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Assessing genetic diversity of domestic populations of channel catfish (*Ictalurus punctatus*) in Alabama using AFLP markers

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Abstract

Amplified fragment length polymorphism (AFLP) was used to analyze the genetic diversity of domestic stocks of channel catfish (*Ictalurus punctatus*). Sixteen populations within Alabama (USA) were surveyed using five primer combinations. A total of 454 reproducible, polymorphic bands were detected from 282 individuals, with an average heterozygosity of 0.135. The percentage of polymorphic loci varied greatly within distinct populations, ranging from 18.26% to 100%. Research populations tended to have a lower percentage of loci polymorphic than farm populations. The estimated average F_{st} value across all loci was 0.4456. However, this value dropped to 0.1763 when the Hicks Farm stock was excluded from the analysis. Cluster analysis by Jaccard's pairwise similarity coefficient indicated high similarity among individuals, with some population-specific clusters and with the Hicks Farm stock being highly distinctive. Nei's population-wise identity measures found all populations except the population from the Hick's Farm related with 96% similarity. The Hick's Farm stock was 34% similar to the other populations. The genotypic information derived from this study is consistent with the historical origins and management of catfish broodstocks. The results of this study suggest that many channel catfish farm strains in Alabama are genetically similar, but some very distinct differences exist. Such information has implications for future broodstock selection and

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management and for further analysis of interactions between domestic and wild populations of channel catfish.

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

1. Introduction

The conservation of genetic diversity is important for the long-term interest of any species (Hamrick et al., 1991). Molecular markers have been very useful for analysis of genetic diversity. Among the several marker systems, amplified fragment length polymorphism (AFLP) (Vos et al., 1995) is highly reliable for the assessment of genetic variation among and within populations (Folkertsma et al., 1996; Travis et al., 1996; Keim et al., 1997; Keiper and McConchie, 2000). AFLP does not require any previously known genetic information, a feature especially useful with species for which there is no establishment of polymorphic markers in relation to population identity, or for which there is limited sequence information (Han et al., 2000; Ajmone-Marsan et al., 2001; de Knijff et al., 2001). Another advantage of AFLP is the capability to produce multi-locus fingerprints in a single analysis, significantly reducing the cost of analysis and increasing the possibility of detecting polymorphisms (Vos et al., 1995).

Channel catfish (*Ictalurus punctatus)* is the most important aquaculture species in the United States, accounting for over 68% of all aquaculture production (Naylor et al., 2000; USDA, 2000). Additionally, channel catfish is a widely sought-after game 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 the genetic variation of *I. punctatus* using isozyme markers and in some cases as it related to selection for body weight (Dunham and Smitherman, 1984; Hallerman, 1984; Hallerman et al., 1986; Carmichael et al., 1992). More recent studies have demonstrated the applicability of several PCR-based markers using microsatellites (Waldbieser and Bosworth, 1997, Liu et al., 1999c), RAPD (Liu et al., 1998a, 1999a), and AFLP (Liu et al., 1998b, 1999b). A comprehensive analysis of genetic variation both within and among populations of channel catfish using DNA markers has not been conducted.

Alabama is one of the major catfish aquaculture states, accounting for about 15% of all U.S. catfish production. Understanding the genetic variation within domestic populations is needed for genetic enhancement programs aiming to maximize the benefits of selective breeding while avoiding potential inbreeding, and random genetic drift. In contrast to domestic livestock, for which a detailed genetic lineage is often known, very little is known about the pedigree information of cultured fish. Current strains and lines of channel catfish originated from wild fish and were perpetuated in national, state, private hatcheries, farms, and research institutions (Dunham and Smitherman, 1984).

Understanding the genetic composition of domestic populations is important for the assessment of genetic diversity of natural populations. The number of domestic catfish produced annually is about 500 million, requiring production of about 2 billion fry annually. Wild populations are relatively small, and very few fry survive predation and obtain adequate food to reach adulthood. Flooding, other accidental escapes, or intentional stocking of domestic catfish could potentially have a significant impact on the genetic composition of wild populations. Analysis of genetic resources of catfish is important to establish baseline data for utilization for both genetic enhancement programs and genetic conservation programs. The objective of the present study was to conduct a baseline survey on the genetic composition of domestic catfish in Alabama using the AFLP technique. This survey is a preliminary step in a comprehensive genetic analysis of channel catfish within and between domestic and natural populations.

2. Materials and methods

2.1. Fish samples

Three hundred fish from 16 populations were collected across the state of Alabama, representing many of the major suppliers of channel catfish fingerlings and different geographic regions. Considerations were also given to the locations of sites within Alabama watersheds, such that the impact of domestic catfish on wild catfish populations may be addressed in future studies. The locations and the abbreviations for the populations are listed in Table 1. Thirty fish each were obtained from seven major fingerling suppliers located in Barbour, Blount, Cherokee, Covington, Geneva, and Hale counties. Ten fish

Table 1
Population numbers, abbreviations, and collection locations (all are names of Alabama counties, except for AU, the Auburn University hatchery, located in Lee County, Alabama)

Number	Abbreviation	County	Farm/hatchery name
1	GENF	Geneva	Lake Geneva Fish Hatchery
2	PETF	Blount	Petit Farm
3	COOF	Cherokee	Davis Farm
4	TOMF	Hale	State Cattle Ranch
5	BLKF	Hale	Blackbelt Aquaculture
6	M	Lee	Auburn University
7	AF	Lee	Auburn University
8	ARMK	Lee	Auburn University
9	T	Lee	Auburn University
10	G	Lee	Auburn University
11	A	Lee	Auburn University
12	MK	Lee	Auburn University
13	TA	Lee	Auburn University
14	AR	Lee	Auburn University
15	CHOF	Barbour	Easterling Farm
16	YELF	Covington	Hicks Farm

each from nine different strains were collected from the Auburn University Catfish Genetics Research Unit: Auburn, Albino Forks, Auburn–Rio Grande, Auburn–Rio Grande–Marion–Kansas, Goldkist, Marion–Kansas, Marion Random, Tishomingo, and Tishomingo–Auburn (Table 1). All of these research populations had been selected for increased body weight for up to six generations, except for Marion random which was a randomly bred control, and Albino Forks which was selected and maintained for albinism, a recessive trait in channel catfish.

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 Remington (1997). Five primer combinations were used: 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 $r_b < 0.09$. The fragment scoring was checked manually subsequent to computer analysis.

Table 2
Adaptor and primer sequences used for AFLP analysis

	Sequence
Adaptor	
*Eco*RI	CTCGTAGACTGCGTACCCTGACGCATGGTTAA
*Mse*I	GACGATGAGTCCTGAGTACTCAGGACTCAT
Primer	
E-AAG	AGACTGCGTACCAATTCAAG
E-ACA	AGACTGCGTACCAATTCACA
E-ACT	AGACTGCGTACCAATTCACT
M-CAC	GATGAGTCCTGAGTAACAC
M-CAG	GATGAGTCCTGAGTAACAG
M-CAT	GATGAGTCCTGAGTAACAT
M-CTC	GATGAGTCCTGAGTAACTC
M-CTG	GATGAGTCCTGAGTAACTG

The five primer combinations used in this research are as described in Materials and methods.

2.4. Data analysis

Fragment data were transferred from AFLP-QuantarPro to a binary (1/0) data matrix. Using the software NTSYS-pc 2.02 (version 2.1; Rohlf, 1998), the data matrix was then used to estimate pairwise genetic similarities by way of two different similarity coefficients: Jaccard (1908) and Dice (1945). Phenograms were constructed based on two different clustering methods: unweighted pair-group method analysis (UPGMA; Sokal and Michener, 1958) and weighted pair-group method analysis (WPGMA; Sneath and Sokal, 1973). To test the goodness of fit, a matrix of cophenetic values was produced from each cluster and compared to the distance matrix on which the cluster was based using the NTSYS software (Rohlf, 1998). The confidence of branch support was then evaluated by way of bootstrap analysis with 1000 replications, performed with the PAUP software package (version 4; Swofford, 2001).

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 (1978) unbiased heterozygosity formula. 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 replications. Genetic distances between populations were calculated by Nei's (1978) unbiased distance and identity measures. 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 and genetic variation

Using five AFLP primer combinations, 454 bands were identified for 282 individuals. Of the 300 original individuals, 18 were omitted because of a high incidence of questionable bands. All 454 bands were polymorphic, with an average heterozygosity of 0.135. The LI-COR automated sequencer using fluorescent labels allowed it possible to analyze a large number of bands from a single run (Fig. 1). The average heterozygosity and percentage polymorphic loci for each population are summarized in Table 3. Great variation was observed among populations with regard to genetic diversity as indicated by the average heterozygosity and the percentage polymorphic loci. The population with the greatest percent polymorphism (100%) and highest average heterozygosity (0.2471) was the Hicks Farm population. The population with the lowest percent polymorphism (18.3%) and the lowest average heterozygosity (0.0609) was Forks albino. The estimated F_{st} value averaged over all polymorphic loci was 0.4456 (99% CI, 0.4100–0.4784) indicative of a strong population structure, in this case, indicating distinct genetic lines. Excluding the Hicks

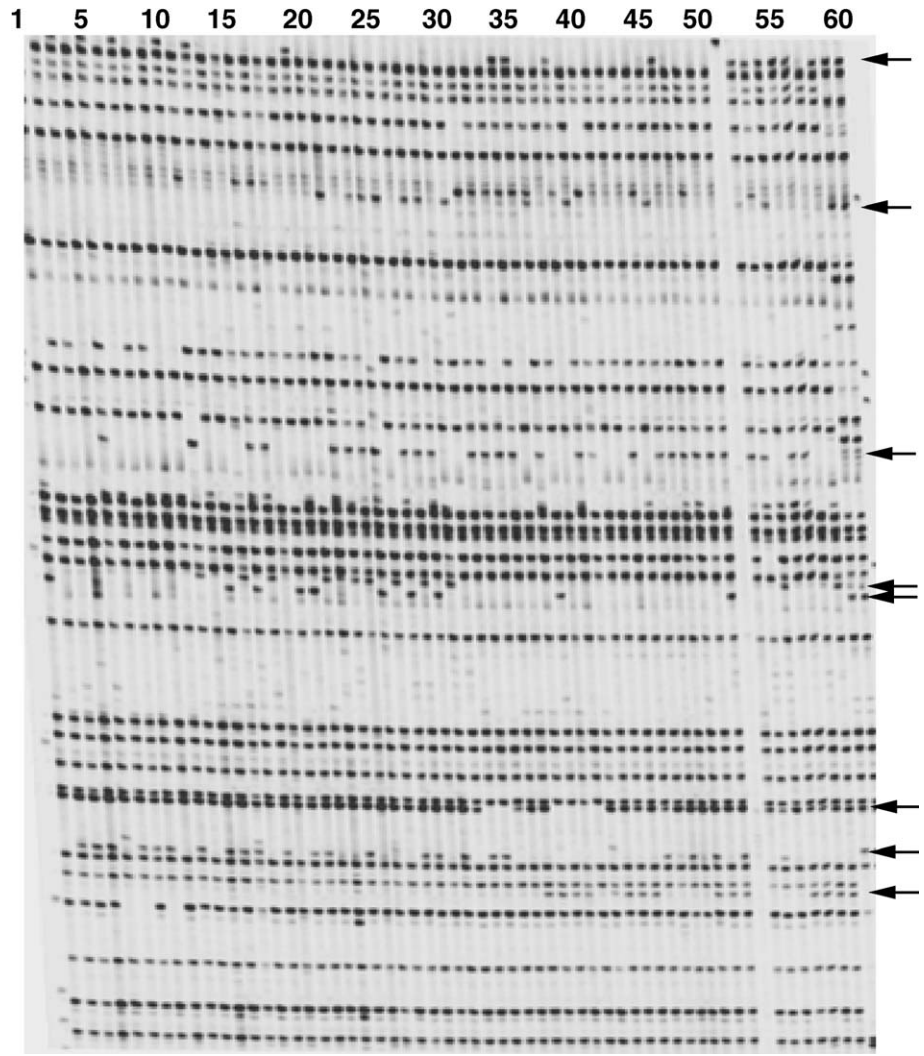

Fig. 1. A portion of an AFLP gel using LI-COR automated sequencer. Each lane represents a single fish with 60 fish shown. Highly polymorphic bands are indicated by arrows on the right margin. All bands were polymorphic when all 282 fish were included (not shown).

Farm population, the estimated F_{st} value across all polymorphic loci was 0.1763 (99% CI, 0.1550–0.2011).

3.2. Population structure

The individual-based similarity trees with the highest cophenetic correlation values, and therefore the best fit between the distance matrix and corresponding tree, were

Table 3
Average heterozygosity and percentage polymorphic loci for 16 populations of *I. punctatus* across 454 AFLP loci

Population ID	*n*	Average heterozygosity	Percentage polymorphic loci
1	30	0.1784	62.56
2	29	0.1243	41.85
3	25	0.2313	70.93
4	30	0.1920	60.13
5	23	0.1611	49.89
6	9	0.0898	27.39
7	10	0.0609	18.26
8	10	0.0905	24.94
9	10	0.0837	22.91
10	10	0.1103	32.16
11	9	0.1003	27.97
12	10	0.0961	26.65
13	10	0.0721	21.80
14	8	0.1063	29.52
15	29	0.2153	68.52
16	30	0.2471	100.00

produced by UPGMA cluster analysis from both the Jaccard's (1908) and Dice's (1945) similarity coefficients (Table 4; Fig. 2). As the correlation value for both of these coefficients was 0.89 and no differences in clustering patterns were revealed, these coefficients were considered equivalent as analytic tools. The Jaccard tree was selected for further analyses.

Cluster analysis revealed an average genetic similarity across all individuals of 79% (± 4.5% S.D.). Within the similarity tree, a number of individuals formed distinct clusters. Forks albino ($n = 10$) from Auburn University, clustered with 89% similarity between individuals, and was supported by a 93% bootstrapping value. Another strong cluster was formed by 25 of 30 individuals from Hicks Farm, which came together with 81% similarity, supported by a bootstrapping confidence value of 100%. Distinct clusters also were formed among other research lines at Auburn University. Auburn–Rio Grande–Marion–Kansas (ARMK) select line ($n = 10$) clustered in a group with 85% genetic similarity, while lines T (Tishomingo) ($n = 10$) and TA (Tishomingo–Auburn) ($n = 10$) came together as a single cluster, also with about 85% similarity between individuals. Seven of eight individuals in AR clustered in a group that had 85% similarity. M (Marion random, $n = 9$) was clustered together with ARMK with 83% similarity. Within this cluster was also grouped one member from MK ($n = 10$). This MK

Table 4
Comparison of cophenetic correlation values obtained from two similarity coefficients and two clustering methods used in analysis of AFLP data

Clustering method	Similarity coefficients	
	Jaccard	Dice
UPGMA	0.89	0.89
WPGMA	0.85	0.86

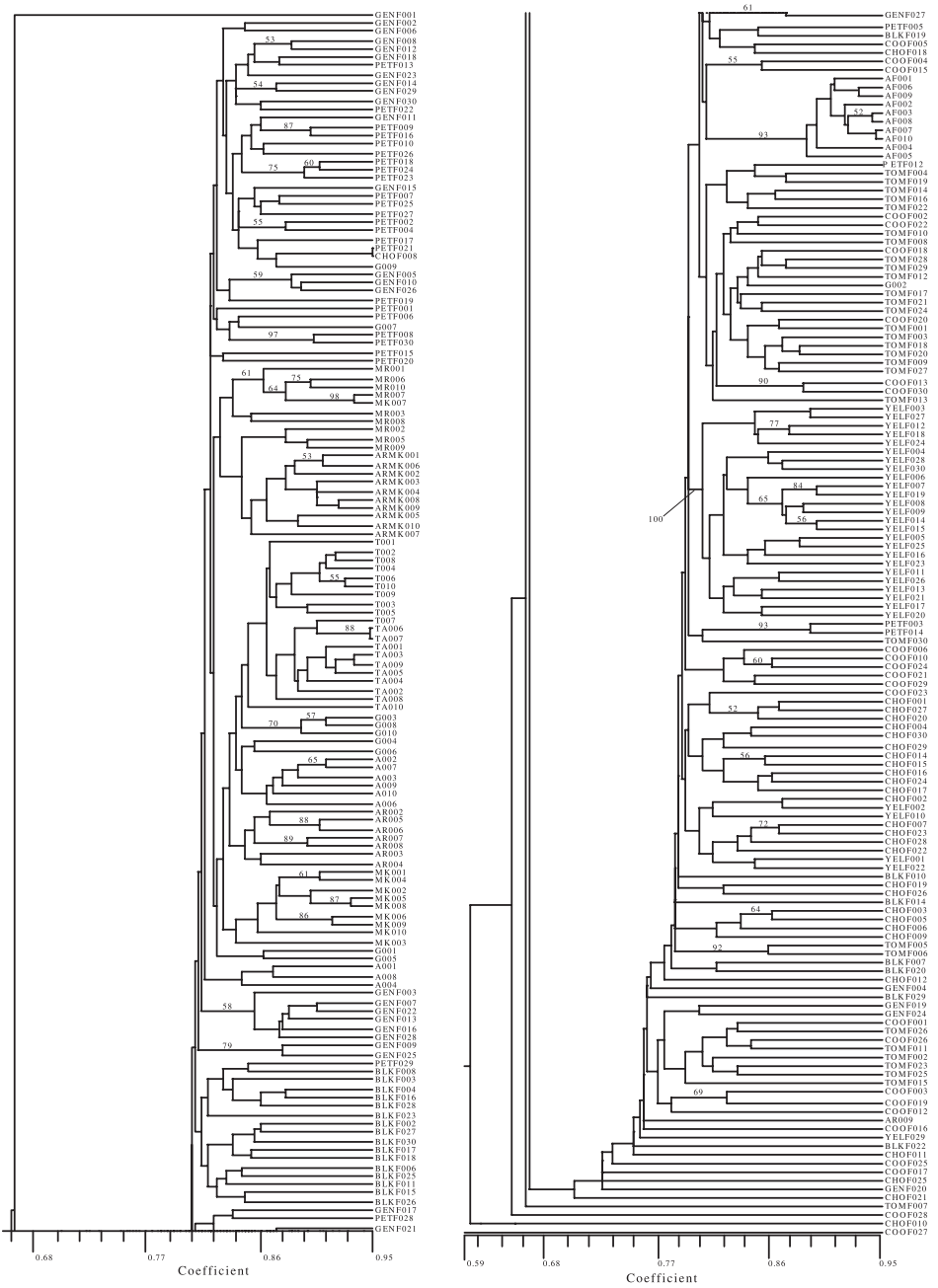

Fig. 2. Indivdiual-wise similarity tree revealed by UPGMA cluster analysis using the Jaccard (1908) similarity coefficient. Significant bootstrapping values are indicated.

individual formed a strong cluster (93% similarity) with a member of M, with a bootstrapping confidence value of 98%. The rest of the MK line clustered together with 84% similarity between individuals. Overall, most individuals from the Auburn research lines clustered together with 82% similarity, with the exception of Forks albino, which was grouped separately (89% similarity). Also excluded from this larger cluster were three individuals from the A line (Auburn) ($n=9$) that fell basally to the main Auburn research lines cluster (82% similarity). Individuals from G line (Goldkist line, first generation fish introduced to Auburn University) ($n=10$) were interdigitated throughout all the groups. The relationships of the nine populations from Auburn could be due to a combination of their small population size, similar historical ancestry, and similar changes induced from selection for growth.

Among the rest of the populations (Geneva Hatchery, Petit Farm, Davis Farm, State Ranch, Blackbelt Farm, and Easterling Farm), there were few distinct, strongly supported clusters. Those that were strongly supported were small compared to the sample size, and individuals tended to be interdigitated throughout the similarity tree, forming small groups intermixed with members of all 16 populations.

The population-wise similarity tree based on Nei's (1978) unbiased identity measures revealed an average population similarity of 88% ($\pm$21% S.D.). Hicks Farm was determined to be only 34% similar to the other populations. Excluding this population, the remaining groups had 96% ($\pm$ 1.3% S.D.) similarity. Forks albino was the most distant group among these remaining 15 populations, with 90% similarity (Fig. 3; Table 5).

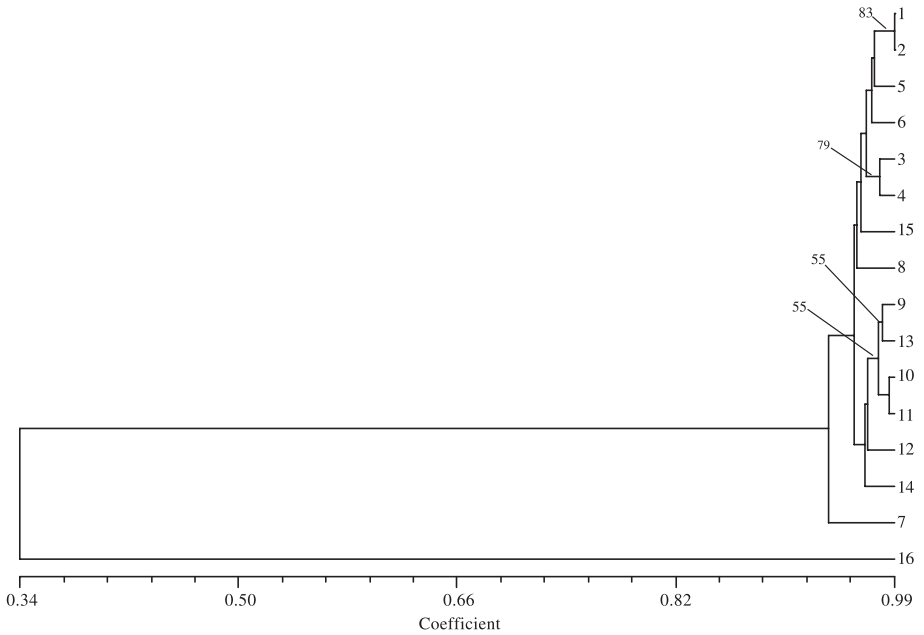

Fig. 3. Population-wise similarity tree revealed by UPGMA cluster analysis using the Nei (1978) genetic identity measure. Population numbers are as given in Table 1. Significant bootstrapping values are indicated.

Table 5
Genetic similarity values based on Nei's (1978) similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1															
2	0.9865	1														
3	0.9695	0.9652	1													
4	0.9754	0.9716	0.9762	1												
5	0.9706	0.9734	0.9645	0.9709	1											
6	0.9654	0.9725	0.9506	0.9567	0.9714	1										
7	0.9447	0.9489	0.9307	0.9405	0.9455	0.9466	1									
8	0.9581	0.9669	0.9440	0.9570	0.9654	0.9673	0.9441	1								
9	0.9647	0.9667	0.9465	0.9528	0.9547	0.9553	0.9322	0.9506	1							
10	0.9759	0.9826	0.9612	0.9686	0.9689	0.9660	0.9450	0.9622	0.9791	1						
11	0.9648	0.9719	0.9520	0.9571	0.9588	0.9576	0.9334	0.9564	0.9667	0.9828	1					
12	0.9587	0.9689	0.9433	0.9480	0.9522	0.9564	0.9312	0.9484	0.9594	0.9751	0.9699	1				
13	0.9597	0.9683	0.9492	0.9547	0.9574	0.9591	0.9298	0.9556	0.978	0.9791	0.9747	0.9645	1			
14	0.9581	0.9641	0.9470	0.9528	0.9527	0.9462	0.9216	0.9616	0.9584	0.9746	0.9634	0.9636	0.9636	1		
15	0.9687	0.9632	0.9685	0.9610	0.9652	0.9431	0.9306	0.9506	0.9455	0.9592	0.9439	0.9401	0.9445	0.9546	1	
16	0.3622	0.3173	0.4253	0.3747	0.3569	0.3130	0.3121	0.3143	0.3068	0.3133	0.3161	0.3166	0.3032	0.3220	0.4138	1

Population numbers are as given in Table 1.

4. Discussion

Genetic analysis of diversity among and within domestic populations of channel catfish in Alabama was conducted using AFLP markers. Our results show the utility of AFLP analysis for assessing within- and among-population diversity in the species without the need for developing species-specific genetic markers. Our findings indicated that some distinct differences exist among and between both some farm strains and research lines of channel catfish in Alabama. The most distinctive population was Hicks Farm in Southeast Alabama. The pattern of genetic diversity observed has practical implications for management of brood stocks and for selective breeding programs. The data can also be used as baseline information for further study of the interactions between domestic and wild populations of channel catfish.

Previously, genetic variation had been studied in channel catfish utilizing isozymes (Dunham and Smitherman, 1984; Hallerman et al., 1986; Carmichael et al., 1992). Hallerman et al. (1986) reported percent polymorphic loci (P), 0–28.6%, and observed mean heterozygosity values (H) 0.000–0.070 (H averaged over all populations: 0.0431) within nine research lines and strains of channel catfish at Auburn University. These values are considerably lower than those reported in the present study (P: 18.3–100%; H: 0.0721–0.2471; H averaged over all populations: 0.135), though similar strains were analyzed here as by Hallerman et al. (1986). The differences are likely due to the ability of AFLP to resolve more loci and to detect greater levels of polymorphism than isozyme analysis, and by the fact that AFLP DNA markers should be neutral, whereas allozyme variation results from differences at coding regions, some of which were inferred to be subject to selective pressures (Hallerman et al., 1986).

If we exclude Hicks Farm, F_{st} averaged over all loci was 0.176. This was surprisingly similar to the result of Hallerman (1984) who found an F_{st} of 0.240 for the ancestral research lines of the research lines examined in the current study. One might expect the allozyme analysis, some of which were selected markers, to yield a more differentiated population structure, but this might be offset by the greater power of the AFLP analysis in finding markers with large frequency differences. Additionally, similarity values were close in magnitude between the two studies. Major clusters in the isozyme study had similarities of 0.96 (Hallerman et al., 1986), the same as the genetic similarity in the AFLP study for Auburn research lines in the two studies. In regards to detecting genetic variation, AFLP analysis indicated more genetic variation; however, in defining overall population structures, the two studies seem to yield similar values of differentiation, although the populations examined were not exactly the same. In regards to certain clusters, the AFLP analysis yielded greater genetic distances. The population-wise similarity analysis done in the present study also reported 96% genetic similarity between all populations except Hicks Farm (34%), with an overall average genetic similarity value of 88%. The Jaccard's individual-wise clustering analysis revealed 79% average similarity, with 82% average similarity among Auburn strains.

The ancestry of a large percentage (42%) of domestic channel catfish can be traced to a single collection from the Red River in Oklahoma, by the Arkansas Game and Fish Commission in 1949 (Dunham and Smitherman, 1984). These fish served as the basis for some of the first farm and hatchery stocks of channel catfish in Arkansas, Louisiana,

Mississippi, and Alabama, including at least half the Auburn line (Dunham and Smitherman, 1984). Most of the farm populations have their origins from the Auburn University hatchery or the Marion hatchery, which is the state-run hatchery in Alabama. The ancestry of these two populations is very similar, as the two hatcheries exchanged broodstock in the past (Dunham and Smitherman, 1984).

The Hicks Farm population in Covington County had the highest diversity (*P*: 100%). While a portion of the broodstock of many of the farm populations in this study were acquired from Auburn University, Hicks Farm population was founded with fish from ponds in Florida. Because the origins of populations 1–15 are fairly similar, and that of Hicks Farm is different from these groups, the similarity value of this population is very low (34%) on the population-wise similarity tree (Fig. 3). Hicks Farm also stands out in that it forms a strong cluster, with a 100% bootstrapping confidence, on the Jaccard's pairwise similarity tree (Fig. 2).

The *P* values (estimates of genetic diversity) reported in the present study were higher (42–100%), for farm populations, than for research populations (18–32%). The greater diversity of the farm populations compared to the Auburn research lines was confirmed by the individual-wise cluster analysis. The farm populations/individuals were highly interdigitated, while the Auburn lines/individuals formed distinct, strongly supported clusters. These differences in genetic diversity may be a reflection of the method of breeding for these populations. The farm populations were randomly bred and had large effective population sizes, while the research lines had small effective population sizes and were selectively bred, except for Marion random. While most Auburn individuals clustered together with 82% individual-wise similarity, Forks albino had an 89% similarity value. The specific selection for albinism probably explains its reported low diversity (*P*: 18%) and relatively high individual-wise similarity value (89% between individuals). This selection for albinism might also explain the relatively lower population-wise similarity value (90% by Nei's unbiased identity measure), as compared to 96% similarity for most of the other populations. Because albinism in channel catfish is a recessive trait, selection for this characteristic tends to lead to increased homozygosity.

Of the nine Auburn University lines, the group with the highest *P* value (32%) was G, Goldkist. G was comprised of first-generation fish from the mating of two select lines transferred to Auburn from Goldkist, which should lead to a higher diversity than in lines with a longer history of both selection and small population size. Indeed, as would be expected from such a breeding history, individuals from this population were interdigitated throughout the individual-wise similarity tree, clustered with individuals from many different populations.

Further clustering relationships of the Auburn research populations are correlated to historical and breeding observations. The Tishomingo line, which originated at the Tishomingo National Fish Hatchery in Oklahoma (Dunham and Smitherman, 1984), clustered together on the Jaccard tree with TA, a line generated by combining Tishomingo and Auburn lines.

When channel catfish were subjected to selection for growth, allozyme polymorphism was reduced compared to control strains, and analyses indicated that some of the reduced diversity was due to selection (Hallerman et al., 1986). In the current study, lines selected

for body weight and one line selected for albinism also exhibited a reduction in AFLP polymorphism compared to randomly bred farm populations, implying that selection for body weight can reduce AFLP polymorphism. However, Marion random, a randomly bred progenitor to most of these selected research lines, had an almost identical reduction in AFLP polymorphism, suggesting that random genetic drift from reduced population numbers had as large or larger effect on polymorphism compared to selection. This is in agreement with the inferences of Hallerman et al. (1986), and is consistent with AFLPs being selectively neutral markers.

Overall, average genetic similarities were 88% by population-wise similarity and 79% by individual-wise similarity. The estimated F_{st} value averaged over all polymorphic loci was 0.4456, which indicates a high degree of genetic differentiation. With the Hicks Farm population excluded from the analysis, the F_{st} was considerably lower (0.1763), indicating a moderate degree of genetic differentiation among the remaining populations.

Channel catfish is the primary aquaculture species in the United States (Naylor et al., 2000), and is an extremely important economic resource in the Southeast, including Alabama. This study revealed significant genetic diversity in Alabama catfish brood stocks. Theoretically, genetic diversity is important because it can give populations the ability to adapt to environmental changes and stressors and to artificial selection. Presently, one of the largest problems in the catfish industry is the prevalence of diseases such as enteric septicemia of catfish (ESC) and columnaris disease (NAHMS, 1995, 1997). While selection for disease resistance is necessary and studies are being undertaken to map genetic markers that may be linked to disease resistance in channel catfish (Liu, unpublished), increased selection pressure without maintaining genetic variation could have long-term adverse effects on the future response of channel catfish stocks to selection. Genetic diversity data can be effectively used to mate individuals or populations having high performance, but having a varied genetic background, in order to maintain genetic diversity for future selective breeding such as preventing catastrophic disease epidemics while maintaining or improving performance for production traits. Additional research is needed to determine the population genetic structure among wild populations of channel catfish, and to determine if domestic farm and research stocks are affecting genetic diversity in wild populations. The baseline data established in the current study should allow the examination of such potential interactions between domestic and wild populations.

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