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Genetic structure and diversity in natural and stocked populations of the mandarin fish (*Siniperca chuatsi*) in China

M. Yang*, C. Tian*, X.-F. Liang, H. Zheng, C. Zhao and K. Zhu

College of Fisheries, Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan, Hubei, China

*These authors contributed equally to this study.
Corresponding author: X.-F. Liang
E-mail: xufang_liang@hotmail.com

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ABSTRACT. The Chinese perch, or mandarin fish (*Siniperca chuatsi*), is a freshwater fish that is endemic to East Asia. In this study, we investigated the genetic diversity and structure of nine natural mandarin fish populations (from the Yangtze River and Amur River basins) and six hatchery stocks (from central and south China) using microsatellite markers. The results show that the genetic diversity of the Yangtze River populations was high and stable, and genetic differences between them were not significant. In contrast, a low level of genetic diversity and strong genetic structure were detected in the Amur River population. These results suggest that the Yangtze River region and the Amur River region should be treated as two separate units in conservation programs. The hatchery stocks exhibited low genetic diversity and significant genetic differentiation compared to natural populations; this may result in a significant impact on the species if escape events occur. Therefore,

a scientific aquaculture management strategy is necessary for the long-term development of hatcheries.

Key words: Genetic diversity; Genetic structure; Mandarin fish; Microsatellite markers

INTRODUCTION

The Chinese perch or mandarin fish (*Siniperca chuatsi*) is a freshwater fish species endemic to East Asia. It is found in most river systems of eastern China, particularly in large, slow-moving rivers (Liang, 1996). However, its natural resources have reduced dramatically since the 1970s, due to habitat degradation and anthropogenic effects (e.g., damming, water pollution, and overfishing) (Li, 1991; Zhang and Zhao, 1999). In the wild, mandarin fish are all but extinct in some regions of north China (Yang, 2003).

In addition, the development of the aquaculture industry and the absence of a scientific or rational management of hatchery stocks is also a threat to the species (Wang et al., 2006). Hence, it is becoming increasingly important to understand the genetic diversity and structure of natural and hatchery populations (Liang, 1996). However, the number of samples and populations used in previous studies has been insufficient for robust quantitative assessments; therefore, information regarding the detailed genetic structure of the mandarin fish is still limited.

In the present study, we selected nine natural populations (from five tributaries and one lake of the Yangtze River basin) and six artificial populations (from the main hatcheries of central and south China) to analyze their genetic diversities and structures using microsatellite markers. This information may provide a scientific basis for the conservation and sustainable use of natural populations, and the high-efficiency utilization of the species' germplasm resources in breeding programs.

MATERIAL AND METHODS

A total of 444 specimens were analyzed, including 273 natural samples from the Yangtze River basin (Yuan River, Xiang River, Ganjiang River, Lushui River, and Dongting Lake) and the Amur River basin (Songhua River). We collected six natural populations from different sites of the Yuan River, the Lushui River, and Dongting Lake (Yuan River, YUAN-A and YUAN-B; Lushui River, LU-A and LU-B; Dongting Lake, DON-A and DON-B). A total of 171 stock specimens were collected from different hatcheries in central and southern China (Central: Hubei Province, Hunan Province, and Jiangxi Province; South: Guangdong Province). As Guangdong Province is the main region for farming mandarin fish in China, we collected three stock populations from different hatcheries in this region (GD-A, GD-B, and GD-C) (Figure 1A). Total genomic DNA was extracted from fin clips using a TIANamp Genomic DNA Kit (Tiangen, Beijing China). DNA was adjusted to a volume of 100 ng/μL and stored at -20°C.

Twelve microsatellite markers were used to estimate the genetic diversity and structure of these populations. Nine primers (SO374, SS55, SS62, SC01, SC80, Sin138, Sin142, Sin166, and SK524) were taken from Qu et al. (2012) and Huang et al. (2013), and the other three primers (FC076, PY55, and SK608) were developed from genomic sequences of *S. chuatsi* in our laboratory: FC076 (F, 5'-TACCCCAGTCGTGTCCCTT-3'; R, 5'-CTTTCCTTATTTATTGACTC-3'); PY55 (F, 5'-TGGGTAGGCTTCATGTGGTA-3'; R,

5'-TGCCACCTTTAGATTTCAGC-3'); and SK608 (F, 5'-GTGGTTTCTACATTTGGGTC-3'; R, 5'-CAGTCAACATTACAGAACCTCA-3'). Microsatellites were multiplexed with up to three fluorescently labeled primers. The polymerase chain reaction (PCR) system and thermal cycling conditions were conducted according to Huang et al. (2013). PCR products were separated using an automatic capillary sequencer (ABI 3130 Genetic Analyzer, Applied Biosystems) at Sangon Biotech (Shanghai, China). Fragment sizes were determined using the GeneMapper® software version 4.0 (Applied Biosystems), by comparing with a GeneScan™ 500 ROX™ (Applied Biosystems) internal size standard.

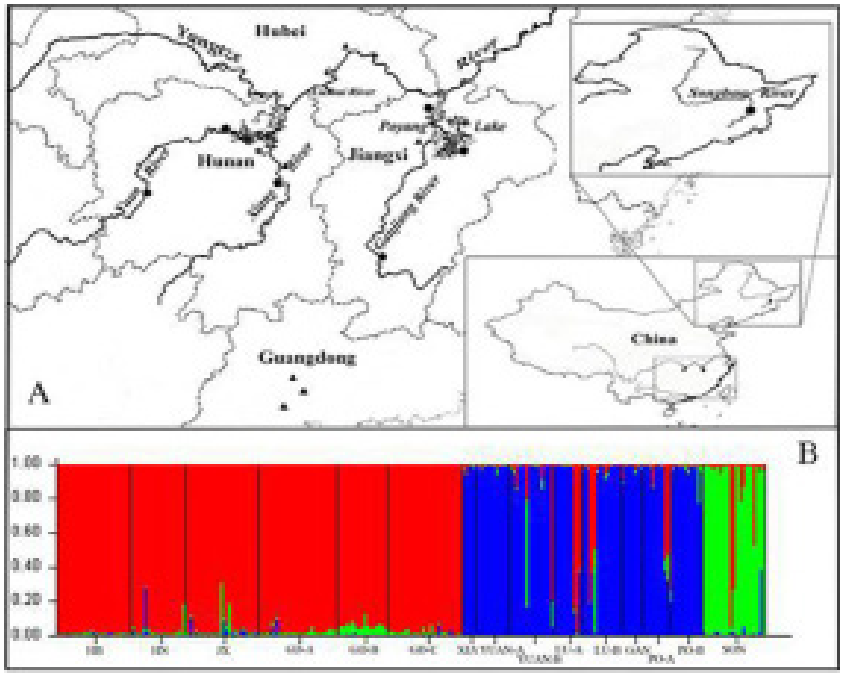

Figure 1. A. Map showing sampling locations. Circles denote wild samples and triangles indicate hatchery samples. **B.** Population genetic structure analysis with the Structure 2.2 software. Each individaul is represented by a single vertical line divided into three colours. Each color represents one gene pool, and the length of the coloured segment shows the individual's estimated proportion of membership in that gene pool. Black lines separate populations that are labelled below the figure.

The presence of null alleles, large allele drop-outs, and scoring errors was evaluated using MicroChecker version 2.2.3 (Van Oosterhout et al., 2004). Genepop version 4.0 (Raymond and Rousset, 1995) was used to test the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium. POPGENE version 1.3 (Yeh, 1997) was used to estimate the observed (H_O) and expected (H_E) heterozygosities. An analysis of molecular variance (AMOVA) was conducted using Arlequin 3.11 (Excoffier and Lischer, 2010); for this analysis, the natural populations were classified as one group and the stock populations were classified as another group. Pairwise F_{ST} values were also calculated by Arlequin. The population genetic structure was analyzed by Structure, version 2.2 (Pritchard et al., 2000). The most probable number of clusters in the dataset was identified based on the probability $K = 2$ to 15, with 1,000,000

Markov chain Monte Carlo repetitions each and a burn-in of 10,000 iterations. Each value of *K* was tested at least 20 times to estimate the deviation between repeated runs. The most probable *K* value was determined according to Evanno et al. (2005).

RESULTS

Three of the twelve microsatellite markers (SC608, Sin142, and PY55) were polymorphisms and were excluded from further analyses. Of the natural populations, the Poyang Lake population exhibited the highest mean levels of genetic diversity [PO-A: N_A (average number of alleles per locus), 6.44; H_O, 0.788; H_E, 0.726; PO-B: N_A, 6.33; H_O, 0.752; H_E, 0.716], and the Amur River population exhibited the lowest mean levels of genetic diversity (SON: N_A, 3.89; H_O, 0.471; H_E, 0.451). All of the hatchery stocks exhibited low mean levels of genetic diversity (N_A, 3.15; H_O, 0.424; H_E, 0.398) (Table 1). The N_A, H_O, and H_E values of the hatchery groups were significantly lower than those of the natural groups ($P < 0.05$).

Table 1. Summary statistics for genetic variation of natural and stocked populations of mandarin fish (*Siniperca chuatsi*).

River	Pop code	Region	Population type	N	N_A	N_E	H_O	H_E
Xiang River	XIA	Yangtze River	Natural	30	4.22	3.00	0.667	0.662
Yuan River	YUA-A	Yangtze River	Natural	33	5.78	3.36	0.590	0.664
Yuan River	YUA-B	Yangtze River	Natural	28	7.44	3.90	0.722	0.730
Lushui River	LU-A	Yangtze River	Natural	31	5.78	4.17	0.606	0.724
Lushui River	LU-B	Yangtze River	Natural	36	6.33	3.78	0.729	0.734
Ganjiang River	GAN	Yangtze River	Natural	28	5.22	3.14	0.694	0.686
Poyang Lake	PO-A	Yangtze River	Natural	31	6.44	3.77	0.788	0.726
Poyang Lake	PO-B	Yangtze River	Natural	31	6.33	3.74	0.752	0.716
Songhua River	SON	Amur River	Natural	25	3.89	2.09	0.471	0.451
Hubei Province	HB	Central China	Stocked	29	3.00	1.84	0.410	0.389
Hunan Province	HN	Central China	Stocked	22	3.00	1.81	0.419	0.375
Jiangxi Province	JX	Central China	Stocked	29	4.22	1.88	0.521	0.418
Guangdong Province	GD-A	South China	Stocked	31	3.22	1.80	0.369	0.361
Guangdong Province	GD-B	South China	Stocked	30	2.78	1.98	0.494	0.372
Guangdong Province	GD-C	South China	Stocked	30	2.67	1.70	0.356	0.311
Mean of natural group				273	5.72	4.44	0.651	0.749
Mean of stocked group				171	3.15	1.91	0.424	0.398
Total				444	4.69	2.94	0.521	0.629

N = number of samples; N_A = average number of alleles per locus; N_E = effective number of alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity.

The pairwise F_{ST} values varied from -0.014 to 0.501, and all of the hatchery stocks exhibited significant genetic differences between each other. Samples from the Amur River also exhibited significant genetic differences from the other populations. However, no significant genetic differentiation was found between the Yangtze River populations. In the natural populations, the lowest value of Nei's genetic distance (0.079) was found between the Ganjiang River (GAN) and Poyang Lake (PO-B) populations, and the highest value (1.1841) was found between the Amur River (SON) and the Yuan River (YUA-A) populations (Table 2). The AMOVA revealed a significant difference between the natural and artificial groups (FCT = 0.219, $P < 0.01$); 68.92% of the genetic variance was between individuals within populations, and 21.86% was between the natural and hatchery groups.

Table 2. Nei's unbiased genetic distances (under the diagonal) and Pairwise F_{ST} (above the diagonal) among populations.

	HB	HN	JX	GD-A	GD-B	GD-C	XIA	YUA-A	YUA-B	LU-A	LU-B	GAN	PO-A	PO-B	SON
HB		0.038*	0.035*	0.055*	0.153*	0.039*	0.406*	0.357*	0.306*	0.285*	0.274*	0.378*	0.326*	0.309*	0.249*
HN	0.037		0.015*	0.089*	0.186*	0.090*	0.412*	0.361*	0.307*	0.296*	0.283*	0.394*	0.334*	0.317*	0.254*
JX	0.036	0.023		0.089*	0.169*	0.094*	0.365*	0.328*	0.277*	0.280*	0.255*	0.364*	0.310*	0.294*	0.265*
GD-A	0.045	0.069	0.074		0.098*	0.016*	0.459*	0.397*	0.346*	0.338*	0.315*	0.430*	0.375*	0.352*	0.231*
GD-B	0.131	0.160	0.157	0.077		0.130*	0.450*	0.382*	0.321*	0.346*	0.303*	0.421*	0.364*	0.334*	0.236*
GD-C	0.030	0.061	0.068	0.016	0.089		0.501*	0.439*	0.379*	0.376*	0.343*	0.471*	0.410*	0.383*	0.247*
XIA	0.945	0.948	0.840	1.138	1.253	1.145		0.062	0.018	0.022	0.034	0.012	0.026	0.003	0.375*
YUA-A	0.790	0.816	0.737	0.902	0.958	0.969	0.265		0.040	0.058	0.062	0.065	0.052	0.047	0.359*
YUA-B	0.685	0.703	0.624	0.802	0.799	0.826	0.171	0.169		0.026	0.015	0.032	0.024	0.002	0.301*
LU-A	0.531	0.576	0.582	0.658	0.832	0.676	0.198	0.236	0.166		0.013	0.010	0.013	0.001	0.290*
LU-B	0.533	0.576	0.520	0.623	0.684	0.618	0.219	0.239	0.120	0.133		0.009	-0.001	0.010	0.271*
GAN	0.878	0.972	0.919	1.079	1.206	1.105	0.180	0.255	0.184	0.143	0.128		-0.014	0.014	0.366*
PO-A	0.706	0.753	0.719	0.857	0.955	0.861	0.211	0.221	0.160	0.148	0.096	0.088		0.008	0.306*
PO-B	0.634	0.680	0.646	0.738	0.779	0.736	0.144	0.195	0.103	0.106	0.114	0.079	0.124		0.289*
SON	0.291	0.295	0.345	0.241	0.266	0.230	1.133	1.184	1.137	0.767	0.721	1.112	0.871	0.766	

*$P < 0.05$.

Bayesian clustering analysis revealed that the highest ΔK value (83.97) was for K = 3, suggesting that all of the individuals fell into three main genetic groups. All of the hatchery stocks were classified into one group, the Amur River population was classified as another group, and the Yangtze River population was the third group. Some stock individuals (sometimes consisting of 80% of the population) were found in natural populations (Figure 1B).

DISCUSSION

The levels of genetic variability in the Yangtze River populations that we found were similar to those estimated by Mei (2010) and Wu et al. (2010), suggesting that the populations of these regions are relatively stable. Genetic diversity in the Amur River samples was very low, suggesting a serious decline of the species, possibly caused by recent habitat destruction and environmental degradation. The low genetic diversity we found in the hatchery populations (both in the central and southern areas) could be explained by several factors: 1) a relatively small effective population size; 2) the effects of genetic drift during the initial stages of transplanting; and 3) intensive directional selection in breeding programs (Doebley et al., 2006; Gross and Olsen, 2010; Karlsson et al., 2010). Therefore, for the genetic improvement of hatchery stocks it is necessary to construct a broad genetic base with a large effective population size, or to regularly introduce individuals from natural populations.

All of the hatchery stocks exhibited high genetic distances and significant genetic differentiation compared to the other populations. The genetic differences between hatchery stocks may have been due to random genetic drift under the stress of artificial selection, which has been well documented in other species (Hedgecock and Sly, 1990; Desvignes et al., 2001; Li et al., 2007; Dawnay et al., 2011), and differences in their environments and management patterns would also cause them to gradually diverge (Liu et al., 2012). The difference between the natural and the artificial populations could be due to a lack of gene flow; their geographical isolation from each other was probably the cause of this. In addition, the different environmental conditions in natural and stock populations also probably contributed to their genetic differences (Liu et al., 2012).

A weak genetic structure was observed in the Xiang River, Yuan River, Lushui River, Ganjiang River, and Poyang Lake populations. All of these tributaries, and the lake, are connected to the Yangtze River, so there is little geographical isolation; the short distances between the populations increased gene flow and probably contributed to their weak genetic structure. By contrast, a strong genetic structure was found in the Yangtze River and Amur River populations; this could be explained by the geographical features of these areas. The Yangtze River and Amur River are south and north, respectively, of the Qinling Mountains and the Huaihe River, which are important geographical boundaries in China. Differences in topography and climate between the south and north may have led to these endemic genetic characteristics. Based on this evidence, we suggest that the Yangtze and Amur River populations should be managed as separate conservation units.

Fish that had been reared in hatcheries were in wild populations, according to Bayesian analysis, suggesting that some individuals may have escaped into the wild gene pool. This is important, because the escape of hatchery stocks may pose a threat to the genetic diversity and/or genetic structure of natural populations (Clifford et al., 1998; Koljonen et al., 2002; Ma et al., 2011). Therefore, to prevent escape events, it is necessary to improve the genetic management and conservation plan for this species.

The data obtained in the present study should be regarded as preliminary, as there are many natural and hatchery resources at other locations yet to be investigated. Therefore, in order to provide a scientific management strategy for the mandarin fish, full-scale analyses should be conducted in future studies.

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