E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(4): 275-279

www.fisheriesjournal.com
Received: 25-05-2019
Accepted: 27-06-2019

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Genetic evaluation of some tilapiine fishes using varying RAPD markers

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Abstract
Genetic variation and divergence within and between the taxonomic groups of interest are usually assessed by the presence or absence of each product, which is influenced by changes in the DNA sequence at each locus. RAPD polymorphisms can occur as a result of base substitutions at the primer binding sites. This study was conducted to evaluate the genetic variation among some cichlid fishes of Epe lagoon, Lagos, Nigeria using different RAPD markers. Genomic DNA was extracted using DNA kit Nucleospin by Macherey Nagel from Germany, following manufacturer's instructions. For DNA amplification using PCR, Primus thermocycler was used and programmed according to required specification. Primers OPC-04 (5' CCG CAT CTA C 3'), OPAC-20 (ACGGAAGTGG) and OPI-16 (TCTCCGCCCT) which were arbitrary primers, were also used for the analysis. With OP-04 a total of 37 PCR products were scored across the gel. A range of 2-7 bands were recognized per sample. With OP-16, there was high level of similarity in the banding pattern within each species. 2-5 bands were characterized ranging from 100-600 base pairs. Concerning primer OPAC 20, 96 bands were distinctly recognized. A range of 1-5 bands were detected in each sample. From the pattern there was a great variability among the four species of cichlid. The DNA banding pattern derived from this primer showed highest similarity between *Tilapia mariae and' wesafu'.* Collating the data of the three primers together, similarity coefficient was calculated. The highest value was obtained between *Tilapia guineensis* and *Tilapia mariae* (0.52992) and lowest between *Tilapia mariae* and *Sarotherodon melanotheron* (0.28925). Genetic similarities among the species of cichlids were thus detected based on bands polymorphisms which were generated by these random primers. The result showed high level of variation among genera. Within the same genera, there was polymorphism, indicating that fish within the same species are not entirely similar.

Keywords: Genetic, Tilapiine, fishes, RAPD, markers

Introduction
Randomly Amplified Polymorphic DNA (RAPD) refers to the random amplification of anonymous loci by Polymerase Chain Reaction (PCR). This method is based on the amplification of regions of arbitrary DNA sequences with short nucleotide sequences. This technique is known to be simple, fast and cost effective. It has high level of polymorphism (variants forms) and only a small amount of DNA is required [10]. There is no need for previous knowledge of the genetic composition of the organism under study [7].
The main disadvantage with this technique is that, the resulting pattern of bands produced is very sensitive to variations which depend largely on reaction conditions, DNA quality and the PCR temperature profile [8]. The use of RAPDs have gained considerable acceptance, particularly in population genetics. Such analysis has been employed to evaluate genetic differences for species, subspecies and population/stock identification in guppy [5], tilapia, brown trout and Atlantic salmon, largemouth trout [12], Ictalurid catfishes [8], common carp and Indian major carps. Naish *et al*., [11] found RAPD useful in detecting diversity which occurs within and between strains of *Oreochromis niloticus.* This study was conducted to evaluate the genetic variation among some cichlid fishes of Epe lagoon, Lagos, Nigeria using different RAPD markers.

Materials and Methods
Genomic DNA was extracted using DNA kit Nucleospin by Macherey Nagel from Germany, following manufacturer's instructions. For DNA amplification using PCR, Primus thermocycler was used and programmed as follows:

Denaturation: (one cycle) at 94 ^{0}C for 4 minutes for separating DNA template.

Annealing: (35 cycles) for annealing of primer to DNA template at 54 ^{0}C for 45s, 37 ^{0}C for 45s and 72 ^{0}Cfor 45s

Extension: (One cycle) at 72 ^{0}C for 10 minutes.

RAPD PCR protocol

Primers OPC-04 (5' CCG CAT CTA C 3'), OPAC-20 (ACGGAAGTGG) and OPI-16 (TCTCCGCCCT) which were arbitrary primers, were used for the analysis. The PCR was performed in a 25 µl reaction mixture containing DNA (10-200ng), 200 µM of each deoxy nucleoside triphosphates (dNTP) (Promega), 2.0mM $MgCl_2$, 1X PCR Buffer, 50pMol of primer, 2 units of *Taq DNA* polymerase (Promega) and sterile water. DNA fragments were separated in a 1% agarose gels and visualized by ethidium bromide staining. 1 kb DNA ladders were used as DNA molecular weight standards [2].

Gel electrophoresis for RAPD

Agarose (1.5%)	1.5g
1XTBE buffer	100ml
Ethidium bromide (1%)	1µl

100 ml of agarose gel was prepared by mixing 20ml of 5X TBE buffer with 80ml double distilled water, then 1.5g agarose was added. Agarose was placed in a 1X TBE buffer, covered with foil paper and boiled in water bath. Ethidium bromide was then added to the melted gel after allowing to cool to 55 ^{0}C. The melted gel was poured into the casting tray and the comb inserted immediately. The comb was removed when the gel became hard and the gel was covered with running buffer. The electrophoresis buffer (0.5XTBE) was made to cover the gel. A 10µl of the amplified product was loaded in each well and run at 100V, 167mA and 17 W for 1hour 15 minutes. The resultant gel from different DNA fingerprints was photographed using Video documentation system.

Results

The results of RAPD analysis using the 3 primers are shown as follows:

PrimerOP-04: With regards to pattern of banding, the result is as shown in Plate 1-3. A total of 37 PCR products were scored across the gel. A range of 2-7 bands were recognized per sample. From the pattern, there were high differences among the species of the cichlids. Even within the same species there was some degree of polymorphism suggesting some level of hybridization among species probably in the freshwater system opening to the Lagoon from Oshun River. Band 21-30 representing *Tilapia guineeensis* showed greater intra-specific similarity.

Primer OP-16: The pattern of primer OPI-16 was presented in Plates 3-4. There was high level of similarity in the banding pattern within each species. 2-5 bands were characterized ranging from 100-600 base pairs.

Primer-OPAC 20: Concerning primer OPAC 20, 96 bands were distinctly recognized. A range of 1-5 bands were detected in each sample. The banding pattern is presented in Plates 4-5. As shown, all samples showed different band pattern with few exceptions. The highest number of bands was detected in *T. mariae* (specimen 1-10). From the pattern there was a great variability among the four species of cichlid. The DNA banding pattern derived from this primer showed highest similarity between *T. mariae* (1-10) and 'Wesafu' (11-20)

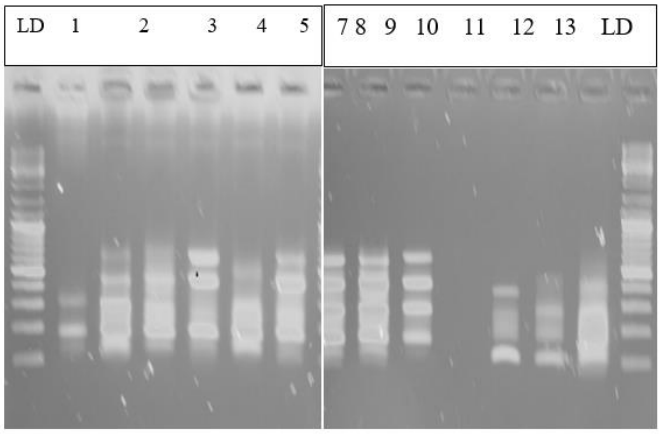

Plate 1: Primer OP-4 where 1-10 (Wesafu) *11-13* (*Tilapiamariae) LD (ladder)*

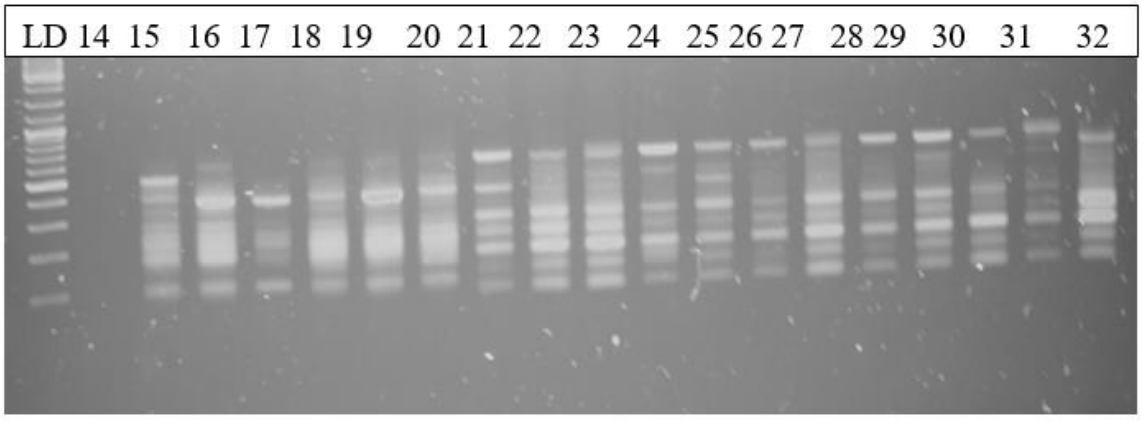

Plate 2: Primer OP-4pattern 14-20 (wesafu), 21-30 (*T. guineensis),* 31-32 (*Sarotherodon melanotheron), LD (Ladder*

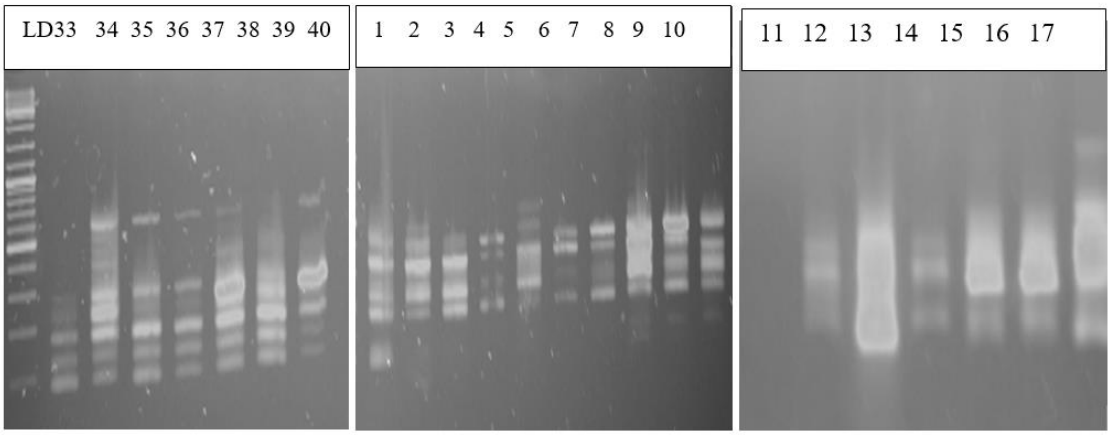

Plate 3: OP 4, 33-39 Primer OPI-16, 1-10 (*T. mariae) OPI 16,* 11-17 (Wesafu) (*S. manelotheron), LD (Ladder)*

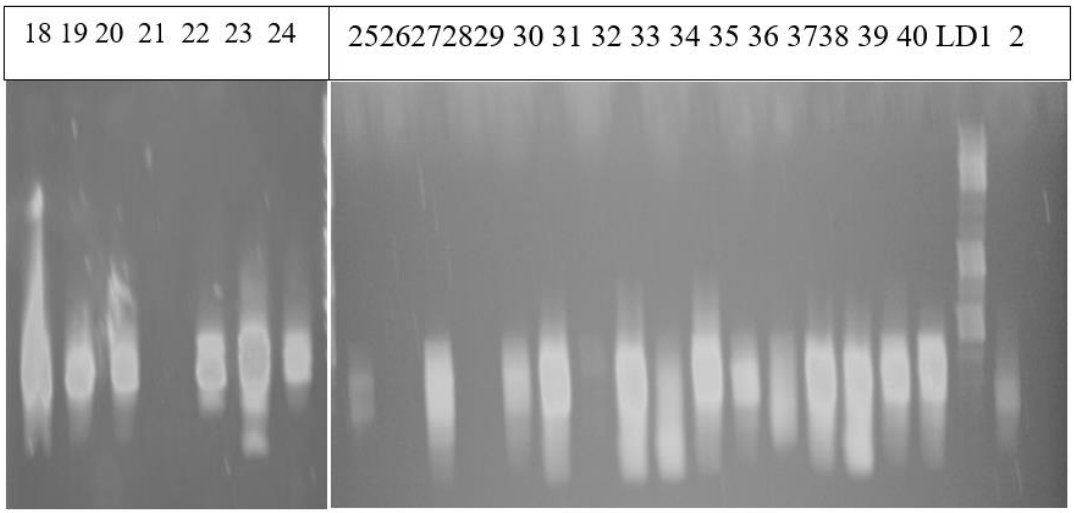

Plate 4: Primer OPI -16: 18-20 (Wesafu), 21-24 (*T. guineensis)* 25-30 (*T. guineensis),* 31-40 (*S. melanotheron*), OPAC 20, Line 1-2 (*T. mariae). LD (Ladder)*

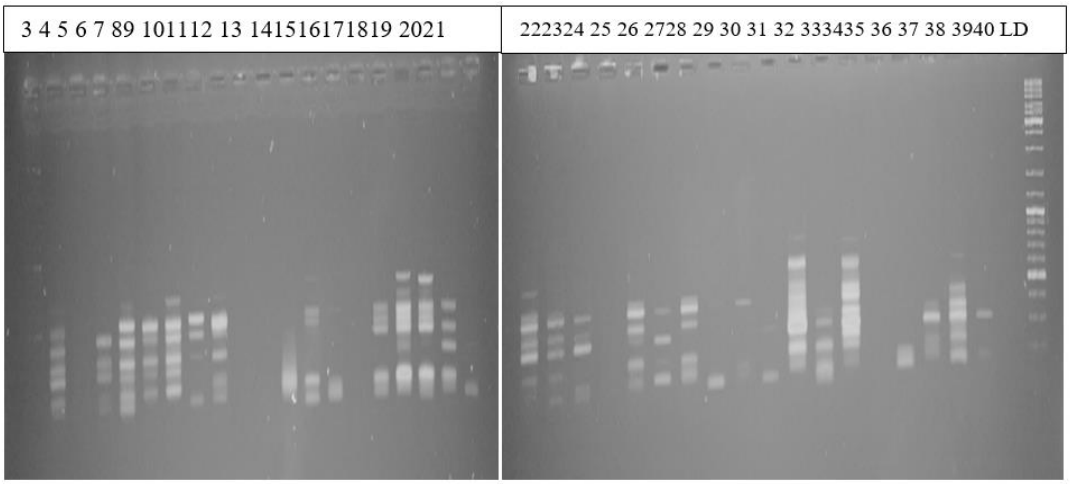

Plate 5: Primer OPAC- 20:3-10 (*Tilapia mariae),* 11-20 ('Wesafu,'), 21-30*T. guineensis*

Dendrogram tree demonstrating the relationship among the four species are shown in Figure 1 which was detected by the three RAPD markers. The results showed two main groups, one group for *T. guineensis* and the other for 'Wesafu', *T. mariae* and *S. melanotheron.*

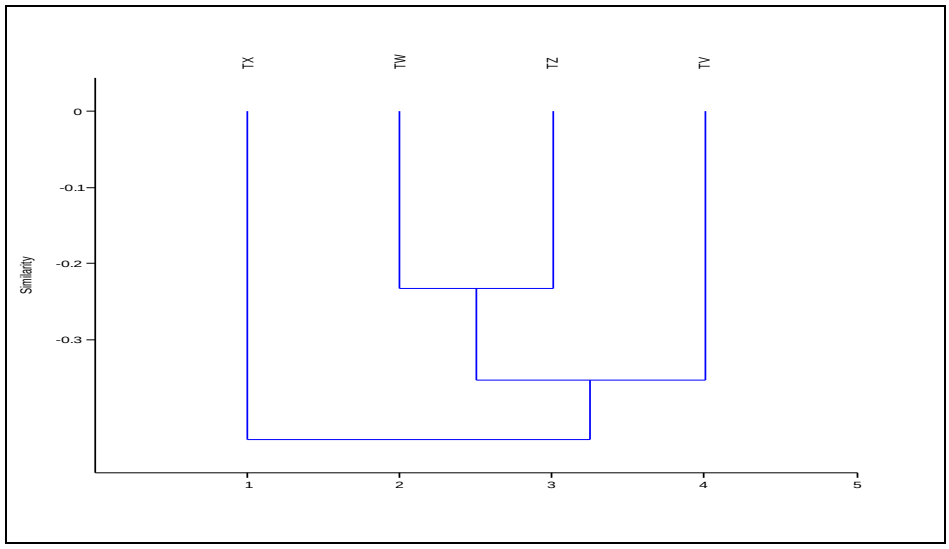

Fig 1: Phylogenetic relationship of the four tilapiine species in Epe Lagoon, Nigeria, where TV represent of *T. mariae*, TW, 'Wesafu', TX, *T. guineensis* and TZ, *S. melanotheron*

Collating the data of the three primers together, similarity coefficient was calculated. The highest value was obtained between *T. guineensis* and *T. mariae* (0.52992) and lowest between *T. mariae* and *S. melanotheron* (0.28925) as seen in table 1.

Table 1: Jukes and Kantors Similarity Distances using 3 primers

	TV	**TW**	**TX**	**TZ**
TV	0			
TW	0.43	0		
TX	**0.53**	0.38	0	
TZ	0.29	0.23	0.38	0

Where TV represents T. *mariae*, TW, 'Wesafu', TX, *T. guineensis* and TZ, *S. melanotheron*

Discussion

Data obtained from molecular analysis (RAPD-PCR) were used to detect the genetic polymorphisms among the tilapiine fishes found in Epe Lagoon. Genetic similarities among the species of cichlids were detected based on bands polymorphisms which were generated by random primers. The study showed that *Tilapia mariae* and *Tilapia guineensis* are genetically more similar than other cichlids in the study. This however is not strange considering the fact that they are from the same generic group. The similarity coefficient equally showed that wesafu is close to *Tilapia mariae* with similarity difference of 0.43. Furthermore, the phylogenetic relationship revealed high degree of similarity between wesafu and *Sarotherodon melanotheron* as they are seen to cluster together suggesting they may likely be from the same generic group. From the banding pattern, there was a great variability among the four species of cichlid. Even within the same species we observed some degree of polymorphism, showing that fish within the same species are not exactly similar. The genetic variability observed in the study can be used to improve production through Marker Assisted Selection (MAS) Although productivity of aquaculture operation can be improved substantially by better management and improved feeding utilization, the future demand for fish cannot be met with this improvement alone. Therefore, genetic variability will serve as an important feature of populations for both short and long term survival of the population. In addition, the genetic variation allows adaptation to changing environmental conditions.

Despite the fact that over 70 species of tilapia have been described, there is considerable argument over whether these species are truly separate species[4]. The taxonomic classification of tilapia is still confusing and has been subject to continuous changes. This is as a result of the similarities and overlap of their morphological characteristics and also partly due to the fact that many of the species hybridize freely in nature.

Conclusion

The result showed high level of variation among the various genera. Within the same genera, there was some degree of The result showed high level of variation among genera. Within the same genera, there was polymorphism, indicating that fish within the same species are not entirely similar. The result showed high level of variation among genera. Within the same genera, there was polymorphism, indicating that fish within the same species are not entirely similar. polymorphism, indicating that fish within the same species are not entirely similar. The result of the present study showed high level of similarity between wesafu and *Sarotherodon melanotheron*, suggesting wesafu might be a species from the genus Sarotherodon or a product of hybridization in the lagoon.

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