Int. J. Aquat. Biol. (2013) (1): 22-27
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Microsatellite variations and genetic structure of common carp (*Cyprinus carpio*) populations in Gomishan bay and Gorganroud River (Southeast of the Caspian Sea)

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Abstract: Common carp (*Cyprinus carpio*) population has been declined in the Caspian Sea in the recent years, mainly due to human manipulation. This valuable species needs to be protected in the Caspian Sea. Considering the commercial value of common carp, its rehabilitation program has been established. In the present study, 8 microsatellite loci were used to assess genetic variation and population structure of common carp in Gomishan bay (GB) and Gorganroud River (GR). These two regions are the most important habitat of common carp. Mean actual (N_a) and expected (N_e) alleles numbers were 15.12 and 11.35 for GB and GR, respectively. Mean observed (H_o) and expected (He) heterozygocity were 0.99 and 0.90 for GB and GR, respectively. Results, also, showed that all investigated loci were polymorphic. Twelve out of 16 tested locus×region combinations showed significant deviation from Hardy-Weinberg equilibrium (HWE) which could be mainly due to increase in He. FST index was found to be 0.011. Hence, AMOVA showed that observed variation was related to within population (99%) as well as between populations (1%). According to the results, it is suggested that studied populations have a great allelic richness and gene flow.

Article history:
Received 3 March 2013
Accepted 11 April 2013
Available online 14 April 2013

Keywords:
Population
Gorganroud River
Gomishan bay
Microsatellite
Genetic structure

Introduction

Nowadays, many fishes are under threat due to destruction of their habitats, over-exploitation, pollution and introduction of predator and competitor species. Hence, they need to be protected via a restocking program (Millennium Ecosystem Assessment, 2005). Restocking programs are a managerial strategy, in which mature individuals are caught from wild and propagated under controlled conditions. Then, fries are released to their natural habitats and this process is repeated next years (Fiumera et al., 1999).

Species ability to survive in the nature is determined by genetic variation that affects their ability to adapt environmental changes. Thus, genetic variation is necessary for the species survival and resistance (Bataillon et al., 1996). Genetic variation management needs the evaluation of genetic structure and separation rate of targeted species stocks (Pujolar et al., 2009). Hence, permanent monitoring of genetic status of species that subjected to rehabilitation program is necessary for their conservation and management.

In the recent decades, little molecular studies were conducted on aquatic organism in compare to terrestrial species (Shabani et al., 2006). A variety of molecular markers are used in population genetic studies, however, among them, the microsatellite markers are widely used. This is mainly due to high frequency in genome, Mendelian inheritance, being semi-dominant, small loci size, ease to determine genotype by polymerase chain reaction (PCR) and great polymorphism (Chen et al., 2008; Dewoody and Avise 2000). Also, this marker has been widely used in fisheries and aquaculture studies, where

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Table 1. Characteristics and sequence of the primers used in microsatellite analyses in common carp.

Locus	Allele number	Allele size	Allele size	Sequence	Temperature (˚C)
MFW2	22	200-228	200-228	F: CACACCGGGCTACTGCAGAG R: GTGCAGTGCAGGCAGTTTGC	64
MFW7	22	160-272	160-272	F: TACTTTGCTCAGGACGGATGC R: ATCACCTGCACATGGCCACTC	62
MFW13	17	188-272	188-272	F: ATGATGAGAACATTGTTTACAG R: TGAGAGAACAATGTGGATGAC	56
MFW16	18	128-204	128-204	F: GTCCATTGTGTCAAGATAGAG R: TCTTCATTTCAGGCTGCAAAG	57
MFW17	25	208-316	208-316	F: CTCAACTACAGAGAAATTTCATC R: GAAATGGTACATGACCTCAAG	57
MFW20	19	208-304	208-304	F: CAGTGAGACGATTACCTTGG R: GTGAGCAGCCCACATTGAAC	60
MFW26	16	108-172	108-172	F: CCCTGAGATAGAAACCACTG R: CACCATGCTTGGATGCAAAAG	60
CypG24	14	112-168	112-168	F: CTGCCGCATCAGAGATAAACACTT R: TGGCGGTAAGGGTAGACCAC	58

inter- and intra-population variation may be limit (Thai et al., 2007).

Cyprinids are one of the important families of fishes, containing more than 2000 species (Kirpichnikov, 1972). Among cyprinids, the common carp (*C. carpio*), a native fish of Eurasia, is commercially valuable species that transferred to different regions of the world (Kohlman et al., 2003). This species, also, inhabits in the Caspian Sea and considered as an important food resource for local people. Although the common carp inhabits in all parts of southern part of the Caspian Sea and inter to its tributaries for reproduction, its population has been declined because of over-fishing and degradation of their spawning ground. Therefore it needs to be conserved (Abdoli and Naderi, 2008).

Currently, stock rehabilitation program of common carp is conducted by releasing artificially propagated fries to the Caspian Sea. Unfortunately, despite the commercial importance and huge market demand for this species, there is no comprehensive information about its population structure in different regions of the Caspian Sea. The available data are related to common carp population genetic in southern Caspian Sea, using mtDNA (Yousefian and Laloei, 2011). Therefore, in the present study, common carp population structure and genetic variation were studied in Gorganroud River (GR) and Gomishan bay (GB) (southeast Caspian) using 8 microsatellite loci.

Materials and Methods

Sampling: A total of 30 specimens were sampled from each study area. Two grams of each fish caudal fin was sampled and preserved in 90% ethanol, separately. Nuclear DNA was extracted according to Hillis et al., (1996). In this protocol, tissue samples were digested by K-proteinase in buffer [100 mM of acidic tris, 10 mM EDTA, 250 mM NaCl and 1% sodium dodecyl sulfate (SDS): pH=8] over 12h at 55 ˚C. The resulted product was purified using phenol-chloroform. DNA was precipitated by adding

Cycle number	Stage	Time	Temperature
1	Denaturation	5	94
	Denaturation	30	94
5	Annealing	30	5 ˚C above the annealing temperature (marked by*)
	Extension	30	72
	Denaturation	30	94
32	Annealing	30	56-64*
	Extension	30	72
1	Final extension	10	72

Table 3. N_a, N_e, H_o, He and pHW of tested loci in Gorganroud River and Gomishan bay.

Area		CypG24	MFW26	MFW20	MFW17	MFW16	MFW13	MFW7	MFW2
GR	N_a	9	15	18	17	14	14	16	18
	N_e	5.64	12.74	14.38	10.96	10.66	10.31	13.17	12.95
	H_o	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00
	H_e	0.823	0.922	0.930	0.909	0.906	0.903	0.924	0.923
	pHW	**	***	***	***	***	ns	***	*
GB	N_a	12	16	18	21	16	14	11	20
	N_e	9.13	12.08	14.63	12.24	12.16	10.46	9.04	14.51
	H_o	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H_e	0.891	0.917	0.932	0.918	0.918	0.904	0.889	0.931
	pHW	***	***	**	***	***	***	***	***

GR=Gorganroud River. GB=Gomishan bay. pHW = Hardy-Weinberg probability test after sequential Bonferroni correction. Ns = not significant. Asterisks show significant difference: * P<0.05; ** P<0.01; *** P<0.001.

cold ethanol (90%) and centrifugation. Then extracted DNA were dissolved in deionized water and kept at –20 ˚C. Quality and quantity of extracted DNA was determined by agarose gel (1%) and biophotometer.

Microsatellite analyses: Eight microsatellite loci MFW2, MFW7, MFW13, MFW16, MFW17, MFW20, MFW26 and CypG24 were chosen based on the previous studies (Crooijmans et al., 1997; Baerwald, 2004) (Table 1).

PCR amplification was carried out in 0.2 ml PCR tubes with an Eppendorf thermal cycler (Table 2). Fifteen µl PCR reactions contained, 0.5 U Taq DNA Polymerase (Fermentas), 1×PCR buffer, 0.2 mMdNTP mix, 1.5 mM MgCl2, 1 µM of each primer set, and about 100 ng template DNA. PCR temperature cycles were as follows: a pre-denaturation at 95 ˚C for 3 minute; followed by 35 cycles of denaturation at 95 ˚C for 30 s, annealing for 30 s and extension at 72 ˚C for 30 s and a final extension at 72 ˚C for 3 min. PCR products were separated on 10% polyacrylamid gels stained with silver nitrate (Rajora et al., 2000). A 50 bp molecular weight marker (Fermentas) was used as the molecular weight standard. Microsatellite allele lengths were estimated using Gel-Pro Analyzer 3.9 software (Gene, USA).

Data analyses: N_a, N_e, H_o and H_e were estimated by Arlequin 2.0 software (Schneider et al., 2000). The reason of deviation from HWE and its significance was determined by FSTAT software. Deviation distribution, genetic distance, genetic identity (Nei, 1978), deviation from HWE and F_{ST} index (AMOVA) were determined by statistical-geneticl software, GenAlex 6.5. Difference in H_o, H_e and allelic variation was determined by Wilcoxon's test using statistical software, SPSS v. 16. To adjust significance level for repetitive tests, sequential Bonferroni correction was used (Rice, 1989).

Results

Results showed that all studied loci were polymorphic. A total of 139 alleles with average of 17.37 alleles were observed in each locus. MFW17 with 23 alleles and CypG24 with 12 alleles had the most and least alleles, respectively. N_a and N_e were 15.12 and 11.35 for GR, while they were 16 and 11.78 for GB, respectively (Table 3). There was no significant difference in N_a and N_e between two studied areas (P>0.05).

Mean H_o was 0.99%, as H_o was found to be 1.00 in all locus exception of MFW20 in GR which was 0.96. Mean H_e was found to be 0.90 which highest and lowest values were observed in MFW20 (0.93) and CypG24 (0.82) in GR (Table 3). There was no significant difference in H_o and H_e values between the studied areas (P>0.05).

Fifteen out of 16 tests (8 locus ×2 areas) showed a significant deviation from HWE (P<0.05), however, only 12 out of 16 tests showed the significant deviation from HWE when sequential Bonferroni correction was performed (P=0.005).

F_{ST} index was found to be 0.011. Hence, AMOVA showed that observed variation was related to within

Table 4. AMOVA for FST.

	df	SS	MS	Est. Var.	%
Between populations	1	4.73	4.73	0.023	1
Within populations	58	231.5	3.99	3.99	99

Table 5. F_{ST} and N_m for tested locus.

	MFW2	MFW7	MFW13	MFW16	MFW17	MFW20	MFW26	CypG24
F_{ST}	0.008	0.009	0.012	0.006	0.020	0.007	0.007	0.020
N_m	29.17	27.58	20.97	40.71	12.23	33.57	36.04	12.43

Table 6. Genetic identity (regular font) and genetic distance (bolded font) of common carp originated from Gorganroud River and Gomishan bay.

Area	Gorganroud River	Gomishan bay
Gorganroud River		**0.25**
Gomishan bay	0.78	

population (99%) as well as between populations (1%) (Table 4). F_{ST} (0.006 - 0.020) and gene flow (N_m) (12.23-40.73) values for each tested locus are presented in Table 5.

Results showed that genetic identity and genetic distance between the studied areas were 0.25 and 0.78, respectively (Table 6).

Discussion

Many fishes have more than one stock which fisheries managements should conserve their genetic variation via their sustainable exploitation. Thus, recognition of genetic information of different stocks is fundamental in fisheries managements (Waldman et al., 1999). Microsatellites markers have provided important information on genetic variation and factors affecting formation of population. These markers encompass a great polymorphism and inheritance (Crooijmans et al., 1997).

Heterozygocity and allele number are of the important parameters in population genetic variation which determine the ability of organism to compete and survive in natural habitats (Hakansson and Jensen, 2005; Frankharn, 2008). In genetic variation studies, allelic richness is more worthy than heterozygocity. In fact, higher allelic richness shows higher effective population size and use of allelic richness is suitable for populations which are treated by selection or conservation programs (Diz and Persa, 2009). In the case of allele number, H_o and H_e, the present study is in line with the previous one on common carp with the similar primers (Ghelichpour et al., 2010). However, the result of this study showed higher rates than that of Crooijmans et al. (1997) on common carp using similar primers. Such contradictories might be related to difference in sample size or higher genetic variation in studied populations compared to the previous one. Crooijmans et al. (1997) stated that higher allele number and heterozygocity could be affected by sample size and studied area. Hence, based on the results, there was no significant difference in allele number and heterozygocity between GB and GR, in the present study.

Twelve out of 16 tests showed a significant deviation from HWE after use of sequential Bonferroni correction. This deviation is mainly due to increase in heterozygocity. As mentioned above, H_o was greater than H_e. Population combination or non-randomized mating (Liu et al., 2005) could cause deviation from HWE, in this study. Generally, a single factor could not cause deviation from HWE, however, combination of some factors such as artificial propagation and stock rehabilitation programs could participate in increase of heterozygocity and deviation from HWE.

Environmental deterrents, life history and mating type could alter populations' genetic structure (Tiedemann et al., 2000). In addition, stock rehabilitation, due to selective propagation, could affect genetic structure. AMOVA, as a statistical analyses, is a suitable means to determine population

structure and between population variation (Grassi et al., 2004). Results of this study showed that observed variation was related to within population (99%) and between populations (1%) and were in line with the previous study on common carp populations which had 99% within population and 1% between populations' variation in the southern Caspian (Ghelichpour et al., 2010).

F_{ST} value (0.011), also, confirmed the little between populations' variation. According to Wright (1978), F_{ST} range of 0–0.05 means small variation. Hence, genetic similarity and distance between the two studied areas were 0.78 and 0.25, respectively. According to value of genetic similarity in same-species populations (0.8-0.9) and in same-genus populations (0.35-0.85), it could be stated that the studied populations belong to same-genus (Thorpe, 1982). On the other hand, the present results showed a great gene flow between the studied areas, which could cause small variation in genetic structure of the areas. This high gene flow might be related to natural fish migration. Also, stock rehabilitation programs could involve in this great gene flow; as produced fries of present restocking program of common carp in the southern Caspian Sea were released into the sea, without considering their parents origin, it could cause great gene flow.

The results of this study suggest that despite of enclosed system of the Caspian Sea and artificial propagation, genetic variation of common carp is considerably high. However, as stock rehabilitation programs are running yet, management programs should be performed to conserve the genetic variation to avoid the problems caused by inbreeding.

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