Microsatellite Variation among Domesticated Populations of Channel Catfish (*Ictalurus punctatus*) and Blue Catfish (*I. furcatus*)

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ABSTRACT

Genetic variability between and within 15 and 5 domesticated lines and strains of channel catfish *Ictalurus punctatus* and blue catfish, *I. furcatus* respectively was examined utilizing eight microsatellite loci. The channel catfish populations were more variable than blue catfish populations. Pairwise comparisons revealed significant genetic differentiation in all population pairs. Substantial genetic differentiation over all population of channel catfish and blue catfish was observed (F_{ST} = 0.2043 and 0.2371 respectively). Among channel catfish populations, there were five main clusters: 1) GK, AS and GKal, 2) S1, S2 and MR, 3) MS, TA and ARMK, 4) T, AF and AR1, and, 5) KR, MK and KS. Among blue catfish, there were two clusters: 1) ARR and DxR, and 2) D, R and AR2. The analysis of molecular variance (AMOVA) indicated slight genetic variability at the group level, channel catfish and blue catfish group (15.05%), population level of channel catfish (20.42%) and blue catfish (23.71%), and individual level of channel catfish (14.16%) and blue catfish (2.13%). Knowledge of the genetic composition of domesticated populations can be applied to maintain and monitor genetic variation, genetic conservation, and be incorporated into breeding programs.

Keywords: microsatellites, population differentiation, channel catfish, blue catfish, genetic variation

INTRODUCTION

Channel catfish, *Ictalurus punctatus*, and blue catfish, *I. furcatus*, are indigenous species to the southern United States with channel catfish being the primary species used commercially in major catfish producing states such as Alabama, Arkansas, Louisiana and Mississippi. Among the several genetic marker systems, microsatellites are a powerful tool to detect genetic variability within and among populations (DeWoody and Avise, 2000). They are widely dispersed in the genome, both in protein-encoding and noncoding DNA. The mutation rate for microsatellite regions is estimated at 10^{-2}-10^{-6} per locus per generation (Ellegren, 2000). The population differentiation detected with microsatellites has been greater than with previous markers such as allozymes (Alarcon *et al.*, 2004). Microsatellites have become a useful tool in many studies involving population structure assessment (Nelson *et al.*,

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1998; Bagley *et al.*, 1999; Beacham *et al.*, 2000; Sekino and Hara, 2001; Salgueiro *et al.*, 2003; Alarcon *et al.*, 2004), broodstock management (Jackson *et al.*, 2003), evolutionary relationships, genetic mapping (Zimmerman *et al.*, 2005), parentage assessment, and population identification (DeWoody and Avise, 2000).

Microsatellites successfully differentiated wild and domestic populations of Atlantic salmon, *Salmo salar* (Norris *et al.*, 1999), and gilthead sea bream (Alarcon *et al.*, 2004). The domestic populations were less variable than the wild (Skaala *et al.*, 2004). Microsatellites are useful for the study in genetic stocks, broodstock selection, constructing dense linkage maps and mapping economically important quantitative traits (Chistiakov *et al.*, 2006). The potential of microsatellites can reveal the differentiation in species-specific level by the private alleles (Norris *et al.*, 1999; Kim *et al.*, 2004). Norris *et al.* (1999) determined that microsatellite variation was smaller in farmed Atlantic salmon compared to wild Atlantic salmon, but mean heterozygosity was similar. Microsatellites were superior to mtDNA analysis for differentiating various domesticated Nile and red tilapia hybrid lines (Romana-Eguia *et al.*, 2004).

Waldbieser and Bosworth (1997) examined microsatellite variation in a single wild population, several commercial populations and selectively bred research line of channel catfish. In general, the wild population had the highest number of alleles. The primary objective of the current study on ictalurid catfish was to contrast genetic variability within and among channel and blue catfish populations utilizing eight microsatellite loci.

MATERIALS AND METHODS

Collection of samples

Blood samples (0.5 ml) from 15 populations (n=325) of channel catfish and 5 populations (n=86) of blue catfish were collected. These populations were maintained at the EW Shell Fisheries Research Unit, Auburn University (Dunham and Smitherman, 1984). Population names, abbreviation and the number of samples used this study are shown in Table 1.

DNA extraction

A 0.5 ml sample of blood collected from each individual from the caudal vein was transferred to a 50-ml centrifuge tube with digestion buffer, containing 100 µg/ml proteinase K (Liu *et al.*, 1998). DNA was extracted from the blood samples using Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). The procedure was done according to instructions from the manufacturer with the following modifications: the DNA was collected in 300 µl of protein precipitation solution and precipitated in 600 µl of isopropanol alcohol. The pellet was washed in 70% cold ethanol. The DNA was resuspended in 50 µl of Tris-EDTA (pH 8.0) buffer and kept at -20°C until PCR amplification.

Microsatellite analysis

Eight microsatellite loci di, tri, and tetranucleotides, used in this study were previously studied in the Catfish Genome Project (Ju *et al.*, 2000). Sequence and primer details are found in Table 2. AU904 is embedded in an unknown spleen gene; AU935, AU936, AU954, AU959 are from unknown brain genes; AU865 is embedded in an unknown skin gene while AU1097 and AU1081 are likely from non-coding regions.

Polymerase chain reaction (PCR) was performed in 384-well plates containing 5 µl reaction mixture. Each reaction mixture consisted of 0.9 µl of 50 ng purified template DNA, 1x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 µl of 100 ng/ml of upper tailed (UT) and lower (L) primers, 1 pmole of IRD-700 or IRD-800 dye labeled tail primer and 1 unit of JumpStart *Taq* DNA polymerase (Sigma, St. Louis MO) and 2.1 µl

Table 1. Species, name of population, number of each population and sample size for blue catfish, *Ictalurus furcatus* and channel catfish, *I. punctatus* analyzed for microsatellite variation.

Species	Number	Name of population	Number of sample
Channel	1	Tishmingo (T)	22
Catfish	2	Albino Forks (AF)	21
	3	Auburn-Rio Grande (AR1)	26
	4	Auburn Select (AS)	21
	5	Goldkist (GK)	14
	6	Goldkist Albino (GKal)	13
	7	Kansas Random (KR)	28
	8	Kansas Select (KS)	34
	9	Marion-Kansas (MK)	25
	10	Marion Select (MS)	11
	11	Tishmingo-Auburn (TA)	5
	12	Auburn-Rio Grande X Marion-Kansas (ARMK)	24
	13	Marion Random (MR)	28
	14	Kansas commercial S1 (S1)	27
	15	Kansas commercial S2 (S2)	26
	Total		**325**
Blue	16	D&B (D)	13
Catfish	17	Rio Grande (R)	11
	18	Auburn X Rio Grande (AR2)	20
	19	9-L (ARR)	12
	20	197 (DxR)	30
	Total		**86**

Table 2. List of primers, primer sequences, repeats in base-pairs, annealing temperature and amplicon size used for microsatellite analysis of blue catfish, *Ictalurus furcatus*, and channel catfish, *I. punctatus*. UT and L at the end of each primer name represent "upper tailed" primer and "lower" primer respectively. Tail sequences are underlined. AU904 is from an unknown spleen gene; AU935, AU936, AU954, AU959 are from unknown brain genes; AU865 is from an unknown skin gene, and AU1097 and AU1081 are likely from non-coding regions.

Primer name	Sequence	Repeated motif	Annealing temperature (°C)	Amplicon size (bp)
AU935UT	GAGTTTTCCCAGTCACGACCCGTTAAGACATAATGAGTAGGACG	$(CAA)_9$	53	118
AU935L	CGTACTGCAAAACATCATTTTCG			
AU936UT	GAGTTTTCCCAGTCACGACAACAGTATAGGGAAACCTGTTGAC	$(ATTC)_6$	53	177
AU936L	GTCACACACACACACATGCA			
AU904UT	GAGTTTTCCCAGTCACGACGGACATTGTTATGGTTTAGTGC	$(TAA)_7$	53	129
AU904L	CGCTGTGTGCGTTGGCTTTGC			
AU954UT	GAGTTTTCCCAGTCACGACAGCCCCTTACTCAGGGACTG	$(GT)_{12}$	53	179
AU954L	GCTGTGTGCGTTGGCTTTGC			
AU959UT	GAGTTTTCCCAGTCACGACACGATTTTCAGTTGAGCCACC	$(GATT)_{11}$	53	177
AU959L	GCAGCGTAAAAAAGAACCGAAGC			
AU865UT	GAGTTTTCCCAGTCACGACTGTTCTGTGTCTAAATGCTGCAC	$(TA)_{13}$	53	169
AU865L	CACTGTTCGATTACAAGTCCGG			
AU1097UT	GAGTTTTCCCAGTCACGACGACAGTGCAGCGTAGTGGAG	$(TG)_{15}$	55	141
AU1097L	CTTCGGTCTTCTCGAAAGTGG			
AU1081UT	GAGTTTTCCCAGTCACGACTGGAGGCGACAGGCAGGTGG	$(ATT)_{13}$	55	208
AU1081L	CATCAACTACAATATCAGCCGCAG			

sterile deionized water, was prepared for DNA amplification in an Eppendorf Mastercycler. Amplification conditions were the following: initial denaturation 3 min at 94°C, 40 cycles of 30 s at 94°C, 30 s at annealing temperature (53°C for AU935, AU936, AU904, AU954, AU959, and AU865 and 55°C for AU1097 and AU1081), 30 s at 72°C and a final extension of 5 min at 72°C. After amplification, reactions were denatured at 94°C for 3 min, loaded on LICOR automated DNA sequencers according to the manufacturer's recommendations. An IRD-700 or IRD-800 labeled size marker was added to each gel to determine relative mobility of amplified fragments from the microsatellite genotype data. Alleles were scored manually.

Genetic and statistical analysis

Allele frequencies and genotypic differentiation in each population were computed in GENEPOP version 3.4 program (Raymond and Rouset, 1995). Allelic richness and F is were computed with FSTAT program. Population differentiation between pairs of populations was evaluated with pairwise F_{ST} values by permutating individuals between samples for all loci combined (Weir and Cockerham, 1984) and populations pairwise (Slatkin, 1995) using FSTAT and ARLEQUIN version 3.01, respectively. The analysis of molecular variance (AMOVA) was used to detect the hierarchical differentiation among populations by ARLEQUIN version 3.01. The genetic distance was computed using PHYLIP version 3.66, including a dendrogram constructed by using the neighbour-joining (NJ) method. The bootstrap test of loci was performed for the NJ tree by recirculating the distance for all loci 1000 times.

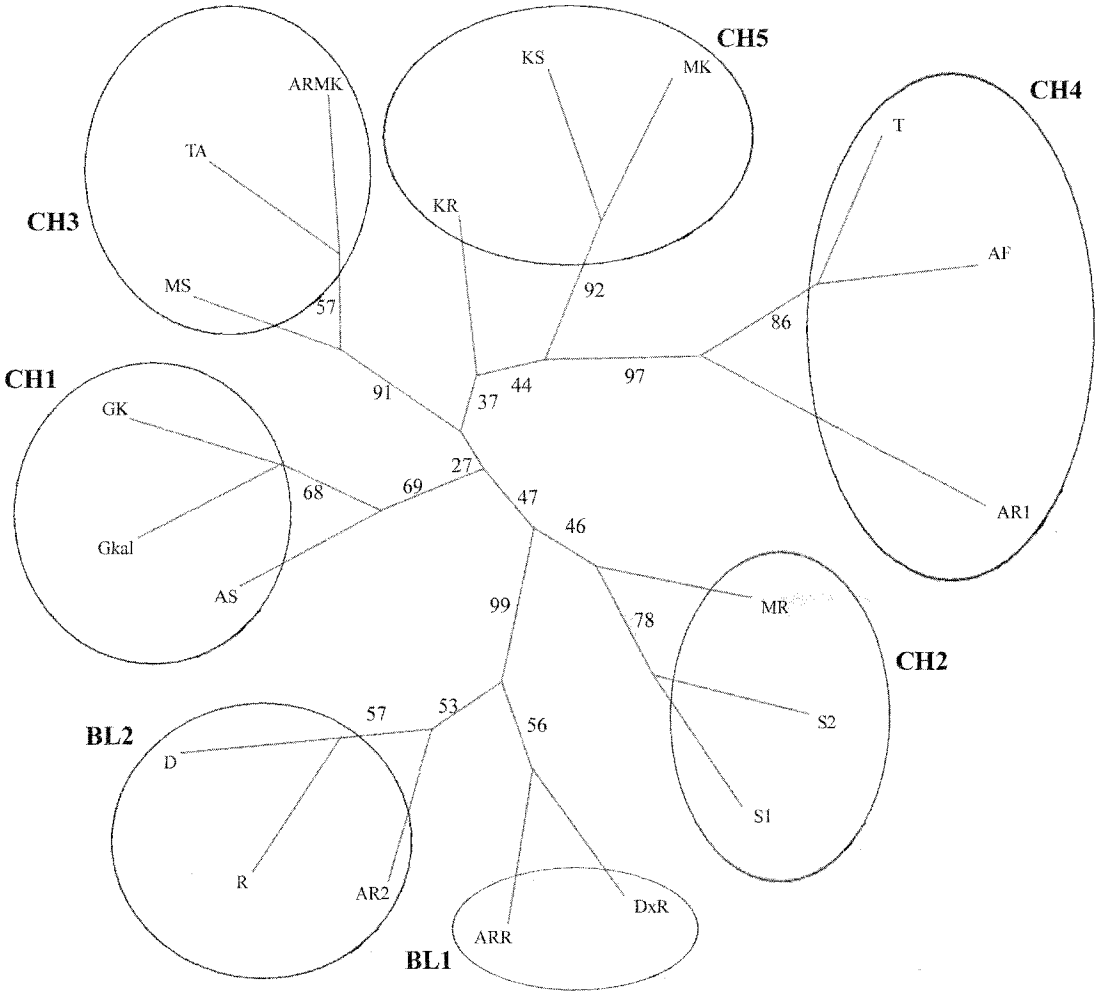

Figure 1. Neighbor-joining tree dendrogram based on Cavalli-Sforza's chord distance. The abbreviations for populations and strains are shown in Table 1.

RESULTS

Genetic variation within populations

The AU 1081 locus had the greatest number of alleles with 18, followed by AU865 (17 alleles), AU954 (15 alleles), AU959 (14 alleles), AU1097 (11 alleles), AU936 (8 alleles), and AU935 and AU904 (7 alleles). Allele size ranged from 121 to 139 bp for AU935, 140 to 196 bp for AU936, 134 to 152 bp for AU904, 191 to 233 bp for AU954, 153 to 209 bp for AU959, 148 to 188 bp for AU865, 146 to 180 bp for AU1097, and 203 to 254 bp for AU1081. Each one of eight microsatellite loci was polymorphic in every population for each species except for AU1081 in Rio Grande blue catfish, AU959 in ARR blue catfish and AU904 in ARMK, MS and MR channel catfish, which were fixed. Blue catfish had a higher observed incidence of fixed loci (5%) compared to channel catfish (2.5%). The number of alleles per locus per population ranged from 1 to 12 in channel catfish and from 1 to 9 in blue catfish. The highest number of alleles per locus was found in the MK line of channel catfish for locus AU1081 (12 alleles) and DxR of blue catfish for locus AU954 (9 alleles). The highest observed number of alleles across all eight loci was in MK line (55 alleles) of channel catfish and DxR (27 alleles) of blue catfish. However, when accounting for sample size, KR had the highest observed allelic richness, 4.12, and MK was third, 3.81, while DxR had the second highest observed allelic richness, 3.03, behind D, 3.08 (Table 3).

Genetic variation among populations

The variation between channel and blue catfish accounted for 15.05% of the genetic variation in the analysis (Table 4). The variation among populations within channel and blue catfish group were 20.42 and 23.71%, respectively. The variation among populations within and between the two species accounted for a cumulative total of 59.28% of the genetic variation. Variation among populations accounted for more of the variation than variation between species or individual genetic variation within populations.

Genetic variation among individuals within population (individual-level variation) in channel (14.16%) and blue catfish (2.13%) was a more minor component of variation (Table 4). F_{ST} value bootstrapped across overall loci exhibited significant population differentiation within the channel (0.2136, 95% CI, 0.131-0.304) and blue catfish (0.2905, 95% CI, 0.204-0.304) (Table 4). All pairwise F_{ST} differentiation analyses showed significant differentiation among lines, and most pairwise comparisons between populations locus by locus were significant. A neighbor-joining dendrogram based on Cavalli-Sforza's chord distance demonstrated clear differentiation between channel catfish and blue catfish species (Figure 1). Among channel catfish populations, there were five main clusters: 1) GK, AS and GKal; 2) S1, S2 and MR; 3) MS, TA and ARMK; 4) T, AF and AR1; and, 5) KR, MK and KS. Among blue catfish, there were two clusters: 1) ARR and DxR, and 2) D, R and AR2.

DISCUSSION

The allelic diversity in the current study had variable values in each population and the pattern of diversity differentiated somewhat compared to other studies on domesticated fish populations using microsatellites (Norris *et al.*, 1999; Was and Wenne, 2002; Lundrigan *et al.*, 2005). In this study, the MK population in channel catfish and the D, DxR and ARR populations of blue catfish possessed the highest mean number of alleles, while the KR population in channel catfish and the D population in blue catfish showed the highest allelic richness. The number of alleles in channel catfish populations (1-12) was slightly higher than in blue catfish populations (1-9). These numbers were slightly

Table 3. Genetic variability, mean number of alleles across loci (*A*), effective number of allele (*Ae*), allelic richness (*Ar*), and fixation index (*Fis*) for microsatellite loci of blue catfish, *Ictalurus furcatus*, and channel catfish, *I. punctatus*.

Population	*A*	*Ae*	*Ar*	*Fis*
T	5.75±1.98	3.75±1.22	3.70±0.81	0.111
AF	4.63±2.56	2.86±1.67	2.99±1.06	0.168
AR1	6.50±2.07	3.65±1.24	3.74±0.79	0.370
AS	5.25±2.25	3.04±1.24	3.31±1.01	0.158
GK	5.63±2.20	4.14±1.77	3.94±1.18	0.151
GKal	4.88±1.64	3.87±1.15	3.77±0.86	-0.025
KR	6.63±2.07	4.39±1.10	4.12±0.63	0.304
KS	6.13±2.53	3.97±2.01	3.69±1.25	0.306
MK	6.88±3.23	4.09±2.44	3.81±1.26	0.284
MS	4.00±1.41	2.58±0.98	2.96±0.98	-0.012
TA	4.00±1.41	3.16±1.32	3.71±1.23	0.026
ARMK	5.25±2.55	3.60±1.79	3.49±1.30	0.165
MR	4.63±2.13	3.16±1.22	3.20±1.10	-0.041
S1	5.25±2.19	3.04±1.30	3.29±0.86	0.289
S2	5.63±2.56	3.62±2.08	3.47±1.33	0.229
D	3.25±1.04	1.87±0.46	3.08±0.97	0.211
R	2.88±0.84	1.73±0.57	2.75±0.79	0.015
AR2	3.25±0.89	1.99±0.77	2.84±0.97	-0.132
ARR	3.00±1.51	2.24±1.02	2.89±1.43	0.253
DxR	3.38±2.39	2.42±1.44	3.03±1.66	0.211

Table 4. Hierarchical analysis of AMOVA for channel catfish, *Ictalurus punctatus*, and blue catfish, *I. furcatus*.

Source of variation	Variance component	Percentage variation	Fixation indices
Among group (channel and blue)	0.40840	15.05	$F_{CT} = 0.15052$
Among population within channel or blue	0.52484	19.34	$F_{SC} = 0.22772$
Among individuals within Population	0.21548	7.94	
Among population within channel	0.57643	20.42	$F_{ST} = 0.2136$
Among individuals within channel population	0.39966	14.16	
Among population within blue	0.20262	23.71	$F_{ST} = 0.2905$
Among individuals within blue population	0.01823	2.13	

lower compared to the number of microsatellite alleles found in domestic populations of sea trout, *Salmo trutta* (3-15) (Was and Wenne, 2002), Arctic charr, *Salvelinus alpinus* (3-12) (Lundrigan *et al.*, 2005) and Asian Nile (3-20) and red hybrid tilapia (3-13) (Romana-Eguia *et al.*, 2004).

In an earlier study on channel catfish, Waldbieser and Bosworth (1997) found that a wild population had higher number of alleles than six commercial populations and a selectively bred research line of channel catfish. The wild farm and research populations averaged 9, 3.5-7.8 and 5.3 microsatellite alleles per locus, respectively. There appears to be a loss of genetic variation when going from wild to domestic populations and possibly to research populations that were selected. In general, Waldbieser and Bosworth (1997) detected more alleles per locus than in the current study with channel catfish having 1-6 alleles, in general, with a mean of about 3 alleles/locus. Two subsamples of a commercial population, randomly bred research populations, selectively bred research populations and a selected commercial population recently reduced to a research population were represented in the current study. There were no obvious differences in overall allele number among these indicating that the differences in population sizes did not appear to have an effect on allele number in channel catfish. However, species differences were apparent. Channel catfish had about twice as many alleles as blue catfish. Blue catfish are harder to spawn in captivity so there is the possibility of a greater amount of adverse effects on allele number due to founder effects. In support of this, DxR, a cross between two strains did possess the greatest number of total alleles. Additionally, the channel catfish line, MK, with the greatest number of alleles was also of mixed ancestry.

The allozyme analysis of channel catfish ancestral to the current research lines indicated an F_{ST} of 0.240 (Hallerman, 1984) and for AFLP analysis showed that F_{ST} averaged over all loci of domestic populations was 0.176 (Mickett *et al.*, 2003). These results for F_{ST} are remarkably similar to that averaged over all loci of channel catfish (0.21) and blue catfish (0.29) in the current microsatellite study. These various lines appear to be maintaining a similar level of differentiation over time, and isozymes, AFLP and microsatellites appear equally effective in identifying the population structure of channel catfish. F_{ST} for domestic and wild populations of channel catfish in Alabama utilizing AFLP (0.36) (Simmons *et al.*, 2006) appears higher than that for the Auburn research lines. This seems logical as genetic differences would be expected to be greater between commercial channel catfish (whose ancestry for the most part is from outside of Alabama (Dunham and Smitherman, 1984), and wild fish from Alabama than among research lines selected in a common way and their control populations. Additionally, one wild population, Yellow River, (Simmons *et al.*, 2006) was especially unique genetically, which drove the F_{ST} value higher.

The pairwise F_{ST} and genetic distances demonstrated that genetic differentiation among populations was substantial in the current study. However, use of the microsatellites to determine divergence of channel and blue catfish (F_{CT} = 0.15052) showed significance, but the F values were relatively small considering that these are two separate species. There was a large number of shared alleles between the two species that probably accounted for this result. Other genetic markers such as isozymes may actually be more powerful for distinguishing ictalurid catfish species. The cumulative differences were substantial enough that the dendrogram revealed separate clusters for channel and blue catfish.

Genetic differentiation (as indicated from the source of variation) among populations within channel catfish (20.42%), within blue catfish (23.71%) and within both (15.05%) was the major component of genetic variation (total =59.18%). Variation among populations accounted for more of the variation than variation between species or individual genetic variation within populations (14.16%) for channel catfish and 2.13% for blue catfish.

Future research might more thoroughly examine wild channel catfish and blue catfish to verify some of the hypotheses generated from the current data set. Microsatellite variation from neutral sites in the genome and those embedded in genes should also be contrasted for their variation and how they have responded to differing breeding histories.

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