



## Genetic relationships among different *Clarias gariepinus* strains in South-Western Nigeria

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### Abstract

The conservation of aquatic animal genetic resources is essential and necessary for effective breeding programmes. In this study, samples of *Clarias gariepinus* were obtained from different locations - Abeokuta, Ogun; Ile-Ife, Osun and Ikotun, Lagos States, for genetic relationships using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis of their serum proteins with standard molecular marker. Results obtained showed varying sizes of polymorphic bands of the serum proteins including  $\beta$ -galactosidase, bovine serum albumin, ovalbumin, Carbonic anhydrase, and  $\beta$ -lactoglobulin. The similarity coefficients within the *C. gariepinus* were relatively high for Ile-Ife, Osun State (47%) and Ikotun, Lagos State (54%) compared with Abeokuta, Ogun State with highest value of 70%. The genetic relationship showed close relationship between strains from Lagos and Ile-Ife at genetic distance of 0.12 compared with Abeokuta at 0.31. This indicated a very high level of genetic similarity and general loss of genetic variation; hence, careful breeding programmes should be planned using breeding population with non-similar history but with unique features.

### INTRODUCTION

The study and conservation of animal genetic resources are indispensable for good breeding programmes and sustainable economic systems. These had led to great contribution to genetic and productive improvements in breeding programmes and formation of mechanism whereby species exchange genetic resources with the goal and possible outcome of strengthening fitness among others (Dunham, 2004).

Aquatic animal genetic resources necessarily need adequate consideration. The information on the genetic constituents of species in between and within other species or strains, helps in the conservation of these genetic resources and biodiversity. It further helps the study of genetic variations and measurements that are directly related to protein products that really affects performance e.g. disease resistance, temperature and salinity tolerance, growth performance, etc in very particular organisms (Liu and Cordes, 2004).

The state of global food fish insecurity due to continuous decline in capture fisheries and fish stock genetic resources depletion usually resulted from over-fishing and environmental degradation, among others in addition to increasing demand for fish and its products by ever-increasing population (Dunham, 2004;

FAO, 2009). Hence, good breeding programmes with high genetic merit stock is necessary for aquaculture development towards sustainable productions especially in the less developed or developing countries of Africa and Asia.

Strain is one of the terms being used to describe organisms with close but similar history. It is being used to describe genetic relationships between a group of fish exploited by a specific method, or existing in a particular area. It is essential to realize that there is a continuum of genetic differentiation. In some groups there may be clearly genetically distinct groups, while in others (or in other parts of their range) there will be groups which are virtually indistinguishable genetically (Beaumont and Hoare, 2003). However, mating of close but related individuals (inbreeding) reduces viability and performance, but hybrid vigour or heterosis can often be consistently expressed where highly heterozygous offspring exhibit increased fitness, which is an important goal of hatchery production (Beaumont and Hoare, 2003).

In Nigeria, genus *Clarias* is more popular among *Clariidae*, and *C. gariepinus* is the most among its numerous species. This is perhaps due to its ready availability (Fagbenro *et al.*, 1993; Adebayo and Fagbenro, 2004; Olaniyi, 2015); and being vastly cultured and subjected to

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intensive research because of its good growth rate, resistance to diseases, tolerance to high stocking density, temperature fluctuations, low dissolved oxygen and salinity; and most importantly good commercial value (Bovendeur *et al.*, 1987; Young *et al.*, 1989; Anderson and Fast, 1991; Barcellos *et al.*, 2001). It is being used in hybridization with other species like the Thai walking catfish (*C. macrocephalus*) in Thailand to improve their native *Clarias* (Na-Nakorn, 1999). Also, it is of great importance in biomedical research applications (Hightower and Renfro, 1988; Hecht *et al.*, 1996). With these merits, the protection of genetic resources of this species is very essential. Other species in the genus are *C. anguillaris*, *C. mossambicus*, *C. batrachus*, *C. meladerma*, *C. nieuhofii*, *C. teijsmanni* etc. The aim of this study was to investigate and compare the genetic similarity or divergence among the breeder species of *C. gariepinus* in the South-Western part of Nigeria using blood serum protein polymorphism analysis.

## MATERIALS AND METHODS

### Sample collection

One hundred and fifty breeder samples of *C. gariepinus* (weight:  $1.5 \pm 0.2$  kg; length:  $53.75 \pm 3.15$  cm) were obtained from two commercial fish farms with 25 samples per each location in the South-Western States, Nigeria: Abeokuta in Ogun; Ile-Ife in Osun; and Ikotun in Lagos States. These samples were transported live in oxygenated plastic bags, and acclimatized for 2 weeks at the Wet Laboratory of the Department of Animal Sciences, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. They were fed with 6.0-8.0 mm Coppens® (Netherlands) pelleted fish feed (45% crude protein) twice daily at 9.00 hrs and 17.00 hrs. The water pH, alkalinity, dissolved oxygen and temperature measured were  $7.1 \pm 1$ ,  $112.31 \pm 1.14$  mg/l,  $24.5 \pm 0.5$  mg/l and  $27 \pm 2$  °C, respectively.

### Serum protein preparation

Blood samples were drawn from the haemal arch of the fish using sterile hypodermic syringe. Physiological saline (0.9 % NaCl) solution was added at ratio 3:2 blood sample and left at ambient temperature for ~1 h. This was then centrifuged at 3,000 rpm for 10 min. The supernatant which contained serum protein was then pipetted into a clean 2 ml eppendorf microtube and stored at -20 °C freezer for further analysis (Avtalion, 1984; Olaniyi *et al.*, 2017).

### Gel preparation

Gel electrophoresis was carried out using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis using the Bio-Rad Mini Protean II Cell kit of 10 ml capacity. Solutions for 4% stacking gel, 12% resolving gel for SDS-PAGE were then prepared for the analysis (Bio-Rad, 1995).

### Sample preparation electrophoresis

Approximately 40 l  $\beta$ -mercaptoethanol (7.5 %) was added to the sample buffer (370 l) before being used. Hence, in each of 10 l protein sample, 40 - 60 l of mixture of sample buffer plus  $\beta$ -mercaptoethanol was added, at ratio 1:5. These prepared samples were heated at 95 °C for 4 min in a water bath, cooled, and thereafter, 10 l each was loaded in each well of the kit. Equal volume of standard pre-stained protein molecular weight (MW) marker was also loaded into a well. The molecular weights of the standard molecular markers in KDa are: ~120= $\beta$ -galactosidase; ~85=Bovine serum albumin; ~50=Ovalbumin; ~35=Carbonic anhydrase; ~25= $\beta$ -lactoglobulin; ~20=Lysozyme. The separation of protein was carried out with the aid of Bio-Rad Electrophoresis Power Supply Model 200/2.0 in the Bio-Rad Mini Protean II Cell at 150 V for ~45 min.

### Staining and de-staining of gel

After the electrophoretic run, the gels were stained in 0.1% Coomassie blue in glacial acetic 1:4 methanol for ~1 h, and then de-stained with 60% glacial acetic 1:4 methanol solution for ~2hrs (Olaniyi *et al.*, 2017). The gel was then scanned for evaluation, scoring and further analysis.

### Gel scoring and analysis

The scoring of the gel for the presence of protein band was "1" and its absence was "0" to generate the data that were analyzed with PALaeontological STatistics (PAST) software to produce dendrograms (Hammer *et al.*, 2001). The software was employed to determine similarity association between the samples using Jaccard option. The mean value of each fish was then used to generate distance indices data for comparative genetic distance evaluation with Euclidean option.

## RESULTS

The representative gel of the blood serum protein analysis in this study is presented in Plate 1 showing MW in lane 1; and *C. gariepinus*

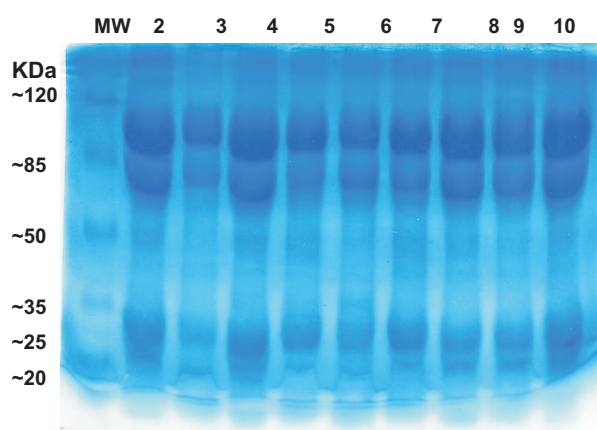


Plate 1 showing MW in lane 1; and *C. gariepinus* samples from, Abeokuta, Ogun State in lanes 2 - 4; Ile-Ife, Osun State in lanes 5 - 7; and Ikotun, Lagos State in lanes 8 - 10. MW: ~120= $\beta$ -galactosidase; ~85=Bovine serum albumin; ~50=Ovalbumin; ~35=Carbonic anhydrase; ~25= $\beta$ -lactoglobulin; ~20=Lysozyme.

samples from Abeokuta in lanes 2 - 4; Ile-Ife in lanes 5 - 7; and Ikotun are shown in lanes 8 - 10. The MW revealed the polymorphic bands of the blood proteins of  $\beta$ -galactosidase, bovine serum albumin, ovalbumin, Carbonic anhydrase, and  $\beta$ -lactoglobulin.

Within the samples, *C. gariepinus* from Abeokuta, Ogun State showed similarity coefficient within the samples of 70.5%; Ile-Ife, Osun State depicted 47% while from Ikotun, Lagos State indicated 54.5%. The similarity

coefficients within the *C. gariepinus* were relatively high except in Abeokuta with highest value.

The genetic relationship between the strains is shown in the dendrogram of genetic distance (Figure 1). The tree showed close relationship between *C. gariepinus* strains from Ikotun, Lagos and Ile-Ife, Osun States at very low genetic distance of 0.12 compared with samples from Abeokuta, Ogun State at 0.31.

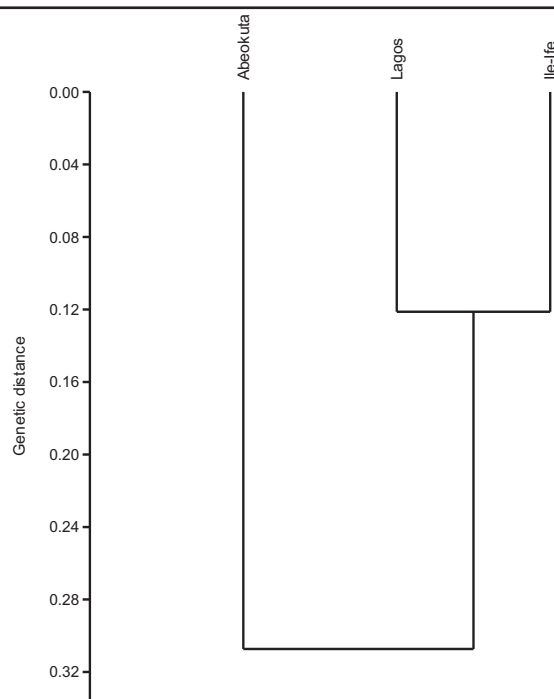


Figure 1 : Dendrogram generated by PAST clustering algorithm (UPGMA) showing genetic relationships of different *C. gariepinus* strains from different locations (Abeokuta, Ogun; Ikotun, Lagos and Ile-Ife, Osun States) in South-Western Nigeria.

## DISCUSSION

The results indicated a very high level of genetic similarity and general loss of genetic variation. This perhaps is as a result of reduced heterozygotes, low polymorphism, and introgression among others (Ruzzante and Doyle, 1990; van der Bank *et al.*, 1992; Bakker, 1994; Galbusera, 1997; Na-Nakorn *et al.*, 2004; Senanan *et al.*, 2004; Betiku and Omitogun, 2006). Moreover, this is usually observed where the geographical barriers are not really distinct, and there may be active gene flow. This is one of the challenges of non-distant hatcheries whereby fish of same history or genetic backgrounds are reared and used for their breeding purposes (Purdom, 1993). This implies inbreeding depression and loss of survival from such progenies that may arise from such breeding practice.

The high level of genetic similarity also depicted the impact of domestication within the population, hence loss of heterozygosity. The close genetic relationship of these strains may be due to proximate or adjacent locations or states; hence farmers need to source for broodstock from afar or reliable sources for increased heterozygosity for better breeding programs. Without this, there may be mating of close relatives (inbreeding) that can lead to general loss of fitness in terms of survival, growth, fecundity and other traits (Purdom, 1993).

## CONCLUSION

*Clarias gariepinus* strains from different locations in South-Western Nigeria are closely interrelated as revealed in the study though with different values. It further confirmed the impact of domestication of the species with cross supply of the samples (breeders, fingerlings etc) both within and between the populations. Therefore, careful breeding programs should be planned using breeding population with non-similar history but with unique features.

**Conflict of interest:** Author indicates that there is no any actual or potential conflict of interest that could inappropriately or possibly influence this work after publication.

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