Evaluation of genetic similarity and distance among seven Clariid fish populations in Kano Rivers, Nigeria

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Abstract

A population can only acquire new gene either through the immigration of individuals from surrounding populations. One of the greatest advantages of microsatellite marker is that DNA sequence information is needed to perform the assay. Thus, a study was carried out to evaluate genetic similarity and distance in strains of Clariid species (*Clarias gariepinus* and *Heterobranchus longifilis*). In achieving this, 25 blood samples were gotten from *Clarias gariepinus* and 10 from *Heterobranchus longifilis* for DNA extraction. DNA were extracted, amplified and scored using seven microsatellite markers. The markers were Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10. The gel images were then subjected to molecular analysis using Genealex software to determine their base pairs and evaluate their genetic variability. The populations examined were *Clarias gariepinus* of River Dudusingaya (CgD), Ghari (CgG), Thomas (CgT), Karaye (CgK), Bagwai (CgB); and *Heterobranchus longifilis* of River Bagwai (HIB) and Ghari (HIG). Among the populations sampled, the genetic similarity ranged from 0.018 for CgD and CgT populations to 0.079 for CgT and HIG populations. The genetic distance observed in the populations ranged from 0.112 for CgG and CgB populations to 0.998 for CgT and CgK populations. The Clariid species that were closer indicated that the river locations shared tributaries and the Clariid species exchanged genetic materials over a long period of time. On the other hand, those that were farther apart indicated that they shared little or no tributaries. It can be concluded that the studied populations were not genetically pure but heterogeneous with varying degrees of genetic similarity and distance therefore, there were no. exhibition of genetic uniqueness and the populations were not isolated.

Keywords: Genetic similarity, Genetic distance, Clariid populations

Introduction

Populations of many species of organisms respond both morphologically and genetically to a changed environment. Within species, genetic diversity is partitioned among and within populations. Long before species decline into extinction, it will suffer a reduction in the level of genetic diversity within and among its populations. Fragmentation causes the formation of small isolated population which are more vulnerable to genetic degradation. While documented

extinctions of marine species are rare, the extinction of population (extirpation) is more common (Musick, 1998; Musick *et al.*, 2000). Genetic variation is important for the long-term survival of species; it ensures the fitness of species or populations by giving the species or populations the ability to adapt to changing environment; and lack of genetic variation or too much of homozygosity are detrimental to survival and fitness (Dunham, 2004). Homozygosity has been correlated with bilateral asymmetry (fluctuating

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asymmetry) which is the unbalanced meristic counts of the right and left halves of the body in fishes (Dunham, 2004). A population can only acquire new gene either through the immigration of individuals from surrounding populations or through the process of random mistakes (mutation) of the codes. Apart from these two processes, the genetic diversity of a population is determined by its size and the selective forces acting on it. In general, mutation occurs rarely, although there are cases where high levels of pollution or radiation have been shown to increase mutation rates (Powles *et al.*, 2000). Genetic improvement of aquaculture species offers a substantial opportunity for increased production efficiency, health, product quality and profitability (Davis and Hetzel, 2000). Genetic improvement techniques for delivering genetic gains include definition of breeding objectives, estimation of genetic parameters that described populations and their differences, evaluation of additive and non-additive genetic merit of individuals or families (Davis and Hetzel, 2000). There is a number of different molecular tagging methods which have been designed to study fish populations. The use of biochemical methods such as isozymes and protein electrophoretic techniques for species identification has been widely applied in fish (Yilmaz *et al.*, 2007; Wei and Musa, 2008). Also, the use of molecular methods such as RAPD techniques (Almeida and Sodre, 2002; Quibai *et al.*, 2006), AFLP techniques (Mickett *et al.*, 2003; Simmones *et al.*, 2006) and microsatellite DNA (Perales *et al.*, 2007; Wachirachaikam and Na-Nakron, 2007) has been widely applied in fish characterization. The greatest advantages for RAPD techniques are that it can potentially sample a large number of loci and that no prior DNA sequence information is needed to perform the assay

(Christopher *et al.*, 2004). Therefore, the objective of the study was to evaluate the genetic similarity and distance in Clariid populations in Kano state rivers with a view to unravel their genetic uniqueness.

Materials and methods

The study was conducted in six selected water bodies in Kano State, namely, River Thomas and River Ghari to the north, River Dudusingaya to the east, River Kano (Tiga section) to the south, River Karaye and Bagwai river to the western part of the state. The first three locations were selected due to their independent flow and their disconnected tributaries. The remaining three locations had the same origin as the Kano River, but the Tiga section of the river flow towards the south while the Karaye river flow towards the western part of the state. The fish samples were identified using an exposition for identification by Moses and Olufeagba, (2009), and confirmed using local names provided by the fishermen. Live fish samples of *Clarias gariepinus* and *Heterobranchus longifilis* from the six locations described above were purchased from commercial catches of the fishermen. Thirty-five blood samples (25 – *Clarias gariepinus* and 10 – *Heterobranchus longifilis*) were transferred to FTA[®] Classic Cards (Whatman Bioscience, Maidstone, UK) and left to dry and subsequently used for DNA extraction. The caudal peduncle of the fish samples was severed to drain out blood into the FTA cards. Genomic DNA was extracted according to Agbebi *et al.* (2013): Five 1.2mm disc of FTA[®] Classic Cards were punched into 1.5 mL Eppendorf tubes. 1000µl of 100mM Tris-base and 0.1% SDS buffer was added and tubes were vortexed gently for 30min. The supernatant was decanted, thereafter, 500 µL of 5M guanidine thiocyanate was added and vortexed for 10min. The supernatant was

decanted again; thereafter, 500 µL nuclease-free water was added and vortexed for 10 min and then the supernatant was decanted. The same volume of nuclease-free water was added and left to stand for 10 min. The supernatant was again decanted and finally, 50µL nuclease-free water was added to the discs. The tubes were heated at 90°C for 10min in a thermocycler to complete extraction. The DNA concentration was further diluted in 1:20 ratio to remove impurities and prevent smearing.

Gel preparation

DNA Amplification and scoring

DNA was amplified using polymerase chain reaction (PCR). PCR was carried out in 10µl reaction volumes containing 20 to 40ng genomic DNA, 2µl 5x Taq mastermix of Taq DNA polymerase, dATP, dCTP, dGTP, dTTP, (NH₄)₂SO₄, MgCl₂, Tween-20, Nonidet P-40, red dye, gel loading buffer, stabilizers, 0.3µl each of the forward and reverse primer sets and 5.9µl of PCR grade water. Seven microsatellite markers isolated by Galbusera *et al.* (1996) were used as primers. The primers were Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10. Amplifications was carried out in a thermocycler as follows: 1 cycle at 94°C (5min) for pre-denaturation, 35 cycles at 94°C (30sec) for denaturation, 35 cycles at 55°C (30sec) for annealing, 35 cycles at 72°C (30sec) for extension and 1 cycle at 72°C (5min) for final extension to complete amplification. PCR condition for each marker was optimized.

Electrophoresis

Electrophoresis was conducted on 2% Agarose gel containing a negative control to detect potential PCR contamination, and each gel contained a positive control using a sample that previously had been genotyped in order to standardize allele scoring among gels. Scoring was done by comparison to 8

bp standard DNA ladder with the aid of gel analyser.

Data analysis

To quantify the extent of molecular variation, locus-by-locus analysis of molecular variance (AMOVA) was performed using Genealex 6.4. The resolve bands were subjected to Genealex 6.4 software to determine their base pairs and for molecular characterization.

Results and discussion

Genetic similarity and relatedness

The similarity values of the population of *Clariid* species studied is presented in Table 1. The table showed that *Clarias gariepinus* from River Dudusingaya were related to those of River Ghari with a similarity value of 0.040, River Thomas with 0.018 and River Bagwai with 0.060. The *Clarias gariepinus* from River Ghari were related to those of River Karaye with a value of 0.040 and River Bagwai with 0.044. They were also related to *Heterobranchus longifilis* of River Bagwai with a similarity value of 0.026. *Heterobranchus longifilis* had a relationship with *Clarias gariepinus* of River Thomas and River Karaye with values 0.070 and 0.066, respectively. The *Clarias gariepinus* of River Thomas were related to those of River Karaye with 0.018 and *Heterobranchus longifilis* of River Ghari with 0.079. Others with values 0.000 were not related meaning that there were no tributaries connecting them. The unbiased genetic similarity values give the relatedness when all sources of error are removed from analysis. Table 2 gives the similarity values and confirmed the values in Table 1 except that the values differed in strength. The result of the Nei's genetic similarity showed that some of the populations were not related at all, indicating that there was no gene exchange between them (0.000). The populations with values ranged from 0.018 to 0.079 had

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some level of genetic similarity among them. These results are in agreement with the findings of Agbebi *et al.* (2013) with *Heterobranchus bidorsalis* and *Clarias gariepinus* strains. The close relationship between the strains had been emphasized by Teugels *et al.* (1990) in a revisionary study using osteological features. Agnese *et al.* (1997b) reported genetic variation at 25 protein and eight microsatellite loci, and

two mitochondrial (mtDNA) segments in two sympatric *Clariid* strains from Senegal River. Results from this experiment were in congruent with those of Rognon *et al.* (1998) in a morphometric and the allozyme study of nine populations of *Clarias gariepinus* and seven populations of *Clarias anguillaris* to quantify their intra and interspecific variation using *Clarias albopunctatus* and *Heterobranchus longifilis* as outgroups.

Table 1: Nei's Genetic Similarity values among the investigated populations

	CgD	CgG	CgB	CgT	CgK	HIG	HIB
CgD	1.000						
CgG	0.040	1.000					
CgB	0.060	0.044	1.000				
CgT	0.018	0.000	0.020	1.000			
CgK	0.000	0.040	0.000	0.018	1.000		
HIG	0.000	0.000	0.000	0.079	0.000	1.000	
HIB	0.000	0.026	0.000	0.070	0.066	0.000	1.000

Cg=*Clarias gariepinus*; Hl=*Heterobranchus longifilis*; D-River Dudusingaya; G-River Ghari, B-River Bagwai, T-River Thomas, K- River Karaye

Table 2: Nei's Unbiased Similarity values among the investigated populations

	CgD	CgG	CgB	CgT	CgK	HIG	HIB
CgD	1.000						
CgG	0.080	1.000					
CgB	0.125	0.107	1.000				
CgT	0.032	0.000	0.043	1.000			
CgK	0.000	0.091	0.000	0.037	1.000		
HIG	0.000	0.000	0.000	0.065	0.000	1.000	
HIB	0.000	0.057	0.000	0.137	0.143	0.000	1.000

Cg=*Clarias gariepinus*; Hl=*Heterobranchus longifilis*; D-River Dudusingaya; G-River Ghari, B-River Bagwai, T-River Thomas, K- River Karaye

Genetic distance

The genetic distance of the population studied are presented in table 3. Nei's genetic distances among all populations ranged from 0.112 (CgG vs CgB) to 0.998 (CgT vs CgK). The Table shows that *Clarias gariepinus* of River Dudusingaya were genetically closer to *Clarias gariepinus* of River Ghari with value 0.223 while they were much more genetically distanced from those of River Tiga with 0.907 and River Bagwai with 0.816. *Clarias gariepinus* of River Ghari had genetic closeness to those of River Karaye (0.214), River Bagwai (0.112) and *Heterobranchus*

longifilis of River Bagwai (0.667). *Heterobranchus longifilis* of River Bagwai shared genetic attributes with *Clarias gariepinus* of River Tiga (0.659) and River Karaye (0.725). *Clarias gariepinus* of River Tiga genetically distanced to those of River Karaye (0.998) and River Bagwai (0.897), but close to *Heterobranchus longifilis* of River Ghari (0.544).

The unbiased genetic distance values give the genetic relatedness when all sources of error are removed from analysis. Table 4 gives the unbiased genetic distance values and confirmed the values in Table 3 except that the values differed in strength. Genetic

distance measures the genetic divergence between species or between populations within a species as indicated by Nei's genetic distance values, Fst values, mean fixation index and hierarchical clustering dendrogram. The results of this study showed that some of the populations were genetically close while some were farther apart indicating that those that were close might have shared some tributaries and connection in terms of the movement or flow of the water bodies. Those that were farther apart might have some level of distinctiveness in terms of their habitat or there were little or no connection between

the water bodies. The results were in agreement with the reports of some authors (Abban and Skibinski, 1988; Agnese, 1989; 1991; Agbebi *et al.*, 2013) on aquatic organisms. Agnese (1989) reported genetic distances varying from 0.271 to 0.916 in the genus *Chrysichthys*; Abban and Skibinski (1988) reported a distance range of 0.88 to 1.14 between *Eutropius niloticus* and *Schilbe mystus*. Agnès (1991) reported range value of 0.003 to 0.112 for populations of *Chrysichthys auratus* while Agbebi *et al.* (2013) reported 0.84 to 0.89 in *Clarias gariepinus* and *heterobranchus bidorsalis*.

Table 3: Nei's Genetic Distance among populations of two clariid strains

	CgD	CgG	CgB	CgT	CgK	HIG	HIB
CgD	0.000						
CgG	0.223	0.000					
CgB	0.816	0.112	0.000				
CgT	0.907	*	0.897	0.000			
CgK	*	0.214	*	0.998	0.000		
HIG	*	*	*	0.544	*	0.000	
HIB	*	0.667	*	0.659	0.725	*	0.000

* = undefined, Cg=*Clarias gariepinus*; Hl=*Heterobranchus longifilis*; D-River Dudusingaya; G-River Ghari, B-River Bagwai, T-River Thomas, K- River Karaye

Table 4: Nei's Unbiased Genetic Distance among populations of two clariid strains

	CgD	CgG	CgB	CgT	CgK	HIG	HIB
CgD	0.000						
CgG	0.523	0.000					
CgB	0.790	0.235	0.000				
CgT	0.429	*	0.140	0.000			
CgK	*	0.401	*	0.307	0.000		
HIG	*	*	*	0.736	*	0.000	
HIB	*	0.857	*	0.971	0.943	*	0.000

* = undefined, Cg=*Clarias gariepinus*; Hl=*Heterobranchus longifilis*; D-River Dudusingaya; G-River Ghari, B-River Bagwai, T-River Thomas, K- River Karaye

Conclusion

The study showed that the populations were not genetically pure but heterogeneous population with varying degrees of genetic similarity and distance. Based on genetic distance and similarity, and high level of heterozygosity, it is better to employ all the loci as basis for selection and cross breeding except Cga09 so as to ensure the highest

gain from selection

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