ELECTROPHORETIC STUDIES OF *Clarias gariepinus* (BURCHELL 1822) AND *Heterobranchus bidorsalis* (GEOFFROY SAINT-HILAIRE 1809) AND THEIR HYBRIDS

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ABSTRACT

The study determined the genetic similarities and differences between *Clarias gariepinus* and *Heterobranchus bidorsalis*. Crosses were carried out on the parents; female (♀) *C. gariepinus* x male (♂) *C. Gariepinus*, ♀*H. bidorsalis* x ♂*H. bidorsalis*, and on the hybrids; ♀*C. gariepinus* x ♂*H. bidorsalis* and ♀*H. bidorsalis* x ♂*C. gariepinus* and two juveniles were analysed electrophoretically for each treatment. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out on the sera of the parents and hybrids. The gel images obtained after electrophoresis were scored and subjected to cluster analysis. The SDS-PAGE gels revealed wide degree of variations in terms of the positions of the bands in the sera protein profiles from the two specimens of each of the parentals and the hybrids. This study concluded that many polymorphic proteins are widely distributed in the investigated fish species.

Keywords: Electrophoresis, Cluster analysis, fitness, similarity

INTRODUCTION

Two fast growing species of *Clarias* are commonly cultured in Nigeria. These are *C. gariepinus* and *C. anguillaris* and fish farmers in Nigeria are in dire need of these species on account of their hardiness and fast growth. Their seeds are, however, not readily available to meet the needs of large scale fish farmers (FAO, 2004) and *C. gariepinus* broodstocks from the wild produce fingerlings with decreased fitness noticeable after only a few generations (Olaleye, 2005). *H. bidorsalis* is in greater demand than *Clarias* because of its exceptional growth rate. The fingerlings of this species are also limited in supply even from wild sources.

Increased productivity of fry and fingerlings with attributes of faster growth rates and better environmental tolerance is *essential* to ensuring fish food security in Africa. Genetic techniques are therefore needed to ensure that a faster growth rate leading to a shorter production cycle as well as a greater tolerance for poor water condition is achieved (Ataguba *et al.*, 2010).

Most morphological traits are polygenic, quantitative or continuous characters and their expression is influenced by environmental conditions. The traditional approach to characterization and evaluation is based on morphological features. However, biochemical analysis of total protein and isozyme markers reveal better diagnostic genetic variations and is usually free from genotype and environment interactions (Lombard *et al.*, 2001; Torkpo *et al.*, 2006). Thus, gel electrophoresis has become a veritable tool for studying variations at the genetic level as well as establishing phylogenetic trees (Buth, 1984).

According to Kirpicknikov (1981), the advantage inherent in the electrophoretic separation of protein variants has led to wide use of this technique in population genetic studies and the rapid development of the biochemical genetics of populations. The occurrence 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. Certain management strategies can however be adopted which allow for the improvement of stock while still maintaining a relatively high genetic variability in broodstock. Many methods are now available for determining genetic variability/diversity in natural populations. Among the most common methods is protein profile analysis through gel electrophoresis.

The study provided information on the total protein profiles of the two investigated fish species and their reciprocal hybrids. It also formed a basis for further studies on producing genetically improved hybrids since proper genetic characterization would help in the selection of suitable strains for aquaculture that could lead to production of new varieties of both fishes to alleviate the problem of short supply of quality fish.

MATERIALS AND METHODS

The broodstocks of *C. gariepinus* and *H. bidorsalis* were obtained from the Teaching and Research Farm; University of Agriculture, Abeokuta, Ogun State and Gureje Aquaculture Centre in Ile-Ife, Osun State. They were obtained from two different farms to prevent the incidence of in-breeding.

The brood stocks were transported in 50 litre- tank opened at the top to the Wet laboratory of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife. They were kept at constant temperature of 25^{0}C in 1000-liter tank and were fed with 37% Crude Protein commercial feed (DURANTE®, Nigeria) three times daily. The water quality was regulated through proper monitoring and replacement weekly. They were fed for three months to allow for proper acclimatization and maturity before their utilization.

Gravid females of *C. gariepinus* and *H. bidorsalis* were selected based on their swollen reddish genital papilla and a well-distended, swollen soft abdomen that oozed out eggs when gently pressed. Sexually matured males of *C. gariepinus* and *H. bidorsalis* were selected based on reddish pointed and vascularised urogenital papillae.

Hypophysation of the specimens was carried out in the Department of Animal Sciences, Wet laboratory, Obafemi Awolowo University, Ile-Ife. The selected female broodstocks of *C. gariepinus* and *H. bidorsalis* (1.0±0.6 kg) were kept separately in hatchery for two days without feeding so that the alimentary track was empty at the time of stripping. Sexually matured males of *C. gariepinus* and *H. bidorsalis* weighing 1.1±0.8 kg were selected and kept in different tanks of 50 litre capacity for two days prior to the time of sperm collection. Oocyte maturation and ovulation in the female broodstocks of the two catfishes was induced by a single intramuscular injection of Ovaprim$^{(R)}$ (SYNDEL, Canada) at a dosage of 0.5ml/kg live weight and then left for 10-17 h latency period depending on the catfish species (as *Heterobranchus has higher latency period than Clarias, Ataguba et al., 2010))* at 25^{0}C-26^{0}C to ensure high hatching rate and low proportion of deformed larvae.

The female broodstock was carefully dried with a clean towel and tightly held at head and tail ends while the eggs were handstripped by pressing their abdomen into clean and dry petridish. The eggs were fertilized with milt obtained from lacerated testes (and kept in physiological salt solution of 0.9%) by using feather to spread the mixture for 1.0 min evenly. The fertilized eggs from each mating combination were spread out in single layer on the screen nets (mesh size of 1mm) placed in the 500-liter hatching tanks at 27^{0}C - 28^{0}C. The following crosses were carried out in replicates:

♀

1. ♀ *C. gariepinus* x ♂ *C. gariepinus* (parental cross)
2. ♀ *H. bidorsalis* x ♂ *H. bidorsalis* (parental cross)
3. ♀ *C. gariepinus* x ♂ *H. bidorsalis* (hybrid)
4. *H. bidorsalis* x ♂ *C. gariepinus* (hybrid).

The electrophoretic analysis was carried out in the Department of Animal Sciences, Biotechnology laboratory, O.A.U., Ile-Ife. A total of 16 adults and juveniles (eight for adults, eight for juveniles) were collected for electrophoresis using a modified method of Laemli (1970). From the caudal region of the broodstock of about twenty four months old and juvenile fish (which had reached the age of six months), three millilitres (3ml) of blood was withdrawn, diluted with two millilitres (2ml) of 0.9% physiological salt solution and expelled from the syringe after removing the needle. Care was taken while allowing blood to run down the side of the centrifuge tube, this is to avoid the inclusion of air bubbles that could be present in the syringe in the blood sample. The sample was left for one hour at room temperature and the serum obtained after centrifuging at 5000 rpm for 15 minutes was decanted and kept at about 5°C until use. The supernatant of each sample was collected and 15µl of each supernatant was electrophoresed in 12% polyacrylamide-bisacrylamide gel. *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)* was carried out according to Laemli, 1970. Bromophenol blue was added to the sera to act as a tracer. Following electrophoresis, the gel was stained with 0.3% Coomassie Brilliant Blue R-250. Destaining in mixture of methanol, acetic acid and distilled water (1:3:5v/v) was done overnight to visualize the protein bands for subsequent scoring.

Seven percent (7%) of β-mercaptoethanol (SIGMA) in sample buffer of 4% stacking gel (including 1.3ml Acrylamide/Bis (SIGMA), 2.5ml Upper Tris buffer of 0.5M Tris-HCl and pH 6.8, 6.1 ml distilled water, 100 µl of 10% (w/v) SDS, 25 µl of 10% (APS) Ammonium per sulphate

(SIGMA), and 10 µl TEMED Tetramethylethylenediamine (ROTH) and 12% resolving gel (including 4.0ml Acrylamide Bis (SIGMA), 2.5ml lower Tris buffer of 1.5M Tris-HCl, pH 8.8, 3.5ml distilled water, 100 µl of 10% (w/v) SDS, 50µl APS and 5µl TEMED) was used for the preparation of each serum sample under a fume hood. To 30 µl sample of each protein extract stored in well-labelled eppendorf tubes in the deep freezer (-20°C), 10 µl of mixture of sample buffer and β-mercaptoethanol was added. These samples were heated at 95°C for 5 min 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. The separation of protein was carried out with the use of Electrophoresis Power Supply Model 200/2.0 in Mini Protean 11 Cell (BIO-RAD) at 150 volts for 1 hour.

When electrophoresis was completed, the gels were removed from the electrode vessels and left in the staining solution (0.1% Coomassie blue, 40% methanol and 10% glacial acetic acid) overnight. The staining was done for 16 hours. After staining, the gels were removed and washed for about two minutes in distilled water. The gels were then rinsed several times with freshly prepared destaining solution (10% glacial acetic acid, 40% methanol and 40% distilled water) until the protein bands were distinct and left in the destaining solution. The gels were scanned and the images were stored in the computer for scoring to compare the degree of similarity of the hybrids with those of parents. Data were collected on the scanned gels by scoring and viewing the presence (1) or absence (0) of protein bands directly from the computer screen (Oladejo *et al.*, 2009).

The protein banding patterns obtained from electrophoretic profiles were subjected to cluster analysis to show the relationship in their clustering patterns using the Unweighted Pair Group Method with Arithmetic means for phenogram or dendrogram grouping (Sneath and Sokal, 1973) using statistics software PAST (Hammer *et al.*, 2006).

RESULTS

The SDS-PAGE electrophoretic profiles of sera of the parental fish and their hybrids in the presence of SDS and β-mercaptoethanol are as shown in Plates 1 and 2. SDS-PAGE gels showed a high degree of qualitative and quantitative intra- and intergeneric variations in terms of the

Plate 1: A representative Coomasie-Blue stained SDS-PAGE gel showing serum protein banding patterns in the adults of *C. gariepinus* and *H. bidorsalis*

C1- ♂ *Clarias*
C2- ♀ *Clarias*
H1- ♂ *Heterobranchus*
H2- ♀ *Heterobranchus*
CM- ♂ *Clarias* used for crossbreeding
HF- ♀ *Heterobranchus* used for crossbreeding
HM- ♂ *Heterobranchus* used for crossbreeding
CF- ♀ *Clarias used* for crossbreeding

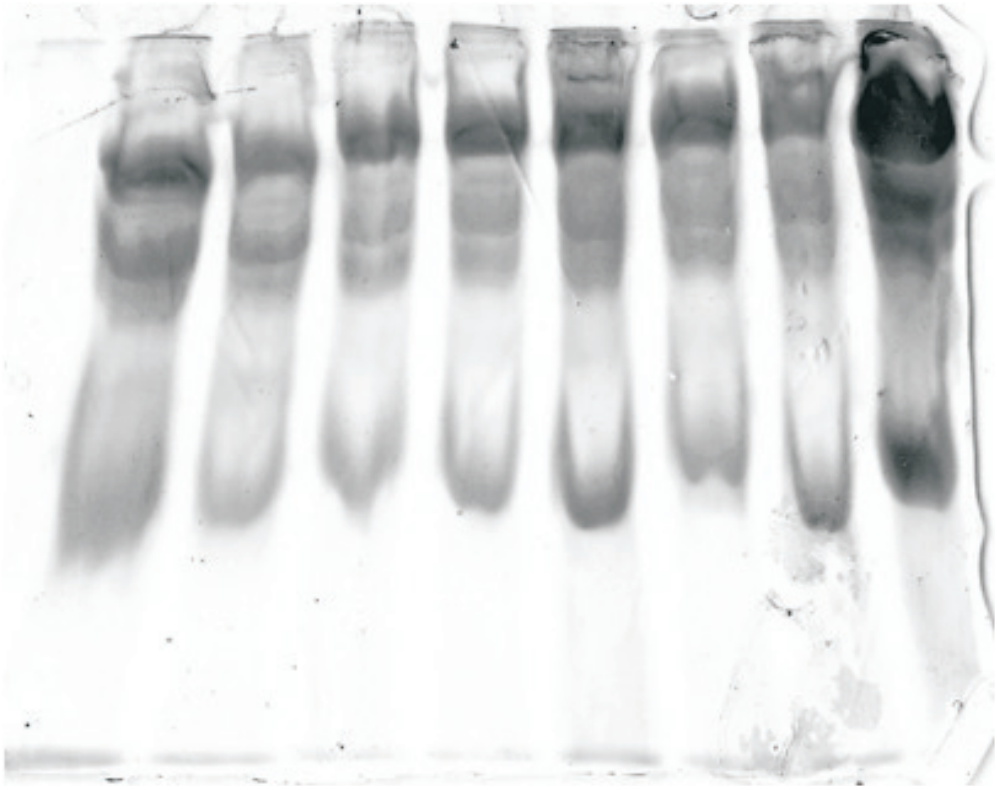

Plate 2: A representative Coomasie-Blue stained SDS-PAGE gel showing serum banding patterns in the juveniles of parentals and the reciprocal hybrids

HH1-♀*H. bidorsalis* x ♂*H. bidorsalis*
HH2-♀*H. bidorsalis* x ♂*H. bidorsalis* } *Parentals*

HC1- ♀*H. bidorsalis* x ♂*C. gariepinus*
HC2- ♀*H. bidorsalis* x ♂*C. gariepinus*
CH1- ♀*C. gariepinus* x ♂*H. bidorsalis* } *Hybrids*
CH2- ♀*C. gariepinus* x ♂*H. bidorsalis*

CC1- ♀*C. gariepinus* x ♂*C. gariepinus*
CC2- ♀*C. gariepinus* x ♂*C. gariepinus* } *Parentals*

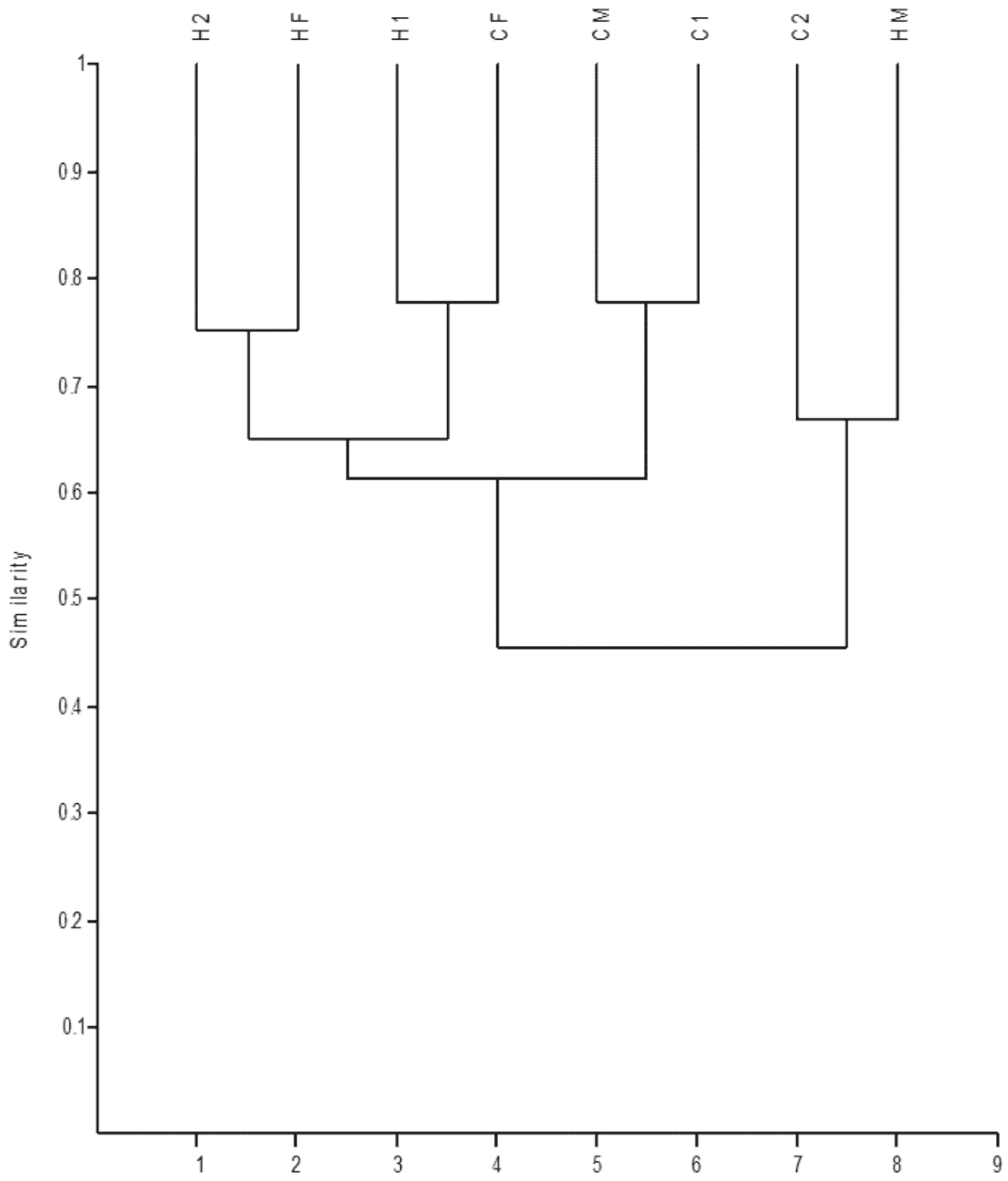

Fig. 1: Dendrogram showing similarity coefficients for adults of the *C. gariepinus* and *H. bidorsalis* based on serum protein banding patterns using the Jackard's clustering algorithm (Oladejo *et al.*, 2009).

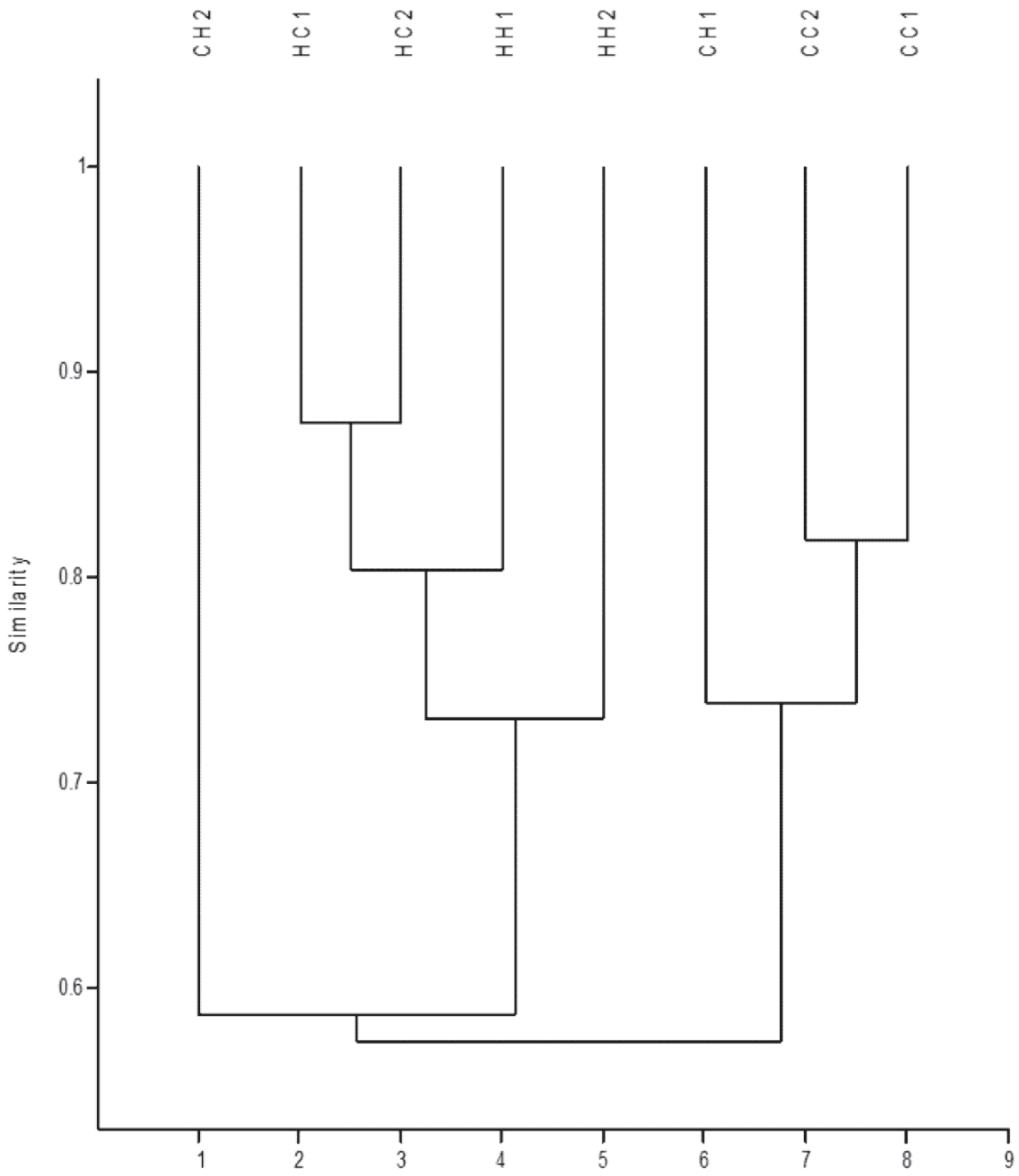

Fig. 2: Dendrogram showing similarity coefficients of *C. gariepinus* and *H. bidorsalis* and that of the reciprocal hybrids based on serum protein banding patterns employing the Jackard's clustering algorithm (Oladejo *et al.*, 2009)**.**

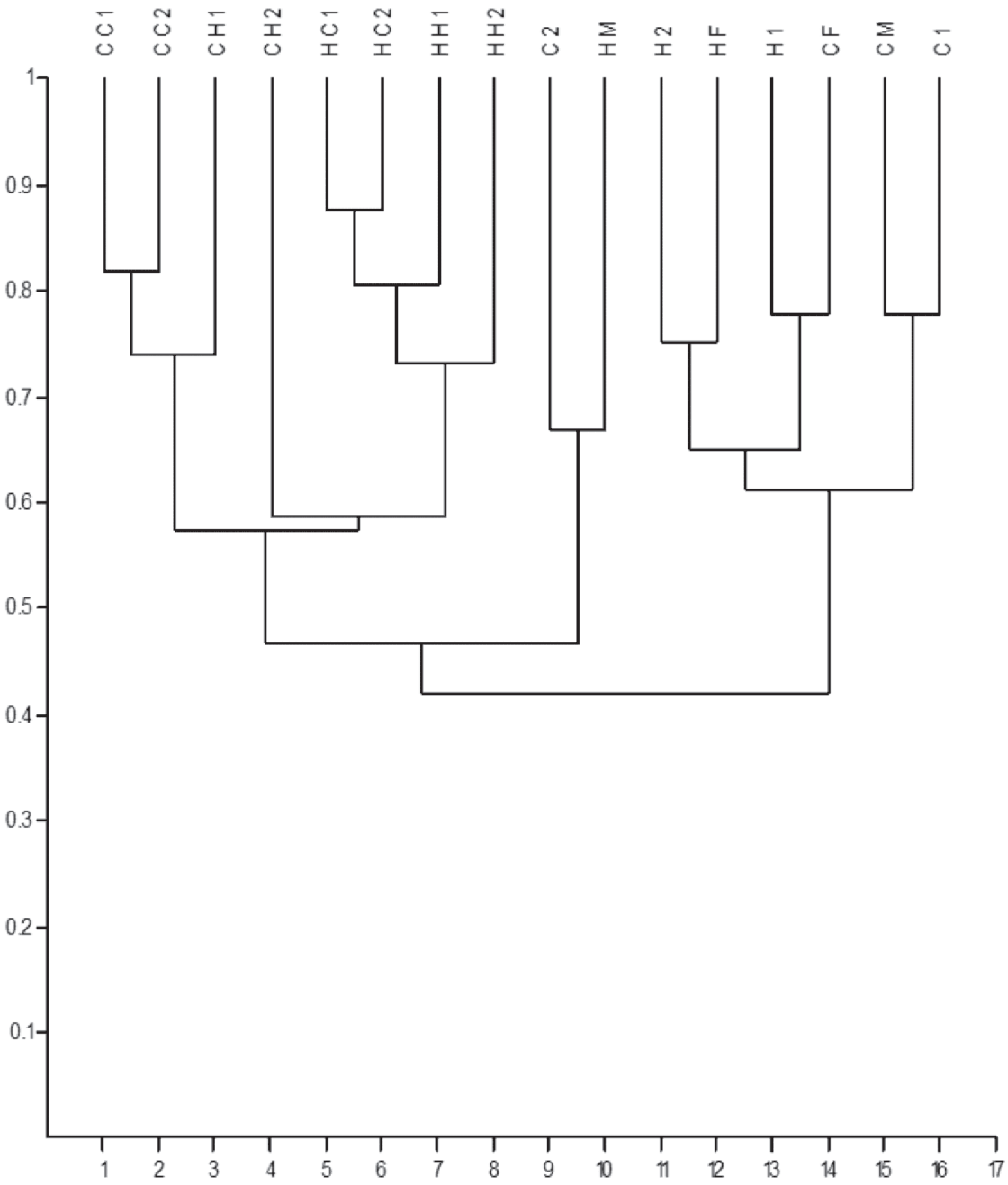

Fig. 3: Dendrogram from combined data on parents and juveniles of *C. gariepinus* and *H. bidorsalis* and their hybrids based on serum protein banding patterns employing the Jackard's clustering algorithm (Oladejo *et al*., 2009).

positions of the band in the protein profiles of the parents as well as those of the reciprocal hybrids. The serum protein banding patterns revealed 15 bands across all the crosses. The protein banding patterns of the juveniles produced four similar bands while the adult ones produced six similar bands.

Figs. 1, 2 and 3 showed the clustering patterns in the dendrogram based on protein banding data. The dendrogram of the parental species in Fig. 1 showed 45% similarity coefficient between the species. The dendrogram for the species in Fig. 2 showed 56% similarity coefficient among the juveniles of *C. gariepinus* and *H. bidorsalis* and their reciprocal hybrids. Fig. 3 is the dendrogram obtained when the data from hybrids and the parentals are combined showed three major clusters. The parental species and juveniles showed 43% similarity coefficient.

DISCUSSION

The electrophoretic profiles of total serum protein were used to analyze the amount of genetic variation within and between the species under study and also to establish the relatedness between them.

The protein profile of an organism is a function of the type of genes the organism has (Aluko, 1986). Borowsky (1977) reported that the alleles of identical electrophoretic mobility in closely related species have similar effects upon fitness. Teugels *et al.* (1992) stated that *H. longifilis* appeared to be closer to *C. anguillaris* and *C. gariepinus* than *C. ebriensis* from the enzymatic polymorphism examined at 13 protein loci in the four African Clariid catfish species and these results corresponded with published karyological and morphometrical data.

In Fig.3 is the dendrogram obtained when the data from hybrids and the parentals are combined showed three major clusters. The parental species and juveniles showed 43% similarity coefficient. This corroborated the relatively low genetic similarity (59%) observed in wild and domesticated Clariid strains from five locations (Betiku, 2006). Teugels *et al.* (1992) studied enzymatic variation in African Clariid catfishes and the electrophoretic results obtained for *C. gariepinus* × *H. 1ongifilis* specimens clearly confirmed hybridization which definitely excludes gynogenesis or androgenesis conditions known to occur in interspecific reproductions.

The importance of genetic variation in aquaculture cannot be overemphasized because genetic variation or heterozygosity was a measure of a population's ability to adapt to environmental change and survive (FAO/UNEP, 1981). In several organisms, individuals possessing the most genetic variation have shown to have better survival rates or higher growth rates. Relatively heterologous individuals appear to be more resistant to environmental variation during developments (Betiku, 2006, Majolagbe, 2010).

Proteins are considered to be direct product of genes and could be taken as markers of these genes (Ladizinsky, 1983). The fact that no two species has completely the same protein profile band in this study could be a reflection of the biochemical differences or genetic variability among the species studied.

The evidence resulting from the different electrophoretic profiles of the parents and hybrids therefore provided clear evidence to the distinct nature of the species and variation existing among them. Illoh *et al.*, (1993) reported that the variation in the total protein band patterns among the parents and hybrids was indicative of genetic diversity. The result from the electrophoretic studies showed that many polymorphic proteins were widely distributed in fish (Kirpichnikov, 1981). Electrophoresis could directly equate variation in protein band patterns to genes encoding them (Gottileb, 1971) as genetic similarity was based on the similarity of the proteins represented by the bands. Illoh *et al.*, (1993) posited that the proteins were under the control of the same genes which have evolved and become fixed over evolutionary time.

Total protein electrophoresis, however does not distinguish all the genetic variability at a given structural gene locus due to the low levels of variation in the coding sequences relative to the other regions of the genome (Arranz *et al.*, 1996; May, 1992; Adeola 2009). Aluko (1986) reported that the total number of bands might not be taken as the total number of proteins in these species because it was possible for different amino acid sequence to result in proteins that could be distinguished electrophoretically. Routine electrophoresis detected only the amino acid substitutions that results in differences in the net charge of proteins (Ayala and Kiger, 1980). This represents only a third of all the amino acid substitutions, as only 30% of all amino acid substitutions on the exterior of the molecule results in a charge shift (May, 1992). Additionally,

only the variability in the coding portions of the genome (which constitutes about 10% of the eukaryotic genome) can be sampled using protein electrophoresis, hence, a further characterization at the genome level is necessary.

Although both catfish species presently belong to different genera, nevertheless the results of this study suggested that the two species are closely related hence, their ability to interbreed. This study could be useful in designing the appropriate crosses that could achieve maximum utilization of breeding resources. High genetic variation is very important in any breeding programme.

In conclusion, the protein profile analysis revealed high genetic variation in the parental species. This could explain the heterosis observed in the growth and survival rates of the hybrids. The electrophoretic profiles showed that there was a large amount of genetic variability in the populations of the two species. This indicated the possibility of maximizing the benefits of hybridization and selective breeding. High genetic variation among catfishes capable of interbreeding determines their adaptive features, hence the need to maintain and improve them by crossbreeding.

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