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Genetic Diversity in Cultured and Wild Population of *Clarias gariepinus* (Burchell, 1822) in Nigeria Using Random Amplified Polymorphic DNA (RAPD) and Microsatellite DNA

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Abstract

The population structure and genetic diversities of *Clarias gariepinus* from the cultured population at Chi Farm (Ajanla) and wild population at Asejire Reservoir (Asejire) were analysed using Random Amplified Polymorphic DNA (RAPD) and Microsatellite DNA markers. Using a CTAB protocol, genomic DNA was extracted from the caudal fins of 20 samples of live specimen collected from each population. Seven RAPD primers and seven pairs of microsatellite DNA primers were used to amplify different loci on the extracted genomic DNA by Polymerase Chain Reaction and the resultant DNA fragments were analysed on agarose gel. The RAPD primers amplified a total of 474 loci with 697 bands in all samples for the seven primers studied. The cultured population from Chi farm showed a total of 366 bands, while the wild population from Asejire Reservoir displayed 331 bands. The cultured population showed a negative inbreeding coefficient (F) of -0.173 ± 0.209, which statistically suggests excess heterozygosity, while a positive but low inbreeding coefficient of 0.042 ± 0.243 was estimated for the wild population. The Analysis of Molecular Variance (AMOVA) for both genetic markers indicated significant difference (p=0.01) between the two populations. The result of the study suggests a loss or on-going loss of genetic variability, which needs conservation intervention in the two populations studied.

Keywords: *Clarias gariepinus*; Microsatellite DNA; RAPD; Genetic diversity; Cultured; Wild

Introduction

C. gariepinus (Burchell 1822) belongs with the ray-finned (Class Actinopterygii) and air-breathing (Family Clariidae) catfishes (Order Siluriformes). The family Clariidae is naturally distributed across Africa, south and south-east Asia with the highest genetic diversity in Africa [1]. Genetic diversity is necessary for the survival of species because it confers better adaptability to changing environment [2-4]. Genetic diversity at a level below the species leads to formation of groups referred to as stocks, which are fundamental units of evolution. They are used by fishery biologists as a basis to manage commercially important marine organisms [5]. Patterns of genetic diversities between stocks provide clues to the histories of the populations and also reveal the degree of evolutionary isolation [6].

Genetic diversity is a critical measure in population studies because, by hinting on the evolutionary history of a population, it reveals the current and future health of the population [7]. Low levels of genetic diversity causes inbreeding depression in the short run and reduced evolutionary potential in the long run [7]. Inbreeding is marked by increasing homozygosity. This leads to loss of fitness because homozygous advantage is lost and deleterious recessive genes are unmasked. The evolutionary potential, defined as the ability of a species to adapt to novel selective pressures, also declines with decreasing genetic diversity, because the limited gene pool diminishes the likelihood of the existence of adaptive alleles in the genome of affected species [7-9].

Molecular genetic markers, such as Microsatellite DNA and Random Amplified Polymorphic DNA (RAPD), have been used extensively to study genetic diversity of cultured and wild stock [10,11]. RAPD is a multilocus [12] genetic marker based on Polymerase Chain Reaction (PCR). It possesses the benefit of simplicity and speed [12], because unlike other PCR-based technologies [13], it does not require prior knowledge of the genome or the gene sequence in the organism that is being genetically interrogated. However, RAPD produces some complex and un-reproducible band patterns which make comparison of results difficult. For this reason, RAPD are unsuitable for database purposes [6].

Microsatellite DNA as a PCR-based molecular marker is more robust than RAPD. It is a single locus marker and the results are highly reproducible. This makes archiving in databases and sharing of results between laboratories possible. However, the development and isolation of the loci require prior knowledge of the genome which has now been made easier by the development of the Next Generation Sequencing (NGS) methods [14].

The study of genetic diversity of the Nigerian stocks of *C. gariepinus* is important because the species, as an important source of animal protein, commands high commercials values due to its high fecundity, high palatability, resilience, disease resistance and rapid growth [15]. The need to monitor the levels of genetic diversity is profound because genetic diversity is closely linked to the evolutionary potential and the survival of the species.

To effectively manage brood stocks for optimum productivity, it is important to compare the genetic composition of cultured with the wild population because significant loss of genetic variation, attributable to low effective number of parents, domestication selection

or the mating design, among other factors, have been reported in hatcheries. Moreover, the feral introgression of the cultured into the wild as a result of escapee fishes has a great tendency of eroding the genetic diversity of the wild, especially if the genetic composition of the cultured population had not been properly managed [16].

Chi farm, Ajanla was established in 1986 on over a hundred hectares of land and is a major producer of aquaculture, poultry and cattle products in Nigeria. The location of the farm is about 40 km from Asejire Reservoir; a wild fishery which supplies Ibadan and its environs. In this present study, seven RAPD and seven microsatellite DNA markers were used to reveal and compare the genetic structure of cultured and wild populations of *C. gariepinus* from Chi farm, Ajanla, and Asejire Reservoir respectively.

Materials and Methods

Sampling and study areas

Twenty samples each of *C. gariepinus* were collected from Chi Farm, Ajanla (7° 14' N, 3° 49' E) and Asejire Reservoir (7° 24' N, 4° 8' E), South-western Nigeria (Figure 1). The sample was identified in the laboratory using fish identification keys by Olaosebikan [17]. The caudal fins, from which DNA was extracted were excised and preserved in 80% ethanol until needed.

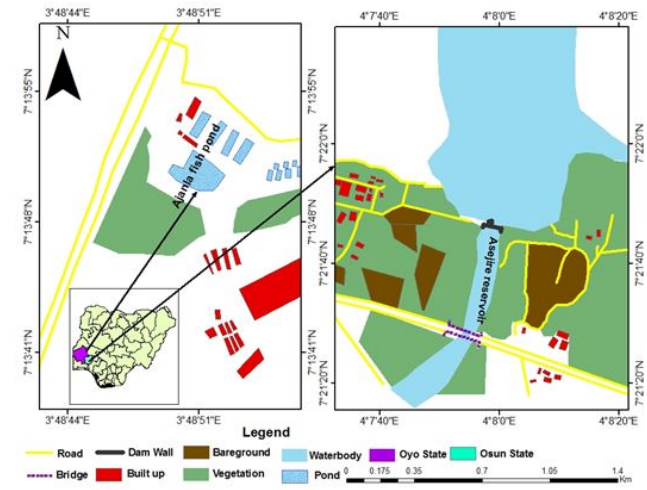

Figure 1: Map showing the study locations.

DNA isolation

Small sections (0.1 g) of the stored fin samples were cut, rinsed, rehydrated in distilled water, and then transferred into micro-centrifuge 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 1% agarose gel.

PCR and electrophoresis

A 10 µl reaction comprising the following was set up for each sample DNA: 5 µL of MyTaq Master mix, 1µL of 10 µM Primer (0.5 µL of each of Forward and Reverse Primers for microsatellite DNA) and 3 µL of Nuclease free water. The set up was prepared on ice. All PCRs were run with the following programme on the thermal cycler: initial denaturation at 95°C for 3 mins, denaturation at 94°C for 30 sec, annealing for 30 sec, and extension at 72°C for 30 sec and final extension at 72°C for 10 mins. The annealing temperature varied according to melting temperatures of the primers. A low annealing temperature of 37°C was employed for all the RAPD primers. See Tables 1 and 2 for primer sequences and the annealing temperatures for the microsatellite DNA primers. The amplified fragments were resolved by gel electrophoresis on 2% agarose SFRTM (Super Fine Resolution) procured from VWR, Canada.

Data analysis

A 100 bp DNA ladder (Norgen PCR Sizer 100 bp DNA Ladder) loaded along with the gels was used for band sizing. For microsatellite DNA, bands were manually scored and the sizes estimated by semi-log plot. RAPD bands were scored as binary data using GelQuest [18]. The data generated were analysed using the Genalex 6.502 [19,20].

Primers	Sequence (5'→3')
OPA 02	TGC CGA GCT
OPA 03	AGT CAG CCA C
OPC 02	GTG AGG CGT TC
OPB 08	GTC CCA CAC GG
OPC 11	AAA GCT GCG G
OPA 12	TCG GCG ATA G
OPA 19	CAA ACG TCG G

Table 1: List RAPD markers used in the study.

Primer	Sequence (5'→3')	Annealing Temperature (°C)

Cga 01 F	GGC TAA AAG AAC CCT GTC TG	53
Cga 01 R	TAC AGC GTC GAT AAG CCA GG	
Cga 02 F	GCT AGT GTG AAC GCA AGG C	53
Cga 02 R	ACC TCT GAG ATA AAA CAC AGC	
Cga 03 F	CAC TTC TTA CAT TTG TGC CC	49.1
Cga 03 R	ACC TGT ATT GAT TTC TTG CC	
Cga 05 F	TCC CAC ATT AAG GAC AAC CAC CG	56.9
Cga 05 R	TTT GCA GTT CAC GAC TGC CG	
Cga 06 F	CAG CTC GTG TTT AAT TTG GC	54
Cga 06 R	TTG TAC GAG AAC CGT GCC AGG	
Cga 09 F	CGT CCA CTT CCC CTA GAG CG	55.8
Cga 09 R	CCA GCT GCA TTA CCA TAC ATG G	
Cga 10 F	GCT GTA GCA AAA ATC CAG ATG C	54.4
Cga 10 R	TCT CCA GAG ATC TAG GCT GTC C	

Table 2: List of microsatellite primers utilized and their sequences.

Results and Discussions

Random amplified polymorphic DNA

The random amplified polymorphic DNA fingerprints (Figure 2) were scored as binary matrix according to the specification of Genalex 6.502 and accounting for missing values wherever found. A total of 474 loci with 697 amplified bands were scored in all samples for the seven primers studied. The cultured population accounted for 366 bands, while the wild populations produced 331 bands.

Popoola et al. [16,21-23] reported percentage polymorphic loci of 68.5, 100, 89.9 and 74.7 for cultured), and ~93% (and 100% for cultured) respectively, which are higher than the 47.9% and 60.8% observed in this study for the wild and cultured populations respectively. In this present study, the cultured population showed a higher level of inherent genetic diversity and allele richness than the wild 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). The Figures respectively recorded for these statistics in this study are: 60.8%, 1.215 ± 0.045, 1.083 ± 0.004, 0.141 ± 0.006, 0.071 ± 0.003 for the cultured population, and 47.9%, 0.958 ± 0.046, 1.076 ± 0.005, 0.120 ± 0.006, 0.063 ± 0.004 for the wild (Table 3). These Figures are lower than reported in similar studies [16,21,23-25].

The AMOVA with a fixation index, ΦPT=0.028, indicated that the sampled populations (cultured and wild) are significantly different from each other in the levels and composition of their genetic diversities at p=0.01, but not at higher probability levels (Table 3). 97% of this variation in genetic diversity came from within the populations, while only 3% is between the populations.

In accordance with the findings of Thorpe et al [26] that 98% of populations of the same species have genetic similarity above 0.85, the Nei's genetic identity (genetic similarity) of 0.998 were observed between the wild and the cultured populations in this study (Table 4).

This lower genetic diversity in both cultured and wild compared to what was recorded in similar studies could be an outcome of several factors which include the types of RAPD primers employed [23] or it may indicate a loss or an on-going loss of variability in both the cultured and wild species which may need to be stemmed by conservation interventions [27].

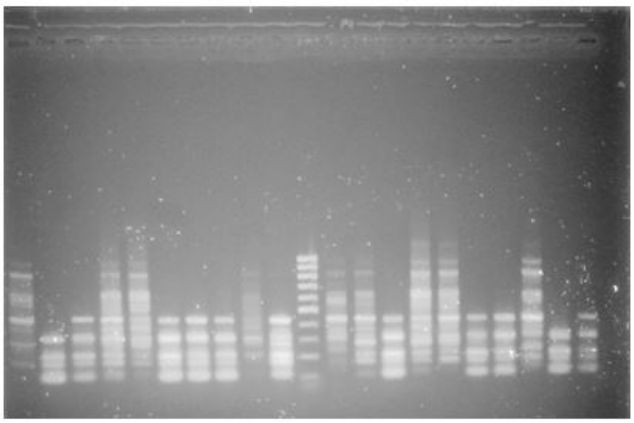

Figure 2: RAPD fingerprint of 20 samples using OPA 02 primer (100bp DNA ladder in the middle).

Populations	%P	Na	Ne	I	h	NI
Ajanla	60.8	1.215 ± 0.045	1.083 ± 0.004	0.141 ± 0.006	0.071 ± 0.003	0.998
Asejire	47.9	0.958 ± 0.046	1.076 ± 0.005	0.120 ± 0.006	0.063 ± 0.004	
Mean	54.3	1.086 ± 0.032	1.079 ± 0.003	0.130 ± 0.004	0.067 ± 0.002	

Na=No. of Different Alleles, Ne=No. of Effective Alleles, I=Shannon's Information Index, h=Diversity, %P=Percentage of Polymorphic Loci, NI=Nei's Genetic Identity.

Table 3: Basic indicators of genetic variation across populations for the RAPD data.

Source	df	SS	MS	Est. Var.	%	ΦPT	No. of permutations
Among Pops	1	26.575	26.575	0.491	3%		
Within Pops	38	636.45	16.749	16.749	97%		
Total	39	663.025		17.24	100%	0.028	999

df=degree of freedom, SS=Sum of Squares, MS=Mean Squares, Est. Var.=Estimated Variance, ΦPT=Fixation Index.

Table 4: Analysis of Molecular Variance (AMOVA).

Microsatellite DNA

The microsatellite DNA fingerprints (Figure 3) were scored using the base pairs of the bands; homozygotes with single bands scored as a single base pair value repeated twice and heterozygotes with double bands as two different base pair values, in accordance with the requirement of Genalex 6.502. Observed heterozygosity and expected heterozygosity, number of alleles, effective number of alleles, deviations from Hardy Weinberg equilibrium and all other statistics were computed in Genalex 6.502 [20].

All the assayed loci are polymorphic in both populations. The cultured population (Ajanla) has a higher heterozygosity of 0.419 ± 0.133 than the wild (Asejire) with 0.387 ± 0.152. Analysis across populations revealed a higher mean number of alleles in the cultured population (3.000 ± 0.724) than the wild (2.714 ± 0.286) with effective number of alleles at 1.705 ± 0.205 and 1.733 ± 0.230 respectively (Table 5). The mean heterozygosity of 40.3% observed in this study is similar to the 44.3% observed by Agbebi et al. [22] in a study comparing *C. gariepinus* and *Heterobranchus bidorsalis*, while the expected heterozygosity (0.361 ± 0.053) is lower than the 0.896 ± 0.111 reported by the same author. The lower expected heterozygosity may suggest reduced evolutionary potentials in these populations. The Shannon's Information index (I=0.629 ± 0.143 and 0.591 ± 0.115) observed in this study, which are lower than previously reported Figures from similar studies [22,28] may suggest a lower or loss of genetic diversity in the studied populations.

The Fixation Index (F), or Inbreeding coefficient, or Heterozygosity deficit, showed that the cultured population possessed excess heterozygosity with a negative coefficient of -0.173 ± 0.209, perhaps due to selection for heterozygotes through negative assortative mating. The wild population has a low rate of inbreeding (F=0.042 ± 0.243), but not an excess of heterozygotes (Table 5). In the wild and cultured populations respectively, 71.4% (5 out of 7) and 42.9% (3 out of 7) of the loci are in disequilibrium (Table 6). In the Analysis of Molecular Variance (AMOVA), the ΦPT for the total population is 0.719 (Table 7). This statistically demonstrates that a high level of genetic differentiation exists between and within the two populations studied.

Selection, mutation, migration (gene flow), genetic drift and population sub-structuring (Wahlund effect) are factors which can independently or unanimously cause disequilibrium. The variation in the frequencies of loci in disequilibrium between the cultured and the wild suggests that the two populations are under different selective pressures.

The disequilibrium in the wild population may be accounted for by any or a combination of poaching, population subdivision (Wahlund effect), genetic drift (Sewall Wright effect and Founder effect) and natural selection. The genetic drift resulting from overfishing may account for the low diversity recorded in the wild population. The community around the Asejire Reservoir is rural with a handful of artisanal fishermen who depend on the catch from the reservoir for their livelihood. These artisanal fishermen may resolve to overfishing in order to make ends meet and without proper enforcement of existing fishery conservation laws, the erosion of the gene pool of the Asejire reservoir is imminent. Garg et al. [27] reported that in capture fishery, excessive exploitation, combined with poor fishery management result in the depletion of the fishery stocks; such depletion can result in loss of total gene pool.

The higher number of loci in Hardy-Weinberg equilibrium may suggest that the Ajanla farm, by accident or by design, have maintained a constant genotypic and gene frequencies. A laudable explanation is the fact that the farm management may be constantly selecting for the same set of specific traits that are of economic importance. This form of artificial selection could keep the frequency of the genes for these beneficial traits, and other linked genes and sequences, constant in the farm stock. It may also be inferred that no alien breed has been allowed into the stock in the recent generation.

The existence of an artificial selection program that focuses on breeding specific desirable economic traits (Heterosis and Complementarity) may also explain the excess heterozygosity recorded in the cultured population, because the desired combination of these traits are most likely found in the heterozygotes due to hybrid vigour (heterozygous advantage). This could also explain the reason for a lower level of genetic diversity in the cultured population, because

according to Barasa et al. [29] mixed ancestry of source populations poses risks to animal via outbreeding depression. Moreover, in order to prevent segregation to undesired traits, the farm management may have limited the parental stocks to few known signatures, while also actively preventing introgression of any alien breed. The low level of statistical gene-flow (Nm=0.098) between the cultured and the wild population (Table 7) underscores the point that the cultured population is actively guarded against introgression. This genetic isolation of the cultured population could limit the evolutionary potential of the stock and leave the fishes susceptible to an epidemic or any other harsh environmental conditions to which they have not adapted.

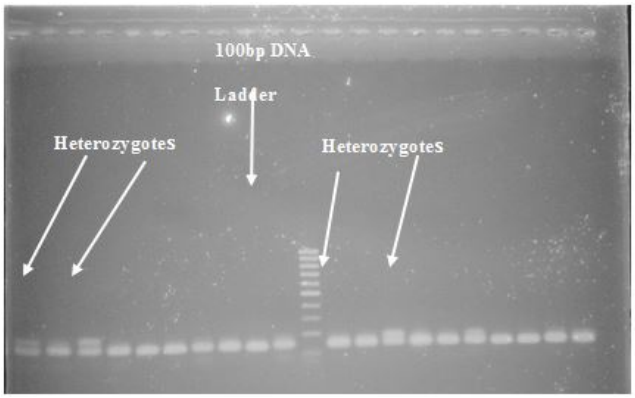

Figure 3: Microsatellite DNA fingerprints of 20 samples showing heterozygotes as double bands and homozygotes as single.

	%P	Na	Ne	I	Ho	He	F
Ajanla	100	3.000 ± 0.724	1.705 ± 0.205	0.629 ± 0.143	0.419 ± 0.133	0.36 ± 0.075	-0.173 ± 0.209
Asejire	100	2.714 ± 0.286	1.733 ± 0.230	0.591 ± 0.115	0.387 ± 0.152	0.361 ± 0.082	0.042 ± 0.243
Mean	100	2.857 ± 0.376	1.719 ± 0.148	0.610 ± 0.088	0.403 ± 0.097	0.361 ± 0.053	0.371 ± 0.055
Ho=Observed Heterozygosity, He=Expected Heterozygosity.							

Table 5: Basic indicators of genetic diversities for Microsatellite loci.

Pop	Locus	Degree of freedom	Chi square	Probability	Significance
Ajanla	Cga 01	1	0.131	0.717	ns
	Cga 02	21	60.34	0	***
	Cga 03	6	8.5	0.204	ns
	Cga 05	1	8.889	0.003	**
	Cga 06	1	0.131	0.717	ns
	Cga 09	1	20	0	***
	Cga 10	1	0.263	0.608	ns
Asejire	Cga 01	3	18.034	0	***
	Cga 02	6	20.147	0.003	**
	Cga 03	3	10.457	0.015	*
	Cga 05	1	2.99	0.084	ns
	Cga 06	1	0.013	0.909	ns
	Cga 09	1	19	0	***
	Cga 10	3	18.017	0	***
Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001.					

Table 6: Summary of Chi-Square tests for Hardy-Weinberg equilibrium.

Source	df	SS	MS	Est. Var.	%	ΦPT	Nm
Among pops	1	165.3	165.3	8.107	72%	0.719*	0.098
Within pops	38	120.2	3.163	3.163	28%	-	-
Total	39	285.5	-	11.27	100%	-	-

Table 7: Analysis of molecular variance (AMOVA).

Genetic variations, which may be considered 'the raw materials for evolution,' are important for the survival of species. There is a continuous need to improve and maintain a healthy level of genetic variability in organisms, particularly in economically important species like *Clarias*. The result of this study suggests a possible loss of variability in both the cultured and the wild populations. This loss, in the wild, may be attributed to overfishing, poaching, population subdivision, genetic drift and natural selection, while the loss of genetic diversity in the cultured population may be a result of strict breeding programs which may have genetically isolated the stock from alien populations. There is a need to deliberately inject new breeds into both the cultured and the wild populations in order to boost the evolutionary potentials of these populations. To avoid trading one woe for another, the introduction of these new genotypes must be done systematically, because outbreeding depression is an imminent risk, if the genetic distance between the populations and the new breeds is too large.

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