Genetic Differentiation Studies among Natural Populations of *Tilapia zillii*

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**Abstract**

The population structure of *Tilapia zillii* (Gervais 1848) from three reservoirs in Nigeria, Osun State (Opa, Osu and Igun) was determined by employing morphological and molecular (Random Amplified Polymorphic DNA) methods. For morphological studies, 25 morphometric measurements and six meristic counts were recorded on 40 individuals within each population. Principal Component Analysis (PCA) was performed on the morphometric and meristic data using the PAST software. For RAPD studies, genomic DNA was extracted from caudal fin tissue using CTAB method and five primers were used to initiate PCR amplifications. All the clusters produced by the Principal components analysis (PCA) of the morphometric and meristic parameters overlapped indicating a low level of genetic differentiation between the three populations of *T. zillii* studied. The UPGMA cluster diagram from RAPD analysis identified two major genotypic groups with inter and intra group relationships. All individuals in the first cluster were from the Osu reservoir, while individuals from Opa and Igun reservoirs constituted the second cluster. Nei’s unbiased measure of genetic distances was 0.8532, 0.7321 and 0.7111 for Osu, Igun and Opa populations respectively. This revealed that Opa and Igun populations were genetically closer, while Osu populations is distant from them. The results suggest that the RAPD technique could be used to differentiate populations of *T. zillii*. However, additional methods such as microsatellite and sequence analysis can be used to maximize the efficiency of genetic differentiation studies.

**Keywords:** genetic distance, morphometric, PCA, RAPD, UPGMA, variation

**Introduction**

*Tilapia zillii* (Gervais 1848) commonly referred to as red belly Tilapia is an important food fish and also one of the most aqua cultured fresh water fish (Adesulu and Sydenham, 2007). It is native to Africa, endemic to Nigeria and widely distributed in Nigerian waters (Agbabiaka, 2010). According to Adesulu and Sydenham (2007) it is the fourth most cultured *Tilapia* fish which has broadened its base from being a subsistence oriented technology to being a component of world commerce; as a high-quality fillet product of the fish has been exported to Europe and the US. It has been cultured intensively for more than four decades; however, its genetic resources have been poorly managed (Omitogun, 2005). Understanding the genetic diversity and differentiation within the species range would be of great importance for the sustainable utilization of the species as an aqua cultural candidate, the protection of the endangered populations and for bio geographical inferences.

In developing management and conservation strategies of any species, knowledge on the biology and population structure of that species is a prerequisite (Turan et al., 2006) and may be useful for studying short-term and environmentally induced variation. Knowledge of the population structure of *Cichlids* is economically important for several issues pertinent to fishery management and future development of aqua cultural strains. Genetic differentiation and level of gene flow are typically used to distinguish one population from another. Differences in morphology (morphometric and meristic study) can also be used, as they are vigorous tools for measuring discreetness of the same species and are also helpful for separating closely related genera, species and even populations within them (Cadrin, 2000).

Morphometric characters are generally being used in discriminating many fish species in several parts of the world (Gunawickrama, 2007; Murta, 2000), in Africa (Teugels, 1992; Hassanien et al., 2011) and also in Nigeria (Eyo 2002, 2003; Anyanwu and Ugwumba, 2003; Edema and Osagiede, 2011; Kuton and Adeniyi, 2014). However, morphological description alone has proved to be unreliable in establishing genetic relationships within and between species.

The ability to understand the genetic relationships within species at the molecular level has greatly increased through the application of Random Amplified Polymorphic DNA (RAPD) techniques (Sabir et al., 2012). This technique has also been successfully exploited for stock identification and population analysis in fish (Welsh and McCeland, 1990; Williams et al., 1990) as well as in differentiating individuals or breeding stocks with a given species or among different species. The advantage that RAPD technique has over other
systems of genetic documentation includes its use of universal sets of primers, no preliminary work such as probe isolation, filter preparation, or nucleotide sequencing is necessary and relatively low cost.

RAPD markers have been used to evaluate the genetic diversity in numerous organisms and on fish populations belonging to the same family or genus (Cooper, 2000; Ali et al., 2004; Usman et al., 2013; Megbowon and Fashina-Bombata, 2013; Asagbra et al., 2014) RAPD markers can be used for species and sex identification in animals (Appannavar et al., 2003; Huang et al., 2003).

In the present study, the genetic variation and relatedness between and among natural populations of *Tilapia zillii* from three different reservoirs in Osun State was determined using morphological and Random Amplified Polymorphic DNA (RAPD) analysis.

**Materials and Methods**

**Collection of samples**

Samples of *Tilapia zillii* were collected from Opa reservoir, Osu reservoir and Igun reservoir (all in Osun State, Nigeria) over a period of six (6) months (July-December 2013). The fishing methods employed for collecting the specimens were gillnetting and cast netting. All collected specimens were packed in polythene bags and plastic container and transported to the laboratory for further analyses. Fority *T. zillii* specimens from each reservoir were preserved in 10% formalin (e.g. 4% formaldehyde solution) for morphometric and meristic studies and fresh *T. zillii* samples were preserved in 95% ethanol for isolation of genomic DNA. Identification was done following identification keys prepared by Paugy et al. (2003) and Adesulu and Sydenham (2007).

**Description of study area**

Opa Reservoir is located at Ife central local government area of Osun State, Nigeria. It lies between longitudes 4º30’ to 4º40’ East of the Greenwich and latitudes 7º27’ to 7º35’ North of the Equator.

Osu reservoir is newly impounded in 1995, located at Osu, Atakumosa West Local government area of Osun State, Nigeria. It lies between longitude 004º38.32’ E to 004º38’49.1’ E and latitude 07º35.3’ N and 07º35’17.9’ N.

Igun reservoir is located in an abandoned gold mine area of Igun village in Atakumosa West Local Government area of Osun State, Nigeria. It extends over longitudes 4º30’ E to 4º45’ E and latitude 07º35’ N to 07º38’ N.

Osu and Igun reservoirs are closer geographically, as they belong to the same local government while Opa reservoir is distant from them. The geographical locations of the three reservoirs on the map of Osun State is shown in Fig. 1.

**Morphometric and meristic studies**

Twenty five measurements as in Dunz and Schliewen (2010) were recorded in 40 individuals within each population as shown in Fig. 2. Six meristic counts were also recorded as follows: number of scales on lateral line, dorsal spine, dorsal ray, gill rakers, anal fin ray and anal fin spine.

**Morphometric data analysis**

Measurements of each morphometric character were standardized to fish size (SL) in accordance with Reist (1985) to remove size-effect using percentage standard length as it follows: 

\[ Mn = \left( \frac{Mo}{SL} \right) \times 100 \]

where: \( Mo \) is the original measurement; and SL is the standard length.

The morphometric measurements were then transformed to common logarithms because linearity and normality are usually more closely approximated by logarithms than by original variables (Hair et al., 1998). Size-corrected data were analysed by multivariate method. Morphometric and meristic characters were analysed separately, since these variables are different both statistically (the former are continuous while the latter are discrete) and biologically (the latter are fixed.
early in development, while the former are more susceptible to the environment) (Allendorf et al., 1987). Principal component analysis (PCA) on morphometric and meristic data was performed using the software, PAST (Hammer et al., 2001). To find out the morphometric factors that can discriminate among the three populations, Principal Component Analysis (PCA) was used in which factor loadings based on eigens values were used to determine the morphometric factors.

**RAPD/PCR**

Total genomic DNA was extracted from caudal fin tissue (1 cm³) following standard CTAB method (Saghai et al., 1984). DNA concentration of all the samples was measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined. The quality of DNA was also detected by agarose gel electrophoresis. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source. The DNA extracts were subvisulated and photographed on UV light source. The DNA was then denatured at 95°C for 10 minutes. The DNA samples were then loaded into 1.5% agarose gel and run at 80 V for 2 hours. The integrity of the DNA was determined. The quality of DNA was determined. The quality of DNA was determined. The quality of DNA was determined. The quality of DNA was determined.

Results

The Principal Component Analysis (PCA) diagram of the 25 morphometric measurements of T. zillii from the three reservoirs of study is shown in Fig. 3, while the PCA diagram for the six meristic counts is shown in Fig. 5. All the clusters produced by the Principal Components Analysis (PCA) of the morphometric measurements overlapped. The character most responsible for variation among the three studied populations of T. zillii was lower jaw width (loading 0.6981) as shown in Fig. 4. The PCA diagram of the six meristic counts showed no specific pattern of differentiation.

**RAPD results**

All five primers employed in this study produced good RAPD fragments with varying bands. The bands ranged in molecular size from approximately 200 to 4500 bp. No artifacts were observed and the number of fragments amplified per primer varied. A total of 320 individual bands were obtained from the five primers, of which 243 bands were polymorphic and 77 bands were monomorphic (Table 2). Primer OPF-05 (250bp-2500bp) produced the highest number of fragments among the primers used, with an average of 141 bands, while primer OPF-04 produced the lowest number of fragments with an average of 18 bands. Result of the phenogram analysis for polymorphism in each primer is shown in Table 3. The total number of RAPD bands produced in Opa, Osu and Igun populations were 69,
The Principal Component Analyses (PCA) diagram of the six meristic counts of *T. zillii* from the three reservoirs of study showed homogeneity of characters (Fig. 3). The differentiation pattern shown by the meristic characters is not informative, as all the samples from the three reservoirs clustered together. Usually, morphometric characters reveal much greater differences among groups than the meristic. This reduced importance of the meristic differences is stressed by the lack of validation by the Principal Component Analysis. Morphometric characteristics showed considerably greater discriminatory power to distinguish the *T. zillii* populations than did the meristic characters.

Several authors have reported evidence of genetically determined morphometric and meristic characters: Carscadden and Leggett (1975), Ihssen *et al.* (1981b), Murta (2000). However, in several situations the patterns of differentiation shown by meristic characters have been considered worse than morphometrics for these purposes (Misra and Carscadden, 1987; Murta, 2000). The number of dorsal fin rays, dorsal fin spine, anal fin rays and anal fin spine from the three locations were constant, indicating identity in parental stock. The fairly constant values of fin rays observed in the three populations agree with the findings of Holden and Reed (1972) and Reed *et al.* (1967) that fin rays of the tribe *Tilapiini* do not vary much.

The morphometric results are insignificant to establish the genetic structure of the population which often leads to taxonomic uncertainty. This is consistent with the report of Daniel (1997), Ponnian and Gopalakrishnana (2000) and Garg *et al.* (2009b). RAPD method uncovered 30 polymorphic loci from five primers selected for population analysis. The result showed that RAPD primers were polymorphic and were able to detect private allele in the studied populations. This is consistent with the fact that the RAPD technique, being able to screen more easily a larger part of the nuclear genome than some other genetic markers (such as allozyme), may assess higher levels of genetic variation. Therefore, the maintenance of genetic variability is very important for the conservation of a species (Barroso *et al.*, 2005). The UPGMA cluster diagram of individual’s genotype from the three populations showed that *T. zillii* population from Opa and Igun reservoirs are more similar genetically than those of the Osu reservoir population.

Nei’s (1978) unbiased genetic distance values also showed that Opa and Igun reservoir populations were closer genetically, while Osu reservoir populations was distant from them. The geographically closest populations (Igun and Osu as shown in Fig. 1) were not the most genetically similar as we had predicted. This is consistent with the findings of Szitenberg *et al.* (2012) in Israel. They reported that the population structure of *T. zillii* did not match the geography of the Israeli water-basins, based either on molecular or morphological data collected. In additional to geographical distance, other factors influencing the genetic relatedness of populations include anthropogenic and environmental factors (Wright, 1993). The principal components analysis (PCA) diagram based on six meristic counts of *T. zillii* from Opa Reservoir, (red) Igun Reservoir, (purple) and Osu Reservoir (blue) showing homogeneity of characters.
Extrinsic factors, such as habitat heterogeneity, climatic and geological events also shape the large-scale population differentiation pattern. The environmental conditions of Igun and Opa reservoirs are similar, as both populations have suffered severe bottleneck events due to heavy pollution for the past few decades. Reports show the presence of high concentration of heavy metals in Igun reservoir as a result of the mining activities going on around it (Aderinto, 2013) and studies also revealed that Opa reservoir is heavily polluted in terms of heavy metal contamination (Adesakin, 2013). Assessment of heavy metals contamination in Osu

Table 1. List of the used primers and their sequences

<table>
<thead>
<tr>
<th>#</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPAE 05</td>
<td>CCTGTCAGTG</td>
<td>250bp-2500bp</td>
</tr>
<tr>
<td>2</td>
<td>OPAF 09</td>
<td>CCCCTCAGAA</td>
<td>200bp-4500bp</td>
</tr>
<tr>
<td>3</td>
<td>OPAF 07</td>
<td>GGAAGCGGTC</td>
<td>200bp-3500bp</td>
</tr>
<tr>
<td>4</td>
<td>OPAD 09</td>
<td>TCGCTTCTCC</td>
<td>200bp-2500bp</td>
</tr>
<tr>
<td>5</td>
<td>OPAE 04</td>
<td>CCAGCACCCTC</td>
<td>200bp-2500bp</td>
</tr>
</tbody>
</table>

Table 2. Total bands scored by the primers and their percentage polymorphism

<table>
<thead>
<tr>
<th>Primers</th>
<th>Total bands</th>
<th>Monomorphic bands</th>
<th>Polymorphic bands</th>
<th>% polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPAE 05</td>
<td>141</td>
<td>35</td>
<td>106</td>
<td>75</td>
</tr>
<tr>
<td>OPAF 09</td>
<td>52</td>
<td>13</td>
<td>39</td>
<td>75</td>
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<td>OPAF 07</td>
<td>63</td>
<td>14</td>
<td>49</td>
<td>78</td>
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<tr>
<td>OPAD 09</td>
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<td>6</td>
<td>40</td>
<td>88</td>
</tr>
<tr>
<td>OPAE 04</td>
<td>18</td>
<td>9</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>TOTAL</td>
<td>320</td>
<td>77</td>
<td>243</td>
<td>76</td>
</tr>
</tbody>
</table>

Table 3. Total bands scored in each population

<table>
<thead>
<tr>
<th>Population</th>
<th>Total bands scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opa</td>
<td>69</td>
</tr>
<tr>
<td>Osu</td>
<td>157</td>
</tr>
<tr>
<td>Igun</td>
<td>94</td>
</tr>
<tr>
<td>Combined population</td>
<td>320</td>
</tr>
</tbody>
</table>

Table 4. Pair wise comparison of Nei’s genetic identity of the three populations of *T. zillii* studied with genetic distance (below diagonal) and index of similarity (above diagonal)

<table>
<thead>
<tr>
<th>Population</th>
<th>Opa</th>
<th>Osu</th>
<th>Igun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opa</td>
<td>****</td>
<td>0.15</td>
<td>0.29</td>
</tr>
<tr>
<td>Osu</td>
<td>0.85</td>
<td>****</td>
<td>0.27</td>
</tr>
<tr>
<td>Igun</td>
<td>0.71</td>
<td>0.73</td>
<td>****</td>
</tr>
</tbody>
</table>

Fig. 6. UPGMA dendrogram, summarizing the data on differentiation between *Tilapia zillii* populations, according to RAPD analysis. Samples 1-7 is from Opa Reservoir, samples 8-14 is from Osu Reservoir and samples 15-21 is from Igun Reservoir.
reservoir has shown that the concentration of heavy metals in the reservoir are within permissible limits (Obayemi, 2013).

Genetic drift and natural selection are two main evolutionary mechanisms that cause population differentiation (Hufford and Mazer, 2003). Natural selection by ecological factors leads to development of ecological adaptation or ecotypes. However, there is need to determine whether the observed population differentiation pattern in this study resulted from any natural selection.

Conclusions

The present investigation contributes to the knowledge on morphological and genetic variation of T. zillii species. The comparative results from both morphological and genetic analysis revealed a reasonable degree of variation in populations of T. zillii from Opa, Osu and Igun reservoirs in Osun state. The findings in this study would therefore have wide application in utilization and management of genetic resources of T. zillii species. Further studies involving large number of samples and primers need to be conducted to get more precise information about the genetic structure of the three reservoir stocks of T. zillii. In future, additional methods such as microsatellite and sequence analysis can be used to maximize the efficiency of genetic differentiation studies.

References


Obayemi OE (2013). Preliminary study of heavy metal concentration in the gills, fillet and liver of Parachanna obscura (Gunther, 1861) in Osu Reservoir in Atakummosa West Local Government, Osun State, Nigeria. BSc Thesis. Obafemi Awolowo University.


