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(RESEARCH ARTICLE)

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Genetic differentiaton of populations of *Oreochromis niloticus* in Nigeria

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Abstract

The differentiations in the genomes of selected cultured and wild populations of Oreochromis niloticus from New Bussa and Ikorodu were assessed in this study. Twenty specimens of O. niloticus were obtained from Kainji Lake and Usman farms at New Bussa and also Majidun River and Toga farms in Ikorodu. DNA was obtained from clipped fins using CTAB based procedure with the purified DNA being analysed in a multigene gradient thermocycler using 8 microsatellite markers. The amplicons generated were subjected to fluorescent genotyping and the corresponding allele scored. The data obtained was used to determine genetic parameters such as inbreeding coefficient, observed and expected heterozygosities, allelic richness, effective number of allele and Nei genetic distance. The average expected heterozygosities for Kainji Lake (CAPK) was 0.7488 while that of Usman farms (CUPN) was 0.7043 in New Bussa. Also, the average expected heterozygosities for Majidun River (CAPI) was 0.5953 and that of Toga farms (CUPT) was 0.4533 respectively. On the average, three populations (CAPK, CUPN and CAPI) were inbred while one population (CUPT) was outbred. The analysis of genetic distance among the populations showed two major clusters; CAPK and CUPN and then CAPI and CUPT with CAPK and CUPN being significantly differentiated However CAPK and CAPI largely overlap while CUPN and CUPT are differentiated.

Keywords: Differentiation; DNA; Genomes; Microsatellites

1. Introduction

Broodstock productivity is reported to represent the most significant constraint on commercial tilapia production. This has been confirmed locally (Anyanwu, *et al*., 1991) and globally (Rana, 1997). Locally, there is a heavy dependence on fry and fingerlings from the wild, and fish farmers are usually confronted with the problem of identification of good strains of tilapia "seeds" for cultivation. Improvement in the methodologies of screening of cultivar stocks is clearly needed, given the increased interest in fish farming and its potential contribution to the food fish supply in Nigeria. Better understanding of the factor modifying broodstock output is thus of great significance to the added growth of tilapia culture.

This study is therefore aimed at contrasting the levels of heterozygosity and hence the genetic distances of selected natural and cultured populations of Nile Tilapia in order to assess the usage of modern population genetic tools in aquaculture practices being employed (especially in the broodstock selection) on the cultured populations.

Broodstocks or base populations used for artificial propagation in Nigerian fish farms are obtained from sources that have little or no genetic databank. Such genetic information are needed for an assessment of the suitability of the broodstocks for sustainable aquaculture, hence this study.

The specific objectives of this study are to:

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- Determine the level of genetic differentiation among the populations; and
- Contrast the expected genetic diversity in the populations;
- Contrast the proportions of the microsatellite loci that are heterozygous in the populations;
- Contrast the proportions of the microsatellite loci expected to be heterozygous under hardy weinberg equilibrium in the populations;

1.1. Genetic differentiation in populations

An assessment of the genetic differentiation among related populations is essential to determining the extent of gene flow and genetic drift between such populations. With the goal of cultured populations being bridging the gap between supply from natural captured populations and the demand for the fish resources and for its sustainable development, it is imperative to be able to determine the extent of genetic differentiation thus determining the managerial measures and to what extent it is needed in such populations.

There have been several studies of genetic differentiation among cultured populations and their source captured populations. For example, El-Zaeem *et al.* (2012) found the wild populations of *O. niloticus* to be highly differentiated from the cultured populations in Egypt. In other studiesRodriguez *et al.* (2008) compared wild boar and domestic pig populations and found the former to possess more genetic diversity than the latter. Similarly, Hara and Sekino (2001), Sekino*et al.* (2002), Li *et al.* (2004), Li *et al.* (2009) and Swart (2012) found significant differentiation between the cultured populations and wild populations of the Pacific abalone, the Japanese flounder, the Pacific abalone, Chinese pearl mussel and the abalone, *Haliotis midae* respectively. Swart (2012) opined the reason for this to be the differing selective pressures in the two environments while Lundrigan *et al.* (2005) showed that there were differentiation between the cultured and the wild populations in the study on North American Arctic Char but there were differentiation in all the populations based on their geographic differences as well with each aquaculture and wild population in each location clustering together. However, in Nigeria, Usman *et al.* (2013) attempted a molecular characterization of *Tilapia guineensis* and *Sarotherodonmelanotheron* from wild and cultured populations showing 82% and 88% similarities respectively in these differentially produced populations of the two species.

2. Methodology

Twenty *Oreochromis niloticus* specimens were collected from both wild and cultured sources of two locations (New Bussa (Niger State) and Ikorodu (Lagos State)) for the study. The fish samples from the wild were bought from landings of artisanal fishermen while the cultured samples were obtained from the fish farms described above. Caudal fin clips of the samples were harvested and kept in 95% ethanol. They were them transferred to a refridgerator in the fish culture laboratory of the Department of Zoology, Obafemi Awolowo University, Ile- Ife, Nigeria set to a temperature of 4^{o}C until DNA extraction.

DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen, 2006). The microsatellites primers used in this study were selected based on the level of polymorphism and excellent amplification obtained from previous studies (Kunkle et al, 2010).The microsatellite loci used were GM 166, GM201, GM284, GM385, UNH 919, UNH 934, UNH 937, UNH 974 (Kunkle et al., 2010). Electrophoresis and genotyping were carried out using ABI Genetic Analyzer. The data obtained was used to determine genetic variation parameters that include observed Heterozygousity (H_o), expected heterozygosity (H_e), allelic richness (A_e), effective number of alleles (N_e), inbreeding coefficient (F_{IS}) and Nei's genetic distance. The genetic distance and the corresponding dendrogram were computed using the Fstat Software Version 2.9.3 (Goudet, 2002). Other parameters were determined using Popgen version 1.31 (Yeh et al., 1999)

3. Results

Table 1 Mean observed and expected heterozygousities, observed and effective number of alleles and inbreeding coefficient of the populations across all the loci

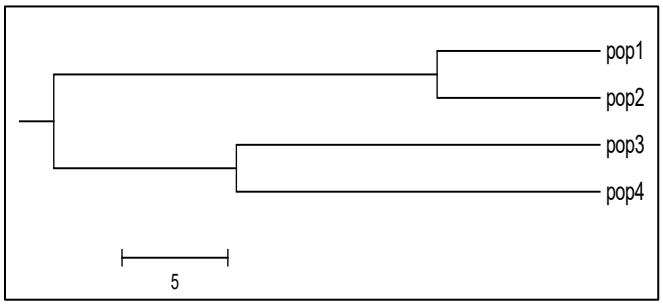
	Captured New Bussa	**Cultured New Bussa**	**Captured Ikorodu**	**Cultured Ikorodu**
N	15	13	9	14
Ho	0.6004	0.6042	0.4125	0.5179
He	0.7488	0.7043	0.5954	0.4533
Na	5.5000	4.7500	3.2500	2.7500

Ne	3.4872	3.5640	2.4519	2.0878
FIS	0.2180	0.1620	0.3330	-0.1560

N= Sample size, Ho= Observed heterozygousities, He = Expected heterozygousities, N_a=Allelic richness, N_e= Effective number of alleles and F_{IS}= Inbreeding coefficient

3.1. Measure of genetic differentiation (inter population genetic variation)

The level of population differentiation is indicated by means of the dendrogram in Figure 1. The four populations clustered into two major clades; the New Bussa populations and the Ikorodu populations.



Where pop1 = Captured New Bussa, pop2 = Cultured New Bussa, pop3 = Captured Ikorodu and pop4 = Cultured Ikorodu

Figure 1 Dendrogram (with genetic distance) of inter-population differentiation using molecular characters

4. Discussion

4.1. Heterozygosity, inbreeding and genetic differentiation

Genetic diversity is one of the most important attributes of any population. Environments are constantly changing, and genetic diversity is necessary if populations are to evolve continuously and adapt to new situations. Low genetic diversity typically leads to increased levels of inbreeding, which can reduce the fitness of individuals and populations. An assessment of genetic diversity is therefore central to population genetics and has extremely important applications. The populations studied showed a pattern of distribution of level of microsatellite heterozygosity where the northernmost samples of New Bussa had higher heterozygosity while the southernmost samples of Ikorodu showed reduced values. This same pattern is depicted in the values obtained for allelic richness and effective number of allele.

Freshwater habitats are known to exhibit higher level of genetic differentiation because of restrictions to gene flow among various sub-populations. The populations assessed in this study are miles apart and hence, are not linked for any genetic exchanges to take place. With such isolation, each population is subjected to different levels of anthropogenic impact and evolutionary forces. Genetic drift for example, changes a population's allele frequency from generation to generation. Genetic drift will eventually drive each allele to either fixation or extinction within a relatively short period of time, and therefore its overall effect is to decrease genetic diversity. Natural selection may or may not fix an allele while inbreeding will increase the proportion of homozygosity in the population.

On the other hand, the cultured populations generally show a lower value compared to the wild populations. This pattern has also been previously reported in a number of studies (Cross and King, 1983; Verspoor, 1988; Evans et al, 2004 and Li et al, 2004). However, in other studies, no significant differences in genetic diversity have been observed between wild and hatchery populations (Gosling, 1982; Dillon and Manzi, 1987). Hedgecock and Sly (1990) pointed out that heterozygosity is insensitive to substantial genetic changes that may occur in cultivated aquaculture stocks within the first several generations of isolation suggesting that loss of alleles is probably a more meaningful measure of how well genetic variation is being maintained in a hatchery stock. Genetic drift in allele frequencies and loss of rare alleles without significant reduction in heterozygosity has been observed in nursery stocks of hard clams with small breeding population sizes (Dillon and Manzi, 1987, Mgaya et al, 1995. According to Mgaya *et al* (1995), genetic differences between wild and hatchery stock can be attributed to a number of factors, including differential survival of larvae under hatchery conditions, degree of inbreeding, and genetic drift, the magnitude of which is related to the effective size of the breeding population.

Furthermore, the cultured populations from Ikorodu have the lowest value of expected heterozygosity despite having the lowest value of inbreeding; that is; though mating was random, the level of heterozygosity obtained was low. This

suggest a low or reduced population size; that is; the size of the base population of the cultured populations from Ikorodu is small (founder effect). When population size is reduced allelic diversity usually decreases. This is often associated with a drop in expected heterozygosity because fewer alleles typically lead to reduced expectations of heterozygosity under HWE. At the same time, observed heterozygosity values may not deplete and in fact there may be a temporary increase in observed heterozygosity compared with expectations under HWE. In some cases, observed heterozygosity excess can be a useful indicator of past bottlenecks, although this test requires a large number of polymorphic loci and can detect only relatively recent bottlenecks (Luikart and Cornuet, 1998). On the other hand, the high level of inbreeding in the wild populations may be because of the reproductive behaviour of Nile Tilapia. Female Nile Tilapias spawn asynchronously (Coward and Bromage, 2000) and the operational sex ratio, therefore, is male biased (operational sex ratio is the sex ratio of animals actively participating in reproduction). A male-biased sex ratio together with the aggressive nature of males is expected to result in strong reproductive competition leading to high variance in male reproductive success. These differences in male reproductive success were reported to lead to increase in the level of inbreeding in the populations of the Nile tilapia (Trong et al, 2013, Mohammadi et al, 2025).

5. Conclusion

The values of heterozygosity of the cultured populations were generally not different from those of the wild populations indicating that the cultured populations have been well managed unknowingly or knowingly in terms of broodstock selection. The level of genetic diversity of base populations should importantly be always considered before utilizing them with an adequate number of individuals from genetically differentiated populations used as founder stock in establishing a culture population.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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