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Original Research Article

Population Genetic Structure of Feral and Cultured African Catfish (*Clarias gariepinus)* inferred from Random Amplified Polymorphic DNA in Kano, Nigeria

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ABSTRACT

Information on the genetic structure of fish is a useful means for optimizing identification of stocks, stock enhancement, breeding programs, management of sustainable yield and preservation genetic diversity. The population genetic structure of African Catfish, *Clarias gariepinus* (Burchell, 1822) in cultured population and feral populations from Rivers Guzuguzu, Fada and Magaga (Kano State) were investigated using RAPD (Random Amplified Polymorphic DNA). Using a CTAB protocol, genomic DNA was extracted from the caudal peduncle of 157 samples of live specimen collected from each population. Five RAPD primers were used to amplify different loci on the extracted genomic DNA by Polymerase Chain Reaction (PCR) and the resultant DNA fragments were analyzed on agarose gel. A total of 406 reproducible bands were obtained in four populations for five primers. The dendrogram separated the four *C. gariepinus* populations into two distinct clades, Guzuguzu and Fada populations being in one clade, while Magaga and Cultured populations belonged to the other clade. The results based on RAPD-PCR profile ranged from 0.012 in Magaga/Cultured to 0.089 in Guzuguzu/Cultured. The genetic identity of *C. gariepinus* from four populations also ranged between 0.885 in Magaga/Guzguzu to 0.998 in Magaga/Cultured. The Nei's genetic distance and identity also confirmed the above information with the following ranges: 0.002 in Fada/Cultured to 0.102 in Magaga/Guzuguzu, and 0.903 in Magaga/Guzuguzu to 1.000 in Magaga/Cultured respectively. In conclusion, the genetic diversity and allele richness of the feral and domestic fish populations were comparable.

Keywords: Farmed fish, Genetic Variation, Gene Flow, RAPD, Kano, Nigeria.

Introduction

Fish is one of the least expensive source of high-quality animal nutrients available for the world's millions of underprivileged families.[1] Wild stock levels have been dropping, leading to a loss in biodiversity as a result of exploitation, human activities, pollution, and global warming.[2] Despite the increased demand for aquatic animal protein, the capture production of fish has been constant since 2001 at about 90.4 million tons. [3]

Genetic variation is beneficial and important for the long-term survival of natural populations as it ensures the provision of high fitness levels, allowing populations to adjust to new environmental conditions,[4] and it has resulted in a fascinating phenomenon that is anticipated to be the consequence of mutation or migration to a genetically dissimilar population.[4] Genetic degradation is caused by a lack of understanding in the field of fish farming. It happens as a consequence of inbreeding, negative selection and hybridization, resulting in an excess of homozygosity in the population and a decline in productivity. The escape of hatchery-bred fish has been reported to cause a loss of genetic diversity in the wild population as a result of hybridization and competition between escaped cultured fish and the feral stock or species.[5]

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The common methodologies used in the identification and characterization of catfishes have been morphological, i.e. meristic, and morphometrics with anatomical features.[6] Despite this, there is significant ambiguity due to morphological resemblance, which has led to some contested species identification hypotheses.[6-7] It was found that molecular tools can provide valuable data for accurate systematics, confirmation of systematic circumstances and phylogeny.[8] The degree of genetic variation in a population clearly specifies what kind of changes it might have experienced in the past, what the current situation is and what the probability of sustenance is in future.[9] It was reported that low levels of genetic diversity have a negative correlation with the potential for adaptation to changing environmental conditions, and that the survival of endangered species may be threatened. Inbreeding is a common scenario in fish hatcheries [10] and the offsprings produced which are genetically inferior are used to stock ponds, and in most cases unintentionally escape into river bodies, causing feral gene introgression into pure wild stocks.[11-12] It reviewed genetic variability in *Clarias gariepinus* and found strong evidence of inbreeding, founder effects and genetic drift in most captive populations.[13] Thus *C. gariepinus* may be prone to loss of genetic diversity and variability due to the extinction of genetically distinct wild populations as a result of the escape of hatchery reared fish or the ranching of fry, as in carps.[14]

Molecular genetic markers, such as Random Amplified Polymorphic DNA (RAPD), has been used extensively to study genetic diversity of cultured and feral stock. RAPD is a multilocus genetic marker based on Polymerase Chain Reaction (PCR). It possesses the benefit of simplicity and speed, because unlike other PCR-based technologies, it does not require prior knowledge of the genome or the gene sequence in the organism that is being genetically interrogated.[15-16]. This study aimed to establish population genetic similarity between feral and cultured African Catfish inferred from Random Amplified Polymorphic DNA in Kano, Nigeria

Materials and Methods

Study location

The study was conducted in three selected water bodies and a commercial fish farm in Kano State between January and December, 2022. These are Rivers Guzuguzu, Fada and Magaga to the western part of the state so also the commercial fish farm. Kano State is located in the semiarid area of North-western Nigeria. It has a population of 9,383,682 comprising of 4,844,128 males and 4,539,534 females.[17] Kano State is the commercial nerve centre of Northern Nigeria. It is located between latitude 10º33' and 12º27'North of the equator and longitude 7º34' and 9º29' East of the Greenwich meridian and as such it is part of Sudano-Sahelian vegetation zone of Nigeria.

Sample collection

The feral fish samples were identified using an exposition for identification[18] and confirmed using local names provided by the fishermen. Live fish samples of *C. gariepinus* (97) from the three locations described above were purchased from commercial catches of the fishermen. Sixty cultured samples of the same specie were equally purchased from a commercial fish farm in the same area. The samples were adults and were transported in large bowls to Animal Science Department of Bayero University Kano.

Sample preparation

At the laboratory of the Department of Animal Science, Bayero University, the fish samples were severed at the caudal peduncle to remove the tissue and placed in whitish rubber containers (2.5 radius) with covers and 20mls of Ethanol (99 %) for preservation. The prepared samples were later transported by air to the laboratory of African Bioscience Ltd in Ibadan, Oyo state for analysis.

Laboratory analysis

Small sections (0.1 g) of the preserved caudal peduncle were cut, rinsed, rehydrated in distilled water, and then transferred into microcentrifuge tubes containing pre-warmed CTAB lysis buffer (60°C) in preparation for homogenization. The constituents of the CTAB buffer include; 2% CTAB (hexadecyl trimethyl ammonium bromide), 100 mM TrisHCL pH=8, 20 mM EDTA, 1.4 M NaCl, 0.2% β- mercaptoethanol (added before use), 0.1 mg/ml proteinase K (added before use). The mixtures of samples and buffer were homogenised and incubated at 60°C for 30 minutes with continuous shaking. They were then allowed to cool before the addition of 200 µl Chloroform. The micro-centrifuge tubes were capped and inverted several times to mix. The mixtures were then spun for 10 minutes at 14000 g in Biologix High Speed Micro-centrifuge tubes, after which the aqueous upper phase containing DNA was transferred into fresh tubes. To precipitate the DNA from the aqueous phase, 300 µL of Isopropanol was added and mixed. The tubes were thereafter left on ice overnight.

The mixture was spun at 14000 g for 10 minutes on the second day and the supernatant was discarded, leaving behind the DNA pellets to which 10 µL of RNase A was added. The samples were incubated again for 30 minutes at 37°C. After cooling, 500 µL of ethanol was added to the samples, and incubation at room temperature (25°C) was allowed for 30 minutes. The samples were spun at 14,000 g for 10 minutes, supernatant decanted and the pellets left to dry for 30 minutes before re-suspending in 100 µL of sterile water. The integrity and purity of the genomic DNA isolates was checked by loading on 2% agarose gel. The extracted DNA were stored at -20ºC for 3 days.

PCR Amplification and Electrophoresis

RAPD PCR set-up

DNA samples were diluted to 1:10 solutions to ensure diluting out of any PCR inhibitors. Thermo-cycling conditions of initial denaturation, 95ºC for 5 minute; denaturation, 95ºC for 45 second; annealing, 37ºC for 1 minute; extension, 72ºC for 2 minutes and final extension of 72ºC for 5 minutes x 45 cycles.

A series of optimization experiments were conducted using the protocol described[19] to determine which conditions produced the strongest and most reproducible patterns. Five commercially available decamer primers (OPA 10, OPA 09, OPA 11, OPA 03 and OPAB 06) from Operon technologies (Alameda, CA, USA) were used for this study. The amplification reactions were performed in volumes of 25uL, containing 50µg of genomic RAPD, 2mM MgCl2, 100uM of dATP, dCTP dGTP and dTTP each, 0.2um of the primer and 0.5 units of Taq DNA polymerase. The total volume of the PCR products were evaluated in 2% agarose gels and visualized by ethidium bromide staining. After electrophoresis, DNA bands profiling were observed under UV light, and the images were documented. The pictures from the gel were used for the analysis of the amplified products.

Data analysis

Amplified fragments were scored as binary data, i.e. presence as 1 and absence as 0. Only data generated from reproducible bands were used for statistical analysis. The number of polymorphic loci, percentage of polymorphic loci (%P), observed number of alleles (ne) and Nei's gene diversity (H) were estimated using the GenAlEx 6.41 software which gives an unbiased estimate of allele frequencies from RAPD data. Genetic distance and identity were estimated using GenAlEx 6.41 software. It was also used to construct an unweighted pairgroup method with average (UPGMA) dendrograms based on the estimated genetic distances.

Results and Discussion

Five primers (OPA-03, OPA-11, OPA-09, OPA-10 and OPAB-06) generated reproducible bands. A total of 406 reproducible bands were obtained in four populations for five primers (Table 1) while the allele attribute are shown in Table 2-4. The random amplified polymorphic DNA fingerprints were scored as binary matrix according to the specification of GenAlEx 6.41 and accounting for missing values wherever found.

The Nei's genetic distance and identity of *C. gariepinus* from four populations with the unbiased Nei's genetic distance and identity were presented in Tables 5 and 6. The results based on RAPD-PCR profile ranged from 0.012 in Magaga/Cultured to 0.089 in Guzuguzu/Cultured. The genetic identity of *C. gariepinus* from four populations also ranged between 0.885 in Magaga/Guzguzu to 0.998 in Magaga/Cultured. The Nei's unbiased genetic distance and identity also confirmed the above information with the following ranges: 0.002 in Fada/Cultured to 0.102 in Magaga/Guzuguzu, and 0.903 in Magaga/Guzuguzu to 1.000 in Magaga/Cultured respectively. In the findings of Thorpe and Sole-Cava,[20] that 98% of populations of the same species have genetic similarity above 0.85, the Nei's genetic identity (genetic similarity) of 0.988 and 0.998 were observed between the feral and the cultured populations in this study.

Table 1: Size and molecular weight of fragments amplified by RAPD primers

RAPD Primer	Sequences (5'to 3')	Size Range	% GC Content	Mol. Weight (g/M)
OPA 10	GTGATCGCAG	200bp - 2500bp	60	2957
OPA 09	GGGTAACGCC	200bp - 1500bp	70	2930
OPA 11	CAATCGCCGT	200bp - 2500bp	70	3002
OPA 03	AGTCAGCCAC	250bp - 2500bp	60	2948
OPAB 06	TGCTCTGCCC	250bp - 3000bp	60	3074

Table 2: Allele Frequencies and Sample Size by Population (Total Haploid Data)

Locus	Allele No	Guzuguzu	Magaga	Fada	Cultured	Total
OPA03	N- (1)	29 (1.000)	8 (1.000)	7 (1.000)	8 (1.000)	52 (1.000)
OPA11	N- (1)	29 (1.000)	3 (1.000)	7 (1.000)	4 (1.000)	43 (1.000)
OPA09	N- (1)	28 (1.000)	10 (1.000)	9 (1.000)	10 (1.000)	57 (1.000)
OPA10	N- (1)	27 (1.000)	8 (1.000)	9 (1.000)	10 (1.000)	54 (1.000)
OPA06	N- (1)	30 (1.000)	9 (1.000)	10 (1.000)	9 (1.000)	58 (1.000)
% P	SE (5%)	80%	80%	80%	60%	Mean (75%)

Table 3: Sample size, No of Allele, No of Effective Allele, Information index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, Diversity and Unbiased Diversity for Guzuguzu, Fada and Magaga Rivers

Locus	N	Na	Ne	I	H	Uhe
OPA03	30.00	2.00	1.07	1.15	0.06	0.07
OPA11	30.00	2.00	1.07	1.15	0.06	0.07
OPA09	30.00	2.00	1.14	0.25	0.12	0.13
OPA10	30.00	2.00	1.22	0.33	0.18	0.19
OPA06	30.00	1.00	1.00	0.00	0.00	0.00
Mean	30.00	1.80	1.10	0.17	0.09	0.09
SE	0.00	0.20	0.04	0.06	0.03	0.03
OPA03	10.00	2.00	1.47	0.50	0.32	0.36
OPA11	10.00	2.00	1.72	0.61	0.42	0.47
OPA09	10.00	1.00	1.00	0.00	0.00	0.00
OPA10	10.00	2.00	1.47	0.50	0.32	0.36
OPA06	10.00	2.00	1.22	0.33	0.18	0.20
Mean	10.00	1.80	1.38	0.39	0.25	0.28
SE	0.00	0.20	0.20	0.11	0.07	0.08
OPA03	10.00	2.00	1.72	0.61	0.42	0.47
OPA11	10.00	2.00	1.72	0.61	0.42	0.47
OPA09	10.00	2.00	1.22	0.33	0.18	0.20
OPA10	10.00	2.00	1.22	0.33	0.18	0.20
OPA06	10.00	1.00	1.00	0.00	0.00	0.00
Mean	10.00	1.80	1.38	0.37	0.24	0.27
SE	0.00	0.20	0.20	0.11	0.08	0.09

Na=No. of Different Alleles, Ne=No. of Effective Alleles, I=Shannon's Information Index, h=Diversity, Uhe=Unbiased Diversity

This lower genetic diversity in both cultured and feral compared to what were recorded in similar studies could be an outcome of several factors which include the types of RAPD primers employed or it may indicate a loss or an on-going loss of variability in both the cultured and feral species which may need to be stemmed by conservation interventions. [21-22]. The higher value of RAPD fragments observed in the 157 individuals showed a reasonable degree of genetic diversity within and between the populations. Five random primers for DNA fingerprinting of *C. gariepinus* generated a total of 406 bands from the 157 individuals in the four populations. 80% of feral and 60% of cultured *C. gariepinus* were polymorphic. The percentage of polymorphic loci was higher than that observed in the same species in Egypt, which was 69.5%.[23] This might be due to the level of cultivation or high level of mixing between the feral and cultured *C. gariepinus*. The values for feral and cultured *C. gariepinus* fall between those reported in *C. batrachus* (86.66%) in India,[22] and in *H. fossilis* (83.87%) in India.[24] However, the values were higher than those in two populations of *H. fossilis* (18.75%),[22] and three populations of *C. batrachus* (25-35.5%) reported in India. [25] Polymorphic of 44% to 64% in five populations of *D. labrax* (L.) was also reported. [26] A higher value recorded in this study might be due to species differences. A higher percentage of polymorphic loci obtained in the feral populations of *C. gariepinus* (80%) indicated a relatively higher level of genetic variation. However, a lower percentage of polymorphic loci (60%) in the cultured population could be an indication of inbreeding in the hatchery population compared with the respective natural populations. The same percentage of polymorphic loci (64.52%) was also reported in the hatchery population of *H. fossilis*, *C. catla* and *L. rohita* using RAPD marker analysis. [24,27-28]

This study recorded percentage polymorphic loci of 80% for all the feral populations and 60% for the cultured population. These are in agreement with number of reports with similar percentages. [5, 21,23] In this study, the cultured and the feral populations showed a similar level of inherent genetic diversity and allele richness as revealed by indicators such as the percentage of polymorphic loci (%P), Number of Alleles (Na), Number of effective alleles (Ne), Shannon Information index (I), and expected heterozygosity (Nei's gene diversity) (Tables 3a and 3b). These Figures were in the same range with the ones reported in similar studies. [5, 21,23, 29]

Phylogenetic similarity between and within the four populations of *C. gariepinus* was depicted by UPGMA dendrogram (Figure 1) which

separated the four *C. gariepinus* populations into two distinct clades, Guzuguzu and Fada populations being in one clade, while Magaga and Cultured populations belonged to the other clade.

Conclusion

In this study, the cultured and the feral populations showed a similar level of inherent genetic diversity and allele richness as revealed by indicators such as the percentage of polymorphic loci (%P), Number of Alleles (Na), Number of effective alleles (Ne), Shannon Information index (I), and expected heterozygosity (Nei's gene diversity).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 4: Sample size, No of Allele, No of Effective Allele, Information index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, Diversity and Unbiased Diversity for Cultured Type

Locus	N	Na	Ne	I	H	Uhe
OPA03	10.00	2.00	1.47	0.50	0.32	0.36
OPA11	10.00	2.00	1.92	0.67	0.48	0.53
OPA09	10.00	1.00	1.00	0.00	0.00	0.00
OPA10	10.00	1.00	1.00	0.00	0.00	0.00
OPA06	10.00	2.00	1.22	0.33	0.18	0.20
Mean	10.00	1.60	1.32	0.30	0.20	0.22
SE	0.00	0.25	0.17	0.13	0.09	0.10
Grand Mean	15.00	1.75	1.29	0.31	0.19	0.21
SE	1.99	0.10	0.07	0.07	0.04	0.04

Na=No. of Different Alleles, Ne=No. of Effective Alleles, I=Shannon's Information Index, h=Diversity, Uhe=Unbiased Diversity

Table 5: Nei's Genetic Distance (below) and Nei's Genetic Identity (above diagonal) between the populations of *Clarias gariepinus* based on RAPD-PCR

	Guzuguzu	Cultured	Magaga	Fada
Guzuguzu	----	0.915	0.885	0.970
Cultured	0.089	----	0.988	0.967
Magaga	0.122	0.012	----	0.947
Fada	0.031	0.033	0.054	----

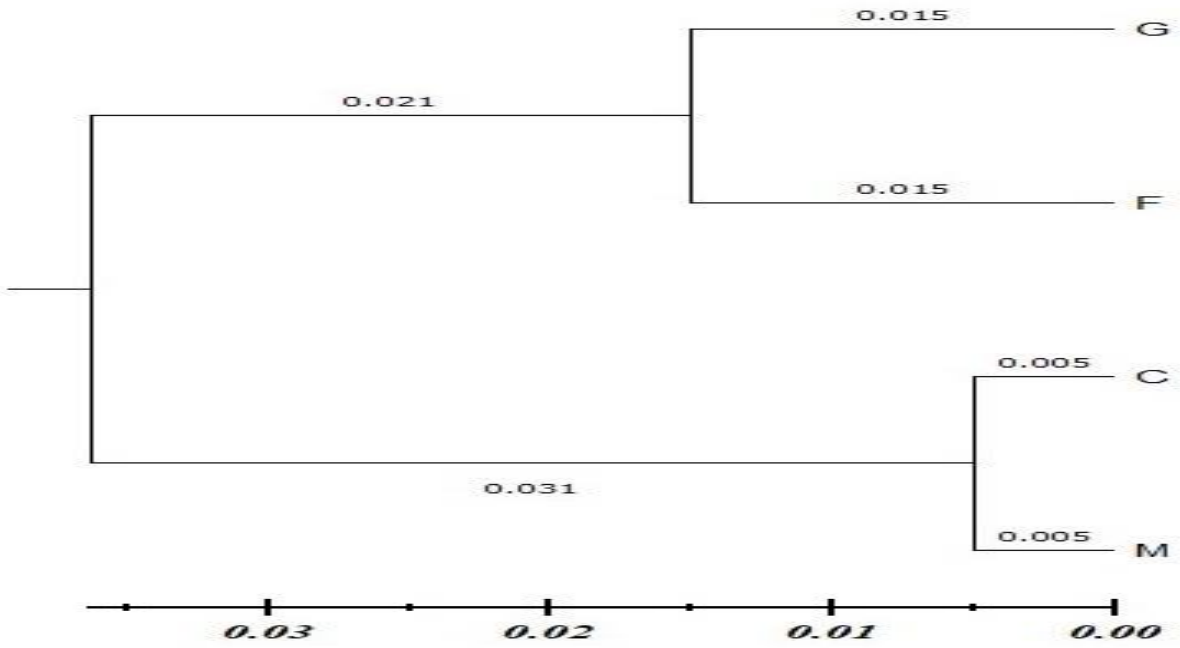

Figure 1: UPGMA Dendrogram of four populations of *C. gariepinus*

Table 6: Nei's Unbiased Genetic Distance (below) and Nei's Unbiased Genetic Identity (above diagonal) between the populations of *Clarias gariepinus* based on RAPD-PCR

	Guzuguzu	Cultured	Magaga	Fada
Guzuguzu	----	0.929	0.903	0.989
Cultured	0.074	----	1.000	0.998
Magaga	0.102	0.000	----	0.982
Fada	0.011	0.002	0.018	----

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