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Genetic Characterization of Selected Domestic Populations of Channel Catfish (*Ictalurus punctatus*) using Microsatellites

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Authors' Contribution
ZS conceived and designed the experiments. QM and BW performed the experiments, analyzed the data and wrote the manuscript. ZS and JJ reviewed and revised the manuscript.

Key words
***Ictalurus punctatus*, Domestic populations, Genetic characteristics, Microsatellite, Population management**

ABSTRACT

In the present study, the genetic variability and relationships of the domestic populations of channel catfish (*Ictalurus punctatus*) in Sichuan Province, China were studied to determine their genetic background and provide essential data for its artificial breeding. Eight microsatellite markers were used to analyze genetic characteristics of eight channel catfish populations (POPA, POPB, POPC, POPD, POPE, POPF, POPG, and POPH). The average polymorphic information content (PIC) ranged from 0.525 (POPD) to 0.733 (POPA) indicating high polymorphism (PIC > 0.5). The average observed heterozygosity (*Ho*) and expected heterozygosity (*He*) in the eight populations ranged from 0.504 (POPH) to 0.767 (POPG) and from 0.6 (POPD) to 0.781 (POPA), respectively. The results showed that the eight populations possess considerable genetic diversity. The largest genetic differentiation (F_{ST}) among the eight populations was 0.233 (between POPB and POPD) and the lowest F_{ST} was 0.04 (between POPA and POPE). Furthermore, population structure analysis and UPGMA dendrogram suggested that there were close genetic relationships between POPA and POPE, POPA and POPG, and POPF and POPH, respectively. POPH showed serious inbreeding based on F_{IS} analysis.

INTRODUCTION

The channel catfish (*Ictalurus punctatus*) is native to the United States and northern Mexico (Wellborn, 1988; Liu, 2009). It was first introduced to Hubei Province of China as an aquaculture species in 1984 (Liu, 2009). Due to its fast growth, high environmental adaptability, strong disease resistance, and delicious taste, channel catfish has become one of the most important aquaculture species in China and been cultivated in over 20 provinces (Liang *et al*., 2007; He *et al*., 2008a; Bi *et al*., 2010; Cui *et al*., 2012). The production of channel catfish not only meets domestic consumption in China but is exported back to the United States (Qin *et al*., 2011).

However, with the development of large commercial production, reduced genetic diversity appeared in some channel catfish populations after years of breeding because of a limited number of parental fish (He *et al*., 2008a, 2008b). The cultured populations usually come from a small population in the breeding programs and have limited genetic variability which may cause reduction in genetic diversity and changes in gene frequencies (Loukovitis *et al*., 2015; Howard *et al*., 2017). Based on microsatellite DNA analysis, Parra-Bracamonte *et al*. (2011) found there is a risk for increased inbreeding of channel catfish in Mexico due to heterozygote deficiency and low effective number of breeders. In some other fish species, it was identified that the reduced genetic variability was caused mainly by the inbreeding depression associated with small effective population sizes and limited broodstocks in cultured populations. The genetic diversity of bighead catfish (*Clarias macrocephalus*) in hatchery was lower than that of wild conserved populations based on microsatellite analysis (Duong and Scribner, 2018). Zhou *et al*. (2017) reported that for *Sipunculus nudus*, the genetic diversity of cultured populations was slightly lower than that of the wild populations inferred from mitochondrial DNA control region sequences.

The source of parental fish was further narrowed down due to a plan to control germplasm resources of channel catfish launched by the United States in 1998 (Xiao *et al*., 2015). In order to resolve the lack of

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germplasm resources for channel catfish in China, the National Fisheries Technical Extension Center and other research institutions implemented the project "The Joint Breeding and Demonstration Program of Channel Catfish" (hereafter called breeding program). The breeding program is designed to improve reproductive technology by establishing sustainable reproduction and breeding system with the existing germplasm resources in China by now (Xiao *et al*., 2015). Because of the advantage of geographical location and great demand for the channel catfish, the provinces of Hunan, Hubei, Jiangxi, Anhui, and Sichuan in China were selected as the main breeding areas in the breeding program.

In the process of breeding program, one of the most important problems is maintaining the genetic variation among populations. This variation permits flexibility and survival of a population in face of changing environmental circumstances (Willi and Hoffmann, 2009; Pavlova *et al*., 2017). It is necessary to understand the genetics of cultured populations to conserve the genetic resources and prevent the genetic decline. Several studies have reported the genetic diversity of channel catfish populations in Hunan, Hubei, and Jiangxi provinces (Liang *et al*., 2007; He *et al*., 2008a; Bi *et al*., 2010). However, no investigation of genetic diversity was conducted for the species in Sichuan area. Thus, it is vital to characterize the genetic resources of channel catfish for its breeding management in Sichuan area.

Due to high polymorphism (Liu *et al*., 2019), microsatellite markers were usually used to investigate genetic variation of breeding stocks in farmed fish (Luo *et al*., 2015; Guja *et al*., 2016), which could be used to prevent inbreeding (Ponzoni *et al*., 2008; Wang *et al*., 2017) and direct mating when creating the base population of breeders for breeding programs (Fernández *et al*., 2014; El Moutchou *et al*., 2018). In the present study, the genetic variations of eight domestic channel catfish populations in Sichuan Province, China were evaluated using polymorphic microsatellites. The goal is threefold: (1) to evaluate the genetic variability of different channel catfish populations, (2) to analyze the genetic relationships among these populations, and (3) to provide scientific and practical suggestions for the breeding program and ensure sustainable commercial production.

MATERIALS AND METHODS

Sample collection and DNA extraction

In this study, 8 populations (each consisting of 30 samples) were analyzed and all the individuals were cultured in Tongwei Original and Fine Aquatic Species Farm, Chengdu, Sichuan, China. Two introduced populations (POPA and POPE) originated from America and were used as parental fish. Except for POPA and POPE, other populations were offspring obtained through breeding program in China and the parental fish were introduced from different areas of USA in different years. POPB, POPC, and POPD populations, POPF population, and POPG population were founded from the breeding program in 2009, 2011 and 2012, respectively. One population (POPH) was a breeding line with red patches on the skin and established in 2012.

The fin tissues of the individuals were sampled and preserved in 100% ethanol at 4 °C. Total DNA was extracted using TIANamp Marine Animals DNA kit (Tiangen, China).

Microsatellite amplification and genotyping

Eight microsatellite loci (Ip077, Ip265, Ip266, Ip268, Ip384, Ip565, Ip607, and IpTr09) (GenBank nos.: AF114755, AF114782, AF114781, AF114780, AF114771, AF114762, AF114758, and AF114769) previously isolated by Tan *et al*. (1999) were used for genetic investigation of the eight populations. Each forward primer was labeled by fluorescent dyes (FAM, TAMRA or HEX) for fragment visualization. All PCR reactions were carried out in a 25 μL reaction volume comprising of approximately 50 ng genomic DNA, 2.5 μL 10×reaction buffer (TaKaRa, China), 2 mM $MgCl_2$, 1U of *Ta*q polymerase (TaKaRa, China), 50 μM of each dNTP, 0.5 μM of each forward and reverse primers. Amplifications were performed using the following PCR procedure: an initial denaturation at 95 °C for 5 min, 35 cycles of a denaturation at 94 °C for 30 s, an annealing at 54 °C to 56 °C for 50 s, an elongation at 72 °C for 30 s, and a final extension at 72 °C for 10 min.

All amplifications were initially examined on 1.5% agarose gels, and then were genotyped and analyzed by Beijing Microread Genetics Co., Ltd (Beijing, China). The genotyping data were read manually from chromatograms.

Data analysis

Genetic diversity

Null alleles were tested using the software MICRO-CHECKER 2.2.3 (Van Oosterhout *et al*., 2004). The mean number of alleles per locus and per population (*A*), polymorphic information content (PIC), observed heterozygosity (*Ho*), and expected heterozygosity (*He*) were determined using CERVUS 3.0 (Marshall *et al*., 1998) to characterize the genetic variability. Private alleles were derived from CONVERT 1.31 (Glaubitz, 2004). Inbreeding coefficients (F_{IS}) per locus and per population were analyzed by Fstat v2.9.3 (Goudet, 2001). Exact tests were implemented in GENEPOP version 4.0.5.3 (Rousset,

2008) for assessment of deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between the loci.

Genetic relationship

The genetic differentiation index between populations, pair-wise genetic statistic F_{ST}, was calculated using GENEPOP 4.0.5.3 (Rousset, 2008), and a random effect model with 1 $\times 10^4$ permutations was used to test the significance of each F_{ST} value. Nei's genetic distance (Nei, 1978) was estimated using POPGENE 1.32 (Yeh *et al*., 1997). Then the UPGMA phylogenetic tree based on genetic distance was reconstructed by the MEGA program (Kumar *et al*., 2008).

To characterize the genetic relationship among eight channel catfish populations, the model-based Bayesian clustering method was applied to infer population substructure based on the software program Structure version 2.1 (Falush *et al*., 2003). The admixture model with correlated alleles was chosen to sort specimens into different clusters. Different numbers of assumed clusters (K) were evaluated (from K=2 to K=10) with each run of 1 $\times 10^6$ Markov Chain Monte Carlo (MCMC) replications after burn-in with 1×10^5 iterations. The most plausible number of population clusters was determined by calculating the distribution of the ΔK statistic as described by Evanno *et al*. (2005). When calculated for each K, this method shows a peak at the most likely K.

RESULTS

Genetic diversity

No genotyping errors due to shutting or null alleles were found through MICRO-CHECKER. All individuals were screened with eight microsatellites loci and generated 110 alleles ranging from 5 (Ip266) to 19 (Ip265, Ip268, Ip565 and Ip607) per locus (*mean* = 13.75). The mean number of alleles per population varied from 3.625 (POPD) to 8.625 (POPA) (Table I). Among the 8 populations, the lowest mean value of PIC (0.525) appeared in POPD, while the highest (0.733) was observed in POPA. The mean value of PIC was 0.599 in all populations (Table I). The lowest mean observed heterozygosity (*Ho*) and expected heterozygosity (*He*) was observed in POPH (0.504) and POPD (0.600), while the highest in POPG (0.767) and in POPA (0.781), respectively (Table I). Additionally, the mean value of observed heterozygosity (*Ho* = 0.683) was slightly higher than expected heterozygosity value (*He* = 0.661) across all populations, suggesting an excess of heterozygosity. Overall, 48 private alleles were detected: 24 in the POPE population, 12 in the POPA, 6 in the POPF, 2 in the POPB and POPG, and 1 in the POPD and POPH, respectively. No private alleles were found in POPC (Table I).

The average F_{IS} values were negative in five populations (POPB, POPC, POPD, POPE, and POPF). The highest F_{IS} value was observed in POPH (F_{IS} = 0.183). No loci significantly ($P < 0.05$) deviated from HWE and no significant linkage disequilibrium was detected at any of the eight loci after Bonferroni correction ($P > 0.05$).

Mean values of *A*, PIC, *Ho* and *He* were higher in two introduced populations POPA and POPE (mean value of *A* = 7.5, PIC = 0.698, *Ho* = 0.717, and *He* = 0.751) compared to the five cultured populations from breeding program (POPB, POPC, POPD, POPF, and POPG) (mean value of *A* = 4.5, PIC = 0.567, *Ho* = 0.705, and *He* = 0.634) (Table I).

Genetic relationship

The average F_{ST} across all populations was 0.145. The lowest differentiation (F_{ST}= 0.040) appeared between

Table I. Genetic diversity of the eight channel catfish populations inferred from microsatellite markers.

	Population	N	A	Pr	*Ho*	*He*	PIC	Fis
Introduced populations	POPA	30	8.625	12	0.692	0.781	0.733	0.116**
	POPE	30	6.375	24	0.742	0.72	0.662	-0.031
	Average	30	7.5	18	0.717	0.751	0.698	
Breeding populations	POPB	30	4	2	0.738	0.641	0.573	-0.153
	POPC	30	4	0	0.75	0.605	0.53	-0.245
	POPD	30	3.625	1	0.637	0.598	0.525	-0.068
	POPF	30	5.375	6	0.633	0.644	0.595	0.017
	POPG	30	5.5	2	0.767	0.682	0.612	-0.127
	Average	30	4.5	2.2	0.705	0.634	0.567	
	POPH	30	5	1	0.504	0.615	0.558	0.183**

N, number of individuals sampled; A, mean number of alleles per population; Pr, number of private alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; PIC, polymorphic information content; F_{IS}, inbreeding coefficients per population; **, P < 0.01; POPA, POPB, POPC, POPD, POPE, POPF, POPG, POPH are abbreviations of population A, B, C, D, E, E, G.

Table II. Pairwise F_{ST} values among the eight populations of channel catfish inferred from microsatellites.

Population	POPA	POPB	POPC	POPD	POPE	POPF	POPG	POPH
POPA	-							
POPB	0.112**	-						
POPC	0.110**	0.226**	-					
POPD	0.115**	0.233**	0.163**	-				
POPE	0.040**	0.168**	0.170**	0.184**	-			
POPF	0.103**	0.157**	0.202**	0.190**	0.166**	-		
POPG	0.063**	0.105**	0.166**	0.132**	0.098**	0.125**	-	
POPH	0.125**	0.157**	0.155**	0.186**	0.205**	0.081**	0.119**	-

** $P < 0.01$. For abbreviation, see Table I.

POPA and POPE and the highest differentiation (F_{ST} = 0.233) was detected between POPB and POPC (Table II). In addition, there were relatively low genetic differentiation between POPA and POPG (F_{ST} = 0.063), POPE and POPG (F_{ST} = 0.098), and POPF and POPH (F_{ST} = 0.081), respectively (Table II).

The UPGMA dendrogram displayed five major clusters (Fig. 1). The POPA and POPE, POPF and POPH, and POPC and POPD were clustered into clade I, IV, and V, respectively (Fig. 1). POPG and POPB was clustered as an independent clade (II and III), respectively, indicating a relatively high level of differentiation with other populations (Fig. 1).

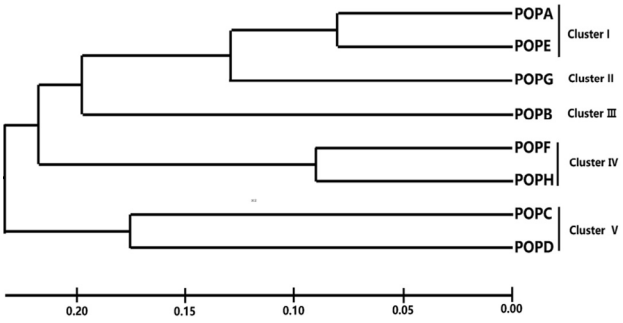

Fig. 1. UPGMA phylogenetic tree based on Nei's genetic distance among eight populations of channel catfish.

Bayesian analyses of population structure revealed the presence of six distinct clusters (Fig. 2). The POPB, POPC, POPD, and POPG formed distinct genetic groups, respectively. The two other clusters included the POPA and POPE populations, and the POPF and POPH populations, respectively (Fig. 2).

DISCUSSION

Genetic diversity is of great importance to the sustainability of many species (Hamrick *et al.*, 1991). The evaluation of genetic diversity and relationships might provide guidance for germplasm resource protection and utilization during breeding for channel catfish. Zhao *et al.* (2011) investigated the genetic diversity in five channel catfish populations introduced from USA using 8 microsatellite loci and found that the populations had high level of genetic diversity (PIC = 0.576-0.687; *He* = 0.634-

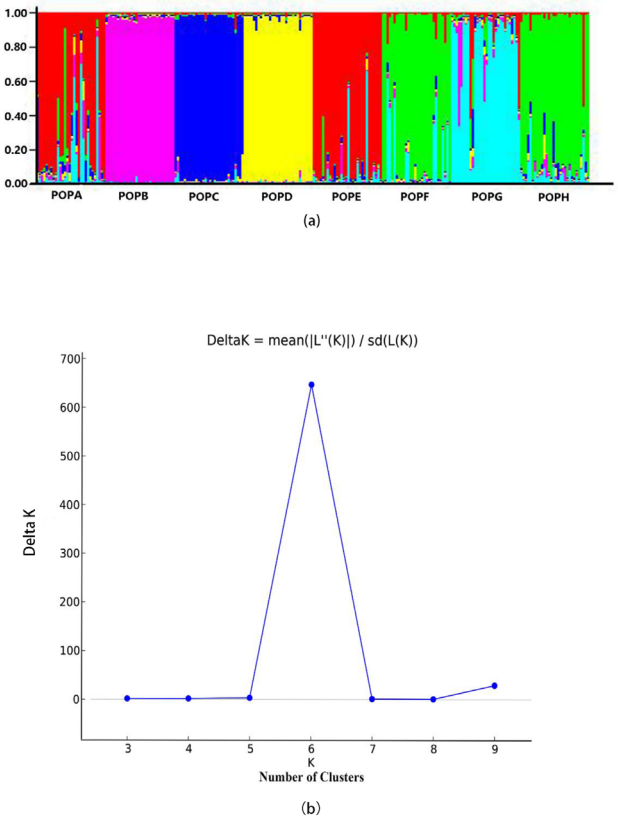

Fig. 2. Results of Bayesian analysis (structure) of channel catfish populations based on eight microsatellite loci. (a) Strucure clustering analysis with K=6. (b) Delta K values from structure analysis.

0.732). Cui *et al*. (2012) concluded that three channel catfish populations introduced from USA had great potentials as parental fish in breeding (PIC = 0.614-0.726; *He* = 0.739-0.757). Qin *et al*. (2011) also reported that the genetic diversity index (haplotype diversity = 0.92857) of five populations introduced from abroad was high based on mitochondrial DNA control region. The above studies suggested that the channel catfish populations introduced from USA had a high level of genetic diversity and could be served as parental fish in breeding program. In our study, the level of genetic diversity (PIC = 0.662-0.773, *He* = 0.720-0.781) of the two introduced populations (POPA and POPE) is very similar to that of the previous studies for the introduced populations of channel catfish. Thus, POPA and POPE populations possess high genetic variability and have potentials as parental fish. It was further supported by the presence of 24 private microsatellite alleles in POPE and 12 in POPA. However, for POPA population, the value of *Ho* (0.692) was lower than *He* (0.781), and its' F_{IS} value is positive, which indicates a deficiency of heterozygotes. Thus, the inbreeding events might have occurred in POPA. Although limited data was obtained for the effects of inbreeding in POPA, it should be considered as an important factor to threat the persistence of the population. Therefore, POPA should be enlarged to effective population size to avoid inbreeding depression.

Multiple generations and limited parental fish will result in changes of genetic diversity during breeding processes. Simmons *et al*. (2006) reported that wild channel catfish populations in Alabama, United States exhibited higher levels of polymorphisms and heterozygosities than the domestic populations. The genetic diversity of channel catfish cultured in China is relatively lower than that of the wild populations in USA when compared with previous studies (He *et al*., 2008b). In the present study, the five populations (POPB, POPC, POPD, POPF, and POPG) (mean value of *A* = 4.5, PIC = 0.567, *Ho* = 0.705, and *He* = 0.634) from the breeding program have a lower degree of genetic diversity than the two introduced populations (POPA and POPE) (mean value of *A* = 7.5, PIC = 0.698, *Ho* = 0.717, *He* = 0.751) (Table I). As reported for other fishes (Kubota and Watanabe, 2003), poor-breeding practices with few numbers of parental fish in reproduction appeared to be the main explanation for the reduced genetic variation of the five populations. Nevertheless, POPB, POPC, POPD and POPG populations represented the heterozygote excess (*Ho* > *He*), indicating the breeding effect in some degree in channel catfish. However, for a successful breeding program, using appropriate sets of breeders to establish founder broodstock is critical for maintaining the genetic diversity in the hatchery stocks (Lorenzen *et al*., 2012). It is still necessary to found appropriate broodstocks to enhance the genetic variations of channel catfish cultured in China.

Knowledge of the genetic relationships among different populations is also useful for the breeding projects. Select mating parental fish according to the genetic relationships could avoid inbreeding effectively and ensure populations have long-term genetic diversity for future generations (Domingues, 2018). In the present study, F_{ST} values were found to be significantly different ($P < 0.01$) among all populations. Wright (1984) divided the range of F_{ST} values into four layers: 0-0.05 (weak genetic differentiation), 0.05-0.15 (moderate genetic differentiation), 0.15-0.25 (high genetic differentiation), and above 0.25 (great genetic differentiation). Regarding these, weak genetic differentiation was found between POPA and POPE (F_{ST} = 0.04), which was in accordance with the results that POPA and POPE had closer relationship inferred from UPGMA dendrogram and structure analysis. It is probably related to similar brood stocks' sources during its founding in the USA. The result was consistent with that the genetic relationships for channel catfish populations introduced from abroad had no obviously geographical differentiation (Qin *et al*., 2011). Also, closer genetic relationship between POPF and POPH might be attributed to the same source of the parental fish in the breeding program.

According to the genetic relationships among the populations, we suggested that POPA and POPE, POPE and POPG should not be mated with each other in order to avoid inbreeding depression. However, POPB, POPC, and POPD could be mated with POPA, POPE and POPG. In addition, POPH population as a variant of channel catfish is not appropriate for breeding due to serious inbreeding and lower genetic diversity compared to POPB, POPC, POPD and POPG.

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Statement of conflict of interest

The authors declare no conflict of interest.

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