shilat), every year. The wild carp for inland aquaculture is under investigation. Understanding the genetic variations and genetic structure of Caspian common carp is important for protecting the genetic resource, conservation gene hank and restraining the genetic decline.

Extensively investigation by using different molecular marker e.g. allozyme, RAPD and microsatellites were used [1-3]. Among different method introduced for genetic of fish, Microsatellite technique seems to be the most suitable once. Microsatellite is variable nuclear genetic markers, which are inherited co-dominantly in a mendelian fashion. Microsatellites have been found suitable for a variety of application in fisheries and aquaculture [4].

In the present study, polymorphic microsatellites were applied to study genetic variations among common carp population from Caspian Sea. Although suffering of over fishing and human impacts for many years in Caspian Sea, it is hope to preserve the gene bank of valuable fishes of this unique lake of the word including common carp.

MATERIALS AND METHODS

Fish Sample and DNA Isolation: A total of 54 adult common carp (*Cyprinus carpio* L.) was sampled in 2008-2009 from South Caspian Sea, Golestan province, Gomishan, Iran. Fins of live fish clipped and immediately soaked in 95% ethanol while traveling and then stored at -20°C until DNA isolation. Total genomic DNA was extracted from ethanol-preserved fin tissues using traditional proteinase-K digestion and a protocol of phenol-chloroform extraction with slight modifications [5].

Amplification of Microsatellite Loci: Five pairs of microsatellite primers designated for common carp, were used in this study. Polymerase chain reaction (PCR) amplifications were performed in a 12.5 µl volume containing 10-50 ng DNA, 1×PCR buffer (10 mmol/l tris–Cl pH 8.3, 1.5 mmol/MgCl2, 50 mmol/l KCl), 120 µmol/l dNtps, 0.15 µmol/l primers and 0.5 U taq-DNA polymerase. The reaction were performed by thermal cycle and the cycle were as follows: a pre-denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 0.5 min, annealing at proper temperature (Table 1) for 40 s and elongation at 72°C for 40 s and a final elongation at 72°C for 10 min. PCR products were separated on 7.5% non-denaturing polyacrylamide gels using TBE buffer in the gel and reservoirs at 200 V for 2–3 h according to alleles size, stained with ethidium bromide in water and visualized with ultraviolet.

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Table 1: Characteristics of microsatellite markers in common carp

Loci	Primer sequence	Annealing temperature °C	References
Syp4	CACACCGGGCTACTGCAGAG		
	GTGCAGTGCAGGCAGTTTGC	58	Crooijmans *et al*., 1997
Ca3/4	GGACAGTGAGGGACGCAGAC		
	TCTAGCCCCCAAATT TTACGG	56	Dimsoski *et al*., 2000
Lidll	ATCAGGTCAGGGGTGTCACG		
	TGTTTATTTGGGGTCTGTGT	55	Turner *et al*., 2004
Z9/10	CGTCTGACAGCCTGCATG		
	CTCGGCGCAGTAGGGAAC	56	Turner *et al*., 2004
Loc5	TTACACAGCCAAGACTATGT		
	CAAGTGATTTTGCTTACTGC	58	Turner *et al*., 2004

Statistical Analysis: Indices of genetic diversity for populations, e.g. The observed number of alleles (A), effective number of alleles frequency (Ne), allele frequency (P), observed heterozygosity (Ho), expected heterozygosity (He) [6], gene diversity, were calculated and deviations from Hardy–Weinberg Equilibrium (HWE) was estimated $\chi 2$ test, Fst and Nm between populations were given by the software of GeneAlex.

RESULTS

All five pair primer Syp4, Ca3/4, LidII, Lco5 and Z9/10 related to microsatellite sites were applicable and polymorphic in wild common carp. These loci all were variable in population (Table 2). A total of 45 different alleles rang in size from 124 to 464 bp were found over the five loci. The number of allele ranged from 5 at LidII to 13 at Ca3/4. The highest allelic frequency was 0.382 in female spawners at Loc5 loci and 0.433 in male spawners at syp4 loci.

Genetic variations within population in experimental group (male and female) for each locus were tested and average observed hetrozygosity ranged from 0.412 to 1. The lowest observed genetic variation was in female at Loc5 and highest at Syp4.

Average expected hetrozygosity ranged from 0.696 to 0.856, but the lowest was at LidII and highest at Z9/10 in males (Table 3). The exact test for fitness to Hardy-Weinberg equilibrium (HWE) on all loci indicated that, Ca3-4 and Z9/10 in male were found to be in HWE

Table 2: Allele frequencies at each locus in two sex groups

locus	Allele	Female	Male	locus	Allele	Female	Male
Syp4	140	0.029	0.000	Ca3/4	220	0.059	0.067
	156	0.324	0.433		232	0.000	0.067
	160	0.118	0.000		244	0.029	0.133
	164	0.059	0.067		248	0.206	0.133
	168	0.265	0.133		252	0.176	0.000
	172	0.000	0.133		260	0.088	0.200
	184	0.088	0.000		268	0.000	0.400
	188	0.029	0.000		292	0.059	0.000
	192	0.088	0.067		296	0.206	0.367
	196	0.000	0.167		304	0.059	0.000
Lidll	432	0.118	0.433		308	0.029	0.000
	436	0.235	0.133		316	0.000	0.033
	456	0.176	0.000		320	0.088	0.000
	460	0.294	0.167	Z9/10	124	0.088	0.167
	464	0.147	0.267		128	0.029	0.033
Loc5	200	0.000	0.000		132	0.265	0.100
	236	0.059	0.067		136	0.088	0.067
	240	0.088	0.133		140	0.265	0.267
	244	0.029	0.133		144	0.059	0.067
	260	0.118	0.000		152	0.059	0.067
	284	0.147	0.200		160	0.088	0.100
	288	0.382	0.400		164	0.059	0.100
	300	0.176	0.000		168	0.000	0.033

Table 3: The expected (He) and observed heterozygosity (Ho)at five microsatellite loci of common carp

	Female brood N=17		Male brood N=15	
Loci	Ho	He	Ho	He
Syp4	1.000	0.791	1.000	0.740
LidII	1.000	0.791	0.867	0.696
Loc5	0.412	0.775	0.533	0.756
Ca3/4	0.882	0.856	0.933	0.769
Z9/10	0.941	0.825	0.867	0.856

Table 4: effective number of alleles (Ne) in five loci of common carp

	Female brood n=17		Male brood n=15	
Loci	Ne	Na	Ne	Na
Syp4	4.777	8.000	3.846	6.000
LidII	4.777	6.000	3.285	4.000
Loc5	4.446	7.000	4.091	6.000
Ca3/4	6.964	10.000	4.327	8.000
Z9/10	5.723	9.000	6.923	10.000

Table 5: The results of test of Hardy-Weinberg at different loci in common carp

Female brood	df	Chi-square	Prob.	Sig.
SYP4	28	58.212	0.001	***
LIDII	15	28.730	0.017	*
LOC5	21	41.670	0.005	**
Ca3-4	45	82.668	0.001	***
Z9/10	36	57.716	0.012	*
Male brood				
SYP4	15	45.000	0.000	***
LIDII	6	29.343	0.000	***
LOC5	15	37.500	0.001	**
Ca3-4	28	31.121	0.312	ns
Z9/10	45	61.433	0.052	ns

Table 6: The estimate Fst nd Nm between the loci

Loci	SYP4	LIDII	LOC5	Ca3-4	Z9/10	Mean
Nm	7.750	4.35	11.696	10.59	22.817	8.619
Fst	0.031	0.055	0.021	0.023	0.011	0.029

(P>0.01). The other loci significantly deviated from HWE (P<0.05) in male and female (Table 5). Another characteristics for determining polymorphism of loci is the real and effective number of allele. The highest effective allele in males and females were 6.923 and 6.96 and the lowest were 3.285 and 4.446, respectively. The highest real allele was in case of Ca3/4 and Z9/10 (Table 4).

In study of Population genetic differentiation, gametal correlation coefficient (Fst), also known as coefficient of inbreeding and Gene flow (Nm) were computed to estimate the differences between populations. All Fst were larger than the standard with no genetic differentiation between two groups of male and female.

The average of Fst was (0.028) and the average of Nm between male and female was 8.619 (Table 6). The difference between two sexes was 98% and within each group was 2%. The genetic relationship among samples analyzed by multidimential scaling analysis of Nei, [7], analysed by GeneAlex and the value was 0.794 shows the two groups of sex were distinct.

DISCUSSION

Recently, microsatellite were used for Dutch carp [1], French and Czech carp [2], Hungarian carp [3], Chinese carp [8] and a few studies involved in Iranian common carp by protein electrophoresis [9] and PCR-RFLP molecular analysis [10]. The Caspian Sea is the most important habitat for native, common carp. Although over fishing has been a serious problem in Caspian Sea, yet there is a large scale stock releasing or resource enhancement for common carp in Caspian Sea in Iran. For this unique stock of common carp, genetic variation and population structures is little known. In the present study, polymorphic microsatellites were applied to study genetic variation, among common carp population of Caspian Sea.

Genetic diversity is important to both natural and cultured populations because it provides the necessary spectrum of genotypes for adaptive response to changing conditions and heterozygous individuals usually are superior to less heterozygous individuals in many economically important characteristics like growth, fertility and disease resistance [11]. Therefore, there has been increasing attention being paid to loss of genetic diversity in natural resources fish stocks including carp. There are several report of analyzing the microsatellite variation in common carp [2, 3, 8, 12]. In most of these studies, there have limitations, due to the sampling of restricted number of populations or the used of small sample sizes such as the present study, therefore the levels of variation detected are broadly similar to the results of this study.

Loss of variations in closed hatchery populations can occur during establishment (founder effects) and over subsequent generations though genetic drift arising from low effective broodstock number [13]. The large reduction in genetic variability in the experimental lines observed in this study, Thai *et al*. [14] and Kohlmann *et al*. [12] indicated the potential negative impact of captive breeding on domesticated common carp stocks in Vietnam and elsewhere. Thus, the low levels of genetic variations most likely reflect the difficulties in genetic management of brood stock leading to low Ne. Destruction of natural spawning ground, lead to artificial propagation of the native common carp of Caspian Sea, releasing in partial mating of population (a small part).

The procedure employed allowed the application of the microsatellite technique on a few fish scales to assess polymorphism pattern through an economic, rapid and non-invasive method and without a quality loss in PCR products.

It is important that the observed heterozygosity were relatively low, which are represented by the significant departure from HWE at maturity of microsatellite loci. Actually several factors will lead to deviation from HWE [15]; the first is over fishing and heavy fishing pressure of large fishes. In this regard, the common carp in the Caspian Sea suffered from excessive exploitation and resources were severely declined. It has been recognized that habitat integrity and stability are imported for fish and common carp population may highly damaged the sharp change of ecosystem of Caspian Sea but more due to human activity have been greatly changing the environment for common carp native to the main river of this lake. At present many Iranian ecologists are worried about negative impacts of sharp change of ecosystem on reproduction of common carp and some endangered species in the river, particularly with migratory characteristics. However there are several attempt to improve the recruitment of the rivers and improve the hydrological changes such as optimum current velocity, that are influence on reproduction of native common carp of Caspian Sea. The understanding of genetic diversity is one of the most important steps in managing fisheries resources and aquaculture selective breeding programs [16-18].

The data of present study might act as the base information of the genetic variations and population structure, use in future for comparing the population divergence and for conservation. It is also useful for illustration of the effect of restocking on genetic structure of common carp in Caspian Sea.

ACKNOWLEDGEMENT

The author thank Ch. Makhtomi, F. Laloei and M.J. Tagavi for sample collection and technical assistance. This study was support by Caspian Sea Ecology Center of Mazandran, Iran.

REFERENCES

1. Tanck, M.W.T., H.C.A. Baars, K. Kohlmann, J.J. Van der Poel and J. Komen, 2000. Genetic characterization of wild Dutch common carp (*Cyprinus carpio* L.). Aquaculture Res., 31: 779-783.
2. Desvignes, J.F., J. Laroche, J.D. Durand and Y. Bouvet, 2001. Genetic variability in reared stocks of common carp (*Cyprinus carpio* L.) based on allozymes and microsatellites. Aquaculture, 194: 291-301.
3. Bartfai, R., S. Egedi, G.H. Yue, B. Kovacs, B. Urbanyi, G. Tamas, L. Horvath and L. Orban, 2003. Genetic analysis of two common carp brood stocks by RAPD and microsatellite markers. Aquaculture, 219: 157-167.
4. Thai, B.T., C.P. Burridge and C.M.. Austin, 2007. Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. Aquaculture, 269: 174-186.
5. Moritz, C. and D. Hillis, 1990. Molecular Systematics: Context and Controversies Sinauer Associates, Sunderland, MA.
6. Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
7. Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

8. Liao, X., X. Yu and J. Tong, 2006. Genetic diversity of common carp from two largest Chinese lakes and the Yangtze River revealed by microsatellite markers. Hydrobiologia, 568: 445-453.
9. Yousefian, M., 2004. Morphologic and electrophoretic comparison of common carp *Cyprinus carpio* L. of water resources of North of Iran. Iranian Scientific Fisheries J., 3: 179-189.
10. Laloei, F.S. Rezvani Gilkolaei, S.M.R. Fatemi and M.J. Taghavi, 2008. Investigation of population genetic structure of common carp in the south Caspian Sea using mtDNA Method (PCR-RFLP). Iranian Scientific Fisheries J., 17: 89-102.
11. Beardmore, A.L., C.G. Mair and C.G. Lewis, 1997. Biodiversity in aquatic systems in relation to aquaculture. Aquaculture Res., 28: 829-839.
12. Kohlmann, K., P. Kersten and M. Flajshans, 2005. Microsatellite-based genetic variability and differentiation of domesticated, wild and feral common carp (*Cyprinus carpio* L.) populations. Aquaculture, 247: 253-266.
13. Allendorf, F.W. and S. Phelps, 1980. Loss genetic variation in hatchery stock of cutthroat trout. American Fisheries Society, 109: 537-543.
14. Thai, B.T., A.T. Pham and C.M. Austin, 2006. Genetic diversity of common carp in Vietnamusing direct sequencing and SSCP analysis of the mitochondrial DNA control region. Aquaculture, 258: 228-240.
15. Castric, V., L. Bernatchez, K. Belkhir and F. Bonhomme, 2002. Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus Fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity, 89: 27-35.
16. Beaumont, R.A. and K. Hoare, 2003. Biotechnology and Genetics in Fisheries and Aquaculture. Blackwell Publishing, Oxford.
17. Dunham, A.R., 2004. Aquaculture and Fisheries Biotechnology Genetic Approaches. CABI Publishing, Oxfordshire, pp: 115.
18. Ward, R.D. and P.M. Grewe, 1995. Appraisal of molecular genetic techniques in fisheries. In: Carvalho, G.R., Pitcher, T.J. (Eds.), Molecular Genetics in Fisheries. Chapman and Hall, Great Britain, pp: 29-54.
19. Crooijmans, R.P.M., V. Bierbooms, J. Komen, J.J Vanderpoel and M. Groenen, 1997. Microsatellite markers in common carp (*Cyprinus carpio* L). Animal Genetics, 28: 129-134.
20. Dimsoski, P., G.P. Toth and M.J. Bagley, 2000. Microsatellite Characterization in (Cyprinidae). Molecular Ecol., 9: 2187-2189.
21. Turner, F., T.E. Dowling, R.E. Broughton and J.R. Gold, 2004. Variable microsatellite marker amplify across divergent lineages of Cyprinidae fish. Conservation Genetic, 5: 279-281.