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Polymorphism of the ND5/6 Gene in mtDNA of Strains of Nile Tilapia, *Oreochromis niloticus*

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Abstract

Restriction fragment length polymorphism (RFLP) analysis was conducted for the ND5/6 gene in mtDNA of different strains of *Oreochromis niloticus*. The size of ND5/6 gene fragment in mtDNA was about 2,393 ± 144 bp. The indices of haplotype diversity and nucleotide diversity of *O. niloticus* were 0.6879 ± 0.0978 and 0.02659 ± 0.0978, respectively. The range of these strains was Sudan 78 > GIFT > Egypt 92 > Egypt 88. There were genetic differences among the four strains. The estimated phylogeny of the different strains indicated that Egypt 88 was nearest to Egypt 92 and Sudan 78 was the most distant from the other strains. The results suggest that genetic bottlenecks and genetic introgression could be explained for mtDNA differentiation among strains of *O. niloticus*. Sudan 78 possessed several unique haplotypes that might be a result of past mating with *O. aureus* females. GIFT line also had several unique haplotypes which was probably a result of its diverse ancestry. The predominant haplotype was also different for the GIFT line suggesting selection for dams that differ in origin from the other three strains.

Introduction

Isozyme variation is low in Nile tilapia, *Oreochromis niloticus* (Rognon et al. 1996, Agnese et al. 1997, Li et al. 2001). This makes strain characterization, genetic marking, study of the impact of domestic populations on wild populations and other such genetic marking research difficult or impossible in Nile tilapia utilizing isozyme analysis. Similar lack of isozyme variation has been found in other fish species such as striped bass, *Morone saxatillis* (Dunham 1996).

The analysis of mtDNA variation is an alternative to study population genetics in fish (Capili and Skibinski 1996, Agnese et al. 1997). In cases such as striped bass where isozyme variation was minimal, significant

mtDNA variation was observed (Waldman et al. 1997). The purpose of this experiment was to ascertain variation in the ND5/6 mtDNA sequences of the NADH dehydrogenase gene in Nile tilapia cultured in China.

Materials and Methods

Sample collection

Samples of Sudan 78 strain, Egypt 88 strain and GIFT line of Nile tilapia, *O. niloticus* were collected from the experiment station of Shanghai Fisheries University. Egypt 92 strain was sampled from Huzhou Fish Farm (Zhejiang Province, eastern China). Livers were collected from the live fish and kept in 95% ethanol until DNA purification.

DNA extraction and mtDNA amplification

Total DNA was purified following the method described in Bernatchez et al. (1992). Two mtDNA sequences of the ND5 and ND6 subunits of the NADH dehydrogenase were amplified by the polymerase chain reaction (PCR) using a Perkin-Elmer thermal cycler (Model 480) and the primers C-Glu and C-leu3 (Cronin et al. 1993).

Restriction enzymes digestion

Six endonuclease restriction enzymes (DdeI, HaeIII, HhaI, HinfI, RsaI, TaqI) were used to digest the amplifed gene fragments of ND5/6. Digestion conditions followed the manufacturers recommendations. Resulting digested fragments were electrophoretically separated on 1.2% agarose gel, stained with Ethidium bromide and photographed under UV light. A 20 µl mixture of lambda-DNA cut with HindIII and lambda-DAN double digested with EcoRI and HindIII was used as size standard.

DNA analysis

Endonuclease phenotypes were named A, B, C, ..., respectively. All endonuclease phenotypes in one fish constituted its haplotype. The digest sites were derived from the numbers and sizes of the fragments digested by enzymes. One (1) and zero (0) denoted presence or absence of the recognition site, respectively (Bernatchez and Dodson 1991).

A haplotype file was constructed with endonuclease enzymes and haplotype data derived by the endonuclease enzyme digestion. Enzyme file was constructed with digestion recognition sequence, nucleotide divergence distance, haplotype diversity and nucleotide diversity estimated (Nei and Li 1979) with GENERATED D and DA programs of the Restriction Enzyme Analysis Package (REAP). The geographical heterogeneity of mtDNA genotypes among the different strains was determined using chi-square random-

ization tests with 1,000 permutations performed by the MONTE program of REAP Software (Roff and Bentzen 1989). An unweighted pair group method of analysis (UPGMA) map of different strains was constructed according to Sneath and Sokal (1973).

Results

Endonuclease enzyme digestion

The mtDNA gene ND5/6 of *O. niloticus* was digested by six endonucleases, (DdeI, HaeIII, HhaI, HinfI, RsaI, TaqI). Twenty-one different restriction fragment patterns were observed. The gene size of ND5/6 in mtDNA was about 2,393 ± 144 bp, which is similar to that of trout (2,400 bp) (Bernatchez and Osinov 1995). The mtDNA haplotypes and restriction fragment patterns of *O. niloticus* are shown in table 1.

Haplotype diversity

Average haplotype diversity of *O. niloticus* was 0.6879 ± 0.0978, and average nucleotide diversity was 0.02659 ± 0.0978 (Table 2). Average haplotype and nucleotide diversity indices of the different strains are shown in table 3. The diversity index of Sudan 78 strain was the highest, followed by GIFT and Egypt 92 and Egypt 88 strain was the lowest.

Haplotype distribution and genetic distance

Seven haplotypes were observed in the GIFT strain, three in Egypt 88 strain, five in Egypt 92 strain and nine in Sudan 78 strain. The haplotype TN3 was the dominant haplotype in all strains except GIFT strain.

Table 1. MtDNA haplotype and patterns of O. *niloticus*

Genotype	DdeI	HaeIII	HhaI	HinfI	RsaI	TaqI
TN1	A	B	A	A	A	A
TN2	B	A	B	B	B	B
TN3	A	B	A	C	A	A
TN4	C	A	A	A	C	A
TN5	A	A	A	A	A	A
TN6	B	B	B	B	B	B
TN7	B	A	B	B	A	B
TN8	C	C	B	D	C	C
TN9	A	A	A	A	C	A
TN10	A	A	A	C	A	A
TN11	C	C	B	C	A	C
TN12	A	C	B	C	C	C
TN13	A	B	C	A	A	A
TN14	C	B	A	A	A	A
TN15	C	B	C	A	D	A
TN16	A	B	A	B	A	A
TN17	A	A	B	A	B	B
TN18	D	A	B	B	B	B

Chi-square tests of the haplotype frequencies in different strains of *O. niloticus* indicated that there were significant genetic differences among the populations ($p < 0.05$). The genetic distance (nucleotide divergence) of the four strains of *O. niloticus* is illustrated in table 2, and a dendrogram of these relationships (UPGMA) is constructed (Fig.1).

Discussion

The nucleotide diversity index sequence indicated that Sudan 78 strain was more diverse > GIFT strain > Egypt 92 strain > Egypt 88 strain. The highest haplotype diversity in Sudan 78 strain may be caused by the genetic introgression with other tilapia species. If this explanation is true, *O. niloticus* males must have hybridized with females from other species in the past in China. Previously, isozyme analysis indicated that introgression occurred in Sudan 78 strain of *O. niloticus* in Nanjing Tilapia Fish Farm by hybridization with *O. aureus* (Li and Cai 1995) supporting this hypothesis.

GIFT strain was selectively bred by the International Center for Living Aquatic Resource Management (ICLARM) after crossbreeding among four African strains and four Asian strains. Therefore, it is not surprising that this line showed a high genetic diversity.

The lowest haplotype diversity in Egypt 88 strain was probably a result of a severe genetic bottleneck since its founded stock was only eight females and one male (Li and Cai 1995). There were significant differences among the different strains of *O. niloticus* for the mtDNA genotype, which reflected their different genetic background. Further study should be undertaken to investigate the correlation between the performance and the mtDNA differences revealed. Genetic similarity between Egypt 88 strain and Egypt 92 strain was likely because of their common country of origin. Egypt 88 strain was introduced to China from Nile River in Egypt in 1988, while Egypt 92 strain introduced from the Philippines also came from Egypt.

Sudan 78 possessed several unique haplotypes that might be a result of past mating with *O. aureus* females. GIFT line also had several unique haplotypes, which was probably a result of its diverse ancestry. The predominant haplotype was also different for GIFT line suggesting selection for dams that differ in origin from the other three strains.

Variation in nucleotide sequence for mtDNA genes is greater in Nile tilapia than isozyme variation. The mtDNA techniques in the current study detected more genetic variation for Nile tilapia than Capili and Skibinski (1996) and Agnese et al. (1997). Expansion of the analysis may allow tracing of ancestry and marking families or strains in Nile tilapia breeding programs. However, nuclear DNA markers would be needed to trace the sire lineage.

Table 2. Haplotype and nucleotide diversity indices of mtDNA in different strains of *O. niloticus*

Strain	Haplotype diversity	Nucleotide diversity
GIFT	0.7607±0.0667	0.04113
Egypt 88	0.5217±0.0988	0.02154
Egypt 92	0.5684±0.1189	0.02982
Sudan 78	0.8333±0.0519	0.05698

Table 3. MtDNA haplotype frequency in different strains of *O. Niloticus*

Population	N	TN1	TN2	TN3	TN4	TN5	TN6	TN7	TN8	TN9	TN10	TN11	TN12	TN13	TN14	TN15	TN16	TN17	TN18
GIFT	26	0.46	0.19	0.15	0.04	0.08	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Egypt 88	24	0.17	0.17	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Egypt 92	20	0.00	0.10	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.15
Sudan 78	29	0.04	0.10	0.35	0.00	0.00	0.00	0.00	0.14	0.04	0.04	0.04	0.04	0.17	0.07	0.07	0.04	0.00	0.00

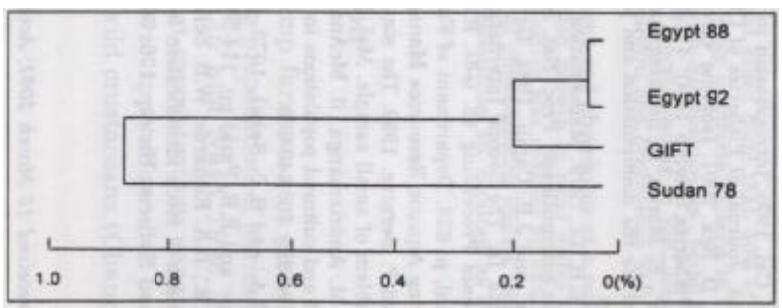

Fig. 1. mtDna dendrogram (UPGMA) of different strains of *O. niloticus*

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