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Comparison of domestic and wild channel catfish (*Ictalurus punctatus*) populations provides no evidence for genetic impact

Micah Simmons, Kathryn Mickett, Huseyin Kucuktas, Ping Li, Rex Dunham, Zhanjiang Liu *

Fish Molecular Genetics and Biotechnology Laboratory, 203 Swingle Hall, Department of Fisheries and Allied Aquacultures and Program of Cell and Molecular Biosciences, Aquatic Genomics Unit, Auburn University, Auburn, AL 36849, USA

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Abstract

Genetic diversity of wild channel catfish (*Ictalurus punctatus*) populations was analyzed using AFLP markers and compared to that of domestic catfish populations to determine the genetic impact of domestic catfish on wild catfish populations. Fourteen wild populations within various watersheds of Alabama were analyzed using five AFLP primer combinations. A total of 396 polymorphic bands were detected from 269 individuals, with an expected mean heterozygosity of 0.16. The percentage of polymorphic bands varied greatly among populations, from 32.2% to 85.0%. The estimated level of population differentiation as measured by average F_{ST} value across all loci was 0.36. The 14 tested wild populations were related with 88% similarity as revealed by Nei's (Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590) unbiased population-wise identity measures. In order to analyze the interactions between domestic and wild populations of channel catfish, 191 polymorphic bands were used to evaluate 569 individuals from 31 populations. The wild populations exhibited higher levels of polymorphisms and heterozygosities than the domestic populations. Strong genetic structures were associated with the geographical distribution of samples, with all samples from a single watershed being closely related. The domestic populations were all related to one another, forming a single branch in the phylogenetic analysis, while all but the Tennessee River populations of many wild populations were more related to one another than to domestic populations. Genetic identities of wild fish from proximal and distal sites were similar, while both wild populations differed from the nearby domestic catfish populations, providing no molecular genetic evidence for apparent impact of domestic catfish on wild populations.

Keywords: Genetic resource; Fish; AFLP; Diversity; Polymorphism; Marker; Catfish; Population

1. Introduction

Molecular markers are useful for population genetic studies to assess influences of various factors on genetic diversity and population structure, such as historic and demographic factors (Avise, 1994) anthropogenic stressors (Whitehead et al., 2003; Bagley et al., 2001; Anderson et al., 1994; Bickham and Smolen, 1994; Fox, 1995) and artificial stocking (McCracken et al., 1993; Englbrecht et al., 2000). Among many types of molecular markers (for a recent review, see Liu and Cordes, 2004), amplified fragment length polymorphism (AFLP) and microsatellites have been demonstrated as useful tools for population genetic studies of fish (Whitehead et al., 2003; Liu, 2003). We selected

* Corresponding author. Fax: +1 334 844 9208.
E-mail address: zliu@acesag.auburn.edu (Z. Liu).

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AFLP because it is easy, fast, inexpensive and robust. In addition, use of AFLP markers with the same primer combinations would allow comparison of results of this study with previously published results regarding genetic diversity of domestic channel catfish *Ictalurus punctatus* (Mickett et al., 2003). In spite of their occasional distribution in clusters in the catfish genome, the large numbers of AFLPs can provide good coverage of the genome (Liu et al., 2003) that would be otherwise costly using other molecular marker systems (Liu and Cordes, 2004). AFLP markers have been used to assess genetic variation in *Morone* and *Thunnus* species (Han and Ely, 2002) and in Asian arowana (Yue et al., 2002). They have also been used to study introgression and hybridization (Liu et al., 1998b; Rogers et al., 2001; Congiu et al., 2001; Young et al., 2001), maternal contribution (Felip et al., 2000), and analysis of strains, morphotypes, and species (Chong et al., 2000; David et al., 2001; Kai et al., 2002) in various fish species.

Genetic diversity is of great importance to the sustainability of populations (Hamrick et al., 1991). Genetic variation within populations can be lost through genetic drift (Allendorf et al., 1987), a process intensified when population size becomes small. Genetic variation among populations can be lost when previously restricted gene flow between populations is increased by stocking or removal of natural barriers, causing differentiation between populations to be lost as a result of the homogenization of two previously distinct entities (Altukhov and Salmenkova, 1987; Campton, 1987; Utter, 2003).

The primary two procedures for maintaining genetic diversity have been the establishment of ex situ gene banks and maintenance of in situ natural genetic resources. However, conservation of genetic diversity through the use of gene banks has been hindered by many factors including limited facilities, expense, and difficulties involved in the preservation of female gametes. While storage of sperm has been relatively straightforward, cryopreservation of fish eggs has been difficult due to their large sizes (Tiersch et al., 1994). It is believed that sustainability of natural genetic resources is fundamentally important for the preservation of genetic diversities.

The integrity of natural fish populations can be significantly affected by anthropogenic activities. Not only anthropogenic stressors can have a significant impact on populations (Palumbi, 2001), but also do routine human activities such as artificial stocking or accidental release of domestic populations into the wild environment. Direct genetic interactions between wild and hatchery fish have been demonstrated in many studies, with the best examples being from studies with salmon. Hatchery-propagated Atlantic salmon were found to compete directly with native salmon for resources such as space, food or mates, alter predation regimes and transfer diseases and parasites (Fleming et al., 2000). The genetic impact of cultured Atlantic salmon (*Salmo salar*) was manifested as a loss of genetic diversity among wild populations (Hindar, 2001). Hatchery-released chum salmon (*Oncorhynchus keta*) account for the vast majority (90%) of salmon caught by Japanese fishermen. These hatchery fish also had much impact on the performance of the wild populations (Hindar, 2001). Rivers heavily supplemented with hatchery salmon have shown a steady decrease in egg size (Heath et al., 2003). A reduction in fecundity and productivity of wild populations was also reported (Brodeur and Busby, 1998). Wild coho salmon (*Oncorhynchus kisutch*) populations off the Oregon coast have suffered reductions in productivity and genetic diversity because of hatchery supplementation (Dehart, 1999). Introgression of straying hatchery chinook salmon (*Oncorhynchus tshawytscha*) has threatened the spring-run population in the Sacramento River (Banks et al., 2000). These cases demonstrated problems that may rise in other species as well (Utter, 2003).

Channel catfish (*I. punctatus*) is the most important aquaculture species in the United States, accounting for more than 60% of all aquaculture production (USDA, 2001). It is also an important game fish (ranking within the top three sport fish), with a broad geographic range encompassing a variety of habitats (Dunham and Smitherman, 1984). Despite its economic and ecological importance, documentation of genetic diversity for this species has been minimal. Early studies focused on its genetic variation using isozyme markers, and in some cases as it related to selection for body weight (Dunham and Smitherman, 1984; Hallerman et al., 1986; Carmichael et al., 1992). More recent studies have demonstrated the applicability of several PCR-based markers, including microsatellites (Liu et al., 1999c; Serapion et al., 2004), random amplified polymorphic DNA (RAPD) (Liu et al., 1998a, 1999a), and amplified fragment length polymorphism (AFLP) (Liu et al., 1998b, 1999b). In a recent study, we determined the level of genetic variation both within and among populations of domestic channel catfish using AFLP markers (Mickett et al., 2003). While greater heterozygosities were found among aquaculture stocks than previously reported, perhaps as a result of the greater differentiating power of AFLP markers, most aquaculture stocks shared high genetic similarities, suggesting that the domestic catfish stocks were narrow in genetic

background. The rather homogeneous domestic catfish population, when coupled with numbers of over a billion fish produced annually, raised concerns of potential impact of the domestic catfish on wild populations. Smitherman and Dunham (1993) noted the possibility that escape of cultured catfish with flooding natural waters into aquaculture facilities may cause genetic influences and competitive interactions on indigenous populations of channel catfish (Smitherman and Dunham, 1993), though evidence for such interactions was lacking. The objectives of the current study was to determine the level of genetic variation among wild channel catfish populations and to compare genetic similarities of the domestic and wild populations to assess potential interactions and impact of domestic channel catfish on wild populations. Here we report that the wild channel catfish populations harbor a greater level of genetic variation than their domestic counterparts, and that there is no evidence of apparent impact of domestic channel catfish on wild populations.

2. Materials and methods

2.1. Wild and domestic fish samples

In order to determine potential impact of domestic channel catfish on the genetic make-up of the wild populations, samples were collected from both domestic and wild populations. Domestic catfish were collected from major aquaculture production sites, as well as major fingerling supplier sites as previously reported (Mickett et al., 2003). For each domestic catfish site, two wild sites were selected, whenever possible: one was "proximal" to the domestic catfish sites, and the other "distal" to the domestic catfish sites. In selection for the sampling sites, not only the distances between the wild populations and the catfish farms were considered; considerations were also made to include distal sites with migration barriers such as dams. A proximal site was selected downstream from the domestic catfish farms. Two distal sites were selected upstream of the

Table 1
Summary of all samples from wild and domestic populations (all are Alabama counties and watersheds, except for AU, the Auburn University hatchery, located in Lee County, AL)

Population number	Population name	Nature of population	Number sampled	Watershed/farm/hatchery
1	Cahaba	Wild	23	Cahaba River Watershed
2	Geneva	Wild	11	Coastal Plain Watershed
3	Pea River	Wild	19	Coastal Plain Watershed
4	Guntersville	Wild	29	Tennessee Watershed
5	Wheeler	Wild	30	Tennessee Watershed
6	Weiss-below-dam	Wild	30	Coosa Watershed
7	Weiss-above-dam	Wild	15	Coosa Watershed
8	Judy creek	Wild	16	Choctawhatchee Watershed
9	Sougahatchee	Wild	23	Tallapoosa Watershed
10	Frank Jackson	Wild	10	Coastal Plain Watershed
11	Yellow River	Wild	8	Coastal Plain Watershed
12	Black Warrior	Wild	30	Tombigbee/Black Warrior
13	Lake Martin	Wild	26	Tallapoosa Watershed
14	Demopolis	Wild	25	Tombigbee/Black Warrior
15	Marion Hatchery	Domestic	30	Marion State Hatchery
16	Geneva Hatchery	Domestic	30	Lake Geneva Fish Hatchery
17	Petit Farm	Domestic	29	Petit Farm
18	Davis Farm	Domestic	25	Davis Farm
19	State Cattle Ranch	Domestic	30	State Cattle Ranch
20	Blackbelt Farm	Domestic	23	Blackbelt Aquaculture Farm
21	Marion Random Strain	Domestic	9	Auburn University Hatchery
22	Albino Fork Strain	Domestic	10	Auburn University Hatchery
23	Auburn–Rio Grande × Marion–Kansas	Domestic	10	Auburn University Hatchery
24	Tishmingo Strain	Domestic	10	Auburn University Hatchery
25	Goldkist Strain	Domestic	10	Auburn University Hatchery
26	Auburn Strain	Domestic	9	Auburn University Hatchery
27	Marion–Kansas	Domestic	10	Auburn University Hatchery
28	Tishmingo–Auburn	Domestic	10	Auburn University Hatchery
29	Auburn–Rio Grande	Domestic	8	Auburn University Hatchery
30	Easterling Farm	Domestic	29	Easterling Farm
31	Hicks Farm	Domestic	30	Hicks Farm

domestic catfish farm and, wherever possible, upstream of a migration barrier. A total of 539 fish from 31 populations were collected across the state of Alabama representing eight watersheds and many of the major suppliers of channel catfish fingerlings within these watersheds. Of these fish samples, 295 were wild catfish and the remaining 244 were domestic catfish. The domestic catfish used in this study were those used for analysis of genetic resources in aquaculture catfish (Mickett et al., 2003). The locations and the abbreviations for the populations are listed in Table 1. Domestic fish were obtained from each of the seven major fingerling suppliers located in Barbour, Cherokee, Covington, Geneva, and Hale counties. Nine domestic strain samples were collected from each of the nine different strains at the Auburn University Hatchery: Auburn, Albino Fork, Auburn–Rio Grande, Auburn–Rio Grande–Marion–Kansas, Goldkist, Marion–Kansas, Marion Random, Tishomingo, and Tishomingo–Auburn (Table 1). These research populations had all been selected for growth for up to 6 generations, with the exception of two populations: Marion Random, which was randomly bred, and Albino Fork, which was selected for albinism.

Wild population samples were collected from eight major watersheds in Alabama (Table 1), and their geographical relationships with the domestic sites are shown in Fig. 1. Sample numbers varied mainly due to relative abundance in particular areas and sampling gear. Sampling gears used consisted of hook and line, hoop netting, backpack shocking, gillnetting, and boat shocking.

2.2. DNA isolation

Blood samples were collected from the caudal vein of each fish using 1-ml syringes. Each blood sample was immediately transferred to a 50-ml centrifuge tube with digestion buffer (Liu et al., 1998b) containing proteinase K at 100 μg/ml. The lysate was stored at room temperature until isolation of DNA. DNA was isolated using the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN), following manufacturer's instructions.

2.3. AFLP analysis

AFLP procedures were performed as described in the Gibco BRL AFLP Protocol (Life Technologies), with some modifications according to Liu et al. (1998b, 1999b). Five primer combinations were used (E=*Eco*R1, M=*Mse*1): E-AAG/M-CAC, E-AAG/M-CAT, E-ACA/M-CAG, E-ACA/M-CTC, and E-ACT/M-CTG (Liu et al., 1998b; Table 2). AFLP products were analyzed on LI-COR automated DNA sequencers. Images were analyzed using AFLP-Quantar™-Pro 1.0 (KeyGene Products, Netherlands). Based on a comparison to manual scoring, parameters within the QuantarPro program were set so that fragments were considered present (+) if the band ratio r_b was such that $r_b \geq 0.25$, and absent (−) if $r_b < 0.09$. Fragments that did not fall within these parameters were considered questionable data (?) and not used. The fragment scoring was checked manually subsequent to computer analysis.

2.4. Identification of potentially useful AFLP markers for population identification

The robustness of AFLP allowed the generation of several hundreds of bands for evaluation. In order to identify AFLPs characteristic of certain populations, we initially attempted to identify fixed AFLP markers within the studied populations, i.e., the AFLPs specific to certain populations. It turned out that there were no such AFLPs among the several hundreds of AFLP bands evaluated. Therefore, we took a quantitative approach for the identification of highly differential AFLPs. If matching AFLPs have highly differential allele frequencies among populations, then they could prove valuable for identification of the populations if used in combination. We used two criteria for the identification of potentially useful AFLPs: first, the highest allele frequency of the AFLP in a population must be at least 0.5 among the 14 populations studied; and second, this allele frequency (≥ 0.5) should be at least five times more frequent in this population than in any other population, with an allowance for two exceptions if two populations were collected from a single watershed.

2.5. Data analysis

Fragment data were transferred from AFLP-QuantarPro to a binary (1/0) data matrix. The confidence of branch support was then evaluated by way of bootstrap analysis with 1000 replications, performed with the PAUP software package (Swofford, 2001, version 4).

Average heterozygosities (H), percent polymorphic loci (P), and F_{ST} values were estimated using the TFPGA program (Miller, 1997). Average heterozygosity estimates were calculated for each locus and then averaged over loci according to Nei's unbiased

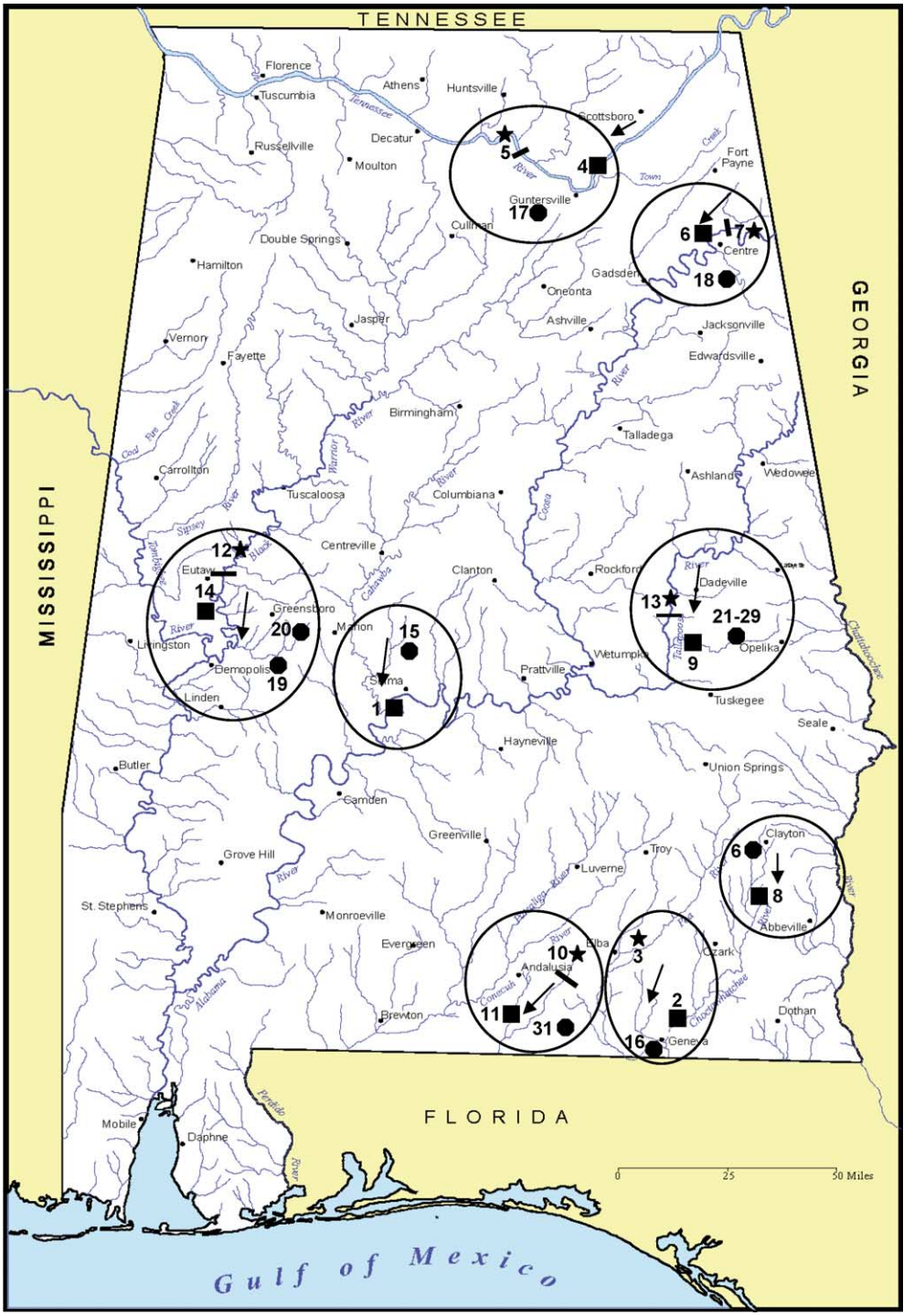

Fig. 1. Schematic presentation of sampling sites within various watersheds (open circles). Solid circles indicate domestic populations; solid squares indicate wild populations proximal to the catfish farms; stars indicate wild populations distal to the catfish farms. Arrows indicate directions of water flow; black bars indicate location of physical barrier (dams).

heterozygosity (Nei, 1978). The percentages of polymorphic loci were estimated based on the percent of loci not fixed for one allele. F_{ST} estimates were calculated following the method of Weir and Cockerham (1984). Confidence intervals were generated by bootstrapping analysis at the 99% confidence level with 1000

Table 2
Primer combinations and primer sequences used for AFLP analysis

Primer combination	Primer sequences
*Eco*RI-AAG/*Mse*I-CAC	AGACTGCGTACCAATTCAAG/ GATGAGTCCTGAGTAACAC
*Eco*RI-ACA/*Mse*I-CAG	AGACTGCGTACCAATTCACA/ GATGAGTCCTGAGTAACAG
*Eco*RI-ACT/*Mse*I-CTG	AGACTGCGTACCAATTCACT/ GATGAGTCCTGAGTAACTG
*Eco*RI-AAG/*Mse*I-CAT	AGACTGCGTACCAATTCAAG/ GATGAGTCCTGAGTAACAT
*Eco*RI-ACA/*Mse*I-CTC	AGACTGCGTACCAATTCACA/ GATGAGTCCTGAGTAACTC

replications. Genetic distances between populations were calculated by Nei's unbiased distance and identity measures (Nei, 1978). The similarity matrix produced by TFPGA was then imported into the NTSYSpc software (Rohlf, 1998) to produce a similarity tree showing the relationships between sampled populations.

3. Results

3.1. AFLP polymorphism within wild populations

Five AFLP primer combinations were used to genotype 295 catfish collected from 14 wild populations from eight watersheds (Fig. 1), of which 26 fish were omitted from analysis because of a high incidence of questionable bands. A total of 396 bands were identified across the remaining 269 fish. All 396 bands were polymorphic, with an expected mean heterozygosity of 0.17. The expected mean heterozygosity and percentage polymorphic loci for each population are summarized in Table 3. Large variations were observed among populations with regard to genetic diversity. The population with the greatest percentage polymorphic loci (85%) and highest expected mean heterozygosity (0.23) was the Guntersville Reservoir population collected from the Tennessee watershed in northern Alabama. The population with the lowest percentage polymorphism (32.2%) and the lowest expected mean heterozygosity (0.12) was the Judy Creek population collected from the Yellow River watershed in the coastal plain watershed in southern Alabama. Higher genetic variation is not surprising because the Tennessee River has a larger catfish population than Judy Creek.

For wild channel catfish populations, the polymorphic loci and the heterozygosities were calculated by using both sets of 396 AFLP markers and 191 AFLP markers. For domestic populations, the polymorphic loci and the heterozygosities were calculated by using only the 191 AFLP markers that could be screened for their genotypes on both the wild and the domestic populations. The polymorphic loci and the heterozygosities calculated by using 396 markers and by using 191 markers were very similar, indicating that 191 markers had a good coverage of the genome for population genetic analysis (Table 3).

3.2. Genetic structure of wild populations

A total of 396 AFLP markers were used to determine the genetic structure of wild populations. Calculated θ values and results from statistical analysis indicated significant structuring of genetic diversity among sampled populations of wild channel catfish. The estimated

Table 3
Expected mean heterozygosity and percentage polymorphic loci for 14 wild and 17 domestic populations of *I. punctatus* across 191 AFLP loci (for both wild and domestic populations), and 396 AFLP loci (for wild populations only)

Population number	*N*	*H* (191 markers)	% polymorphic loci (191 markers)	*H* (396 markers)	% polymorphic loci (396 markers)
1	23	0.1398	42.4	0.1414	43.2
2	11	0.1727	60.2	0.1772	58.4
3	19	0.1290	41.4	0.1411	44.9
4	29	0.2174	87.4	0.2277	85.4
5	30	0.1530	60.2	0.1575	61.1
6	30	0.2131	73.2	0.2133	72.4
7	15	0.1983	63.3	0.1992	62.5
8	16	0.1645	50.8	0.1656	51.8
9	23	0.1459	50.3	0.1387	46.1
10	10	0.1064	31.9	0.1208	34.2
11	8	0.1195	31.9	0.1181	32.2
12	30	0.1800	69.1	0.1788	71.4
13	26	0.1099	53.4	0.1186	53.7
14	25	0.2039	80.1	0.2059	79.5
15	30	0.1420	52.9		
16	30	0.1647	49.2		
17	29	0.1519	46.1		
18	25	0.1834	75.4		
19	30	0.1675	64.9		
20	23	0.1516	48.7		
21	9	0.1160	29.8		
22	10	0.0736	20.4		
23	10	0.0998	28.3		
24	10	0.0870	26.7		
25	10	0.1341	36.6		
26	9	0.1095	31.4		
27	10	0.1087	29.8		
28	10	0.0955	25.7		
29	8	0.1324	36.1		
30	29	0.1600	67.5		
31	30	0.1418	47.1		

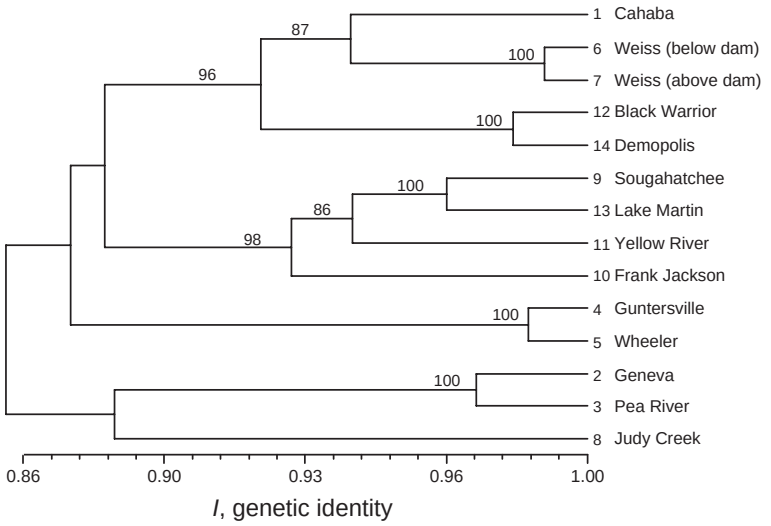

Fig. 2. Wild population-wise similarity tree revealed by UPGMA cluster analysis using the Nei (1978) unbiased genetic identity measure. Significant bootstrapping values are indicated.

F_{ST} value averaged over all polymorphic loci was 0.36 (99% C.I.; 0.39–0.32). A dendrogram reflecting genetic relatedness is shown in Fig. 2. Populations from the same watershed were more similar to one other than to other populations. For instance, populations 6 (Weiss population below dam) and 7 (Weiss population above dam) were the most related populations. Similarly, populations 12 (Black Warrior River population) and 14 (Demopolis population), populations 9 (Sougahatchee Creek) and 13 (Lake Martin population), populations 10 (Frank Jackson) and 11 (Yellow River), populations 4 (Guntersville) and 5 (Wheeler), and populations 2 (Geneva) and 3 (Pea) were more related to each other within than across watersheds. This finding suggested the existence of population differentiation among various watersheds. In almost all cases, the populations from the same watershed clustered together in the phylogenetic tree (Fig. 2).

3.3. Potential useful markers for population identification

No fixed AFLPs were found specific to any population. However, we observed AFLPs that had highly differential allele frequencies among various populations (Fig. 3). Therefore, two criteria were used to qualify potentially useful AFLPs: (1) the AFLPs must have an allele frequency of 50% in the characteristic population, and (2) this allele frequency must be at least five times greater than those in any other population with an allowance for two exceptions. As summarized in Table 4, 23 AFLPs met these criteria. Clearly, many

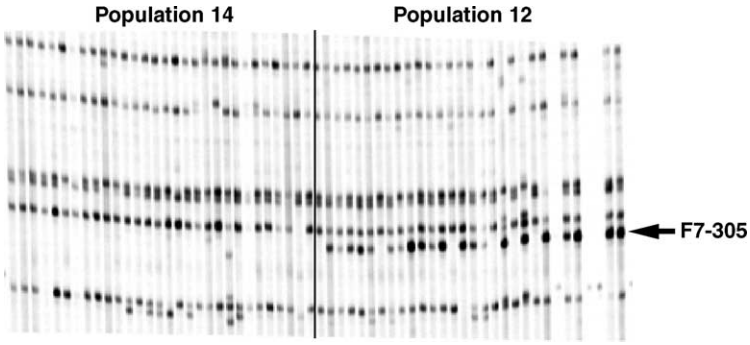

Fig. 3. An example of AFLP markers with highly differential allele frequencies. Shown on the left panel are 30 samples from population 14 and on the right are 30 samples from population 12, separated by a vertical line. The AFLP band (F7-305) is frequent among fish from population 12 but was rare in other populations, with only population 14 shown here.

Table 4
AFLPs with highly differential allele frequencies among 14 wild populations

Watershed	Cahaba	Coastal plain		Tennessee		Coosa		Cho	Tallapoosa		Coastal plain		Tombigbee	
Locus	1	2	3	4	5	6	7	8	9	13	10	11	12	14
B2-70	0.000	0.051	0.000	0.068	0.000	0.039	0.000	0.000	0.000	0.099	0.000	0.500	0.035	0.000
B2-176	0.000	0.106	0.030	0.022	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.646	0.000	0.000
B4-240	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.622	0.000	0.061	0.000	0.065	0.000	0.101
B2-63	0.000	0.000	0.030	0.045	0.018	0.039	0.036	0.080	0.000	0.032	0.000	0.646	0.053	0.097
F7-305	0.000	0.000	0.000	0.045	0.000	0.039	0.036	0.000	0.000	0.000	0.000	0.000	0.778	0.000
F7-173	0.000	0.397	0.657	0.045	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039
F7-218	0.000	0.574	0.358	0.091	0.017	0.060	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.039
F7-354	0.000	0.047	0.030	0.068	0.034	0.060	0.074	0.635	0.000	0.000	0.000	0.000	0.051	0.191
C3-360	0.000	0.000	0.000	0.639	0.342	0.038	0.036	0.000	0.000	0.000	0.000	0.000	0.087	0.077
B4-70	0.000	0.478	1.000	0.049	0.017	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.017	0.060
C3-189	0.000	0.047	0.000	0.068	0.000	0.019	0.000	0.000	0.000	0.030	0.074	0.000	0.817	0.728
F7-226	0.000	0.000	0.000	0.045	0.000	0.060	0.000	0.000	0.074	0.000	0.000	0.000	0.817	0.804
F7-127	0.000	0.047	0.030	0.022	0.000	0.019	0.036	0.184	0.000	0.000	0.000	0.000	1.000	0.723
F7-199	0.000	0.000	0.000	0.068	0.000	0.039	0.000	0.034	0.000	0.000	0.000	0.000	1.000	1.000
B4-379	0.000	0.000	0.000	0.100	0.000	0.020	0.039	0.000	0.074	0.030	0.000	0.000	1.000	1.000
F7-283	0.000	0.000	0.000	0.705	1.000	0.191	0.114	0.034	0.000	0.000	0.000	0.000	0.144	0.191
B4-83	0.000	1.000	1.000	0.000	0.017	0.000	0.000	0.036	0.000	0.030	0.244	0.000	0.034	0.060
B2-232	0.024	0.293	0.657	0.091	0.000	0.060	0.155	1.000	0.000	0.065	0.000	0.000	0.053	0.097
B4-79	0.000	0.699	1.000	0.049	0.034	0.000	0.000	0.000	0.000	0.030	0.622	0.000	0.069	0.080
B4-168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.782	0.160	1.000	0.646	0.000	0.000
B4-218	0.000	1.000	1.000	0.100	0.000	0.000	0.039	0.402	0.074	0.000	0.000	0.065	0.051	0.060

The watersheds from which samples were collected are indicated in the first row: Cahaba, Cahaba River watershed; Coastal plain, Coastal plain watershed; Tennessee, Tennessee watershed; Coosa, Coosa watershed; Cho, Choctawhatchee watershed; Tallapoosa, Tallapoosa watershed; Tombigbee, Tombigbee/Black Warrior watershed. The numbers indicate population numbers as detailed in Table 1.

of these 23 AFLPs are characteristic of certain populations. For instance, seven AFLP markers had high allele frequencies in populations 2 and 3, both of which were from the same watershed (Fig. 1). Two of the seven AFLPs, F7-173 and F7-218, were produced by F7 primer combination; four of the seven AFLPs, B4-70, B4-79, B4-83, and B4-218, were produced by B4 primer combination; and the remaining one AFLP, B2-232, was produced by B2 primer combination. Therefore, the primer combinations of B2, B4, and F7 collectively should produce AFLP fingerprints highly characteristic of populations from the Coastal plain watersheds. Similarly, AFLPs C3-360 and F7-283 were highly characteristic of populations 4 and 5; B4-240, F7-354, and B2-232 were characteristic of population 8; F7-305 was characteristic of population 12; and C3-189, F7-226, F7-127, F7-199, and B4-379 were highly characteristic of population 12 and 14, both of which were from the Tombigbee/Black Warrior watershed. Hence, it appears that B2, B4, and F7 primer combinations are quite useful for the generation of AFLPs characteristic of populations. Although these AFLPs were not population-specific markers, their differential allele frequency would lend opportunity for potential use in combinations for the identification of populations from various watersheds.

3.4. Comparison of genetic similarities of domestic populations with their proximal and distal wild populations

In this study, the wild channel catfish populations were found to contain significantly higher percent polymorphic loci (31.9–87.4% vs. 25.7–75.4%, $P<0.05$, Fig. 4) and expected mean heterozygosity values (0.1064–0.2131 vs. 0.087–0.1834, $P<0.05$, Fig. 5) than the domestic populations (Table 3, Mickett et al., 2003). For the majority of watersheds, the wild populations had equal or greater heterozygosities than their domestic counterparts (Fig. 5). The noticeable exception was the Coastal plain watershed containing the Hicks farm, where a greater heterozygosity was observed for the domestic population than for the wild population. In the case of the Tallapoosa watershed, much lower percentages of polymorphic loci were observed with the domestic populations. However, much of this lower heterozygosity was attributed to the research stocks of various strains at Auburn University, making the difference larger between the wild populations and the domestic populations.

One of the objectives of this research was to provide initial determination of the potential impact of domestic channel catfish on their wild populations.

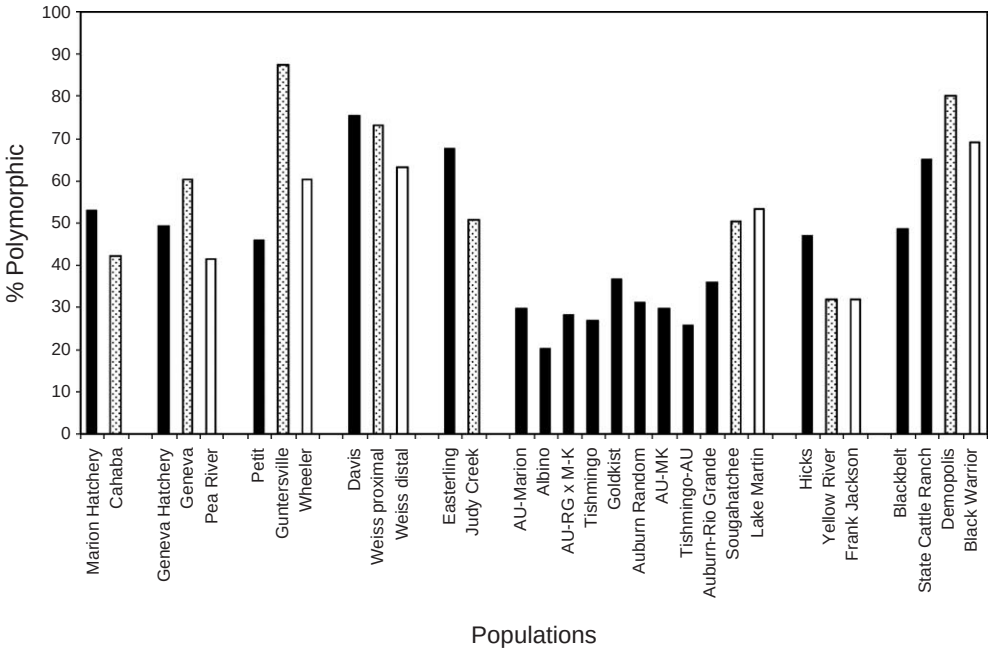

Fig. 4. Comparison of polymorphism rates of AFLPs (in percentage of polymorphic bands) of domestic catfish populations (solid bars) with those from wild populations proximal (dotted bars) or distal (open bars) to the catfish farms. Details for the populations are as described in Table 1.

Our hypothesis was that if large numbers of domestic catfish were released as a result of intentional stocking, or of accidental release from flooding, then the wild catfish populations proximal to the catfish farming centers should be genetically more similar to the domestic populations than the wild catfish populations

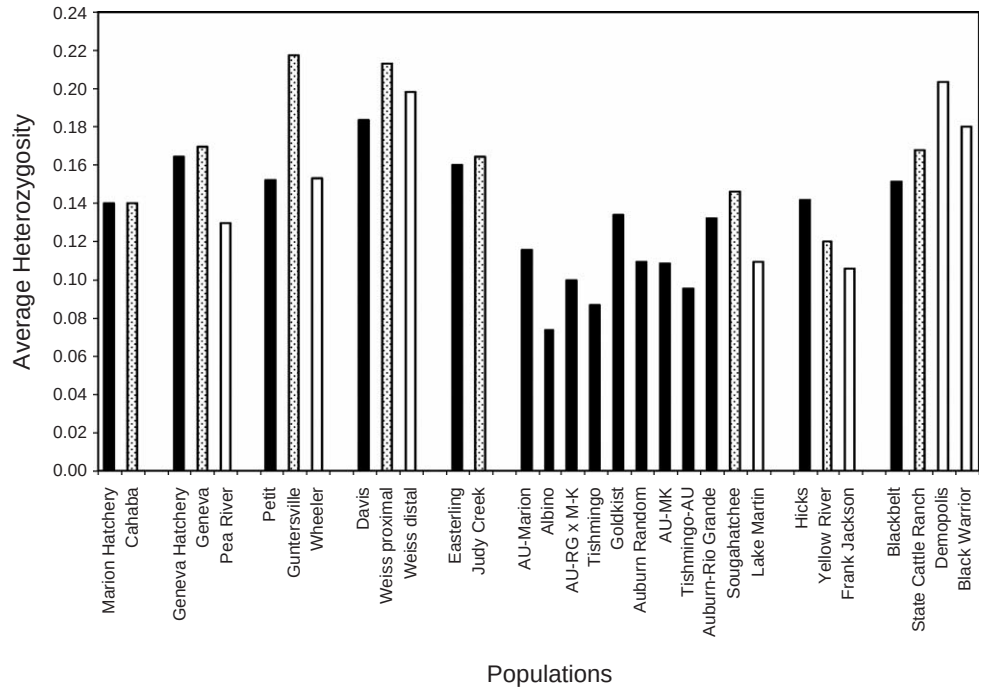

Fig. 5. Comparison of heterozygosities of AFLPs of domestic catfish populations (solid bars) with those from wild populations proximal (dotted bars) or distal (open bars) to the catfish farms. Details for the populations are the same as described in Table 1.

distal to the farming centers; this should be especially true if there is a physical barrier such as dams between the distal wild populations and the domestic fish farms. Catfish can swim/flow downstream, but not upstream against physical barriers such as dams unless they have navigation locks as in the Tombigbee and Tennessee systems. A direct comparison of "distal" populations with "proximal" populations and the domestic populations should reveal the degree of similarities among them. A greater similarity of proximal wild populations to domestic populations would demonstrate impact of domestic fish on wild populations; in contrast, if the proximal and distal populations are similar, but different from the domestic fish, then there is no evidence to support a significant impact of domestic fish on wild populations. Analysis of the AFLP bands produced by the five primer combinations indicated 191 bands shared by the domestic and wild populations. These frequencies of these bands were analyzed to determine the genetic relatedness of both wild and domestic populations.

Two tests were conducted for the genetic similarity analysis using 191 AFLP markers. The first was a genetic similarity analysis using Nei's genetic distance with UPGMA method. As shown in Fig. 6, all the domestic fish, regardless of their location within the watershed, were clustered as a single clade (lower portion of figure), indicating their common origin from a limited number of stocks raised at the aquaculture operations (Mickett et al., 2003). In contrast, the wild fish populations were clustered into clades more or less according to their origin of watersheds, as already discussed above. In the second approach, the Fisher's exact test was conducted to test the significance of genotype and allele frequency differences. No significant differences in genotype and allele frequencies were

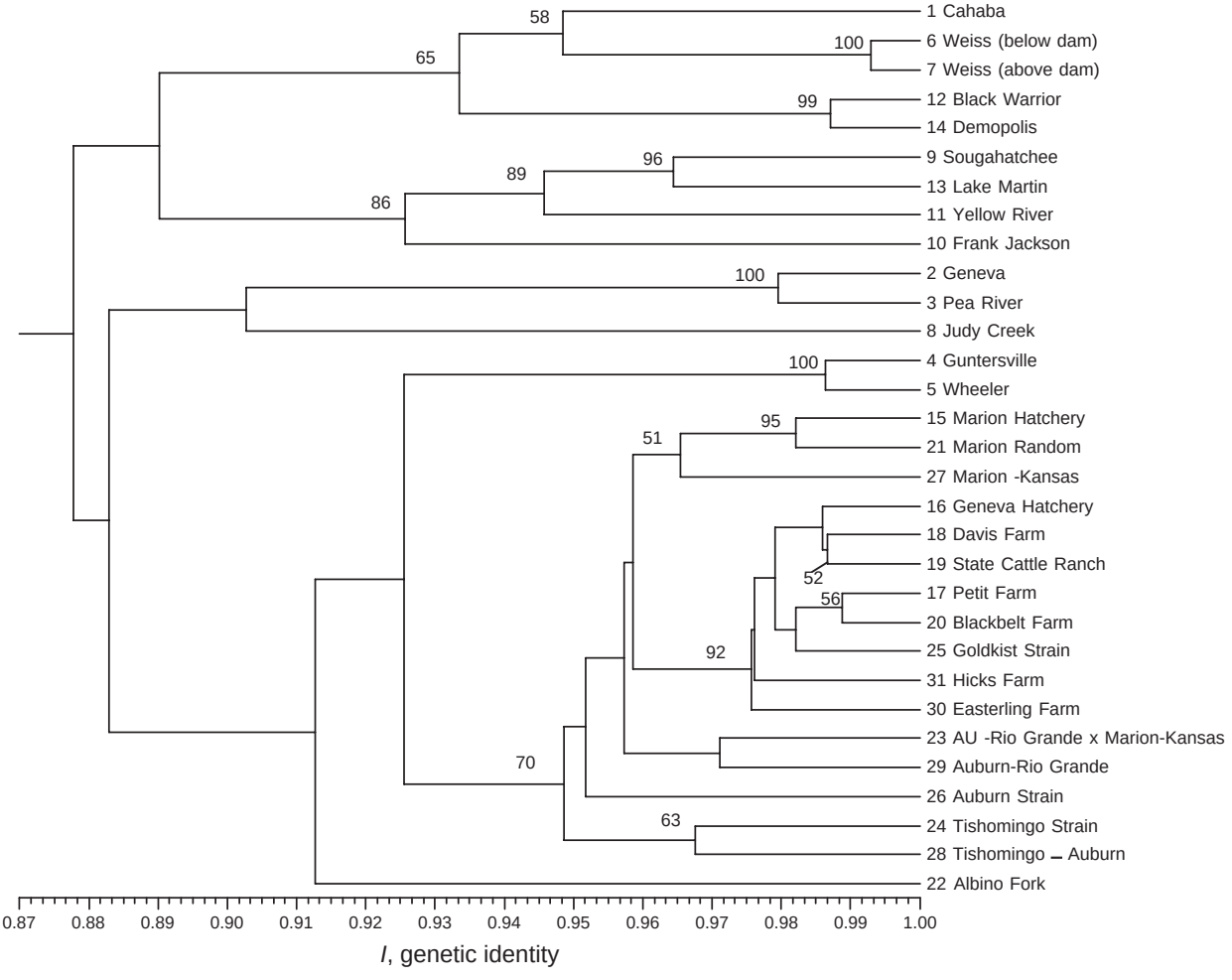

Fig. 6. Similarity tree of domestic and wild channel catfish populations constructed by using 191 AFLP markers based on the Nei's genetic similarities. Details of the populations are the same as explained in Table 1.

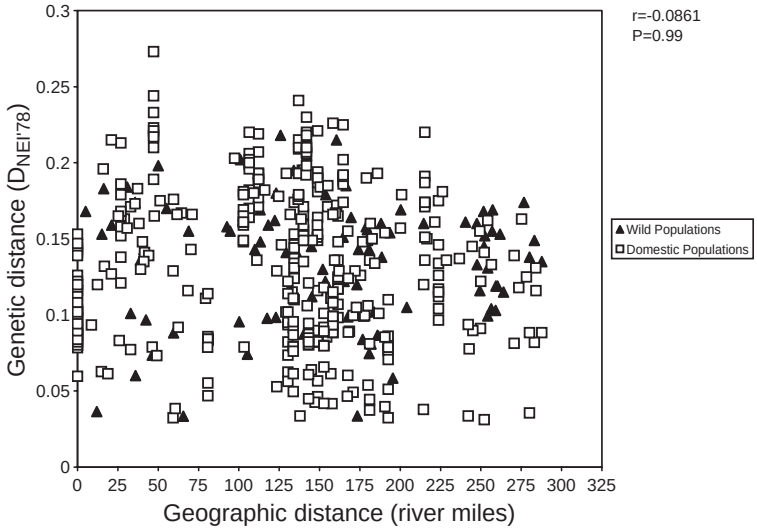

Fig. 7. Plot of genetic distances (*Y*-axis) over geographical distances (*X*-axis).

found between wild populations distal or proximal to the domestic fish farming centers, but significant differences were found between proximal wild populations and domestic populations, as well as between the distal wild populations and the domestic populations. Results of these tests indicated that there are no statistical differences among pairs of wild populations within watersheds, but differences between domestic and wild populations. We also conducted analyses of the relationship between genetic and geographical distances between all population pairs. This test is particularly meaningful for population samples from a single watershed. As shown in Fig. 7, there were no correlations between genetic distances and the geographical distances. Taken together, we conclude that there is no evidence for significant impact of domestic populations on wild populations.

4. Discussion

This research is the first using DNA markers for the analysis of natural genetic resources in channel catfish. Although some initial analysis of genetic resources were conducted using allozyme markers among research strains of domestic catfish (Hallerman et al., 1986), and initial analysis of genetic resources among domestic catfish in Alabama using AFLP markers (Mickett et al., 2003), no previous research has been conducted to address the issue of genetic diversity among wild catfish populations.

We found a greater level of genetic diversity of wild catfish populations than in domestic populations, an expected but a novel result. An average heterozygosity was found to be 0.16 among the tested watersheds. A greater level of heterozygosity is likely if wider geographical areas were included in the samples. Previous research already indicated significantly, different phenotypes existed among various geographical areas (Mickett et al., 2003). As we previously reported (Mickett et al., 2003), the aquaculture stocks are quite narrow in genetic background. This research showed the additional reservoir of genetic variability contained within natural populations, which may be useful for additional genetic/genomic input into aquaculture stocks.

Population genetic structure of the channel catfish populations as revealed by AFLPs supported both the historic and biogeographical hypotheses. In the wild populations, the genetic patterns were well explained by biogeographical factors. Regardless of the distance or presence of barriers to the domestic populations, samples collected from the same watershed shared a high level of genetic similarities, suggesting sufficient flow of genes among upstream and downstream populations. The only noticeable exception was the wild channel catfish population collected from the Wheeler Lake, which showed relatedness with the domestic channel catfish from Marion Hatchery. A survey of stocking history revealed that the domestic catfish from Marion Hatchery was used to stock Wheeler Lake (Maurice Jackson, Alabama Department of Conservation, personal communications).

There were no significant differences in the levels of heterozygosity between distal populations and proximal wild populations, even though a higher level of heterozygosity was observed in most distal populations. Similar results were found with *Catostomus occidentalis* in

western U.S. watersheds by Whitehead et al. (2003) using both AFLP and microsatellite markers, with European cyprinids using mitochondrial markers based on the complete nucleotide sequence of cytochrome *b* gene (Zardoya and Doadrio, 1999), and with South American *Hypostomus* catfish using mitochondrial D-loop haplotypes (Montoya-Burgos, 2003). In comparison with the situation of the wild populations, the similarity of domestic catfish populations was explained by historic factors, as expected from known gene flow in breeding histories of aquaculture stocks (Mickett et al., 2003).

The large number of loci (396 for the study of wild populations and 191 for the comparison of wild and domestic populations) used in this study should help reducing interlocus variance. While interlocus variance can be reduced with increased number of loci, intralocus variance can only be reduced by increasing the number of individuals sampled. In this regard, the allele frequency differences could be potentially over-estimated or under-estimated in some populations because of the small sample sizes. For instance, only 8 fish were collected from the Yellow River wild population; 10 fish were collected from the Frank Jackson wild population; and 11 fish collected from the Geneva wild population. Substantial effort was devoted to collect samples; numerous fishing trips were made with the assistance of the Alabama State Department of Conservation, but we were unable to collect larger samples in several selected sampling sites. Our goal was to collect 20–30 fish from each wild population, but this objective was not met as a result of scarceness of channel catfish in selected sites within certain watersheds. However, the small samples of several sites should not significantly affect our ability to obtain a reasonable estimation of heterozygosities. As stated by Gorman and Renzi (1979), even a few individuals are sufficient for estimating H if the number of loci examined is large (Nei, 1987). However, we recognize the effect of small sample size on intralocus variation. For instance, 23 AFLPs were identified that have highly differential allele frequencies among certain populations. Potentially, these AFLPs could represent highly differential AFLPs for population identification, but additional research is needed to determine their usefulness for this purpose. As the sample sizes increases, the frequency of alleles could change.

This research assessed the potential impact of the domestic catfish industry on wild populations for the first time. Because of the large size of the catfish industry, with over one billion fish raised annually, the potential risks for accidental releases and subsequent genetic impact could be tremendous, especially over time. To date, there is no evidence showing significant impact of the domestic catfish on wild populations in Alabama. This result may have implications for the use of non-indigenous or genetically improved strains of catfish in earthen ponds. Considering that the catfish industry has been in large-scale production since the mid-1980s, accidental release can be avoided, whatever the mechanism. We believe that a number of factors may have made this success possible. The location of earthen ponds distant from rivers may be the major factor. This is in great contrast of the situation for salmon raised in net pens where significant genetic impact has been reported (Hindar et al., 1991; Fleming et al., 2000).

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References

Allendorf, F., Ryman, N., Utter, F., 1987. Genetics and fishery management: past, present, and future. In: Ryman, N., Utter, F. (Eds.), Population Genetics and Fishery Management. Washington Sea Grant Publications/University of Washington Press, Seattle, pp. 1–19.

Altukhov, Y.P., Salmenkova, E.A., 1987. Stock transfer relative to natural organization, management, and conservation of fish populations. In: Ryman, N., Utter, F. (Eds.), Population Genetics and Fishery Management. Washington Sea Grant Publications/ University of Washington Press, Seattle, pp. 333–343.

Anderson, S., Sadinski, W., Shugart, L., 1994. Genetic and molecular ecotoxicology: a research framework. Napa Conference on Genetic and Molecular Ecotoxicology, 12–15 October, 1993; Yountville, CA, USAEnvironmental Health Perspectives, vol. 102, pp. 3–8.

Avise, J.C., 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York, USA. 511 pp.

Bagley, M.J., Anderson, S.L., May, B., 2001. Choice of methodology for assessing genetic impacts of environmental stressors: polymorphism and reproducibility of RAPD and AFLP fingerprints. Ecotoxicology 10, 239–244.

Banks, M.A., Rashbrook, V.K., Calavetta, M.J., Dean, C.A., Hedgecock, D., 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of Chinook salmon (*Oncorhynchus*

tshawytscha) in California's Central Valley. Canadian Journal of Fisheries and Aquatic Sciences 57, 915–927.

Bickham, J.W., Smolen, M.J., 1994. Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. Environmental Health Perspectives 102, 25–28.

Brodeur, R.D., Busby, M.S., 1998. Occurrence of an Atlantic salmon (*Salmo salar*) in the Bering Sea. Alaska Fishery Research Bulletin 5, 64–66.

Campton, D.E., 1987. Natural hybridization and introgression in fishes: methods of detection and interpretation. In: Ryman, N., Utter, F.M. (Eds.), Population Genetics and Fishery Management. Washington Sea Grant Program. University of Washington Press, Seattle, pp. 161–192.

Carmichael, G.J., Schmidt, M.E., Morizot, D.C., 1992. Electrophoretic identification of genetic markers in channel catfish and blue catfish by use of low-risk tissues. Transactions of the American Fisheries Society 121, 26–35.

Chong, L.K., Tan, S.G., Yusoff, K., Siraj, S.S., 2000. Identification and characterization of Malaysian river catfish, *Mystus nemurus* (C&V): RAPD and AFLP analysis. Biochemical Genetics 38, 63–76.

Congiu, L., Dupanloup, I., Patarnello, T., Fontana, F., Rossi, R., Arlati, G., Zane, L., 2001. Identification of interspecific hybrids by amplified fragment length polymorphism: the case of sturgeon. Molecular Ecology 10, 2355–2359.

David, L., Rajasekaran, P., Fang, J., Hillel, J., Lavi, U., 2001. Polymorphism in ornamental and common carp strains (*Cyprinus carpio* L.) as revealed by AFLP analysis and a new set of microsatellite markers. Molecular Genetics and Genomics 266, 353–362.

Dehart, D.A., 1999. Affidavit to State of Oregon, Case Number 99-6265-HO. Oregon Department of Justice, Salem, OR.

Dunham, R.A., Smitherman, R.O., 1984. Ancestry and Breeding of Catfish in the United States, Cir. 273. Alabama Agricultural Experiment Station, Auburn, AL.

Englbrecht, C.C., Freyhof, J., Nolte, A., Rassmann, K., Schliewen, U., Tautz, D., 2000. Phylogeography of the bullhead *Cottus gobio* (Pisces: Teleostei: Cottidae) suggests a pre-Pleistocene origin of the major central European populations. Molecular Ecology 9, 709–722.

Felip, A., Martinez-Rodriguez, G., Piferrer, F., Carrillo, M., Zanuy, S., 2000. AFLP analysis confirms exclusive maternal genomic contribution of meiogynogenetic sea bass (*Dicentrarchus labrax L.*). Marine Biotechnology 2, 301–306.

Fleming, I.A., Hindar, K., Mjolnerod, I.B., Jonsson, B., Balstad, T., Lamberg, A., 2000. Lifetime success and interactions of farm salmon invading a native population. Proceedings of the Royal Society of London. B 267, 1517–1523.

Fox, G.A., 1995. Tinkering with the tinkerer: pollution versus evolution. Environmental Health Perspectives 103, 93–100.

Gorman, G.C., Renzi, J., 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 2, 242–249.

Hallerman, E.M., Dunham, R.A., Smitherman, R.O., 1986. Selection or drift: isozyme allele frequency changes among channel catfish selected for rapid growth. Transactions of the American Fisheries Society 115, 60–68.

Hamrick, J.L., Godt, M.J.W., Murawski, D.A., Loveless, M.D., 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, pp. 75–86.

Han, K., Ely, B., 2002. Use of AFLP analyses to assess genetic variation in *Morone* and *Thunnus* species. Marine Biotechnology 4, 141–145.

Heath, D., Heath, J., Bryden, C., Rachel, J., Charles, F., 2003. Rapid evolution of egg size in captive salmon. Science 299, 1738–1740.

Hindar, K., 2001. Interactions of cultured and wild species. In: Tlusty, M.F., et al. (Eds.), Marine Aquaculture and the Environment. Cape Cod Press, Falmouth, MA, p. 50.

Hindar, K., Ryman, N., Utter, F., 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48, 945–957.

Kai, Y., Nakayama, K., Nakabo, T., 2002. Genetic differences among three colour morphotypes of the black rockfish, *Sebastes inermis*, inferred from mtDNA and AFLP analyses. Molecular Ecology 11, 2591–2598.

Liu, Z., 2003. A review of catfish genomics: progress and perspectives. Comparative and Functional Genomics 4, 259–265.

Liu, Z.J., Cordes, J., 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238, 1–37.

Liu, Z., Li, P., Argue, B.J., Dunham, R.A., 1998a. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F_1, F_2 and backcross hybrids. Animal Genetics 29, 58–62.

Liu, Z., Nichols, A., Li, P., Dunham, R.A., 1998b. Inheritance and usefulness of AFLP markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F_1, F_2 and backcross hybrids. Molecular and General Genetics 258, 260–268.

Liu, Z., Li, P., Argue, B.J., Dunham, R.A., 1999a. Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. Aquaculture 174, 59–68.

Liu, Z., Li, P., Kucuktas, H., Nichols, A., Tan, G., Zheng, X., Argue, B.J., Yant, R., Dunham, R.A., 1999b. Development of amplified fragment length polymorphism (AFLP) markers suitable for genetic linkage mapping of catfish. Transactions of the American Fisheries Society 128, 317–327.

Liu, Z., Tan, G., Li, P., Dunham, R.A., 1999c. Transcribed dinucleotide microsatellites and their associated genes from channel catfish *Ictalurus punctatus*. Biochemical and Biophysical Research Communications 259, 190–194.

Liu, Z.J., Karsi, A., Li, P., Cao, D., Dunham, R., 2003. An AFLP-based genetic linkage map of channel catfish (*Ictalurus punctatus*) constructed by using an interspecific hybrid resource family. Genetics 165, 687–694.

McCracken, G.F., Parker, C.R., Guffey, S.Z., 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Transactions of the American Fisheries Society 122, 533–542.

Mickett, K., Morton, C., Feng, J., Li, P., Simmons, M., Dunham, R.A., Cao, D., Liu, Z.J., 2003. Assessing genetic diversity of domestic populations of channel catfish (*Ictalurus punctatus*) in Alabama using AFLP markers. Aquaculture 228, 91–105.

Miller, M.P., 1997. Tools for Population Genetic Analysis (TFPGA), Version 1.3. A Windows Program for the Analysis of Allozyme and Molecular Population Genetic Data. Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

Montoya-Burgos, J.I., 2003. Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. Molecular Ecology 12, 1855–1867.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Palumbi, S.R., 2001. Humans as the world's greatest evolutionary force. Science 293, 1786–1790.

Rogers, S.M., Campbell, D., Baird, S.J., Danzmann, R.G., Bernatchez, L., 2001. Combining the analyses of introgressive hybridisation and linkage mapping to investigate the genetic architecture of population divergence in the lake whitefish (*Coregonus clupeaformis*, Mitchill). Genetics 111, 25–41.

Rohlf, F.J., 1998. NTSYS, pc. Version 2.10p. Applied Biostatistics, Setauket, NY, http://www.exetersoftware.com/cat/ntsyspc/ntsyspc.html.

Serapion, J., Kucuktas, H., Feng, J., Liu, Z.J., 2004. Bioinformatic mining of type I microsatellites from expressed sequence tags of channel catfish (*Ictalurus punctatus*). Marine Biotechnology 6, 364–377.

Smitherman, R.O., Dunham, R.A., 1993. Relationships among cultured and naturally occurring populations of freshwater catfish in the United States. In: Marcia, R.C., James, P.M. (Eds.), Interactions Between Cultured Species And Naturally Occurring Species In The Environment: Proceedings of the Twenty-second U.S.–Japan Aquaculture Panel Symposium, 21–22, August 1993, Homer, AlaskaUJNR Technical Report, vol. 22.

Swofford, D.L., 2001. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods) Version 4. Sinauer Associates, Sunderland, MA.

Tiersch, T.R., Sheryl, A.G., Carmichael, G.J., 1994. Cryopreservation of channel catfish sperm: storage in cryoprotectants, fertilization trials, and growth of channel catfish produced with cryopreserved sperm. Transactions of the American Fisheries Society 123, 580–586.

USDA, National Agricultural Statistics Service (NASS), 2001. Catfish Production. U.S. Department of Agriculture, Washington, DC.

Utter, F., 2003. Genetic impacts of fish introductions. In: Hallerman, E. (Ed.), Population Genetics, Principles and Applications for Fisheries Scientists. American Fisheries Society, Bethesda, MD, pp. 357–378.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358–1370.

Whitehead, A., Anderson, S.L., Kuivila, K.M., Roach, J.L., May, B., 2003. Genetic variation among interconnected populations of *Catostomus occidentalis*: implications for distinguishing impacts of contaminants from biogeographical structuring. Molecular Ecology 12, 2817–2833.

Young, W.P., Ostberg, C.O., Keim, P., Thorgaard, G.H., 2001. Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). Molecular Ecology 10, 921–930.

Yue, G., Li, Y., Chen, F., Cho, S., Lim, L.C., Orban, L., 2002. Comparison of three DNA marker systems for assessing genetic diversity in Asian arowana (*Scleropages formosus*). Electrophoresis 23, 1025–1032.

Zardoya, R., Doadrio, I., 1999. Molecular evidence on the evolutionary and biogeographical patterns of European cyprinids. Journal of Molecular Evolution 49, 227–237.