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GENETIC VARIATION IN THREE CAPTIVE POPULATIONS OF TWO STRAINS OF COMMON CARP (*CYPRINUS CARPIO* L.) IN BANGLADESH REVEALED BY MICROSATELLITE DNA MARKERS

Md. Nurul Alam and Md. Samsul Alam*
Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, 2202 Bangladesh
*E-mail: samsul_bau@yahoo.com

ABSTRACT
The genetic variations of three captive populations of scaled carp and mirror carp (*Cyprinus carpio*) viz. Sagor Matsho Khamar, Adorsho Matsho Khamar and Anil Fish Farm, all located in the district of Mymensingh in Bangladesh, were studied using microsatellite DNA markers. Three microsatellite loci (*MFW13, MFW17* and *MFW28*) were amplified by polymerase chain reaction and the PCR products were resolved on 6% polyacrylamide gel and the alleles were visualized by silver nitrate staining. All three loci were found to be polymorphic in all the populations. The average number of alleles ranged from 4-5 per locus. The average observed heterozygosity (H_o) ranged from 0.54 to 0.67 which were lower than the respective expected heterozygosity in all the populations. Based on allelic richness and heterozygosity levels, the genetic variation in the scaled carp population of the Sagor Matsho Khamar was the highest while that in mirror carp sample of the same farm was the lowest. Significant deviations from Hardy-Weinberg expectations (HWE) were detected in 2/3rd of the tests. The F_{ST} value was highest (o.153) between the mirror carp population of Anil Fish Farm and Sagor Matsho Khamar. Nei's genetic distance value was also highest (0.462) between these two populations. The lowest F_{ST} value (0.001) was found between the scale carp and mirror carp populations of Sagor Matsho Khamar. The study revealed a relatively low level of genetic variation at microsatellite loci in the scale carp and mirror carp captive populations which can be attributed to inbreeding and nonrandom mating.

KEY WORDS: Genetic variation; common carp; microsatellite; inbreeding.

INTRODUCTION

Common carp (*Cyprinus carpio* L.) is probably the oldest domesticated and most extensively cultured species on the globe (Balon, 2006). They have been farmed for about 4,000 years in China and for several hundred years in Europe (Wohlfarth, 1984). The world annual aquaculture production of common carp in 2009 was 3, 216, 203 MT compared to 2, 457,378 MT of Salmonids (FAO, 2011).There are numerous strains of common carp with three general groupings based on different scale patterns, viz, i) scaled carp, *C. carpio* var. communis ii) mirror carp, *C. carpio* var. specularis iii) leather carp, *C. carpio* var. nudus. Common carp varieties have been developed through a combination of forces including geographical isolation, adaptation, accumulation of mutations and natural as well as human selection pressure (Hulata, 1995). Scale pattern in common carp is controlled by the S and N genes which are produced through a type of dominant epistasis where N gene is the epistatic locus but it is a dominant lethal epistatic gene (Tave, 1993). Scale pattern in common carp is of great importance because market demands and price depend to a large extent on the scale patter. For example, the demand and market price of mirror carp are higher in Europe while scaled carp is more desired in Asia (Wohlfarth *et al.*, 1963).

Although Bangladesh is quite rich in endemic aquatic genetic resources (260 fresh water fish and 24 prawn species; 475 marine water fish and 36 shrimp species) introduction of different varieties of fish species has been occurring since 1960 (Rahman, 1985). As many as 15 exotic culturable fishes have been deliberately introduced into Bangladesh with the objectives of boosting aquaculture production and for insect and weed control (Ali, 1998). Scaled common carp was first introduced in Bangladesh by the Department of Fisheries in 1960 from China and then a second batch was brought from Vietnam in 1995 (Rahman, 1985; Hussain. 1997). The mirror carp was first introduced in Bangladesh in 1979 from Nepal and then second and third batch from Hungary in 1982 and 1996 respectively (Hasan, 1990; Rahman, 1985; Hussain and Mazid, 2001). Among the common carp strains, the scaled carp and mirror carp are more popular in Bangladesh and are being extensively cultured throughout the country. In Bangladesh, common carp strains are bred repeatedly in captivity with relatively lower number of effective parents compared to the major carp species. As a result genetic erosion may have occurred through inbreeding, genetic drift and bottleneck effect in the captive populations. The objective of the present study was to asses the genetic variation within and among three captive populations of scaled carp and mirror carp.

MATERIALS AND METHODS

Collection of sample and genomic DNA extraction

Fingerlings of scale carp and mirror carp were collected from three different establishments located in Mymensingh district viz. Sagor Matsho Khamar (SMK), Adorsho Matsho Khamar (AMK), and Anil Fish Farm (AFF). A small amount of fin tissues was excised from the caudal fin of 30 fish from each strain and each farm and

preserved in 95% ethanol. Approximately 40 mg of fin tissues from each sample was cut into small pieces, homogenized in extraction buffer (100 mM Tris.HCl, 250 mM NaCl, 10 mM EDTA and 1% SDS) and the genomic DNA was extracted by proteinase-K digestion, phenol: chloroform: isoamyl alcohol extraction and isopropanol-ethanol precipitation method as described by Islam and Alam (2004). The concentration of extracted DNA was estimated using a spectrophotometer (Bio Photometer Plus, Eppendorf, Germany).

Microsatellite marker amplification

Three di-nucleotide (CA) microsatellite markers such as *MFW13, MFW17* and *MFW28*, developed by Crooijmans *et al.* (1997) were used in this study. PCR was performed in a 10 µl reaction volume containing 50 ng templates DNA, 0.25 µM of each primer, 0.25mM each dNTPs, 1 unit of Taq DNA polymerase (Genei, Bengalore, India) and 1 µl 10 × reaction buffer containing 15 mM $MgCl_2$. The temperature profile consisted of 3 minutes initial denaturation at 94°C followed by 35 cycles each consisting of 30 seconds at 94°C, 30 seconds at 55°C and 1 min at 72°C. After the 35th cycle the reaction was continued for an additional 5 minutes at 72°C to allow elongation of the amplified fragments.

Gel electrophoresis and data analysis

PCR products were separated on 6% denatured polyacrylamide gel containing 19:1 acrylamide: bis-acrylamide and 7M urea using a 38×30cm vertical gel chamber (Sequi-Gen GT sequencing gel electrophoresis system, BIO-RAD). After completion of electrophoresis, the gel was stained with silver nitrate following Promega (Madison, WI) silver staining protocol. The bands representing particular alleles at the microsatellite loci were scored manually. The sizes of the bands were estimated using the software DNA FRAG version 3.03 (Schaffer and Sederoff, 1981). Allelic variation among the different populations, genetic distance and fit to Hardy-Weinberg proportions were estimated by the software POPGENE (version 1.31) (Yeh *et al*., 1999) with 1000 simulated samples. The software G-Stat (Siegismund, 1995) was used to estimate allelic frequencies and homogeneity test among the different populations. The software *FST*AT version 2.9.3 (Goudet, 2001) was used to calculate F-statistics (F_{ST}) between populations.

RESULTS

Genetic variation in different populations and deviation from Hardy-Weinberg Expectation

The three microsatellite loci were polymorphic and revealed a total of 15 alleles ranging from 3-6 alleles per locus. The sizes of the alleles ranged from 155 to 198 bp at locus *MFW13*, 234 to 277 at locus *MFW17* and 290 to 307 bp at locus *MFW28* (Table 1). Alleles *MFW17*-178 and *MFW28*-307 were found to be private in the scale carp population of Sagor Matsho Khamar and the mirror carp.

TABLE 1. The sizes and frequencies of alleles at three microsatellite loci in three populations of scale carp and mirror carp strains of *C. carpio* (SC: scale carp, MC: mirror carp, SMK: Sagor Matsho Khamar, AMK:Adorsho Matsho Khamar and AFF: Anil Fish Farm).

Allele size (bp)	Populations					
	Scaled carp			Mirror carp		
	SC-SMK	SC-AMK	SC-AFF	MC-SMK	MC-AMK	MC-AFF
MFW13						
198	0.19	0.23	0.26	0.41	0.33	0.10
191	0.17	0.13	0.06	0.09	0.11	0.12
184	0.33	0.44	0.37	0.18	0.43	0.40
178	0.02	0	0	0	0.04	0
167	0.02	0.02	0.09	0.05	0	0.21
155	0.27	0.17	0.22	0.27	0.09	0.17
MFW17						
277	0.19	0.17	0.31	0	0.37	0.60
263	0.19	0.10	0.17	0.27	0.11	0.06
253	0.35	0.33	0.37	0.45	0.24	0.29
243	0.17	0.21	0.06	0.14	0.24	0.04
234	0.10	0.19	0.09	0.14	0.04	0.02
MFW28						
307	0.04	0	0	0	0.04	0
297	0.44	0.27	0.26	0.32	0.24	0.67
294	0.38	0.52	0.43	0.45	0.41	0.29
290	0.13	0.21	0.31	0.23	0.31	0.04
Total nos. of null alleles across the loci	0	2	2	3	1	2

population of Adorsho Matsho Khamar population. The total number of null alleles was the highest in the mirror carp population of Sagor Matsho Khamar followed by the Adorsho Matsho Khamar and Anil Fish Farm of both the strains. The scale carp population of Sagor Matsho Khamar had no null allele (Table 1). The number of alleles ranged from 3-6 in scaled carp and from 3-5in mirror carp. The average number of alleles in the scaled carp population of the Sagor Matsho Khamar was the highest while the average number of allele of the mirror carp population of the same hatchery was the lowest. The average number of alleles in the scaled carp populations of Adorsho Matsho Khamar and Anil Fish Farm and the mirror carp population of Anil Fish Farm was the same (4.33) (Table 2). The observed average heterozygosity (H_o) was highest (0.85) in the scaled carp population of Sagor Matsho Khamar and lowest (0.27) in the mirror carp population of the same hatchery. The expected heterozygosity (*He*) was highest in the scaled carp of Adorsho Matsho Khamar (0.79) and lowest in the Anil Fish Farm (0.47). Out of the 18 test of fit to Hardy-Weinberg Expectation, deviations were found in 6 cases due to the deficiencies in the observed heterozygosity (*Ho*) compared to the expected heterozygosity (*He*). Four deviations were found at locus *MFW28* and one each at the locus *MFW13* and *MFW17*. Among the populations, two deviations were observed in the mirror carp populations of Adorsho Matsho Khamar and Anil Fish Farm while a single deviation was observed in the scaled carp population of the Adorsho Matsho Khamar and mirror carp population of Anil Fish Farm (Table 2).

TABLE 2. Allelic variations (*N*= No. of alleles, H_o= heterozygosity observed, H_e= heterozygosity expected) and deviations from H-W expectation (χ^2-values followed by degrees of freedom in parentheses) at three microsatellite loci in different populations of two strains of *C. carpio*

Parameters	Populations					
	Scaled carp			Mirror carp		
	SC-SMK	SC-AMK	SC-AFF	MC-SMK	MC-AMK	MC-AFF
MFW13						
N	6	5	5	5	5	5
H_o	0.73	0.77	0.70	0.73	0.78	0.62
H_e	0.77	0.72	0.75	0.75	0.70	0.76
1-H_o/H_e	0.03	-0.09	-0.04	-0.02	-0.14	0.17
H-W Test	14.41 NS	6.86 NS	7.85 NS	8.29 NS	2.93 NS	29.21**
	(15)	(10)	(10)	(10)	(10)	(10)
MFW17						
N	5	5	5	4	5	5
H_o	0.85	0.70	0.74	0.64	0.60	0.65
H_e	0.78	0.79	0.74	0.72	0.75	0.57
1-H_o/H_e	-0.10	0.10	-0.02	0.07	0.19	-0.18
H-W Test	9.10 NS	15.43 NS	5.09 NS	5.80 NS	23.16*	9.95 NS
	(10)	(10)	(10)	(6)	(10)	(10)
MFW28						
N	4	3	3	3	4	3
H_o	0.42	0.42	0.52	0.27	0.59	0.35
H_e	0.65	0.63	0.67	0.69	0.69	0.47
1-H_o/H_e	0.34	0.31	0.21	0.57	0.12	0.25
H-W Test	7.70 NS	9.76*	3.68 NS	10.67*	12.59*	51.73***
	(6)	(3)	(3)	(3)	(6)	(3)
Average H_o over loci	0.67	0.63	0.65	0.55	0.65	0.54
Average H_e over loci	0.73	0.71	0.72	0.72	0.71	0.60
Average number of alleles	5.00	4.33	4.33	4.00	4.67	4.33
Polymorphism (P_{95})	1.00	1.00	1.00	1.00	1.00	1.00

Statistically significant values are marked with asterisks. $P<0.05$, ** $P<0.01$, and ***P=0000, NS= Not Significant,

Inter-population genetic structure and genetic distance

The F_{ST} (population differentiation) value between the population MC-AFF and MC-SMK was the highest (0.153) while the F_{ST} value between SC-SMK and MC-SMK was the lowest (0.001). The F_{ST} value was not significant between the scale carp population-pairs. However, the F_{ST} values between all the mirror carp population pairs were significant. The F_{ST} values between six different pairs of scaled cap and mirror carp population were not significant (Table 3). The Nei's genetic distance value between MC- SMK and MC- AFF was also the highest (0.462) across all the studied loci (Table 3). Pair-wise comparisons of six population samples of *C. carpio* using homogeneity tests are shown in Table 4. The population pairs SC-SMK/SC-AMK, SC-SMK/SC-AFF and SC-SMK/MC-SMK were homogenous at all the loci.

TABLE 3. Multilocus F_{ST} (above diagonal) and Nei's (1972) genetic distance (below diagonal) values between pairs of different populations of two strains of *C. carpio* L. across all loci

		Scaled carp			Mirror carp		
		SC-SMK	SC-AMK	SC-AFF	MC-AMK	MC-AFF	MC-SMK
Scaled Carp	SC-SMK	---	0.002	0.009	0.024	0.062*	0.001
	SC-AMK	0.059	---	0.003	0.007	0.105*	0.011
	SC-AFF	0.076	0.058	---	0.003	0.078*	0.011
Mirror carp	MC-AMK	0.119	0.066	0.058	---	0.093*	0.043*
	MC-AFF	0.163	0.280	0.204	0.244	---	0.153*
	MC-SMK	0.093	0.116	0.115	0.206	0.462	---

Statistically significant values are marked with asterisks. * $P<0.05$

TABLE 4. Homogeneity between samples of scaled carp and mirror carp. χ^2 values followed by degrees of freedom in Parentheses

Populations	χ^2-values		
	MFW13	*MFW17*	*MFW28*
SC-SMK / SC-AMK	2.66 (3) NS	3.68 (4) NS	3.46 (2) NS
SC-SMK/ SC-AFF	5.5 (4) NS	5.10 (4) NS	4.88 (2) NS
SC-SMK / MC-AMK	8.10 (3)*	7.24 (4) NS	6.52 (2)*
SC-SMK/ MC-AFF	10.68 (3)*	23.26 (4)***	8.04 (2)*
SC-SMK / MC-SMK	4.35(3) NS	8.32 (4) NS	1.03 (2) NS
SC-AMK / SC-AFF	5.31(4) NS	10.43 (4)*	1.58 (2) NS
SC-AMK / MC-AMK	2.33(3) NS	10.86 (4)*	1.80 (2) NS
SC-AMK / MC-AFF	12.89(4)*	28.84 (4)*****	19.63 (2)****
SC-AMK / MC-SMK	5.65(3) NS	10.72 (4)*	0.28 (2) NS
SC-AFF / MC-AMK	6.03(4) NS	10.41 (4)*	0.06 (2) NS
SC-AFF / MC-AFF	8.18 (4) NS	11.07 (4)*	24.51 (2)*****
SC-AFF / MC-SMK	3.23 (3) NS	13.95 (3)**	0.65 (2) NS
MC-AMK/ MC-AFF	15.87 (4)**	12.87 (3)**	27.67 (2)*****
MC-AMK / MC-SMK	6.38 (3) NS	21.01 (3)***	0.60 (2) NS
MC-AFF / MC-SMK	13.76 (4)**	33.50 (3)******	10.19 (2)**

Statistically significant values are marked with asterisks. *$P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$, ***** $P<0.00001$ and ****** $P=0$

But the population pairs SC-SMK/MC-AFF, SC-AMK/ MC-AFF, MC-AMK/MC-AFF and MC-AFF / MC- SMK were not homogeneous at any locus.

DISCUSSION

Genetic variation is considered as an important parameter of a population's ability to adapt to changing environment or stressed conditions (Allendorf *et al*., 1987). The loss of genetic variation, due to selection, inbreeding, genetic drift and bottleneck effects, results in a decrease of population's adaptive potentiality (Ferguson et al., 1995). We report here the genetic variation within and among three captive populations of scaled carp and mirror carp strains of common carp based on heterozygosity and polymorphism at three microsatellite loci. We also report the population differentiation on the bases of allele differences in allele frequencies measured as F_{ST} between different pairs of scaled carp and mirror carp populations. The average observed heterozygosities (H_o) of all the studied populations varied form 0.54 to 0.67. The average expected heterozygosities (H_e) varied from 0.60 to 0.73. The Ho_s were lower than the He_s in case of all the populations. This means that the populations have lost some heterozygosity over the years. The population SC-SMK had the highest *Ho* (0.67) and average number of alleles (5.00). Thus genetic variability was higher in this population compared to the other populations. The genetic variability of the population SC-AMK and SC-AFF were more or less similar in terms of *Ho* and average number of alleles. The population MC-SMK had the lowest average number of alleles (4.00). The average number of allele obtained in the present study was however lower than those reported by Mondol et al. (2006) at five other loci in scaled carp and mirror carp populations. Kohlman et al. (2005) found lower level of genetic variation with a mean alle of 4.4 in domesticated common carp compared to the feral and wild populations with 8.2 alleles. Loss of allelic variation has been reported for Polish hatchery populations of trout (Was and Wenne, 2002). Similarly, Sekino et al. (200) found that the number of microsatellite alleles was markedly reduced in the hatchery strains compared with the wild populations in Japanese flounder. Microsatellites are generally considered to be selectively neutral and it can be said that the reduction of microsatellite allelic diversity in the population MC-SMK could be related to a possible population bottleneck associated with breeding practice.

There were no deviations from Hardy-Weinberg expectations found in the populations SC-SMK and SC-AFF. That means, these two populations were in equilibrium conditions and were complied with the hypothesis of random mating. Only one population of scaled carp and all three populations of mirror carp had deviation from Hardy-Weinberg expectation at some loci.

Alam and Islam (2005) also found that the population of *Catla catla* was deviated from Hardy-Weinberg equilibrium at a number of loci. Mirror carp populations were found to have more null allele (6) than scaled carp populations (4). That means the mirror carp strain had relatively higher level of loss of allelic variations and loss of heterozygosity than scaled carp strain. A null allele at a microsatellite is any allele that has amplified only weakly or not visible on the plate after electrophoretic separation and staining (O'Connell and Wright, 1997) and is recognized as an important factor in the reduction of observed heterozygosity compared with that expected on the basis of Hardy-Weinberg expectation. Mutation such as deletion and insertion within the priming site is thought to be the major reason for null alleles in microsatellite marker analysis (Callen et al., 1993). A heterozygous individual sometimes may be mistyped as homozygote if a null allele occurs due to nonamplification of an allele due to poor DNA preparation. However, inbreeding is a major factor of causing reduction in heterozygosity in a captive population. The deviation of mirror carp strain from equilibrium might be due to loss of heterozygosity in hatchery populations as a result of inbreeding. Higher level of loss of allelic variation in mirror carp than scaled carp may be due to the fact that scaled carp was introduced into Bangladesh in two batches while mirror carp in three batches and bred repeatedly in the hatcheries with small effective number of broods (*Ne*). As a result genetic erosion might have happened in most of hatchery populations through inbreeding and genetic drift.

Pair wise F_{ST} values were used to estimate the level of differentiation between the population pairs. The F_{ST} value was highest (0.153) between the population MC-AFF and MC-SMK. The F_{ST} value of 0.153 represents a high level of population differentiation. As the captive populations are isolated entities, random changes in gene and genotype frequencies take place independently of each other resulting in differentiation or divergence between the isolated populations unless fishes are manually exchanged among them (Chakraborty and Leimar, 1987). Nei's genetic distance value was also highest (0.462) between these populations. The underlying causes of the highest genetic distance between two mirror carp population instead scaled carp and mirror carp was unknown. On the other hand the lowest F_{ST} value (0.001) was found between SC-SMK and MC-SMK. This value was statistically insignificant. That means, these two populations are genetically similar to each other. The cause may be, these two populations were collected from the same fishery (SMK) and probably they were produced by cross-breeding between scaled carp and mirror carp. Now a days hybridization is a common practice in most of the of the fish hatcheries in Bangladesh. Non-significant population differentiation was also observed in several comparisons within regions, in particular between German pond carp or between Uzbek wild carp (Kohlmann et al., 2003). In contrast, highly significant population differentiation was found between Uzbek wild and Uzbek domesticated Uzbek and German, Uzbek and East Asian, and German and East Asian common carp populations (Murakaeva *et al*., 2003).

CONCLUSION

The study was aimed at revealing the genetic variation within and differentiation among three captive populations of two strains of common carp- scaled carp and mirror carp. All three microsatellite loci examined were polymorphic in all the three captive populations of the two strains. Based on allelic richness and heterozygosity levels, the genetic variation in the scaled carp population of the Sagor Matsho Khamar was the highest while that in mirror carp of the same farm was the lowest. The levels of observed heterozygosity in the populations were significantly lower than those of the expected heterozygosities indicating incidence of inbreeding in the populations. To increase the levels of heterozygosity, we advise to exchange brood fish among the farms.

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