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Genetic diversity of commercial species of the tilapia genus *Oreochromis* in Mexico

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Abstract: Molecular techniques such as the DNA markers are commonly used to examine intra and interpopulation variation among fish strains. The objetive of this work was to analyze the genetic diversity of three tilapia species (*Oreochromis aureus*, *O. mossambicus* and *O. niloticus*) cultured in Mexico, through the DNA markers, genetic and allele frequencies, number of alleles, endogamy, estimated and expected heterozygosity (He), and conformation of Hardy Weingberg, (H-W). After 150 culture days, a total of 24 individuals for each fish species were genotyped using a panel of 8 microsatellite markers. The result showed that the mean number of alleles per locus and heterozygosity for each tilapia species were 19 and 0.442, respectively. The diversity indices showed that *O. aureus* was more variable (He=0.813) followed by *O. niloticus* (H_E=0.789) and *O. mossambicus* (H_E = 0.552). Mean endogamy values fluctuated from 0.314 for *O. niloticus* to 0.436 for *O. aureus*. Highly significant deviations in most of loci of three species, and all diversity parameters suggested non-random mating in populations studied. The results in this study contribute to the knowledge of the genetic pool of the commercial tilapia species in Mexico.

Key Words: Fish, genetic diversity, DNA markers, microsatellites

Introduction

Mexico is one of the most important tilapia producers in Latin America (Ruiz *et al*., 2007) Cichlids are cultured in almost all the country including Sinaloa state, where the interest for tilapia farming industry is increasing (Rodríguez-González, 2009). In 1964, *Tilapia*

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rendalli, *Oreochromis mossambicus* and *O. aureus* became the first species introduced to Mexico (Morales, 1974). Later, another tilapia strains (Nile tilapia, Rocky Mountain, albino and red hybrid) were introduced and held at private and government sectors for rearing and on-growing purposes (Barriga-Sosa *et al*., 2002). However, as in other countries, management practices, hybridization, migration and inbreeding are affecting genetic variability of current stocks. For instance, McAndrew (1993) identified genes from four tilapia species inside the genetic pool of a commercial variety. Morales (2003) pointed out that inbreeding increase organisms deformities, genetic damage, and promote heterogeneous size of fry in tilapia populations, and reduce feed conversion ratio, survival and growth of juvenile and adult fish.

In order to facilitate and improve correct reproductive and genetic management of *Oreochromis* species cultured biotechnological techniques could be applied. Uribe-Alcocer *et al*. (1989) described banding patterns of some tilapia species, and Barriga-Sosa *et al*. (2002) reported the first attempt to describe the genetic variability on tilapia species and strains that are used for aquaculture purposes in Mexico.

Nowadays, molecular techniques such as the DNA markers are commonly used to examine inter and intra population variation among fish strains (Peral, 2003). Specifically, DNA markers have been used for mapping genetic information of *O. niloticus* (Lee *et al*., 2004) to evaluate the genetic variability of two *O. niloticus* strains cultured (Espínola de Souza 2007), study the expected and obtained heterozygosity in several commercial tilapia varieties (Melo *et al*., 2006; Hassanien and Gilbey, 2005) and to compare productive indices among different fish strains related to genetic information (Aranguren-Méndez *et al*., 2005).

Currently, exist an increasing interest for tilapia farming in Mexico due to its great potential in terms of production potential; however, to monitor genetic diversity that assist breeding programs is fundamental and very necessary to avoid genetic variability lost and, in consequence, inbreeding issues. Therefore, the objective of the present work was to assess the genetic diversity of three tilapia species (*Oreochromis aureus*, *O. mossambicus* and *O. niloticus*) cultured in Sinaloa, Mexico, using DNA markers.

Materials and Methods

Sample collection and DNA extraction

Oreochromis niloticus, *O. aureus* and *O. mossambicus* broodfish (6 ± 2 g mean initial weight) from Centro Acuícola de Jala, Colima, were transported and maintained at the Aquaculture laboratory of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa-IPN. For

culturing of tilapia, standard conditions for optimal fish growth were followed (Morales, 2003). After 150 days, 24 muscle samples of the caudal tissue (12 females and 12 males) of each species were obtained and maintained in ethanol at 100% for posterior DNA extraction. Preserved samples were processed and DNA was subsequently extracted using the GenElute Mammalian Genomic DNA Miniprep kit (G1N350; SIGMA-Aldrich®, St. Louis, Missouri).

Microsatellites selection

A panel of eight microsatellites was selected for this study from previous reports, UNH145, UNH155, UNH160, UNH166 and UNH207 (Bhassu *et al*., 2004), UNH190 and UNH208 (Rutten *et al*., 2004), and UNH211 (Hassanien and Gilbey, 2005), most based on the information criterions such as allelic range, number of alleles, polymorphic information content and other available information. All microsatellites were marked with infrared flourescense (IRD800) for further analysis in LI-COR, Model 42001 G sequencer.

Polymerase chain reaction (PCR) and loci typifying

PCR reactions were performed in a final volume of 10 μL composed of 50 ng DNA, 1.25 U Taq DNA polymerase (Promega Co, Madison, WI, USA) and 0.4 mM dNTPs. The MgCl2 concentrations, DNA volumes and temperature profiles varied (Tab. 1). PCR products were denatured at 95°C for 5 minutes and were electrophoresed on a 6.5% polyacrylamide-bisacrylamide gel for 2 h on a LI-COR 42001G sequencer. The allele sizes were determined by SAGAGTTM^{TM} software using the IRDye® 800 Sizing Standard ranging from 50-350 bp.

Genetic diversity analysis

Information of used panel was verified by CERVUS ver. 3.0 software (Kalinovski *et al*., 2007) estimating allele number, allele frequencies polymorphism information content (PIC) and identity individual exclusion probabilities. Using microsatellite allelic frequencies, the average number of alleles (A) and the effective number of alleles were estimated following the proposed formula $Ae = 1/\Sigma x_i^2$, where x_i = frequency of the allele at each locus (Li *et al*., 2004). The Hardy-Weinberg equilibrium (HWE) was tested, and population-diversity parameters were examined. Expected (H_E) and observed (H_O) heterozygosity were estimated using GENEPOP version 4.0 software (Rousset, 2008). An analysis for estimating F_{ST} indices by allele identity was performed additionally at the same software. Complementarity, inbreeding coefficients (F_{IS}) and gene diversity indices (G) were estimated by FSTAT version 2.9.3 (Goudet, 2001).

Results

A panel of microsatellites for joint typifying of the three Tilapia species most used in

Tab. 1: Description of microsatellite loci and PCR conditions used to genotype *Oreochromis* species samples

Loci	Primers (sense/antisense) → 5'3'	PCR conditions		
		$ADN_{\mu L}$	T_m ºC*	$MgCl_2$**
UNH145	CATGCTGAAAGCTGATTT/ ACCCACACCTAAAATTAGAGATA	3.0	55	1.5
UNH155	CGCACTTACTCTTGGCT/ AGAGCTGGAGTCATATGG	2.0	60	3.0
UNH160	CCATTGGCTCTTACATC/ GATAGCATTTCTGTAGTTATGG	1.0		2.5
UNH166	CCCTCACACACACTCTT/ GATAACGACACGACAGTAC	2.0	55	1.5
UNH190	CGCGATCGAGCATTCTAA/ TGTCTGCACGCGCTTTTGT	2.0	55	1.5
UNH207	ACACAACAAGCAGATGGAGAC/ CAGGTGTGCAAGCAGAAGC	2.0	60	1.5
UNH208	CTTCTTGGCCTACAATTT/ CAGATGGGTGATAGCAA	2.0	60	2.5
UNH211	GGGAGGTGCTAGTCATA/ CAAGGAAAACAATGGTGATA	1.5	60	3.0

*Annealing temperature during PCR

**$MgCl_2$ concentration (mM)

farming systems of Mexico was successfully optimized. Descriptive information of microsatellite panel optimized by Tilapia species is presented in Table 2. All microsatellite showed to be informative, except UNH190 for *O. mossambicus* with the lowest average number of alleles (A), and the unamplified UNH208 for *O. mossambicus* and *O aureus*. Individual identity exclusion probabilities for genotyped microsatellites ranged from 0.403 to 0.952 for *O. mossambicus*, 0.784 to 0.989 for *O. aureus*, and 0.716 to 0.982 for *O. niloticus* (Tab. 2). Combined exclusion probabilities were 1.40^{-6}, 1.1^{-9} and 1.9^{-10}, for the three panels optimized for Tilapia species, respectively.

For *O. mossambicus, O aureus* and *O. niloticus* the A obtained was 6.42, 11.71 and 11.25 alleles per loci, respectively; and estimated average Ae for all three species was 2.96, 6.42 and 5.70 alleles per loci.

The standard diversity indices showed that *O. niloticus* was the observed most variable (average H_O= 0.541) followed by *O. aureus* (average H_O = 0.464) and *O. mossambicus* (mean He = 0.351; Tab 3). Similarly gene diversity (G) estimators were 0.868, 0.611 and

0.237 for *O. niloticus*, *O aureus* and *O. mossambicus*, respectively.

For fixation index F_{IS} the highest average level was observed for *O. aureus* population (0.436) and the lowest for *O. niloticus* (Tab. 3). Highly significant deviations ($P < 0.05$) from Hardy-Weinberg equilibrium were estimated for all loci and populations, with exception of UNH145 locus for all populations and UNH166 in *O. niloticus* (Tab. 3).

Tab. 2: Descriptive information of microsatellite panel used for genotyping of three Tilapia species

Loci	Specie	A	Allelic range (bp)	Ae	PIC	P
UNH145	*O. mossambicus*	8	158-178	3.44	0.664	0.871
	O. aureus	11	162-182	8.53	0.871	0.975
	O. niloticus	14	134-204	8.29	0.868	0.974
UNH155	*O. mossambicus*	7	132-146	6.13	0.815	0.952
	O. aureus	10	142-192	5.31	0.787	0.940
	O. niloticus	5	144-188	2.20	0.468	0.716
UNH160	*O. mossambicus*	7	154-182	4.11	0.720	0.904
	O. aureus	7	156-184	2.95	0.602	0.825
	O. niloticus	11	154-200	4.31	0.751	0.930
UNH166	*O. mossambicus*	10	137-173	3.09	0.659	0.878
	O. aureus	18	139-187	12.95	0.918	0.989
	O. niloticus	8	141-221	3.01	0.633	0.855
UNH190	*O. mossambicus*	2	153-155	1.33	0.218	0.405
	O. aureus	12	125-179	4.36	0.749	0.926
	O. niloticus	14	119-205	9.93	0.891	0.982
UNH207	*O. mossambicus*	5	124-162	1.36	0.254	0.449
	O. aureus	17	96-180	8.41	0.872	0.976
	O. niloticus	14	114-178	8.60	0.873	0.975
UNH208	*O. mossambicus*					
	O. aureus					
	O. niloticus	11	52-108	2.91	0.637	0.863
UNH211	*O. mossambicus*	6	54-170	1.30	0.226	0.403
	O. aureus	7	56-138	2.44	0.542	0.784
	O. niloticus	13	54-170	6.36	0.827	0.959

A: Average number of alleles, bp: base, Ae: Effective numer of alleles, PIC: Polyorphism information content, P: Individual identity exclusion probability

Fixation indices analysis by allele identity showed divergence in population structure, amongst the studied *Oreochromis* populations. Estimated pairwise F_{ST} was high between all species; with the higher estimate between *O. mossambicus and O niloticus* (0.2845) and lower between *O. aureus* and *O. niloticus* (0.1651).

Tab. 3: Inbreeding coefficients, observed and expected heterozygosities and Hardy–Weinberg genetic deviation probabilities for three analyzed Tilapia species

Loci	$F_{IS\ 1}$	$F_{IS\ 2}$	$F_{IS\ 3}$	$H_{O\ 1}$	$H_{O\ 2}$	$H_{O\ 3}$	$H_{E\ 1}$	$H_{E\ 2}$	$H_{E\ 3}$	$HW_{1p\text{-}val}$	$HW_{2p\text{-}val}$	$HW_{3p\text{-}val}$
UNH145	-0.154	0.171	0.021	0.792	0.750	0.917	0.651	0.902	0.898	0.0526	0.0890	0.4113
UNH155	0.469	0.351	0.706	0.458	0.542	0.167	0.855	0.829	0.558	0.0003	0.0008	0.0000
UNH160	0.412	0.512	0.261	0.458	0.292	0.583	0.773	0.660	0.785	0.0027	0.0000	0.0010
UNH166	0.403	0.475	0.147	0.417	0.542	0.583	0.691	0.945	0.682	0.0001	0.0000	0.1072
UNH190	0.839	0.582	0.461	0.042	0.333	0.500	0.254	0.787	0.918	0.0009	0.0000	0.0000
UNH207	0.542	0.420	0.172	0.125	0.500	0.750	0.270	0.900	0.902	0.0008	0.0000	0.0000
UNH208			0.570			0.292			0.669			0.0000
UNH211	0.298	0.591	0.376	0.167	0.292	0.542	0.236	0.624	0.861	0.0030	0.0000	0.0000
Average	0.348	0.436	0.314	0.351	0.464	0.541	0.532	0.806	0.784			

F_{IS}: Inbreeding coefficient, H_O: Observed heterozygosity; H_E: heterozygosity, HW: Hardy-Weinberg deviation probability, *O. mossambicus* (1), *O. aureus* (2) y *O. niloticus* (3)

Discussion

Tilapia farming is one of the most important aquaculture productive systems focused to animal origin protein production. In Mexico, Tilapia farming systems use mainly three species, *O. niloticus, O. aureus and O. mossambicus*; however, the intense reproduction characteristics of the species make difficult proper management. Here was proposed and assessed a molecular approach to evaluate genetic diversity of Tilapia including a microsatellite panel for genotyping under comparable conditions. There are several studies for tilapia (Carleton *et al.*, 2002; Bhassu *et al.,* 2004; Romana–Eguía *et al.,* 2004) dealing with genetic diversity and genetic improvement and supporting this approach.

For the three *Oreochromis* species analyzed were distinguished slightly contrasting levels of gene diversity (G) and Ae; and although *loci* distinguish higher levels of polymorphism, there is a considerable amount of unique alleles in heterozygosis, and a large amount combined perhaps in homozygosis as heterozygosis excess complementary HW analysis suggested.

Previous reports on genetic diversity parameters have showed very variable patterns accordingly to the tilapia population analysed. For instance, Espínola de Souza (2007) obtained similar H_O (0.471) for *O. niloticus* and *O. niloticus* Chitralada variety cultured in Brasil, compared with that observed for *O. aureus* (H_O= 0.464) in this study. Melo *et al*., (2006) found a H_O value of 0.610 in five tilapia varieties (Ceara, Chitralada, Israel, Taiwan and red *O. niloticus*) cultured in Brasil, meanwhile Bhassu *et al*. (2004), registered an observed heterozygosity of 0.642 in five tilapia populations of *O. niloticus* and *O. mossambicus*. Both H_O values are similar to that found for *O. niloticus* in the present work (H_O = 0.642). In the case of *O. mossambicus,* the obtained H_O value (0.350) showed low heterozygosity suggesting poor genetic variability for this species (López *et al*., 2007). In general, the obtained H_O values lower compared with wild tilapia populations from the river Nile. Hassanien and Gilbey (2005) mentioned that low H_O in cultured fish could be explained for the reduced variability due to inbreeding among few brood fish. The obtained H_E for *O. niloticus* and *O. aureus* (H_E = 0.784; H_E = 0.806, respectively) in the present study was similar to those reported by Hassanien and Gilbey (2005) for wild *O. niloticus* populations (He = 0.702).

Similarly, the obtained F_{IS} values are higher compared with the work of Rutten *et al.* (2004) and Melo *et al*. (2006) whose reported 0.041 and 0.048, respectively, for domesticated *O. niloticus* populations. Romana–Eguía *et al*. (2005) obtained F_{IS} of 0.170 and 0.192 in cultured *O. niloticus,* after applying a genetic improvement program to wild populations captured in the river Nile.

Highly significant deviations from H-W equilibrium for the three tilapia species suggest non-random mating producing deficit of heterozygotes. Has been suggested that heterozygosity deficit is strongly affected by factors such as management in the applied reproduction programs at the farms (López *et al*., 2007), since number of brood fish and sexual ratio are not adequate to maintain allele frequency in equilibrium. Additionally, Bhassu *et al*. (2004) pointed out that lack of genetic equilibrium in allele frequency is also related to size of sample and population selected. Hence, it is very recommended renewal of brood stock in hatchery farms to avoid inbreeding among fish culture population and reduction of genetic variability.

Finally, genetic distances analysis corroborated divergence of populations when compared with criterion suggested by Hartl and Clark (2007), clarifying that no genetic flow existed between populations and reinforcing importance of molecular techniques to assess genetic isolation of commercial populations to avoid interbreeding and unexpected mixed populations (McAndrew, 1993).

The obtained results showed that the use of

microsatellites is a powerful tool to evaluate the commercial tilapia populations cultured in Sinaloa, Mexico. It is concluded that genetic diversity of the tested species is low, promoted perhaps, by particular management practices and low genetic flow suggesting the progressive close of populations and inbreeding risk. These and periodical molecular data would aid decision making on convenient reproduction management of stocks, assuring the maintaining of low inbreeding levels and the highest productive response.

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