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Assessing genetic diversity of populations of topmouth culter (*Culter alburnus*) in China using AFLP markers

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Abstract

Genetic diversity of five wild populations and a cultured population of topmouth culter (*Culter alburnus*) was investigated using amplified fragment length polymorphism (AFLP). A total of 373 reproducible bands amplified with seven AFLP primer combinations were obtained from 163 fish. The percentage of polymorphic loci ranged widely from 37.0% to 69.2% within distinct populations. The cultured population appeared to have a lower level of polymorphism (37.0%), gene diversity (0.121 ± 0.188) and Shannon's Information index (0.183 ± 0.263) than the wild populations. Analysis of molecular variance (AMOVA) revealed that average F_{ST} value overall loci was 0.2671, and the percentage of variation within population (73.29%) was larger than among populations (26.71%) ($P < 0.01$). The six populations were clustered into two major clades with UPGMA. The results from analysis of population pairwise gene flow indicated moderate gene flow among populations. Our study indicated that the genetic diversity of the cultured population was reduced compared with the wild populations. Geographic isolation, habitat, and artificial selection all may have played important roles in population differentiation. The information may be beneficial to future broodstock selection and defining conservation management for the different populations of topmouth culter.

Keywords: Topmouth culter (*Culter alburnus*); AFLP; Genetic diversity; Genetic resource; Population

1. Introduction

Genetic diversity is of great importance to the sustainability of populations (Hamrick et al., 1991). The level of genetic diversity may reveal information about historical population sizes and structure (Sivasundar and Hey, 2003). For management of a species, knowledge of intraspecific genetic variations may help to assess extinction risks such as inbreeding and evolutionary potential in a changing world (Hedrick, 2001).

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Molecular markers are useful for population genetic studies to assess influences of various factors on genetic diversity and population structure (Englbrecht et al., 2000; Whitehead et al., 2003). Among many types of molecular markers, amplified fragment length polymorphism (AFLP) has been demonstrated as a useful tool for population genetic studies of fish (Liu et al., 2003), particularly for those species whose genome information is lacking. AFLP is highly efficient for the determination of genetic variations as a multi-locus molecular marker technique (Vos et al., 1995). Because it is easy, fast, inexpensive and robust, AFLP has been successfully used to study genetic diversity and relationships in yellowback sea bream (Xia and Jiang, 2006), *Lutjanus Bloch* (Zhang et al., 2004), tilapia (Agresti et al., 2000), flounder (Liu et al., 2005), rainbow trout (Young et al., 1998), and catfish (Liu et al., 1998, 1999; Mickett et al., 2003; Simmons et al., 2006).

The topmouth culter, *Culter alburnus* (Basilewsky), belongs to Cypriniformes, Cyprinidae, *Culter*, and widely distributed throughout large rivers, reservoirs, and lake areas of China (Chen, 1998). The cultured production has greatly increased over the past decades, accounting for about 1500 tons annually. Topmouth culter has already become one of the most important commercial freshwater fish in China. Despite its economic and ecological importance, genetic diversity for this species has not been studied. Early studies only focused on the population genetic structure and diversity of its closely related species cyprinid fish (Bártfai et al., 2003; Salgueiro et al., 2003). Furthermore, wild populations of topmouth culter are declining so quickly that the conservation of its genetic diversity has become an extremely important issue. Therefore, in order to prevent further decline of the genetic resource, an understanding of its genetic diversity, genetic structure, and geographical distribution becomes essential. In this study, we investigated the genetic diversity and structure within and among five wild populations and a cultured population of topmouth culter using AFLP to compare the level of genetic diversity in the wild populations and the cultured population. This study provides baseline information on genetic background of this species, and such information should be beneficial to population conservation and fisheries management in this species.

2. Materials and methods

2.1. Fish samples

A total 163 fish were collected from six locations as shown in Fig. 1 (Xingkaihu Lake, Heilongjiang province; Sanxikou Reservoir, Sichuan province; Taihu Lake, Jiangsu province; Liangzihu Lake, Hubei province; Nanwan

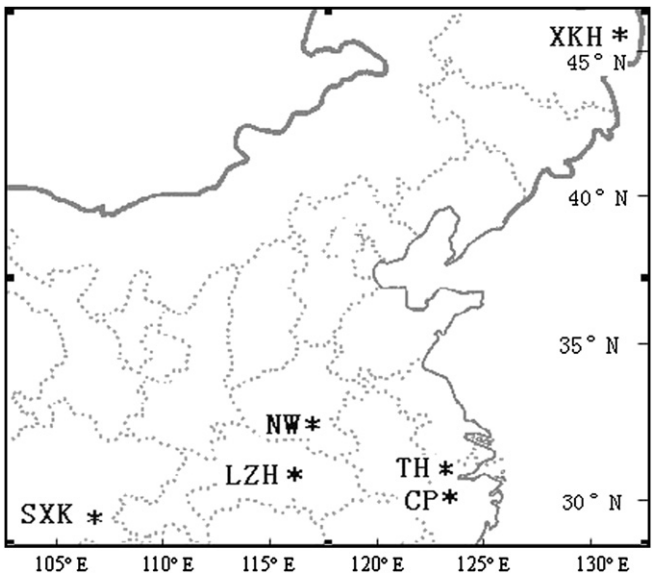

Fig. 1. The map of the locations of samples collected of topmouth culter populations. (XKH: Xingkaihu Lake, Heilongjiang province; SXK: Sanxikou Reservoir, Sichuan province; TH: Taihu Lake; LZH: Liangzihu Lake, Hubei province; NW: Nanwan Reservoir, Henan province; CP: Cultured population, Zhejiang Institute of Freshwater Fisheries, Zhejiang province.)

Table 1
Summary of all samples in this study

Population name	Abbreviation	Nature of population	Number sampled	Location
Xingkaihu	XKH	Wild	20	Heilongjiang province
Sanxikou	SXK	Wild	30	Sichuan province
Taihu	TH	Wild	23	Jiangsu province
Liangzihu	LZH	Wild	30	Hubei province
Nanwan	NW	Wild	30	Henan province
Cultured[a]	CP	Cultured	30	Zhejiang province

[a] Zhejiang Institute of Freshwater Fisheries.

Reservoir, Henan province, and Zhejiang Institute of Freshwater Fisheries, Zhejiang province). Muscle samples from each fish were preserved in 75% ethanol for genomic DNA isolation. The locations and the abbreviations for the populations are listed in Table 1.

2.2. DNA isolation

Genomic DNA was isolated using the DNA Isolation Kit from Sangon (Shanghai, China) following the manufacturer's instructions. DNA concentrations were estimated and standardized using known concentrations of λ DNA on 1.0% agarose gel. DNA concentration was brought to 50–150 ng/μl for AFLP analysis.

2.3. AFLP analysis

AFLP analysis was performed following the protocol described by Vos et al. (1995) with some modifications according to Wang et al. (2000). Briefly, the DNA samples were digested with EcoRI and MseI and then ligated to restriction site-specific adaptors. Pre-amplification was carried out using adaptor-specific primers with a single selective base on each primer. The pre-amplification products were diluted 50-fold, and then used for selective amplification. The selective amplification used primers with three additional selective bases. Of 100 primer combinations, 80 of them were screened using six DNA samples. Seven primer combinations were selected for AFLP analysis of the genetic diversity of topmouth culter populations (Table 2). The products of selective amplification were analyzed on Hoefer SQ3 sequencer (Phamacia Biotech, USA) and separated by 4.5% denaturing polyacrylamide gel electrophoresis at 60 W constant power for 1.5 h. The bands were visualized using silver staining (Bassam and Gresshoff, 1991).

2.4. Data analysis

Clear and unambiguous bands in length ranging from 50 to 1200 bp were considered as usable, and the band sizes were estimated using a standard 100 bp DNA ladder (Dongsheng Biotech). The bands were analyzed using the

Table 2
Adaptors and seven primer combinations sequences used in the study

Adaptors and primers	Sequences
Adaptors	
MesI adaptor	GACGATGAGTCCTGAG/TACTCAGGACTCAT
EcoRI adaptor	CTCGTAGACTGCGTACC/CTGACGCATGGTTAA
Primers	
E-ATC/M-CCT	GACTGCGTACCAATTCATC/GATGAGTCCTGAGTAACCT
E-AGT/M-CAT	GACTGCGTACCAATTCAGT/GATGAGTCCTGAGTAACAT
E-ATC/M-CTA	GACTGCGTACCAATTCATC/GATGAGTCCTGAGTAACTA
E-AGC/M-CAG	GACTGCGTACCAATTCAGC/GATGAGTCCTGAGTAACAG
E-ACC/M-CTT	GACTGCGTACCAATTCACC/GATGAGTCCTGAGTAACTT
E-ATC/M-CTC	GACTGCGTACCAATTCATC/GATGAGTCCTGAGTAACTC
E-ATC/M-CTG	GACTGCGTACCAATTCATC/GATGAGTCCTGAGTAACTG

Table 3
Population genetics parameters (mean ± SD) for the six populations of topmouth culter analyzed by AFLP

Pop.	P	N_a	N_e	H	I
XKH	50.94	1.509 ± 0.500	1.233 ± 0.334	0.141 ± 0.181	0.218 ± 0.260
SXK	42.90	1.429 ± 0.496	1.230 ± 0.349	0.134 ± 0.189	0.202 ± 0.271
TH	51.21	1.512 ± 0.500	1.252 ± 0.371	0.144 ± 0.195	0.218 ± 0.275
LZH	38.34	1.383 ± 0.487	1.207 ± 0.336	0.122 ± 0.183	0.183 ± 0.269
NW	69.17	1.692 ± 0.462	1.288 ± 0.351	0.174 ± 0.186	0.271 ± 0.262
CP	37.00	1.370 ± 0.483	1.213 ± 0.349	0.121 ± 0.188	0.183 ± 0.263
Overall	91.15	1.912 ± 0.284	1.300 ± 0.366	0.178 ± 0.191	0.279 ± 0.261

P, the percentages of polymorphic loci; N_a, observed number of alleles; N_e, effective number of alleles; H, Nei's gene diversity; I, Shannon's information index.

software Cross Checker (version 2.91; Buntjer, 1999) and the fragment data were transformed into a binary (1/0) data matrix. The percentages of polymorphic loci (P), observed number of alleles (N_a), effective number of alleles (N_e), gene diversity (H) (Nei, 1973), Shannon's Information index (I), gene flow [$N_m = 0.5(1 - G_{ST})/G_{ST}$] (McDermott and McDonald, 1993), Nei's genetic distance (Nei, 1978) and coefficient of gene differentiation (G_{ST}) were calculated using the software package of POPGENE 3.2 (version 1.31; Yeh et al., 1999). F_{ST} values were analyzed using ARLEQUIN (version 3.01; Excoffier et al., 2006).

Phenograms were constructed based on unweighted pair-group method analysis (UPGMA; Sokal and Michener, 1958) using the software NTSYS-pc 2.02 (version 2.1; Rohlf, 1998). To test the goodness of fit, a matrix of cophenetic values was produced from each cluster and compared to the distance matrix on which the cluster was based using the NTSYS software (Rohlf, 1998). The confidence of branch support was then evaluated by way of bootstrap analysis with 1000 replications, performed with the PAUP software package (version 4; Swofford, 2001). Molecular variances within and among populations of topmouth culter were estimated by analysis of molecular variance (AMOVA) using software ARLEQUIN (version 3.01; Excoffier et al., 2006).

3. Results

3.1. AFLP polymorphism and genetic variation of the six populations of topmouth cutler

A total of 373 bands were identified using seven AFLP primer combinations from 163 fish across the six populations, 340 bands were polymorphic, and the percentage of polymorphic bands was 91.15%. The values of N_a were from 1.370 ± 0.483 to 1.692 ± 0.462 and N_e from 1.207 ± 0.336 to 1.288 ± 0.351. The population with the greatest percent polymorphic loci (69.17%), the highest gene diversity (0.174 ± 0.186) and Shannon's Information index (0.271 ± 0.262) was the Nanwan population (NW). In the wild populations, the lowest percentage polymorphic loci, gene diversity and Shannon's Information index were in the Liangzihu population (LZH), and they were 38.34, 0.122 ± 0.183 and 0.183 ± 0.269, respectively. However, the cultured population (CP) collected from Zhejiang Institute of Freshwater Fisheries had a lower percentage of polymorphic loci (37.0%), gene diversity (0.121 ± 0.188) and Shannon's Information index (0.183 ± 0.263) than the wild populations. The genetic variation statistics parameters of fish are summarized in Table 3. The estimated G_{ST} value averaged overall polymorphic loci was 0.214. The F_{ST} values estimated between the populations ranged from 0.1786 to 0.3628 (Table 4). The greatest F_{ST} values

Table 4
Population pairwise F_{ST} values (above diagonal) and genetic distance values (below diagonal) (Nei, 1978)

Pop.	XKH	SXK	TH	LZH	NW	CP
XKH	***	0.1993	0.2088	0.2644	0.1786	0.2089
SXK	0.0637	***	0.2433	0.3628	0.3307	0.3183
TH	0.0329	0.0568	***	0.2690	0.2854	0.3175
LZH	0.0601	0.0766	0.0591	***	0.2961	0.3156
NW	0.0306	0.0422	0.0297	0.0621	***	0.2326
CP	0.0550	0.0391	0.0586	0.0449	0.0464	***

Table 5
Population pairwise estimated gene flow (N_m)

Pop.	XKH	SXK	TH	LZH	NW	CP
XKH	***					
SXK	2.4065	***				
TH	4.4898	2.7810	***			
LZH	2.4040	1.9170	2.5443	***		
NW	5.2866	4.0741	5.7859	2.7210	***	
CP	2.6021	3.5593	2.5632	2.9715	3.5454	***

$N_m = 0.5(1 - G_{ST})/G_{ST}$.

were between the Sanxikou (SXK) and LZH populations (0.3628) and the lowest between Xingkaihu (XKH) and NW populations (0.1786) ($P < 0.01$). The F_{ST} value of overall populations was 0.2671.

3.2. Population structures

Nei's genetic distance among the six populations was from 0.0297 to 0.0766 (Table 4). The greatest genetic distance (0.0766) was between the populations SXK and LZH, and the lowest (0.0297) between the populations Taihu (TH) and NW ($P < 0.01$). Between the cultured and wild populations, the largest genetic distance was found between the cultured and TH population (0.0586), and the lowest was between the cultured and SXK population (0.0391) ($P < 0.05$). Population pairwise gene flow estimates (N_m) are shown in Table 5. The largest estimated gene flow (5.7859) was between the populations TH and NW, and between the populations SXK and LZH presented the lowest (1.9170). The gene flow between CP and wild populations ranged from 2.5632 to 3.5593.

The population-wise similarity tree based on Nei's (1978) unbiased identity measures revealed an average population similarity of 85% (±18% SD). The six populations were clustered into two major clades. The first group, including populations XKH, TH and NW, was from locations at higher latitudes than the others; Cultured, LZH and SXK populations were clustered as the second group with locations at lower latitudes (Fig. 2).

Molecular variance within and among topmouth culter populations analyzed with analysis of molecular variance (AMOVA), and revealed that the percentage of variations within population (73.29%) was larger than that among populations (26.71%) and there were significant differences ($P < 0.01$) under the significance tests with 1000 permutations (Table 6).

4. Discussion

Genetic analysis of diversity among and within domestic and wild populations of topmouth culter in China was conducted using AFLP markers. Once again, this work demonstrated that AFLP is one of the most powerful

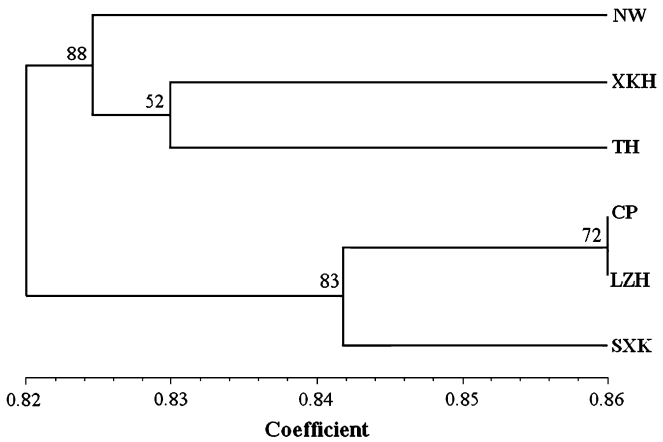

Fig. 2. Population-wise similarity tree revealed by UPGMA cluster analysis using the Nei (1978) genetic identity measure. Population names are as given in Fig. 1. Bootstrap values are indicated.

Table 6
Analysis of molecular variance (AMOVA) within and among the populations of topmouth culter

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*P*
Among populations	5	1431.055	10.483	26.71	<0.01
Within populations	157	4113.442	28.765	73.29	<0.01
Total	162	5544.497	39.248	$F_{ST} = 0.2671$	

d.f., Degree of freedom; significance tests with 1000 permutations.

approaches for the assessment of fish genetic variation among populations, especially for species whose molecular genetic background was not previously available (Lucchini, 2003). Our results demonstrated the utility of AFLP analysis for assessing genetic diversity within and among populations in the species without the need for developing species-specific genetic markers. In addition, our findings indicated that some distinct differences exist among and between both the domestic strain and wild lines of topmouth culter in China, and the most distinctive population was the NW population with the highest genetic diversity. The pattern of genetic diversity observed has practical implications for management of brood stocks and for selective breeding programs. The data can also be used as baseline information for further study of the interactions between domestic and wild populations of topmouth culter in the long-term and for studies involving genetic and environmental interactions.

The results of our study were demonstrated that the genetic diversity of topmouth culter populations was high. The level of polymorphism was similar to that in common carp (Wang et al., 2000). The level of polymorphism and genetic diversity of the cultured population was found lower than that of the wild populations, and is congruent with that of the farmed strain that had considerably lower genetic diversity than the samples collected from wild populations (Skaala et al., 2004; Murakaeva et al., 2003). Because the cultured population was bred with relatively small effective population size, while the wild populations are assumed to have large effective population sizes, the loss of genetic variation likely was attributable to selection pressure (Hallerman et al., 1986) and the limited effective population size (Ryman et al., 1995). Furthermore, the reduction of the genetic diversity may be also due to reproduction and geographic isolation and genetic drift (Ward et al., 2003). These results suggested that the artificially cultured topmouth culter population need to be maintained at moderate effective population size to prevent reduction of genetic diversity. Obviously, one option is to conduct crossbreeding with wild populations to enhance genetic diversity and it must be based on improvement of the germ plasm resources.

The average F_{ST} value of overall populations was 0.2671, which indicates a high degree of genetic differentiation among the populations of topmouth culter, however, is lower than the values estimated in the populations of catfish analyzed using AFLP (Mickett et al., 2003; Simmons et al., 2006) and higher than that of Asian seabass (Zhu et al., 2006). Clearly, quite different genetic differentiations can be found with different species. Despite the fact that AFLP-based F_{ST} value is generally higher than that estimated by using codominant markers (Whitehead et al., 2003), the population pairwise F_{ST} values suggested that there was a moderate degree of genetic differentiation between some populations, such as, NW and XKH population. However, there was a high level of genetic differentiation between most populations of topmouth culter in China.

Our results suggested that gene flow among populations existed, in spite of the fact that samples were collected from closed watersheds and the locations far away from one another. The estimated N_m value decreased with the increasing geographic distance (Table 5), indicating that N_m was influenced by the geographic isolation. However, it presented larger N_m values between XKH, NW and TH populations despite their large geographic distances. The topmouth culter was most famous in the Xingkaihu Lake and Taihu Lake in China. Thus one possibility is that unrecorded topmouth culter introductions may have occurred among these populations. The intentional or unintentional introduction of new species to aquatic systems is considered to be one of the greatest environmental threats facing fish populations around the world (Lowe-McConnell, 1990). Although the topmouth culter of XKH, NW and TH was the same species, their introductions could still have a tremendous impact on genetic resource of the native population.

The results of UPGMA dendrogram similarly indicated gene flows among populations. For instance, XKH, NW and TH clustered in the same group were all from north and east of China and their habitats are more similar to one another than to those of other populations. Therefore, XKH, NW and TH may have come from the same ancestor and the differences were likely to be a result of geographical separation, habitat and artificial selection. This speculation is supported by the management history of the second group containing the CP and LZH populations; CP

population came originally from the LZH population which is located at the middle of China. These populations exhibited the lowest genetic diversity among the wild populations. Attention should be paid to the low genetic variations of the LZH population. As the major elements influencing genetic diversity appeared to be geographic isolation, habitat, effective population size and artificial selection (Ribeiro et al., 2000), crossbreeding should be an effective solution to increase genetic diversity.

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