Genetic Characterization of Four Strains of *Oreochromis Niloticus* Using the Rapidly Amplified Polymorphic DNA (Rapd).

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Abstract

The genetic characterization of four strains of Oreochromis niloticus was carried out using Randomly Amplified Polymorphic DNA (RAPD). Three samples each of four strains of grown O. niloticus in Nigeria, viz; local mixed sex strain, commercially available mixed sex strains from China and a farm in Ibadan, and hormonal sex reversed all male population were used. Genomic DNA was isolated from the flesh tissues of the strains of O. niloticus using the Cetyl Trimethyl Ammonium Bromide (CTAB) method. The banding pattern was transformed into numerical values and statistical analysis was done using Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) to calculate the Nei's similarity coefficients. Canonical classification was also carried out.Out of the twenty five RAPD markers used, 10 were informative on agarose gel, of with 58% polymorphisms. A total of seventy seven bands were amplified. From the cluster analyses, the local strains were shown to be genetically distinct but the other 3 showed various levels of similarities. The Nei's genetic distance among the populations was 47%. Apart from the local strain, others showed greater similarities with variability of 37% among themselves. Within strain diversity were 15%, 32%, 25% and 33% for the local strain, tilapia from commercial farm, China strain and the hormonal sex reversed respectively. The distinctness of the local strain and the narrow variability within its membership showed that the population is not as genetically divers as the other three strains. The future of tilapia stock improvement will rely on appropriate stock choice, development of sound management techniques and selective breeding.

Keywords: Genetic characterization, RAPD, variability, Genomic DNA, *O. niloticus*

INTRODUCTION

Tilapia is now the second most cultured group species after the carps (FAO, 2008). Their favourable attributes include: high tolerance of poor water quality and crowding, good performance on commercial catfish feed, high degree of disease resistance, and mild flavoured, white flesh. Large-scale commercial culture of tilapia is limited almost exclusively to the culture of three species: *Oreochromis niloticus, O. aureus, and O. mossambica*. Of the three species, the Nile tilapia, *O. niloticus*, is by far the most commonly cultured species. Tilapias are yet to reach their full aquaculture potential because of the problems of precocious maturity and uncontrolled reproduction, which often results in the overpopulation of production ponds with stunted fish. Several approaches have been developed to tackle the problem of tilapia. One of such is population control using methods such as monosex culture, sex reversal by androgenic hormones, cage culture, tank culture, the use of predators, high density stocking, sterilization, intermittent/selective harvesting, and the use of slow maturing tilapia species, among others, as have been reviewed by Mair and Little (1991) and Fagbenro, (2002). There is however dearth of information on genetic diversity characterization of local tilapia strains in Nigeria. However, In order to improve the aquaculture fish production in Nigeria, it is imperative that the stocks to be cultured are characterized by the use of molecular genetic markers. The knowledge of genetic structure of populations is also essential for the implementation of management programmes (SoleCava, 2001). This research is aimed to shed light on the genetic diversity of four strains of farmed Nile tilapia in Nigeria.

MATERIALS AND METHOD

Site

The experiment was conducted at the University of Ibadan fish farm.

Experimental fish

The Four strains of *Oreochromisniloticus* used for the genetic characterization were obtained from three sources. The first strain was the indigenous tilapia population from the University of Ibadan fish farm. This population has never been improved genetically through any conscious efforts of man. The second strain was a mixed sex populations from a commercial fish farm in Ibadan. These breeds have been improved upon genetically. The third strain was a mixed sex tilapia population from China. This strain has also been genetically improved and the seeds imported from China. The fourth strain was hormonal sex reversed all male populations from a reputable hatchery in Lagos.

Sample preparation

Three replicates of each fish populations were taken. This made a total of twelve specimens. Scales were removed and discarded from each fish with individual new razor blades while flesh parts were put in labeled sample bottles for immediate refrigeration. The labeling was done as follows: Local strain's samples 1.1, 1. 2, and 1.3; the mixed sex populations from a commercial farm in Ibadan farm were 2.1, 2.2 and 2.3; the mixed tilapia populations from China were 3.1, 3.2 and 3.3 while the hormonal sex reversed all male tilapia were 4.1, 4.2 and 4.3.

Genomic DNA extraction

The genomic DNA of fish tissues were isolated using CTAB method, Stewart and Nura (1993).

Statistical Analysis

The banding pattern was transformed into numerical values, where the presence of a band was scored as 1 and absence of a band was scored as 0. The binary value was transferred for analysis using UPGMA method Rohlf *et al*., (2000). The cluster analyses were performed for the molecular data by Un-weighted Pair-Group Method Using Arithmetic Averages (UPGMA) (Sneath and Sokal, 1973). The dendrogram was generated with the SAHN subroutine of NTSYS-PC to yield the similarity coefficient between the genotypes (Rohlf, 1993). The data was also run on differential function analyses for canonical discrimination.

RESULTS

RAPD markers

Out of 25 markers used, 10 RAPD primers were informative and 9 SSR primers were not informative while 6 primer pairs gave no amplification. Seventy-seven alleles were generated from the markers. Nineteen of the alleles generated were monomorphic while 58 were polymorphic. Table 1 gives the names of the primers, the sequence and the fragment sizes obtained from each primer used they generally ranged from150bp to 5000bp lower and upper range respectively.

Table 1: List of the primers used and their sequences

S/N	Primer Name	Sequence	Fragment size
1	OPAD 09	TCGCTTCTCC	200bp - 2500bp
2	OPC 09	CTCACCGTCC	200bp - 3500bp
3	OPAE 04	CCAGCACTTC	200bp - 2500bp
4	OPAE 05	CCTGTCAGTG	250bp - 2500bp
5	OPAE 09	TGCCACGAGG	250bp - 3000bp
6	OPAF 07	GGAAAGCGTC	250bp - 3500bp
7	OPAF 08	CTCTGCCTGA	250bp - 3500bp
8	OPAF 09	CCCCTCAGAA	200bp - 4500bp
9	OPAF 11	ACTGGGCCTC	200bp - 3500bp
10	OPAF 12	GACGCAGCTT	200bp - 3000bp

Dendrogram showing diversity coefficients within and among strains

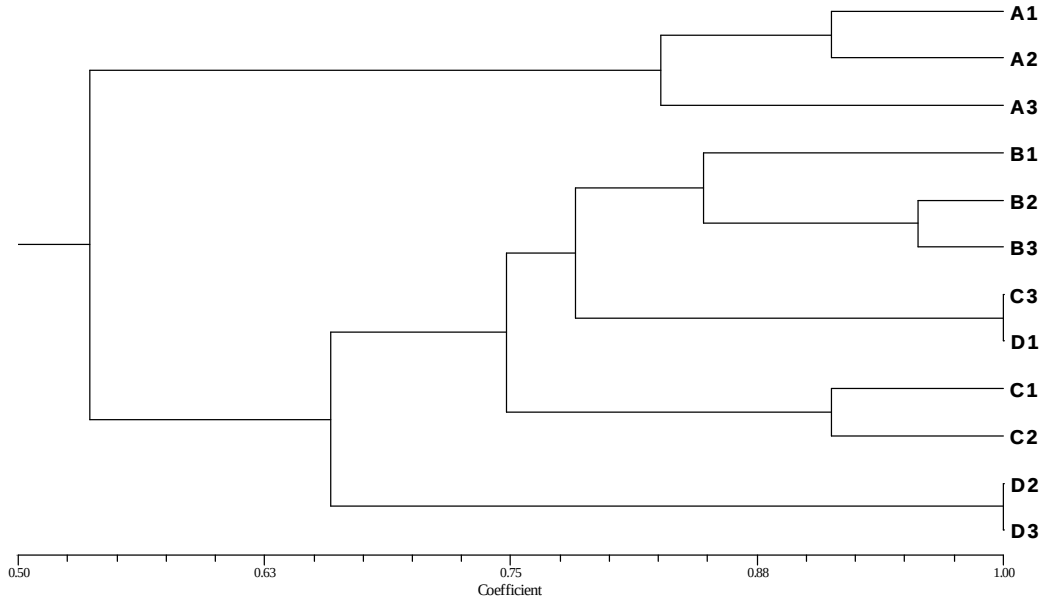

Figure 1: Dendogram for fish samples

Key:
A1-A3 - The local strain
B1-B3 - The mixed sex from commercial farm
C1-C3 - The mixed sex from china
D1-D3 - The hormonal sex reversed

The result of the hormonal sex reversed shows overall diversity of 0.67 to 1.00. This gives respective similarity coefficients of 0.15, 0.31, 0.25 and 0.33.
These values were the calculated Nei's genetic similarity matric of the data scoring for the electrophoresis gel given in table 2 below.

Similarity coefficient and Darwin trees

This showed the actual similarity values among the various individual in the experiment. It further illustrates the dendogram.With this table the similarities between individuals across the populations is seen. This is given in Table 3 below. The Darwin trees further groups the individual across the strains but this time from a defined root. Fig.1 and 2 illustrate this.

Differential Function Analyses

The analyses for the Eigen values and the Wilk's Lamda (Tables 4 and 5), which were run to know if the populations were of the same base species or not showed no significant difference. This means that the populations were same species and not differentiated enough to form sub species

Table 2: Data scoring for electrophoresis gel

		Sample											
		1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3
	cc1	1	1	1	1	1	1	1	1	1	1	0	0
	cc2	1	1	1	1	1	1	1	1	1	1	0	0
	cc3	1	1	1	1	1	1	1	1	1	1	1	1
	cc4	1	1	0	0	0	0	0	0	0	0	0	0
	cc5	0	0	0	0	0	0	1	1	0	0	0	0
	cc6	1	1	1	1	1	1	1	1	1	1		1
	cc7	0	0	1	1	1	1	0	0	1	1	0	0
	cc8	0	1	1	1	1	1	0	0	1	1	0	0
	cc9	0	0	0	0	1	1	1	1	1	1	0	0
	cc10	0	0	0	1	1	1	1	1	1	1	0	0
	cc11	0	0	0	0	0	0	0	0	1	1	0	0
	cc12	0	0	0	0	0	1	1	1	1	1	1	1
L													
	cc13	1	1	1	1	1	1	1	1	1	1	1	1
o	cc14	0	0	0	1	1	1	1	1	1	1	1	1
c	cc15	1	1	1	1	1	1	1	1	1	1	1	1
i	cc16	1	0	1	0	0	0	0	0	0	0	0	0
	cc17	0	0	0	1	1	1	1	1	1	1	1	1
	cc18	0	0	0	0	1	1	0	1	0	0	0	0
	cc19	0	0	0	1	1	1	1	1	0	0	0	0
	cc20	1	1	1	1	0	0	0	1	0	0	0	0
	cc21	0	0	0	0	0	0	0	0	1	1	0	0
	cc22	0	0	1	0	0	0	0	0	0	0	0	0
	cc23	1	1	1	0	0	0	0	0	0	0	0	0

Table 3: Similarities coefficient of fish DNA samples

1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3
1.0000000											
0.9130435	1.0000000										
0.8260870	0.8260870	1.0000000									
0.6086957	0.6956522	0.6956522	1.0000000								
0.4782609	0.5652174	0.5652174	0.8695652	1.0000000							
0.4347826	0.5217391	0.5217391	0.8260870	0.9565217	1.0000000						
0.5217391	0.5217391	0.4347826	0.7391304	0.7826087	0.8260870	1.0000000					
0.5217391	0.5217391	0.4347826	0.7391304	0.7826087	0.8260870	0.9130435	1.0000000				
0.4347826	0.5217391	0.5217391	0.7391304	0.7826087	0.8260870	0.7391304	0.6521739	1.0000000			
0.4347826	0.5217391	0.5217391	0.7391304	0.7826087	0.8260870	0.7391304	0.6521739	1.0000000	1.0000000		
0.6086957	0.6086957	0.5217391	0.6521739	0.6086957	0.6521739	0.7391304	0.6521739	0.6521739	0.6521739	1.0000000	
0.6086957	0.6086957	0.5217391	0.6521739	0.6086957	0.6521739	0.7391304	0.6521739	0.6521739	0.6521739	1.0000000	1.0000000

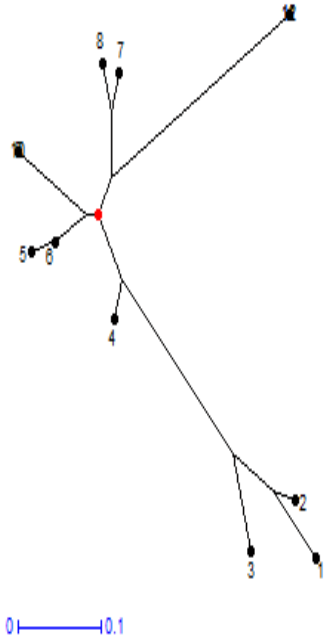

Figure 1: Darwin's radii graph

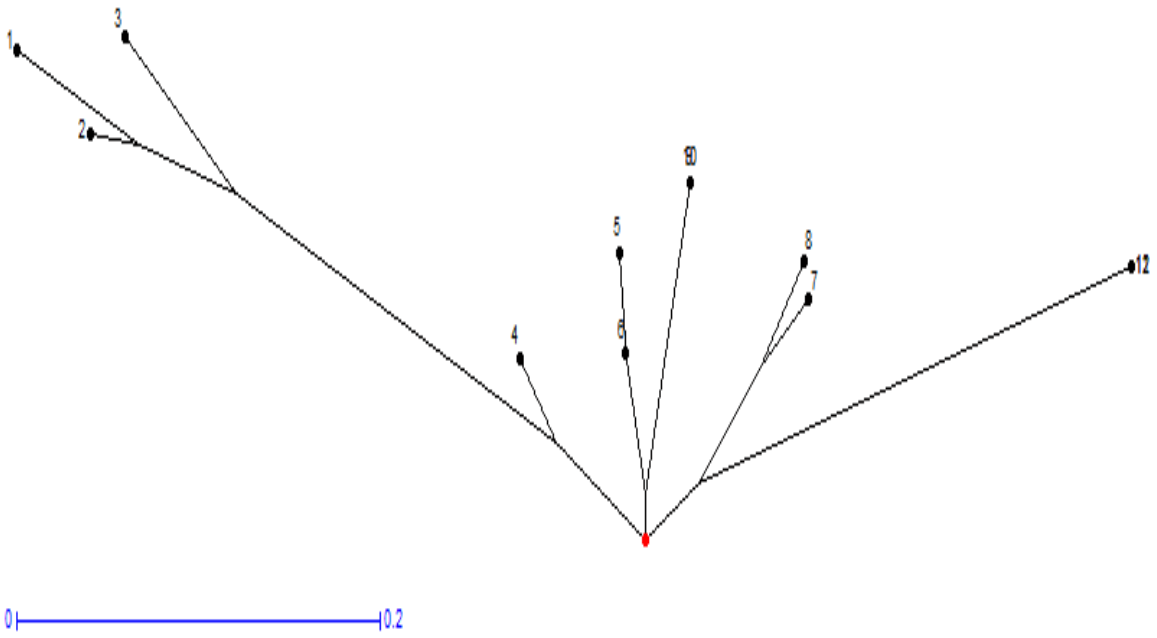

Figure 2: Darwin's hierarchy

Table 4: Eigen values showing Canonical Correlation

Function	Eigen value	% of variance	cummulative %	Canonical Correlation
1	11.4759(a)	76.5	76.5	0.959
2	2.000(a)	13.3	89.8	0.816
3	1.525(a)	10.2	100	0.777

@ 0.95 degree of variation: No significant difference

Table 5: Wilks' Lambda diversity within strains

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	.011	25.017	21	.246
2 through 3	.132	11.137	12	.517
3	.396	5.094	5	.404

DISCUSSION

Inter strain genetic diversity

The study demonstrated that multiple genotypes were found among the studied strains of *O. niloticus*. The distinctness and poor variability within the members of the local strain may be an indication of poor gene flow. This is an indication of a wide genetic distance between the various strains. Among the other three, the degree of variability is high enough to suggest that there is gene flow within these populations. In this situation gene flow is defined as the exchange of genetic traits between populations through movement of individuals (Stiling, 1996). From the analyses done, of the three strains with higher levels of similarities, the china tilapia and the hormonal sex reverse exhibited the most genetic similarities across the strains. However, the Differential Function Analyses done to know if the strains were sufficiently differentiated into subspecies, the Eigen values and Wilk's Lambda shows no significant differences at 0.95 levels of significant (tables 4 and 5). This showed that the strains were not subspecies but a single species of fish.

Genetic diversity within strain

The coefficient of variation within the local strain showed low diversity, with diversity index of 15. This is quite a very narrow range that may not portend too much prospect for the species. This is because this result shows low level of genetic variability within the stock and according to Sousa *etal*., (2011); genetic variability in a population is important for biodiversity because without variability, it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction. This may in the least reduce vigour in the population since they keep breeding among themselves. The intra strain diversity indices of the mixed sex from Durante, the China group and the hormonal sex reversed which indices of .31, 0.25, and 0.33 respectively showed marked increase in genetic variability over the local strain. This higher genetic mixture within those populations maybe responsible for the observed improvement in performance in terms of faster growth rate, more efficient feed utilization, better fillet quality, more attractive colouration among other desirable qualities. Since heterozygosity is reduced in inbred or local populations, the large variability of these populations must be representative of populations that is exhibiting gene flow (Stiling 1996). The distinctness of the local strain and the various levels of similarities shown by the rest may also have to do with their geographical history. The distribution of species or populations and their genetic structure depends not only on biological and environmental but also on historical factors (Agnèse *etal* 1997), these climatic changes (Maley, 1991) could explain the genetic structure of some fish populations in West Africa (Adépo-Gourène et al., 1997).

Implications for future breeding and stock management programmes

The study revealed a low level of genetic diversity among the local cultured strain on the university of Ibadan fish farm. This may not have been the case when they were first introduced to the farm decades ago. Continuous used of same or few parent stocks can deplete genetic variability within any cultured stock. This has been studied by various researchers and found to be of significant effect on productivity and sustainability. Pullin and Capili, (1988) discovered that despite many positive culture attributes, many Asian tilapiine stocks (in particular O. niloticus) are characterized by high

levels of inbreeding and have experienced declines in levels of genetic diversity resulting from the use of small founder populations. Widespread introgression of O. mossambicus alleles into some cultured stocks (particularly O.niloticus) and poor stock management practices have also been highlighted as potential causes for reductions in performance of some stocks in culture (Hulata et al. 1986, Guerrero and Tayamen 1988, Appleyard and Mather 2002.).

CONCLUSION

In animal breeding, it is expected that a cross between two animals originating from two populations with a large genetic distance between them will result in maximum heterosis or hybrid vigour. Then the exchange of genes between populations determines the relative effects of selection and genetic drift (Balloux and Lugon-Moulin, 2002). Genetic variation is the raw material in a species and populations, which enables them to adapt to changes in their environment. Through selective breeding by generation and generation, some economic traits could be improved and stabilized, and as a result, new strains could be created. In history, many good breed were generated by selective breeding (Lou 1999; Hines 1976). The improving of traits is contributed by the natural factors and human factors that may induce mutations, but the selection itself will not create new genes. Selection may change the allele frequencies, which may cause the changes of traits. Finally, some favourable traits could be accumulated and strengthened (Li *etal*., 2008). The implication of this is that the local strain should not be used alone for breeding purposes but careful selection should be done with other strains or populations to improve desirable qualities.

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