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HETEROGENEITY STUDIES OF WILD *CLARIAS GARIEPINUS* (OSTEICHTHYES, CLARIIDAE) USING SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

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Heterogeneity Studies of Wild *Clarias gariepinus* (Osteichthyes, Clariidae) Using SDS-Polyacrylamide Gel Electrophoresis. Ola-Oladimeji, F. A., Idowu, E. O., Adewumi, A. A., Fafowora, K. C. — This study determined the genetic variations that exist in *Clarias gariepinus* obtained from two natural populations in Nigeria, using their serum protein profiles. A total of 51 samples of *Clarias gariepinus* collected from Ado-Ekiti and Ilesa were used for this experiment. Blood was extracted from the caudal vein of each individual fish and electrophoresis was performed based on standard methods. Following this, gel images were taken, scored and subjected to classical cluster analysis using Bray-Curtis similarity index. This showed the presence of variations in *C. gariepinus* between the studied populations and samples from Ado-Ekiti reservoir displayed more diversity than those from Ilesa. Hence, this showed the feasibility for selecting samples from Ado- Ekiti to improve culture of *C. gariepinus* in further breeding studies.
K e y w o r d s: *Clarias gariepinus*, electrophoresis, Bray-Curtis similarity index, diversity.

Introduction

Several morphological studies have been carried out on assessing the variations in *Clarias gariepinus* (Turan et al., 2005; Solomon et al., 2015; Ola-Oladimeji et al., 2017). However, most morphological traits are polygenic, quantitative or continuous characters and their expression is influenced by environmental conditions (Lombard et al., 2001; Torkpo et al., 2006). Thus, Sodium dodecyl sulphate-polyacrylamide gel electrophoresis is a technique in which proteins can be viewed on polyacrylamide gels and it has been used to study variations in different populations of organisms. This is because this biochemical analysis of total protein had been confirmed to display more diagnostic genetic variations, in addition to being free from genotype-environment interaction (Lombard et al., 2001; Torkpo et al., 2006).

The present investigation was therefore, aimed at determining the genetic variations that exist in two populations of *Clarias gariepinus* in Ekiti and Osun States of Nigeria using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Materials and method

Twenty-three (23) samples of *Clarias gariepinus* were collected from Ado-Ekiti reservoir in Ado-Ekiti-Ekiti (7°38´0″ N, 5°13´0″ E), Ekiti State and 28 samples were obtained from Omi Ayo River in Ilesa (7°37´0″ N, 4°44´0″ E) located in Osun State of Nigeria. These were transported to the Biotechnology Laboratory in the Department of Animal Sciences, Obafemi Awolowo University (O.A.U.) in Ile-Ife, Nigeria for further analysis.

Serum collection for analysis

Three millimeters (3 ml) of blood was withdrawn from the tail region of the fish with needle and syringe and was diluted with 2 ml of 0.9 % saline solution having ensured there was no bubble in the tube. The sample was left for one (1) hour at room temperature and the serum obtained after centrifuging at 3000 rpm for 10 minutes was decanted and kept at about 5 °C until use.

SDS-PAGE analysis

The supernatant of each sample was collected and 15 μl of each supernatant was electrophoresed in 12 % polyacrylamide-bisacrylamide gel. Preparation of gel for SDS-PAGE followed the modified method of Laemmli (1970) and Majolagbe et al. (2012). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using Electrophoresis Power Supply Model 200/2.0 in Mini Protean II Cell (BIO-RAD).

Seven percent (7 %) of β-mercaptoethanol (SIGMA) in sample buffer of 4 % stacking gel (including 1.3 ml Acrylamide/Bis (SIGMA), 6.1 ml distilled water, 2.5 ml Upper Tris buffer of 0.5M Tris-HCl and pH 6.8, 100 μl of 10 % (w/v) SDS, 10 μl (TEMED) Tetramethylethylenediamine (ROTH), 25 μl of 10 % (APS) Ammonium per sulphate (SIGMA), and 12 % resolving gel (including 3.5 ml distilled water, 4.0 ml Acrylamide/Bis (SIGMA), 2.5 ml lower Tris buffer of 1.5 M Tris-HCl, pH 8.8, 100 μl of 10 % (w/v) SDS, 5 μl TEMED and 50 μl APS were used for the preparation of each serum sample under a fume hood. To 30 μl sample of each protein extract stored in labelled eppendorf tubes at very low temperature (–20 °C), 10 μl of mixture of sample buffer and β-mercaptoethanol were added. These samples were heated at 95 °C for 5 minutes in a water bath. There were eight wells and each had the capacity to hold 30 μl of each serum. Twenty five (25) μl of the heated sample and sample buffer was loaded in each well while the electrophoretic separation of protein was carried out at 150 volts for 1 hour. Bromophenol blue was added to the sera to act as a tracer. The gels were then carefully removed from the casting plate and placed in a 0.1 % Coomassie blue staining solution overnight. The staining was done for 16 hours. After staining, the gels were removed and washed for about two minutes in distilled water. Following this, the gels were then rinsed many times with freshly prepared de-staining solution (10 % glacial acetic acid, 40 % methanol and 40 % distilled water) until the protein bands were distinct and they were left in the de-staining solution. The gels were scanned and the images were stored in the computer for scoring to compare the degree of similarity of the two populations.

Data analysis

Data were obtained from the scanned gels by scoring the presence (denoting 1) or absence (denoting 0) of protein bands directly from the computer screen (Oladejo et al., 2009). Data collected from the SDS-PAGE were analyzed by means of PAST 3.14 software© 2016 (Hammer et al., 2001) to generate dendrogram of Unweighted Paired Group Method of Algorithm (UPGMA) cluster analysis for standard genetic distances based on band frequencies scores for each population. This was done to determine the genetic similarity (similarity coefficient) between the studied populations (Diyaware et al., 2012). PAST software © 2016 (Version 3.14) was used to determine the differences between the means of bands at each locus in the two populations using T-test while significance for all statistical tests was taken as $p < 0.05$.

Results

There was a high degree of intra-specific variations in terms of the number and positions of the bands in the protein profiles of the two populations.

Samples of *C. gariepinus* obtained from Ado-Ekiti reservoir had 236 bands in sum while 291 bands were estimated in samples from Ilesa. Table 1 shows the means of bands at each polymorphic site and their significance levels. Out of 19 polymorphic sites identified, *C. gariepinus* from Ado-Ekiti reservoir had lower number of bands (at 9 polymorphic sites) compared to those from Ilesa with 10 polymorphic sites though there was lack of bands among samples from Ilesa in the last three polymorphic sites recorded for Ado-Ekiti. Unique bands were identified on the 2nd polymorphic site within Ado-Ekiti reservoir

samples while unique bands were also recorded on the 13th polymorphic site in *C. gariepinus* obtained from Ilesa.

The results obtained from cluster analysis showed that the specimens collected from Ado-Ekiti had 38 % similarity coefficient (fig. 1) while those from Ilesa had 62 % similarity coefficient (fig. 2). In fig. 3, similarity index among all the members of the populations is 30 %. There were two major clusters at 54 % similarity coefficient while other clusters and sub-clusters were present at various levels. Only two catfish, obtained from Ilesa were homogenous at 100 % similarity index while the other individuals showed heterogeneity and various values of similarity coefficient.

Discussion

This study presented the baseline data on the genetic variations of *C. gariepinus* from Ado-Ekiti reservoir and Ilesa. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed polymorphism of serum protein

Table 1. Means of bands at each polymorphic site and significance levels

Polymorphic site	Ado-Ekiti	Ilesa	T-Test
1	0.391	0.821	0.00*
2	1.000	0.464	0.00*
3	0.522	0.643	0.39
4	0.304	0.607	0.03*
5	0.870	0.607	0.04*
6	0.870	0.607	0.04*
7	0.348	0.786	0.00*
8	0.348	0.750	0.00*
9	0.522	0.857	0.01*
10	0.652	0.750	0.46
11	0.700	0.929	0.03*
12	0.522	0.464	0.69
13	0.700	1.000	0.00*
14	0.700	0.357	0.02*
15	0.435	0.534	0.48
16	0.700	0.214	0.00*
17	0.391	0.000	0.00*
18	0.130	0.000	0.05
19	0.174	0.000	0.02*

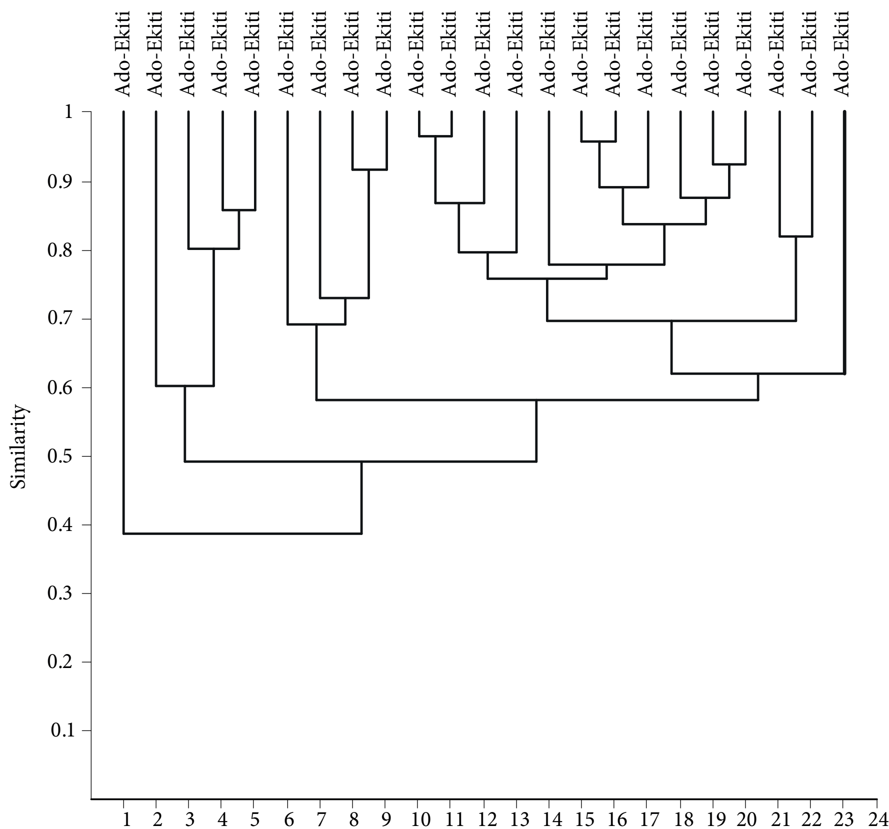

Fig. 1. Dendrogram obtained from Classical Cluster analysis using Paired group Bray-Curtis similarity index on *Clarias gariepinus* in Ado-Ekiti.

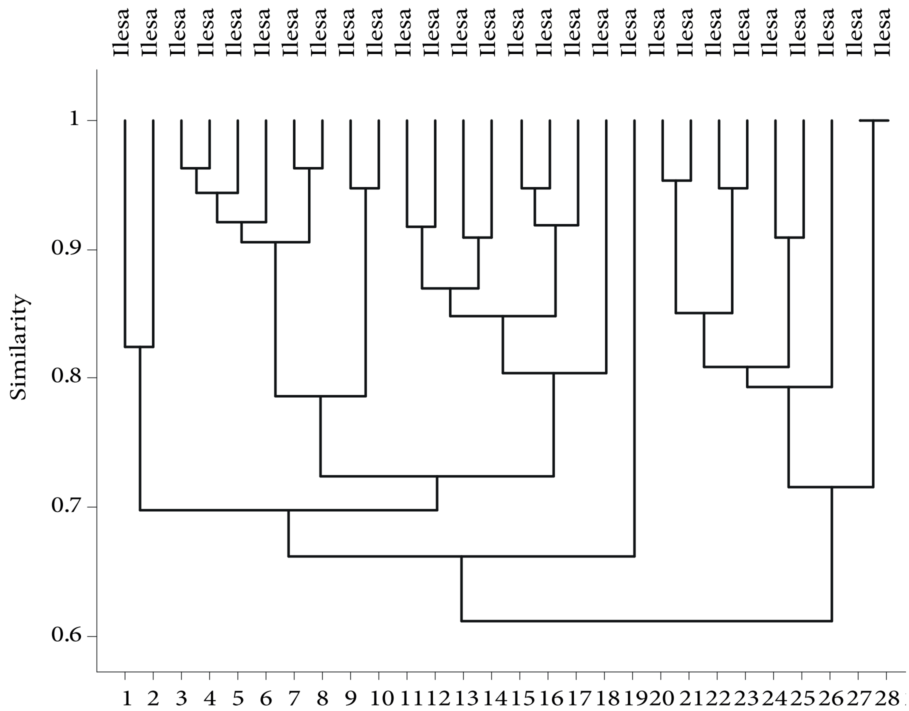

Fig. 2. Dendrogram from Classical Cluster analysis using Paired group Bray-Curtis similarity index on *Clarias gariepinus* obtained in Ilesa, Osun State.

bands between the two populations of *C. gariepinus*. Samples from two populations were polymorphic and heterogenous, although the catfish from Ado-Ekiti were more heterogenous. This is indicative of presence of variations in these catfish. Popoola et al. (2014) also reported high level of genetic heterogeneity among samples of catfish studied. According to Kirpicknikov (1981), the characteristic advantage in the electrophoretic

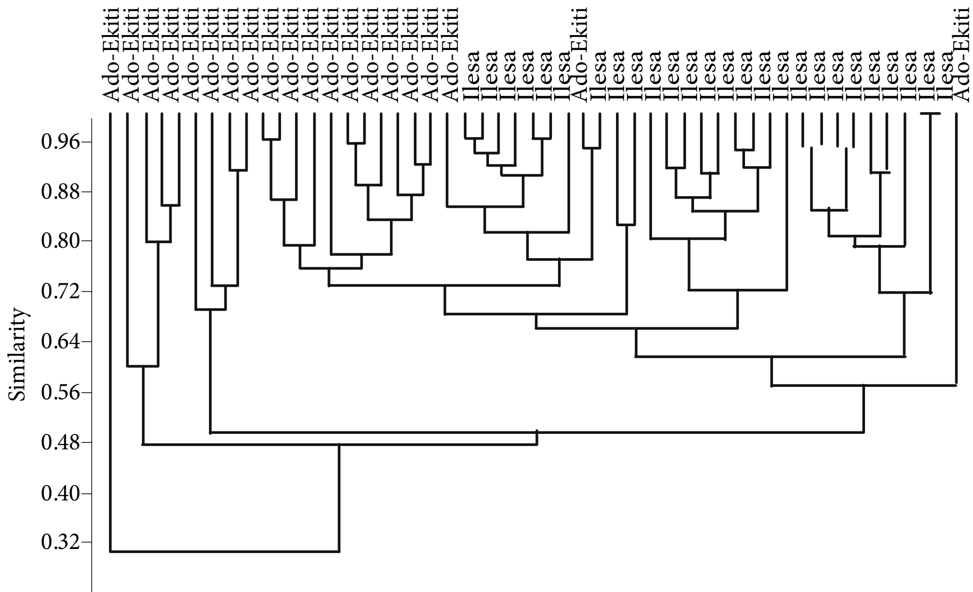

Fig. 3. Dendrogram obtained from Classical Cluster analysis using Paired group Bray-Curtis similarity index on *C. gariepinus* from two natural populations in Ado-Ekiti and Ilesa.

separation of protein variants has resulted in extensive use of this technique in population genetic studies and fast development of the biochemical genetics of population. Also, the existence of polymorphism in species and subspecies is valuable to their survival given that genetic variability promotes fitness in particular environments. Polymorphism also increases adaptability thereby providing for the possibility of genetic change.

In this study, the similarity coefficient (30 %) was very low. This was lower than the reports of Akinwande et al. (2012) on the hybrids of *H. longifilis*, *C. gariepinus* and *C. angularis*, Diyaware et al. (2012), on *Clarias anguillaris, Heterobranchus bidorsalis* and their hybrids, Majolagbe et al. (2012) on *Clarias gariepinus* and *Heterobranchus bidorsalis* parentals and their hybrids and Popoola et al. (2014) on cultured and wild *Clarias gariepinus* populations with similarity coefficients of 92 %, above 80 %, 43 % and 85 % respectively. This variation may be explained based on the origin of these fish samples. Also, this low similarity coefficient of 30 % (which denotes high heterogeneity of 70 %) may be because they were wild strains and were separated geographically, thus gene flow is rare as evident in the result. In addition, the observed high intraspecific variation in this study is different from Saad et al. (2009) who recorded similarity coefficients above 90 % among different populations of *Clarias gariepinus*.

Since electrophoresis can directly equate variation in protein banding patterns to genes encoding these proteins (Gottileb, 1971) genes controlling each character can be determined and separated to perfect and speed up the work of breeders for genetic improvement (Omitogun et al., 2001). More studies should therefore be done to know if the samples will have better fitness traits and the genes responsible may therefore be improved on for further breeding purposes. Also conservation strategies can be implemented to restrict over-exploitation of these catfish in these areas to avoid extinction which had been observed in other populations.

Conclusion

This study has established the presence of variations in *C. gariepinus* in the studied populations and samples from Ado-Ekiti displayed more heterogeneity than those from Ilesa. This indicates that this strain may perform well if improved on in future investigations.

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