Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 25(2): 21 – 35 (2021) www.ejabf.journals.ekb.eg



Population Genetics of Fast- and Slow-Growing Strains of *Clarias gariepinus* (Osteichthyes: Clariidae) as Revealed by Microsatellite Markers

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ARTICLE INFO

Article History: Received: Nov. 16, 2020 Accepted: Dec. 18, 2020 Online: March 12, 2021

Keywords: Clarias gariepinus; Microsatellite; variability; growth; locus

ABSTRACT

This study employed microsatellite DNA markers on cultured fast- and slowgrowing strains of an important freshwater catfish, Clarias gariepinus from four populations in Southwestern Nigeria with a view to providing information on their genetic variability in relation to growth performance. Genomic DNA was extracted from the blood of each individual fish from the selected groups following standard procedures. The DNA sample collected was then amplified in a thermocycler using Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10 markers. The amplicons generated were subjected to genotyping and the corresponding alleles scored. The higher fixation index values identified in the slow-growing strains of adult C. gariepinus indicated the existence of small number of heterozygote genotypes and an excess of homozygote genotypes compared to the fast-growing strains of adult C. gariepinus, suggesting that heterozygosity confers better growth performance. The C. gariepinus fast-growing strains also had higher genetic variability than the slow-growing populations. The Cga02 locus was identified as the specific locus on which significance levels were consistently expressed. Thus, it may be a potential locus for breeding studies of C. gariepinus. Analysis by Chi-square tests across all loci and populations revealed that not all the seven loci conformed to Hardy-Weinberg equilibrium in each population.

INTRODUCTION

Among other fish in Nigeria, *Clarias gariepinus* is mostly reared because of its good properties and these have encouraged farmers to invest in its seed production. However, most *C. gariepinus* farmers use brood-stock of unknown genetic quality and this practice has led to inbreeding resulting in the production of unviable hybrids which subsequently cause low productivity in fish farms. Research to increase the yield of *C. gariepinus* focused mainly on the food and feeding habits (Adewumi and Ola-Oladimeji, 2016; Onura *et al.*, 2018), embryology (Olaniyi and Omitogun, 2014), hybridization (Awodiran *et al.*, 2000; Olaleye, 2005), cytogenetics (Aluko and Awopetu, 1995; Awodiran *et al.*, 2000; Majolagbe *et al.*, 2011) and genetic variability (Popoola *et al.*, 2014). Other genetic techniques that are being used for the improvement of aquaculture fishes are sex reversal, ploidy manipulations, androgenesis and gynogenesis (Dunham, 2011; Olaniyi and Omitogun, 2012).

Variation is genetically determined (**Ikpeme** *et al.*, **2015**). Hence, a wide variety of markers have since been developed for different aquaculture species. One commonly used method to study the genetics of populations is the use of microsatellite DNA markers.

Microsatellite DNA are short DNA sequences that exist in multiple copies repeated in tandem (**Pierce, 2012**). Microsatellites, also known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (SRTs) are composed of tandem repeats of two to six nucleotides that are dispersed across the genomes, both in coding and non-coding regions. Dinucleotide repeats are the most common and they are found in most species compared to other nucleotide repeats (mono, tri, etc.) (Li *et al.*, 2002). Most microsatellite loci are more or less small, ranging from few to a few hundred repeats and microsatellites containing a larger number of repeats are more polymorphic, though polymorphism has been observed in microsatellites with as few as five repeats (Karsi *et al.*, 2002).

Microsatellite markers have become a marker of choice in many genetic studies. Therefore, this study aims to characterize the fast- and slow-growing strains of *C. gariepinus* genetically using microsatellite markers and provide information on their genetic variability.

MATERIALS AND METHODS

1. Collection and identification of fish specimens

Eight-week old fast- and slow-growing strains of *C. gariepinus* produced by specially managed brood-stocks were identified and collected from four selected farms. Samples were collected from the following selected locations; the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Ogun State, (FUNAAB) (7° 09' 20" N, 3° 20' 42" E); Sun Fish Farm, Ibadan, Oyo State, (7° 22' 39" N, 3° 54' 21" E); Ade Aquaculture Centre, Ado-Ekiti, Ekiti State, (7° 37' 23" N, 5° 13' 15" E); and Edens Fish Farm, Ile-Ife, Osun State, (7° 28' 0" N and 4° 34' 0" E) in Southwestern Nigeria (**Fig. 1**). At the sampling sites, each of the 40 fast- and 40 slow-growing strains was separated appropriately and the fish were raised in different tanks simultaneously at Edens Fish Farm, Ile-Ife for 16 weeks.

2. Experimental setup

Clarias gariepinus juveniles in each tank were fed with commercial floating feed, containing 45% crude protein two times daily (7.30 a.m. and 6.30 p.m.). During the culture period, the water temperature was taken with a thermometer weekly while pH and nitrite were also measured weekly using Colombo Aquatest®, 2011 water testing kit to ensure that the environment was within tolerant limits expected for the *C. gariepinus* populations.

3. Evaluation of growth performance

At the end of the 16-week culture period for 320 fast- and slow-growers of *C*. *gariepinus*, five largest fast-growing *C*. *gariepinus* from each experimental grouping were sorted and coded accordingly as follows; FAB 1-5= Fast-growers Abeokuta 1-5, FIB 1-5= Fast-growers



Fig. 1. Map of Nigeria with colour-coded states where the experimental *C. gariepinus* juveniles were collected (**Ola-Oladimeji** *et al.*, 2020)

Ibadan 1-5, FAD 1-5= Fast-growers Ado-Ekiti 1-5, FIL 1-5= Fast-growers Ile-Ife 1-5. The five smallest slow-growers were also labelled as follows; SAB 1-5= Slow-growers Abeokuta 1-5, SIB 1-5= Slow-growers Ibadan 1-5, SAD 1-5= Slow-growers Ado-Ekiti 1-5 and SIL 1-5= Slow-growers Ile-Ife 1-5.

Growth performance of these strains was done by measuring their total length (TL) and body weight (BW) with a measuring board and digital electronic scale (TH-1000, PEC Medical USA) respectively.

4. Blood sample collection for DNA extraction

Genomic DNA was then extracted from the blood of each of the individual fish from the selected groups using NORGEN Blood Genomic DNA Isolation Kit (NORGEN, Biotechnology Corporation, Canada, 2014). The extraction was performed according to **El-Mogy** *et al.* (2016), using spin column chromatography method.

5. Microsatellite PCR and electrophoresis

DNA amplification was done using microsatellite primers (Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10), according to standard but modified methods of **Galbusera** *et al.* (1996). The characteristics of the microsatellite primers were utilized in **Table 1**.

The amplified products (8 μ l) of each sample were separated by electrophoresis in 1% agarose gels buffered with 1X TAE and the polymorphisms among the amplification products were detected by staining with 15 μ l Ethidium Bromide. A PCR Sizer 100 bp DNA ladder (100bp - 1,000bp) (Norgen Biotek Corporation, Canada) was electrophoresed alongside the microsatellite reactions respectively as molecular weight marker. Electrophoresis was run at 100 V for 2.5 h. Finally, the DNA bands were examined under Ultra-Violet (UV) transilluminator system (ZENITH, India), and the stained gels were photographed and saved

 Table 1. Characterization of seven microsatellite primer sets (Galbusera et al., 1996) used in the analysis, including the repeat array, primer sequences and annealing temperatures (Ta(°C))

Repeat array	Primer Sequences $(5' \rightarrow 3')$	Fa (°C)
(GT) ₁₅	F: GGCTAAAAGAACCCTGTCTG	55.0
()15	R: TACAGCGTCGATAAGCCAGG	
$(GT)_{10}N_2(GT)_8$	F: GCTAGTGTGTGAACGCAAGGC	54.6
	R: ACCTCTGAGATAAAACACAGC	
(GT) ₂₁	F: CACTTCTTACATTTGTGCCC	51.1
	R: ACCTGTATTGATTTCTTGCC	
$(GT)_{11}N_2(GT)_2$	F: TCCACATTAAGGACAACCACC	G 57.9
	R: TTTGCAGTTCACGACTGCCG	
$(GT)_5N_2(GT)_9$	F: CAGCTCGTGTTTAATTTGGC	56.0
	R: TTGTACGAGAACCGTGCCAGG	
$(GA)_{3}N_{3}(GT)_{11}N$	F: CGTCCACTTCCCCTAGAGCG	57.8
$(GT)_6N_2(GT)_4$	R: CCAGCTGCATTACCATACATGC	Ĵ
$(GT)_2N_2(GT)_{15}$	F: GCTGTAGCAAAAATGCAGATGC	C 56.4
	R: TCTCCAGAGATCTAGGCTGTCC	
	Repeat array $(GT)_{15}$ $(GT)_{10}N_2(GT)_8$ $(GT)_{21}$ $(GT)_{11}N_2(GT)_2$ $(GT)_5N_2(GT)_9$ $(GA)_3N_3(GT)_{11}N$ $(GT)_6N_2(GT)_4$ $(GT)_2N_2(GT)_{15}$	Repeat arrayPrimer Sequences $(5' \rightarrow 3')$ (GT)_{15}F: GGCTAAAAGAACCCTGTCTG R: TACAGCGTCGATAAGCCAGG(GT)_{10}N_2(GT)_8F: GCTAGTGTGTGAACGCAAGGC R: ACCTCTGAGATAAAACACAGC(GT)_{21}F: CACTTCTTACATTTGTGCCC R: ACCTGTATTGATTTCTTGCC(GT)_{11}N_2(GT)_2F: TCCACATTAAGGACAACCACCC R: TTTGCAGTTCACGACTGCCG R: TTTGCAGTTCACGACTGCCG(GT)_5N_2(GT)_9F: CAGCTCGTGTTTAATTTGGC R: TTGTACGAGAACCGTGCCAGG(GA)_3N_3(GT)_{11}NF: CGTCCACTTCCCCTAGAGCG R: CCAGCTGCATTACCATACATGC GT)_2N_2(GT)_5(GT)_2N_2(GT)_15F: GCTGTAGCAAAAATGCAGATGC R: TCTCCAGAGATCTAGGCTGTCC

on the computer.

6. Scoring of microsatellite bands

Microsatellite bands were scored with ImageJ, image processing and analysis software (NIH image, 2017) and sizes were estimated by semi-log plot.

7. Statistical analysis

Descriptive statistics, Student's t-test, Analysis of variance (ANOVA), Duncan's multiple range test (DMRT) were done with Microsoft Office Excel (2007) and IBM SPSS Statistics (Version 22, 2013) software.

8. Data analyses for microsatellite DNA

Microsatellite allelic data were analysed using GenAlEx 6.502 genetic software (**Peakall and Smouse, 2012**) to produce genetic parameters. Values among groups were subjected to ANOVA and mean comparisons were made using DMRT where there were significant differences. Also, microsatellite genotype frequencies were tested for deviations from the Hardy-Weinberg equilibrium (HWE) with Chi-square test. Significance for statistical tests was taken at p<0.05.

RESULTS

1. Growth performance-related traits of C. gariepinus

Differences in total length and body weight between the fast- and slow-growing strains of *C. gariepinus* used for microsatellite DNA analysis are shown in **Table 2**. The total length was significantly lower at p<0.05 in the slow-growers than in the fast-growing strains. Also, the difference in their body weights was statistically significantly different at p<0.05, with the fast-growers having the higher recorded value.

Table 2. Differences in length and weight between the two strains of C. gariepinus used for

	DNA analysis						
Strains	Total length (cm)	Body weight (g)					
Fast-growing C. gariepinus	40.44 ^a ±0.58	466.66 ^a ±18.66					
Slow-growing C. gariepinus	21.55 ^b ±0.50	81.89 ^b ±5.62					
*Means in the same column superscripted by different letters differed significantly ($P < 0.05$)							

2. Microsatellite DNA Analysis

The microsatellite DNA fingerprints (**PLATE 1**) were scored using the base pairs of the bands; homozygotes with single bands scored as a single base pair value repeated two times and heterozygotes with double bands as two different base pair values (**Awodiran and Afolabi, 2018**) with the aid of GenAlEx 6.502 genetic software (**Peakall and Smouse, 2012**).



PLATE 1 (A and B). Agarose gel electrophoretic profile of microsatellite DNA for the Cga10 primer of adult *C. gariepinus*

Lanes 1-5= FAB (Abeokuta fast-growers), 6-10= SAB (Abeokuta slow-growers), L = DNA ladder, 11-15=FIB (Ibadan fast-growers), 16-20=SIB (Ibadan slow-growers). Lanes 21-25= FAD (Ado-Ekiti fast-growers) and 26-30= SAD (Ado-Ekiti slow-growers), L = DNA ladder, lanes 31-35=FIL (Ile-Ife fast-growers) and 36-40=SIL (Ile-Ife slow-growers).

The summary of homozygosity and heterozygosity obtained after scoring the bands across seven microsatellite loci in the fast-and slow-growing populations of *C. gariepinus* is shown in Table 3. Across all loci, the total percentage homozygosity (37.13 %) was higher in the slow-growers compared to the fast-growers with 35.66 % homozygosity. However, the reverse trend was observed in the heterozygosity analysis with the fast-growers having higher heterozygosity (14.34 %) compared to 12.87 % heterozygosity recorded for the slow-growing populations (**Table 3**).

Table 3. Summary of the homozygosity and heterozygosity obtained after scoring the bands in fast-and slow-growing *C. gariepinus* strains studied at seven microsatellite loci

	Slow-g	rowers	Fast -growers			
Primer (Locus)	Homozygotes	Heterozygotes	Homozygotes	Heterozygotes		
Cga01	16	4	14	5		
Cga02	18	1	14	6		
Cga03	10	9	10	8		
Cga05	18	2	19	1		
Cga06	11	8	14	5		
Cga09	13	6	12	8		
Cga10	15	5	14	6		
Total	101	35	97	39		
Total (%)	37.13	12.87	35.66	14.34		

Results in **Table 4** showed genetic variability estimates for each growth group of adult *C. gariepinus* across the 7 microsatellite loci. Analyses showed that the number of alleles was significantly higher (p<0.05) in fast-growers collected from Abeokuta than in fast- and slow-growers from Ado-Ekiti and Ile-Ife. However, the number of alleles recorded in fast-growers from Abeokuta was non-significantly different (p>0.05) from fast-growing strain from Ibadan and slow-growing strains from Abeokuta and Ibadan respectively. The number of effective alleles in the fast-growing strain from Abeokuta was also found to be significantly higher (p<0.05) than all the other fast- and slow-growing strains of *C. gariepinus*.

The Shannon's information index (I) (Table 4) of the fast- and slow-growing strains from Ado-Ekiti and Ile-Ife and the slow-growing strain from Abeokuta was significantly lower (p<0.05) than those of the fast-growing strain from Abeokuta. The Shannon's information index of the fast-growing strain was however, non-significantly different (p>0.05) from those of the slow- and fast-growing strains collected from Ibadan.

The observed heterozygosity among the different populations were found to be nonsignificantly different (p>0.05) from each other. However, the expected heterozygosity (**He**) was significantly higher (p<0.05) in the Abeokuta fast-growing strain when compared with those of slow- and fast-growing strains from Ado-Ekiti and Ile-Ife. The expected heterozygosity in the slow- and fast-growing strains collected from Abeokuta and Ibadan was however, found to be non-significantly different (p>0.05) from each other. The fixation index (F) among the different populations was non-significantly different (p>0.05) from each other. Analysis however showed that the individual heterozygosity (**H**-indiv.) was significantly higher (p<0.05) in the Abeokuta fast-growing strain when compared with slow- and fast-growing strains from Ado-Ekiti. The individual heterozygosity values in the fast-growing Abeokuta, Ibadan and Ile-Ife strains and those of slow-growing strains from Abeokuta, Ibadan and Ile-Ife were not significantly different (p>0.05) from each other.

merosatemeroer. (Standard error in parenticeses)										
Population	Na	Ne	Ι	Но	He	F	H-indiv.			
Slow-growers										
SAB	5.286 ^{ab}	4.475 ^a	1.530 ^a	0.314 ^a	0.754 ^{ab}	0.608 ^a	0.314 ^{ab}			
	(0.680)	(0.603)	(0.128)	(0.114)	(0.029)	(0.141)	(0.07)			
SIB	5.714 ^{ab}	4.826 ^a	1.625 ^{ab}	0.336 ^a	0.778 ^{ab}	0.582 ^a	0.324 ^{ab}			
	(0.565)	(0.547)	(0.103)	(0.108)	(0.023)	(0.128)	(0.053)			
SAD	4.143 ^a	3.514 ^a	1.328 ^a	0.114 ^a	0.711 ^a	0.834 ^a	0.124 ^a			
	(0.143)	(0.165)	(0.030)	(0.059)	(0.014)	(0.084)	(0.061)			
SIL	4.857 ^a	3.927 ^a	1.416 ^a	0.264 ^a	0.717 ^a	0.640^{a}	0.262 ^{ab}			
	(0.553)	(0.595)	(0.117)	(0.056)	(0.034)	(0.059)	(0.113)			
Fast-growers										
FAB	6.857 ^b	6.270 ^b	1.847 ^b	0.400 ^a	0.829 ^b	0.536 ^a	0.400 ^b			
	(0.705)	(0.616)	(0.112)	(0.123)	(0.021)	(0.142)	(0.07)			
FIB	5.429 ^{ab}	4.500 ^a	1.545 ^{ab}	0.321 ^a	0.754 ^{ab}	0.593 ^a	0.314 ^{ab}			
	(0.685)	(0.570)	(0.133)	(0.095)	(0.032)	(0.112)	(0.083)			
FAD	4.714 ^a	4.032 ^a	1.453 ^a	0.150 ^a	0.743 ^a	0.793 ^a	0.148 ^a			
	(0.286)	(0.337)	(0.063)	(0.059)	(0.019)	(0.084)	(0.045)			
FIL	5.000 ^a	4.149 ^a	1.489 ^a	0.279 ^a	0.747 ^a	0.634 ^a	0.281 ^{ab}			
	(0.436)	(0.357)	(0.089)	(0.077)	(0.024)	(0.102)	(0.092)			

Table 4. Genetic variability estimates of each *C. gariepinus* population across the seven

 microsatellite loci
 (Standard error in parentheses)

*Means in the same column superscripted by different letters were significantly different (P<0.05)

SAB= Slow-growers Abeokuta, SIB= Slow-growers Ibadan, SAD= Slow-growers Ado-Ekiti, SIL= Slow-growers Ile-Ife, FAB=Fast-growers Abeokuta, FIB= Fast-growers Ibadan, FAD= Fast-growers Ado-Ekiti, FIL= Fast-growers Ile-Ife, Na = Number of alleles, Ne = Number of effective alleles, I = Shannon's information index, Ho = Mean observed heterozygosity, He = Mean expected heterozygosity, F = Fixation index, H-indiv.= Individual heterozygosity.

The genetic variability estimates between the fast- and slow-growing strains of adult *C. gariepinus* when microsatellite allelic data based on each microsatellite locus were used for analyses in **Table 5**.

Fast-growing strains had higher number of alleles (**Na**) at all loci except at Cga03 and Cga06. Further analyses however showed that the recorded differences were only significantly higher (p<0.05) at Cga02 locus. Except at locus Cga03, the number of effective alleles (**Ne**) in the fast-growing strains was higher than those of the slow-growers at all the other loci examined. In addition, the number of effective alleles was recorded to be significantly higher (p<0.05) in the fast-growers only at locus Cga02. The Shannon's information index (**I**) values were higher in the fast-growing strains of the fish than in the slow-growing strains. The exception occurred only at Cga03 locus where the slow-growers had higher Shannon's information index values. The higher **I** values recorded in the fast-growers at Cga02 locus.

At all the loci except at Cga03, Cga05 and Cga06 loci, the single locus observed heterozygosity (**ho**) were higher, though non-significantly (p>0.05) in the fast- than in the slow-growing strains of adult *C. gariepinus*. The fast-growing strains of the fish also had higher single locus expected heterozygosity (**he**) than the slow-growing strains. The only locus where the slow-growers had higher **he** value than the fast-growing strains was Cga03. Analysis further revealed that it was only at Cga02 that the recorded higher **he** values was significantly higher (p<0.05) in the fast-growers than in the slow-growing strains. Analysis of the fixation index (**F**) showed that the slow-growing strains of adult *C. gariepinus* had higher values (non-significantly different at p>0.05) than the fast-growing strains. The exceptions occurred at loci Cga05 and Cga06 where the fast-growers had higher **F** value than the slow-growers.

Geı	netic					Pr	imers (L	oci)						
Ind	ices													
	Cg	a01	Cga0	2	Cga0	3	Cga0	5	Cga	106	Cga09)	Cga10	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S
Na	5.000	4.250	6.250*	4.250	5.750	6.750	4.250	4.250	5.000	5.250	6.250	5.500	6.000	4.750
	(0.41)	(0.479)	(0.479)	(0.250)	(0.854)	(0.946)	(0.479)	(0.25)	(1.080)	(0.75)	(1.031)	(0.957)	(1.080)	(0.479)
Ne	4.048	3.557	5.591*	3.763	4.517	6.134	3.974	3.482	4.331	3.972	5.377	4.661	5.327	3.729
	(0.621)	(0.369)	(0.256)	(0.441)	(0.898)	(0.742)	(0.498)	(0.192)	(0.964	4) (0.891) (0.808)	(0.847)	(1.094)	(0.180)
	1.472	1.328	1.770*	1.372	1.585	1.831	1.388	1.339	1.468	1.456	1.722	1.575	1.679	1.423
	(0.115)	(0.107)	(0.061)	(0.084)	(0.159)	(0.152)	(0.126)	(0.051)	(0.205)	(0.186)	(0.167)	(0.165)	(0.183)	(0.074)
10	0.263	0.200	0.300	0.063	0.438	0.450	0.050	0.100	0.263	0.438	0.400	0.300	0.300	0.250
	(0.047)	(0.082)	(0.129)	(0.063)	(0.075)	(0.150)	(0.05)	(0.058)	(0.125)	(0.140)	(0.123)	(0.129)	(0.173)	(0.096)
ne	0.735	0.710	0.820*	0.724	0.757	0.828	0.735	0.710	0.740	0.711	0.800	0.768	0.790	0.73
-	(0.039)	(0.029)	(0.008)	(0.029)	(0.037)	(0.026)	(0.036)	(0.017)	(0.045)	(0.058)	(0.032)	(0.034)	(0.037)	(0.013)
F	0.636	0 726	0.637	0 905	0 425	0 473	0 936	0 857	0.671	0 4 1 9	0 524	0 623	0 644	0 662
-	(0.077)	(0.114)	(0.157)	(0.095)	(0.088)	(0.176)	(0.064)	(0.083)	(0.144)	(0.150)	(0.214)	(0.152)	(0.194)	(0.127)

Table 5. Genetic variability estimates using microsatellite allelic data of the fast- and slow-growing *C. gariepinus* strains based on each microsatellite locus. (Standard error in parentheses)





Fig. 2. Allele frequencies by population over loci

The distribution of genetic diversity in the studied populations (**Fig. 3**) revealed that 63 and 29% of the total variation existed among and within individuals, respectively; 5% among regions and 3% among populations.



Fig. 3. Percentages of Molecular Variance: Input as allelic distance matrix for F-statistics analysis

Analysis by Chi-square tests (**Table 6**) across all loci and populations revealed that not all the seven loci conformed to Hardy-Weinberg equilibrium in each population.

Table 6. Probability values for Hardy-Weinberg equilibrium obtained from Chi-Square tests

Population				Loci				
	Cga01	Cga02	Cga03	Cga05	Cga06	Cga09	Cga10	
Slow-growers								
SAB	0.019*	0.020*	0.247	0.020*	0.172	0.170	0.132	
SIB	0.103	0.029*	0.170	0.020*	0.293	0.050*	0.050*	
SAD	0.113	0.020*	0.062	0.116	0.116	0.062	0.020*	
SIL	0.113	0.062	0.170	0.029*	0.020*	0.103	0.103	
Fast-growers	;							
FAB	0.050*	0.029*	0.170	0.020*	0.17	0.17	0.297	
FIB	0.116	0.092	0.050*	0.019*	0.207	0.337	0.050*	
FAD	0.125	0.050*	0.207	0.029*	0.029*	0.020*	0.020*	
FIL	0.100	0.224	0.100	0.113	0.019*	0.113	0.029*	

* significantly different at p<0.05.

SAB= Slow-growers Abeokuta, SIB= Slow-growers Ibadan, SAD= Slow-growers Ado-Ekiti, SIL= Slowgrowers Ile-Ife. FAB=Fast- growers Abeokuta, FIB= Fast-growers Ibadan, FAD= Fast-growers Ado-Ekiti, FIL= Fast-growers Ile-Ife.

DISCUSSION

Fast-growing *C. gariepinus* strains especially those from Abeokuta location were more heterozygous in most loci probed than the slow-growing strains, however, the slow-growing *C. gariepinus* strains exhibited higher homozygosity than the fast-growers. The result obtained probably confirmed that admixture of stocks might be routinely practised more frequently in the Abeokuta fish farm compared to the selected fish farms. The results obtained could also suggest that heterozygosity might have a positive effect on growth.

Genetic variability estimates for each growth group of *C. gariepinus* across the seven (7) microsatellite loci showed polymorphism in all the loci assayed. **Agbebi** *et al.*, (2013) similarly reported polymorphism of all loci (Cga01, Cga02, Cga03, and Cga05) assayed in the strains of clariid species. Fast-growers from the Abeokuta location was found to have the highest values for all the genetic indices measured across all loci while the slow-growing strain from Ado-Ekiti exhibited the lowest values across all loci except for the fixation index. Without associating growth values of these sub-populations with

the genetic indices, significant differences were observed in number of alleles (Na) between fast-growing strain from Abeokuta and Ado-Ekiti, between the fast-growers from Abeokuta and Ile-Ife on one hand and between the fast-growing strain from Abeokuta and the slow-growing strains from Ado-Ekiti and Ile-Ife respectively. A deviating trend was also recorded for number of effective alleles (Ne). Significant differences were recorded between the fast-growing strain from Abeokuta compared with fast-growers from Ibadan, Ado-Ekiti and Ile-Ife. Significant Ne values were also recorded between the fast-growers from Abeokuta, when compared with those of the slow-growing strains from Abeokuta, Ado-Ekiti and Ile-Ife respectively. The results were probably suggestive of the good quality of fast-growing strain especially the growth group, collected from the Abeokuta location. Pujolar et al. (2005), studying the growth and heterozygosity of European eel, Anguilla anguilla, observed that some slow-growing eel population presented higher observed heterozygosity at some specific loci, but generally fast-growing eels had the higher observed heterozygosity (Ho = 0.726 ± 0.275) when compared to the slow-growing eel (Ho = 0.711 ± 0.253). Likewise, comparisons between fast- and slow-growing strain of adult C. gariepinus from different locations also identified statistical significant differences (p<0.05) in number of alleles, number of effective alleles, Shannon's information index and single locus expected heterozygosity only at Cga02.

With the exception of fast- and slow-growing strains from Ibadan, the individual heterozygosity was observed to be higher in all fast-growing *C. gariepinus* strains than those obtained in the corresponding slow-growing strain from the same location. This could be attributed to the higher number of heterozygote loci (in the fast-growers) used in calculating individual heterozygosity. This same trend was observed in the comparison of the fast- and slow- growing strains of *C. gariepinus* from the same location using other genetic indices. The only exception recorded was for fixation indices where the higher genetic variability recorded in the slow-growers from Ibadan could not be explained.

Similarly, the highest recorded value (0.834) for fixation index (F) / inbreeding coefficient/ heterozygosity deficit was observed in the slow-growing strain from Ado-Ekiti while the least value (0.536) was recorded in the fast-growing strain from Abeokuta across all loci analysed. Generally, fast-growing *C. gariepinus* strains showed smaller value of inbreeding coefficient than the slow-growing ones. The result corroborated of **Ojango** *et al.* (2011) observation that a large value of inbreeding coefficient typifies the existence of a small number of heterozygote genotypes and an excess of homozygote genotypes, while a small value denotes the occurrence of heterozygote genotypes at a higher proportion than the homozygote genotypes. According to **Pierce** (2012), inbreeding coefficients can range from 0 to 1. A value of 0 implies that mating in a large population is random; a value of 1 infers that all alleles are identical by descent.

In addition, a high diversity is not only vital for the enhancement of stocks, but will also increase the ability of the population to resist diseases and allow adaptation to possible environmental changes (**Gamfeldt and Kallstrom, 2007**). This study has identified fast-growing *C. gariepinus* as strains with higher genetic variability than the slow-growing strains. Strain identification is complicated, since fixed, strain-specific markers are not usually available for strains within a species. The amount of genetic variation among strains which might be limited may require DNA markers and techniques with higher resolution than traditional markers such as allozymes or RAPDs. Allele frequencies for each microsatellite locus which were evaluated for each strain and those microsatellites that have highly differential allele frequencies among strains are used for strain identification (**Liu and Cordes, 2004**). Some strains of fish species have been identified through this approach (**David et al., 2001; Mickett et al., 2003**).

The non-conformities to Hardy–Weinberg equilibrium observed probably suggested that at these loci, evolution may be occurring in some of the growth groups. An implication of the Hardy–Weinberg law for the genetic structure of a population is that a population cannot evolve if it meets the Hardy–Weinberg assumptions, because evolution involves change in the allelic frequencies of a population (**Pierce, 2012**). Deviation from HWE was observed mostly at Cga05. This is similar to **Galbusera** *et al.*, (**1996**) who reported significant deviations at loci Cga05 and Cga09 in the characterization of *C. gariepinus*.

CONCLUSION

This study has identified fast-growth catfish as a strain with larger genetic variability than slow-growth strain. Estimates of **Na**, **Ne**, **I**, **He** and **H-indiv.** could be used in future breeding studies as markers of relative growth potential, and these can help in conservation of biodiversity of *C. gariepinus*. Also, Cga02 has been discovered as the specific locus on which significance levels were consistently expressed thus, it should be explored in future studies in larger populations of *C. gariepinus* since it showed promising higher potential for improvement of growth performance in the fish. In spite of these results, studies on genetic-diversity-fitness correlations for culturable species with shooter-phenomenon are recommended.

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