COMPARATIVE STUDIES OF THE GENOMES OF *CLARIAS GARIEPINUS, HETEROBRANCHUS BIDORSALIS,* AND THEIR HYBRID USING HYDROXYAPATITE CHROMATOGRAPHY*

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Abstract

The study investigated the genomes of *Clarias gariepinus, Heterobranhus bidorsalis* and their hybrid. The elution pattern of *C. gariepinus* showed the presence of a low melting (AT rich) region between the temperatures of 60 °C and 70 °C. This very light fraction in *Clarias* showed as a minor shoulder in the DNA of the hybrid. There appeared to be so much integration of the genome of the two parental stocks in the *Heteroclarias* that the early melting fraction of the *C. gariepinus* DNA did not show prominently. The elution pattern of *Heteroclarias* DNA showed an intermediate pattern between those of *H. bidorsalis* and *C. gariepinus*, which however was tilted towards those of the *Clarias* DNA pattern. Also the melting temperatures of 79.2 °C, 83.0 °C and 82.0 °C obtained for *H. bidorsalis, C. gariepinus* and the hybrid respectively showed that the Tm of the DNA of the hybrid is intermediate between those of the parental stock.

Key words: Genome, sonicate, unimodal, melting temperature (Tm)

1. Introduction

Artificial production of hybrid is a powerful tool in fish culture (Purdon, 1976). Inter-generic cross-breeding between the African catfishes *Clarias* species and *Heterobranchus* species is widely used in Nigerian fish culture enterprises (Madu *et al.*, 1992, 1993; Salami *et al.*, 1993; Adeyemo *et al.*, 1994; Aluko, 1995; Nwadukwe, 1995). The possibility of natural hybridization between any two species is an indication that they are genetically very close.

In spite of the widespread culture and commercial importance of *Clarias, Heterobranchus* and their hybrids in African aquaculture, virtually nothing is known about the underlying genetic basis of these economic species and their hybrids (Aluko, 1996). There is ample report of successful production of intra-generic hybrids of Clariid catfishes. Inter-generic hybrids show superior growth advantage over the parent stocks. The F1 hybrid obtained from the cross between such two Clariid species is fertile (Aluko, 1995; Nwadukwe, 1995). The fertility of the F1 hybrids has been exploited by aquaculture practitioners in Nigeria by using these hybrids as breeders for further propagation.

Hybrid fingerlings produced between parental *H. bidorsalis* and *C. gariepinus* are easily identified from parent *Heterobranchus* by the presence of longer anterior dorsal fin and very short adipose fin (Aluko, 1999). Awodiran *et al.*, (2000) also used mitotic chromosomes to distinguish between hybrids produced from parental *Heterobranchus longifillis* and *Clarias anguilarias*.

However, in some inter-generic hybrids investigated, the adipose fin length compared favourably with those of the parental *Heterobranchus* and with continuous hybridization, hybrids with lengths of adipose fin similar to those of the parental offspring could be produced (Nlewadim and Omitogun, 2005) thereby making it very difficult to identify and separate properly the hybrids from the parental especially during breeding exercises.

Investigations carried-out on meiotic chromosomes of *C. gariepinus* and *H. bidorsalis* and *C. anguillaris* showed that 24 bivalents were recorded for *H. bidorsalis* out of which seven were ring chromosomes compared to 27 bivalents and six ring chromosomes recorded in *C. gariepinus*. Two ring chromosomes were recorded for *C. anguillaris* with 26 bivalents. The closeness in the number of ring chromosomes of *H. bidorsalis* and *C. gariepinus* indicated high degree of homology probably needed for precise pairing of chromosomes to form bivalents and consequently influence the viability of the cross (Aluko, 1994).

Hybridization combines useful and desirable traits from both parents into an offspring of superior quality. Combination of traits in the hybrid would lead to superior characteristics both in context of production or marketing over either of the parent species as such

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superior offspring exhibit hybrid vigour (Tave, 1995). Despite the versatility of the species hybridization technique for production of hybrid with superior characteristics to the parental in the African catfishes, there has been no report of simultaneous improvement to so many traits in single line mating. Improvement of such commercially important traits has however been recorded in hybrids formed between the channel catfish *Ictalurus punctatus* and blue catfish *I. functatus* (Michael and Rex, 1998). Understanding of the basic genomic composition is therefore very important for decision making relating to marker development, linkage and physical mapping (Liu, 2003) especially in the closely related African catfish species. Hydroxyapatite crystals (which discriminates between double and single stranded DNA molecules) over a wide range of temperatures (Bernardi, 1965) is a very useful tool for determination of genomic composition. The objective of the current study is to provide information on the genomic compostion of *C. gariepinus*, *H. bidorsalis* and their hybrid using hydroxyapatite chromatography with a view to deducing the organization of at least some components of the genome.

2. Materials and methods

Source and extraction of DNA:

DNA was extracted from the liver of freshly killed C. *gariepinus*, H. *bidorsalis* specimens and their hybrid (*Heteroclarias*), obtained from Akinrinade Farm, Yakoyo, Ile-Ife, Nigeria. Liver samples from the sacrificed specimens were homogenized in about three times their volumes of cold solution of homogenizing buffer (0.15 M NaCl, 0.015 M sodium citrate, supplemented with 0.001 M ethylenediaminetetraacetate, pH 7.0)in an ice bath using a Polytron PT-20-OD set at low speed. Whole cells were precipitated by centrifugation and suspended in 5 ml SSC (0.15 M NaCl, 0.015 M sodium citrate, 0.05 M EDTA and 0.25% sodium lauryl sulphate, pH 9.0) was added. The viscous lysate was then pooled and the NaCl concentration increased to 1.15 M and shaken vigourously until it dissolved.

An equal volume of cold chloroform-isoamyl alcohol (20:1) was then added to the lysate which was shaken vigorously in an ice bath until an emulsion was obtained. The emulsion was centrifuged at 4,000 rpm for 10 minutes, leading to formation of three layers. The top aqueous layer which contained the nucleic acids was carefully removed with a wide tipped pipette. The chloroform-isoamyl alcohol treatment was repeated until there was little or no protein precipitate at the interphase between the top aqueous layer and the bottom layer.

The clear aqueous layer was cooled and twice its volume of 95% ethanol was added slowly by the side of the container so as to precipitate the nucleic acids. The mixture was swirled gently and the precipitate which was normally fibrous was picked with a Pasteur pipette and dissolved in 5-15 ml 1 X SSCE, pH 7.4.

Bovine pancreatic ribonuclease (RNAse) was added to the nucleic acid solution to a concentration of 50 µg of enzyme per ml and incubated for one hour at 60 °C in a Gallenkamp water bath. The solution was left at room temperature for at least 30 minutes. Pronase was added to a concentration of 100 µg of enzyme per ml and the mixture was then incubated at 37 °C in a water bath overnight.

The sodium chloride concentration of the digest was again increased to a final concentration of 1.15 M, and an equal volume of chloroform-isoamyl alcohol (20:1) was added and shaken well in ice bath and centrifuged. The top aqueous layer was collected, and the deproteinization procedure was repeated with chloroform-isoamyl alcohol until there was no precipitate at the interphase between the top aqueous layer and the lower chloroform layer. The aqueous layer was then pooled, cooled, and twice its volume of cold 95% ethanol was added to precipitate the DNA. The white fluffy precipitate (DNA) was picked with a Pasteur pipette and dissolved in 5-15 ml of 0.12 M sodium phosphate buffer (NaP).

DNA fragmentation:

The purified DNA was sheared with an MSE Soniprep 150 (equipped with a regular probe at a near maximum setting of 28 amplitude microns) for 6 minutes, at 2 minutes intervals. The sonicated DNA was dialysed in two changes of 2 L 0.06 M NaP and then 0.12 NaP for at least ten hours for each change. The optical density (OD) of the DNA sample at 260 nm was determined using UV-Spectrophotometer. The optical density was determined from the average of the close readings of the diluted DNA sample.

DNA Reassociation:

Sonicated DNA sample in 0.12 M NaP was heat denatured by boiling at 100 °C for 10 minutes. The denatured DNA was quickly transferred into a Gallenkamp water bath maintained at a constant temperature of 60 °C water bath.

DNA fractionation on hydroxyapatite columns using sodium phosphate buffer:

Hydroxyapatite crystals were prepared in the laboratory using a modified Bernardi (1971) method. The hydroxyapatite crystals were packed in a 1.8 cm diameter water-jacketed column containing 0.12 M NaP in which the crystals were allowed to settle to a height of 10 mm or 1 cm and the column was washed with 40-50 ml of 0.12 M NaP at 60 °C to equilibrate the crystals to the 0.12 M NaP. The temperature of the column was maintained with a HAAKE D3 water circulator. The desired flow rate of the NaP through the column was achieved by a control valve.

Thermal elution of the native and highly repetitive DNA sample of Clariid species and the hybrid:

The sonicated and unsonicated native DNA and the essentially highly repetitive DNA were thermally fractionated on hydroxyapatite column. Each sample was loaded on the hydroxyapatite column equilibrated to 0.12 M NaP at 50 °C. The temperature of the column was increased in steps of 4 °C to 98 °C. The temperature over the crystals was monitored with a mercury in - glass thermometer. The optical density of each fraction was determined using a UV - Spectrophotometer and the amount of DNA per fraction was computed and plotted against temperature. The melting temperatures (T_m) of the various samples were then determined from the profiles obtained.

Isolation of highly repetitive DNA sequences:

The sonicated DNA samples in 0.12 M NaP was denatured by boiling at 100 °C for 10 minutes and was quickly put in a water bath pre-set at 60 °C to anneal to a Cot (Britten and Kohne, 1968) of 50. At the end of the reassociation, the sample was diluted to 0.06 M NaP and quickly loaded on hydroxyapatite column that had been equilibrated to 0.12 M NaP. The single-stranded DNA was eluted by washing loaded hydroxyapatite column with about 20 ml of 0.12 M NaP at 60 °C. The temperature of the column was then increased in steps of 4 °C until the final temperature of 98 °C - at this temperature all DNA molecules would have melted. The thermal elution profile was determined as was done for the native DNA above.

3. Result and Discussion

The basis of thermal stepwise elution of double stranded DNA from hydroxyapatite (HA) is that molecules denature according to the proportion of (A + T) in the molecule and elute from the crystals as single strands, Rice, (1972). The thermal elution experiments carried-out in this study provided a convenient means of determining the melting temperatures of the DNA extracted from the two closely related Clariid species and the hybrid as well as the quantity of the highly repetitive DNA molecules. In 0.12 M NaP, DNA molecules are known to elute from HA as soon as they melt. The lower temperature fractions are presumed to be rich in adenine and thymine (A + T), while the fractions which melt at higher temperature are presumed to be rich in guanine and cytosine (G + C) (Marmur and Doty, 1962; Walker and Maclaren, 1965).

Fig. 1 shows the comparative thermal elution profiles of the sonicated samples of the DNA of *Heterobranchus bidorsalis, Clarias gariepinus* and the hybrid *Heteroclarias*. The result showed that the sonicated DNA of *H. bidorsalis* starts to elute at 70 °C and reaches its peak at 78 °C (Fig. 1.). The pattern of the elution profile is unimodal. The sonicated DNA of *C. gariepinus* on the other hand starts to elute as from a temperature of 54 °C also with a unimodal pattern. The elution pattern of the sonicated DNA of *Heteroclarias* is within a temperature range of between 70 °C and 98 °C and reaches a clearly defined peak at 82 °C.

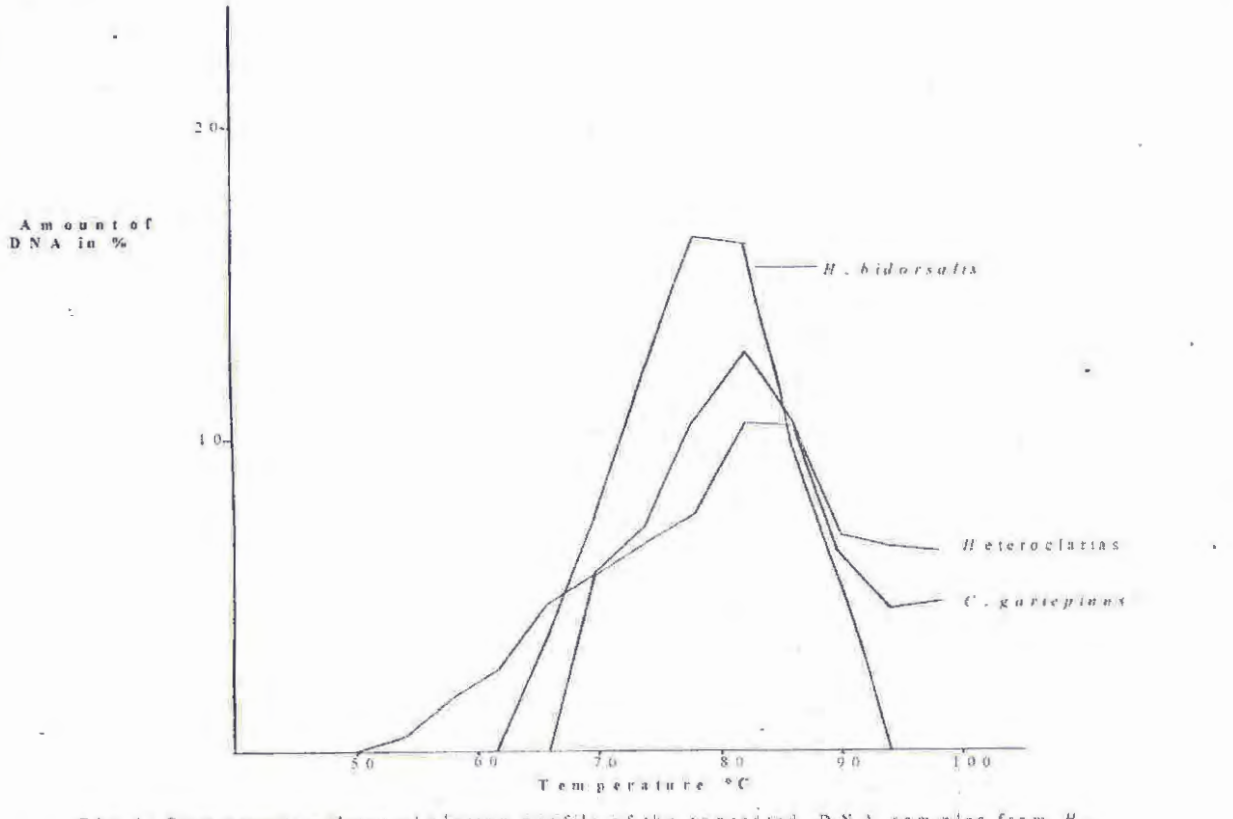

Fig. 1 Comparative thermal elution profile of the sonicated DNA samples from *H. bidorsalis*, *C. gariepinus*, and *Heteroclarias*

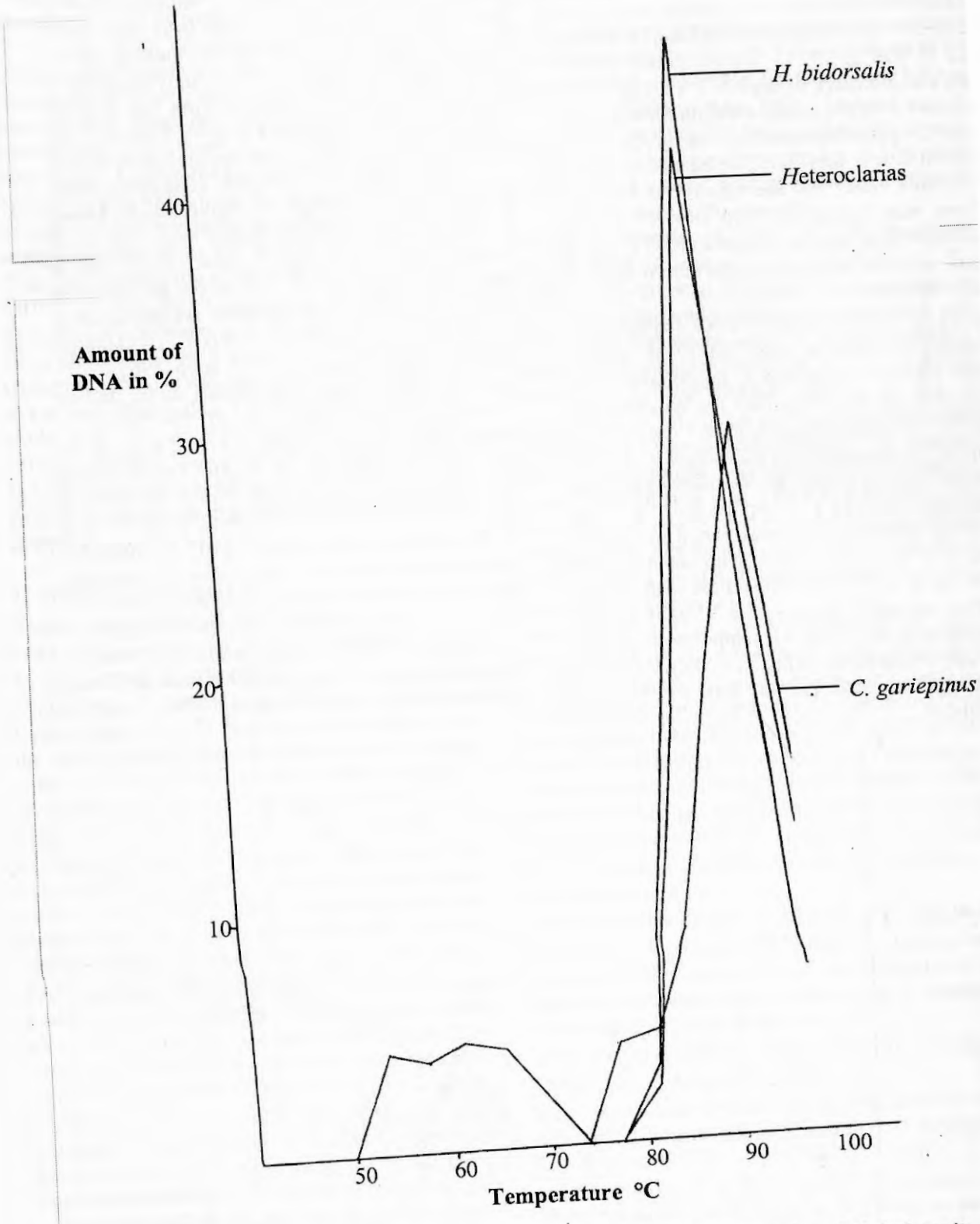

Fig. 2: Comparative thermal elution profile of the DNA samples of *H. bidorsalis*, *C. gariepinus*, and *H*eteroclarias.

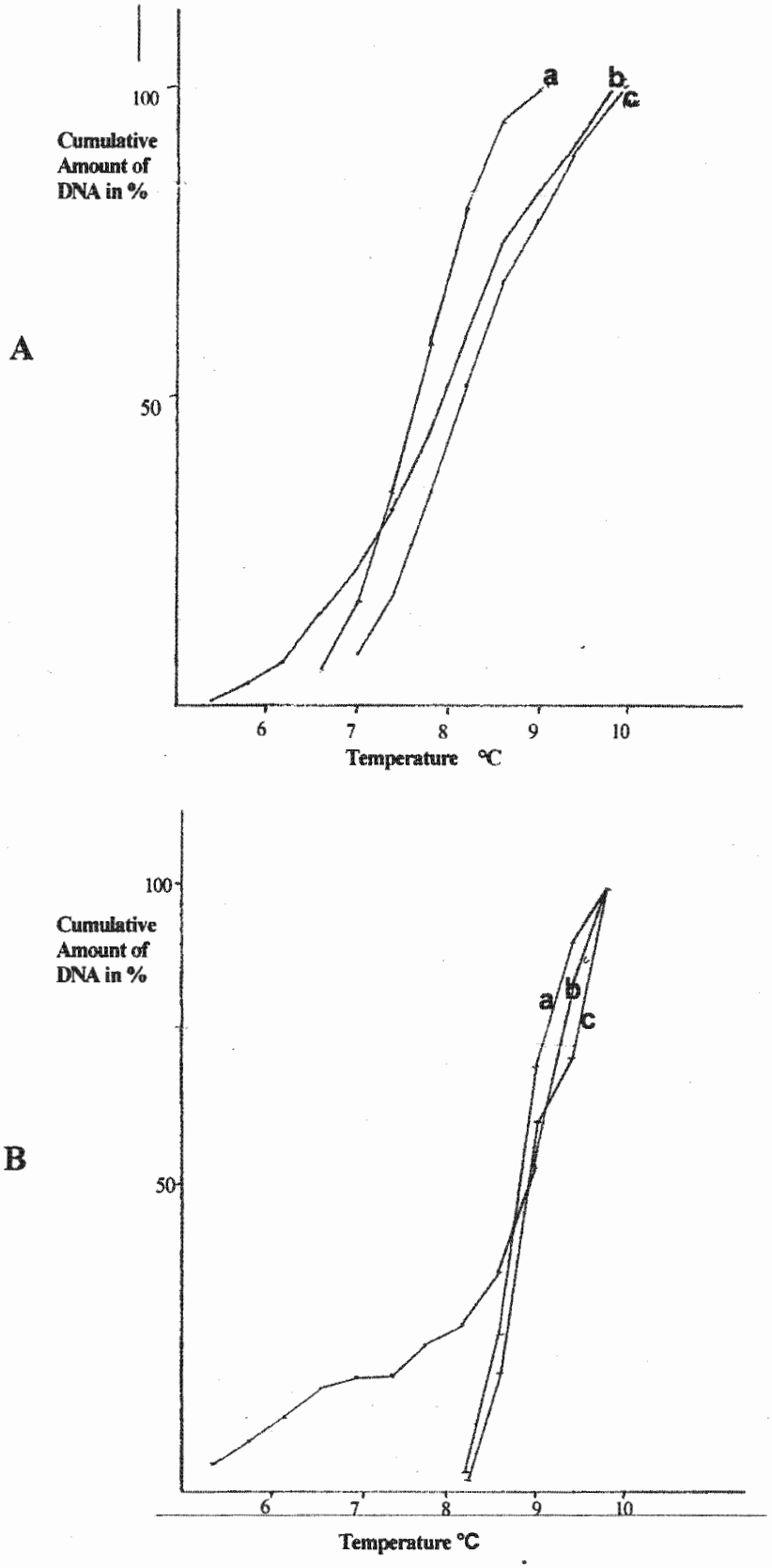

Fig. 3: A. Melting curves of the sonicated DNA samples from (a) Heterobranchus bidorsalis (b) *Clarias gariepinus and* (c) Heteroclarias

B. Melting curves of the unsonicated DNA samples from (a) Heterobranchus bidorsalis (b) *Clarias gariepinus and* (c) Heteroclarias

The unsonicated DNA of *H. bidorsalis* melts within a temperature range of between 82 °C and 98 °C showing the peak at 90 °C (Fig. 2). The elution profile of the unsonicated *C. gariepinus* DNA samples is such that a light component elutes essentially between 59 °C and 74 °C, while a heavy component elutes between 75 °C and 90 °C (Fig. 2). The light fractions appear to be somewhat heterogeneous and constitute no more than 10% of the genome. The major component which is a heavy fraction appears to be more homogenous. The unsonicated DNA of the hybrid *Heteroclarias* melts within a temperature range of 82 °C and 98 °C, reaching the peak at 90 °C (Fig. 2).

There appears to be so mush integration of the genome of the two parental species in the *Heteroclarias* that the early melting fraction of the *C. gariepinus* does not show prominently. This is evidenced in the thermal elution profile of both the sonicated and unsonicated DNA samples. (Fig. 1 and 2). The elution pattern of the sonicated DNA of the hybrid, whose genome represents the combination of the intergeneric cross, shows that although the DNA of *Heteroclarias* tended to have intermediate patterns between *H. bidorsalis* and *C. gariepinus* it is tilted towards the *Clarias* genomic pattern. However, the very light fraction in *Clarias* DNA only shows as a minor shoulder in the hybrid DNA.

The elution profiles also show that the DNA of the two closely related Clariid species and the hybrid had high GC composition.

Comparison of the melting curves of unsonciated and sonicated DNA samples from *C. gariepinus, H. bidorsalis* and *Heteroclarias* is shown in Fig. 3 while the T_ms derived from the elution profiles was used to calculate the base composition for each sample as shown in Table 1. Irrespective of the fish species, unsonicated DNA samples had higher melting temperatures than corresponding sonicated samples. The result also showed that *C. gariepinus* (83 °C) had higher Tm values than *H. bidorsalis* (79 °C) with those of the hybrid (82 °C) in between. The (G + C) base compositions for sonicated samples were lower than those of unsonciated samples irrespective of the fish species (Table 1). In all the fishes, the (G + C) base composition was higher in the unsonicated samples than in those of corresponding sonicated ones. The result also showed that the (G + C) bases of *C.gariepinus* was higher than those of *H. bidorsalis* whether the DNA samples were sonicated or not. The hybrid Heteroclarias had a (G + C) base composition higher than those of *H. bidorsalis* but lower than those of *C. gariepinus* irrespective of the treatment of the DNA.

The thermal stability of the product of the reassocaition of highly repetitive DNA can be used to quantitatively estimate the degree (level) of sequence divergence within a given family. Reassociation analysis of the fragments accomplished by allowing reassociation to occur to Cot 50, isolate the highly repetitive, and possibly some intermediate repetitive DNA sequences in the genome of the fishes. The unimodal elution profile of the repetitive DNA sample from *H. bidorsalis* and *Heteroclarias* were essentially similar. However, the *C. gariepinus* repetitive DNA sample showed a somewhat bimodal pattern of elution.

The presence of a low temperature melting region in the *C. gariepinus* genome suggests that the genome contains runs of AT sequences some of which could be excised during preparation. More of these sequences were however excised during sonication which indicated a higher proportion of the AT rich sequences eluting ahead of the bulk of the DNA. The result therefore shows that the DNA of *C. gariepinus* not only contains an (A + T) rich portion but the bulk of the DNA also contains higher amount of GC than that of *H. bidorsalis*. This very light fraction in *Clarias* shows as a minor shoulder in the DNA of the hybrid giving an impression that it is plenty enough to influence the elution pattern of the combination of the two genomes (in the hybrid). In addition, this light component which obviously is highly repetitive, clearly resolves into a high AT-rich peak and the rest of the repetitive DNA.

If the base sequence of any genome coincides with the sequence of another, the character of the elution profile, melting curve, should be identical. In the present study, a similarity is depicted by the elution patterns of the repetitive DNA samples of the three fishes. However, the DNA of *C. gariepinus* shows a deviation in that its elution pattern resolves the DNA into two components - a light, apparently AT rich and heavier GC rich, components.

Table 1: T_m and corresponding base composition of the sonicated and unsonicated DNAs of *Clarias gariepinus, Heterobranchus bidorsalis* and *Heteroclarias.*

	Unsonicated DNA sample	Sonicated DNA sample	Base composition (Sonicated)	Base composition (Unsonicated)
Clarias gariepinus	92.8 °C	83.0 °C	33.41 (G+C)	57.31 (G+C)
Heterobranchus bidorsalis	90.0 °C	79.2 °C	24.14 (G+C)	50.48 (G+C)
Heteroclarias (Hybrid)	91.2 °C	82.0 °C	30.97 (G+C)	53.41 (G+C)

Britten and Kohne (1968), observed that the longer the period after divergence of two species, the greater the reduction in thermal stability of its highly repetitive sequences. By implication the more thermally stable sequences evolved much earlier than the less thermally stable sequences which are of more recent origin. The level of similarity in the elution pattern of the highly repetitive DNA obtained in this work corroborates the observed morphological similarities among the two species of catfish. This would also give credence to the suitability of highly repetitive DNA sequences as genetic markers for taxonomic or other kind of characterization (Liu and Cordes, 2004) since the two fish species investigated in this project do not look radically different, the differences in their genomes, as can be detected by the method of thermal elution, shows that most of their genomes are similar, including important genes, which have not been affected by the separation of the two fish species. Alternatively it could be surmised that the two genomes are essentially the same, however the perceived differences in the genomic composition was probably due to the evolution of higher copy number of the A + T rich highly repetitive DNA in *Clarias* than *Heterobranchus*.

The T_m of the *Heterobranchus* species (79 °C) in the present study falls in the same range obtained for loach genome (Kuprisanova and Timofeeva, 1974). However, the Tm of the *Clarias* species was slightly higher probably due to the presence of some G + C rich sequences, presumably also occurring in high copy numbers Fig. 3.

The high T_m values obtained for the unsonicated samples might be because of the tendency of native DNA to intertwine or the long length of each DNA piece. However, after sonication, the double stranded DNA fragments binds to the HA respond by way of elution to the stepwise increase in temperature.

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