Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae)

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Introduction

Among all tilapia species, the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), originating in West and East Africa (TREWAVAS, 1983), is commercially the most important. It has been introduced into many Asian and South American countries.

The natural range of *O. niloticus* includes the Senegal, Gambia, Niger, Volta, Benue, Chari, Nile, and Awash Rivers and many lakes like those of the Rift Valley: Edward, George, Albert, Kivu, Tanganyika, Baringo, Turkana. TREWAVAS (1983) described seven subspecies, using morphometrical analysis: *O. niloticus niloticus* from West Africa and the Nile, *O. n. eduardianus* from Lake George, Edward and Tanganyika, *O. n. cancellatus* from the Awash River system in Ethiopia, *O. n. filoa* from the hot springs of

the Awash River, *O. n. vulcani* from Lake Turkana, *O. n. baringoensis* from Lake Baringo, *and O. n. sugutae* from River Suguta in Kenya. SEYOUM and KORNFIELD (1992a) described a new subspecies, *O. n. tana* from lake Tana in Ethiopia, using genetical (mitochondrial DNA) characteristics.

Even though *O. niloticus* has a wide distribution and a real economic importance, little is known about the genetic characterization of natural populations. This could be of great importance for the future development of aquacultural strains, for the protection of small endangered populations (like those of small lakes such as Baringo or the Suguta River), and for biogeographical inferences. In this study, investigations have been done using standardised techniques on natural populations from the major basins (the Senegal, Niger, Volta, Nile, Awash, and Suguta Rivers; Lakes Chad, Tana, Turkana, Edward, Baringo) and representing all the described subspecies.

Materials and methods

Sampling and allozyme study

Specimens of *O. niloticus* were collected from August 1993 to December 1994, in 17 locations : River Senegal at Dagana, River Niger at Selingue, River Niger at Bamako, Lake Volta at Akosumbo, Lake Chad near Karal, River Chari at N'Jamena, Lake Manzalla at Manzalla, River Nile at Cairo, Lake Tana at Bahar Dar, Hot springs of the Awash system at Sodore, Lake Koka at Koka, Lake Zyway at Meki, Lake Awasa at Awasa, Lake Turkana at Loyangalani, River Suguta at Kapedo, Lake Baringo at Kampi ya Samaki, Lake Edward at Mweya.

They were kept at -20°c for a few days and then maintained at -80°c for later analysis except for specimens from Lake Tana which were immediately preserved in alcohol. Specimens of *O. aureus*

come from a farmed strain (Lake Manzalla; Egypt). Standard horizontal starch gel (12%) electrophoresis was carried out to investigate the products of 25 loci. The stain recipes and buffer used were those described in POUYAUD and AGNESE (1995) and PASTEUR *et al.* (1987). The nomenclature is that proposed by SHAKLEE *et al.* (1990).

Amplification of the control region of mitochondrial DNA.

To amplify a 1 Kb fragment in the control region of mtDNA, HN20 and LN20 primers (BERNATCHEZ and DANZMANN, 1993) were used.

Digestion of the amplified products

Five to eight µl of the PCR-amplified control region was digested by 5 units of one of the restriction enzymes in a final volume of 20 µl containing the appropriate buffer.

Microsatellites

A total of 4 different primer sets for *Sarotherodon melanotheron* described by POUYAUD *et al.* (submited) have been used : SMEL1 (Gene Bank number U69153), SMEL2 (X99799), SMEL3 ((X99800), SMEL4 (X99801).

Analysis of the data

To analyze allozymic, microsatellites or RFLP data, different programs from Phylip (Phylip software package, Felsenstein, v. 3.5) were used: Consense, Mix, Genedist, Neighbor, Seqboot.

Results

Allozymes

Sixteen of the 25 loci studied were polymorphic. The rate of observed heterozygosity (H) was between 0.000 (Lake Baringo) and 0.045 (Volta River) and the rate of observed polymorphism (P95%) between 0.00 (Lake Baringo) and 0.08 (all West African and Nile populations except the Niger River at Selingue). These values are comparable to those obtained in previous studies of natural *O. niloticus* populations (SEYOUM and KORNFIELD, 1992b; ROGNON *et al.*, 1996) even if the loci analyzed were not the same as in the present study.

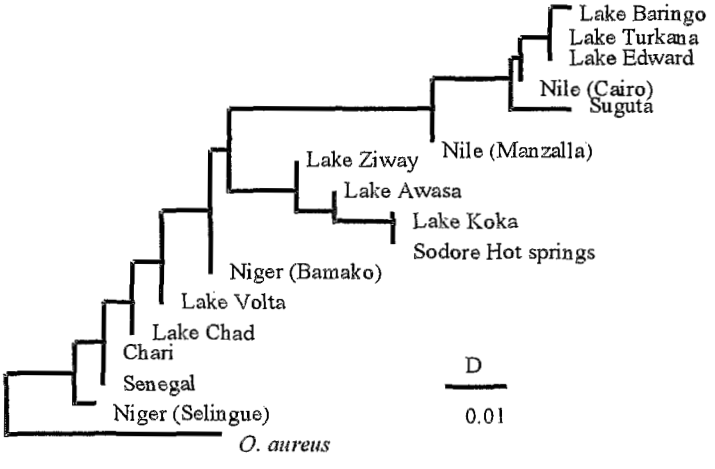

Figure 1
Network produced by Phylip on the 16 populations representing seven subspecies of *O. niloticus*. This is a consensus tree produced using Consense from 1000 trees produced using Seqboot and Neighbor. The number at each junction represent the frequency of its occurrence

To build a genetic network, a total of 1000 randomly modified frequency matrices were obtained using the program Seqboot. These matrices were then transformed into NEI's (1972) genetic distance matrices using the Genedist program. The corresponding trees were built by the programme neighbor and summarized into a single tree using with Consense (Fig. 1).
Populations are clustered in three major groups. One is composed of the Nile drainage (the Nile and Lake Edward), and the Kenyan Rift Valley populations (Lake Turkana, Lake Baringo and the Suguta River). The second major group is composed of the Ethiopian Rift Valley populations (Sodore and Lakes Koka, Awasa, Ziway) and the third group of the West African populations (the Senegal, Niger, Volta, Chari Rivers and Lake Chad).

Microsatellites

The four microsatellites loci were polymorphic with 3 to 27 alleles. West African populations are less polymorphic than East African population (H=0% Bamako and Selingue population and H=52% lake Turkana).

To build a genetic network (Fig. 2), the same procedure as previously described for allozymes was done using Mix program (a parcimony algorithm). Populations from Ethiopia are clusetered together, while populations from West Africa and Nile are on the other side of the network. Populations from Kenyan and ugandan Rift valley are clustered between these two groups.

RFLP mtDNA

Six enzymes (AsnI, HinfI, RsaI, AvaII, MspI, TaqI) gave 13 phenotypes corresponding to nine different haplotypes (Fig. 3). In Sodore, Lake Edward and the Nile, more than one haplotype was found (2, 2 and 4 respectively). Individuals from Suguta and Baringo have private haplotypes. On the contrary, Lake Chad, Volta and the Niger share the same haplotype which is also present in the Nile population and in *O. aureus*. Specimens from Lakes

Turkana, Edward and Tana also shared the same haplotype found in the Nile. Fig. 3 shows the consensus tree calculated from the 18 most parsimonious networks obtained with the MIX program. The mtDNA haplotypes are geographically distributed. At one side of the network, all populations from West Africa and *O. aureus* are clustered (they share the same haplotype), on the other side there are the two Ethiopian Rift Valley populations and between these two groups are the Kenyan and Ugandan Rift Valley populations. Nile population shows affinities with West African populations and with specimens from Lake Tana and Turkana.

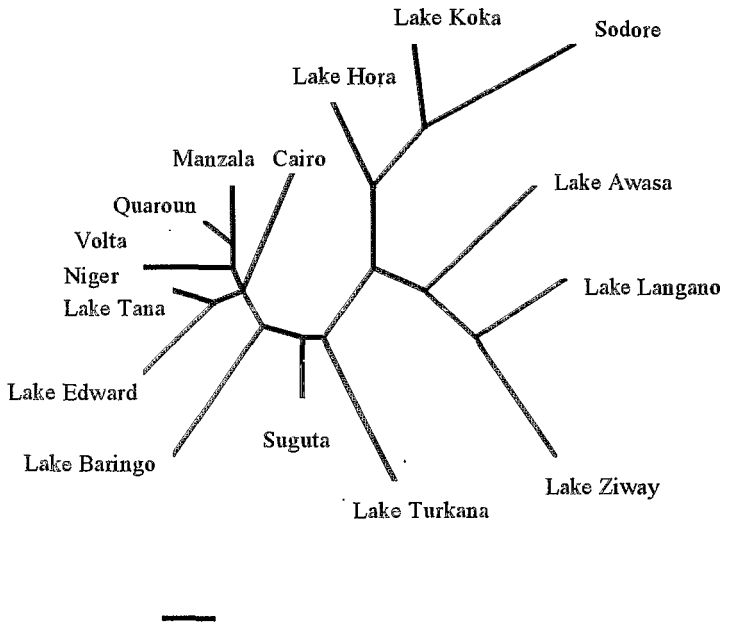

Figure 2
Network produced by Phylip using microsatellites data, on the 16 populations representing seven subspecies of *O. niloticus*. This is a consensus tree produced using Consense from 1000 trees produced using Seqboot and Neighbor.

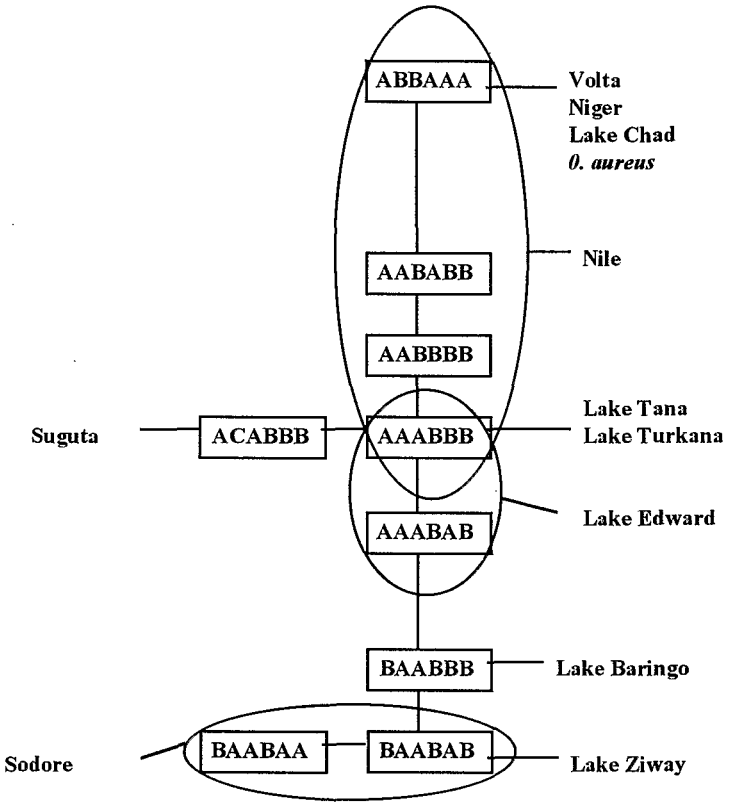

Figure 3
Network produced by Phylip on the 9 mtDNA haplotypes observed. This is a consensus tree produced using Consense from the 18 most parsimonous trees produced using Mix

Discussion

These last results showed that West African *O. niloticus* mtDNA cannot be distinguished from *O. aureus* mtDNA. Two hypotheses can explain why *O. aureus* and *O. niloticus* can share the same

mtDNA. First, this haplotype can be an ancestral one which existed before the two species were isolated. Second, the mtDNA of one species could have been established in the other without nuclear contamination. This phenomenon has already been observed in fishes (DUVERNELL and ASPINWALL, 1995). Tilapia species are well known for their ability to hybridize in captivity (CRAPON DE CRAPONA and FRITZSCH, 1984), in the case of introduced species (DAGET and MOREAU, 1963; ELDER *et al.*, 1971), or in natural conditions (POUYAUD, 1994). TREWAVAS (1983) reported some experiments of hybridization between *O. aureus* and *O. niloticus*. The crosses *O. aureus* male with *O. niloticus* female and *vice versa*, gave a high proportion of males (90 to 100%). In these conditions, transfer of a mtDNA haplotype from one species to the other by natural hybridization is difficult. These observations seem to favour the ancestral DNA hypothesis.

The results obtained by SEYOUM and KORNFIELD (1992a, 1992b), with total mtDNA digestions suggested that *O. n. cancellatus* and *O. n. filoa* form a group independent of all other subspecies. The population of *O. n. tana* also showed, in these studies, a large divergence from other populations. Accordingly, they decided to consider *O. n. cancellatus* and *O. n. filoa* as a new taxon with two subspecies : *O. cancellatus cancellatus* and *O. cancellatus filoa*, respectively. the Population from Lake Tana was then considered as a new subspecies, *O. n. tana*. Our results suggest that the conclusions of SEYOUM and KORNFIELD (1992a, 1992b) have to be considered with reserve.

There are also some differences observed between our results and TREWAVAS' (1983) nomenclature. TREWAVAS (1983) assigned population from Lake Tana to *O. n. cancellatus*, not on a firm morphological basis but because the non-cichlid fishes of Lake Tana were all assigned to Ethiopian species (SEYOUM and KORNFIELD, 1992a). In our study, the Lake Tana mtDNA haplotype is similar to one observed in the Nile population and different from the one observed in the Ethiopian populations. Microsatellites also revealed that population from Lake Tana is closed to *O. n. niloticus* populations. These results could allow to modify the subspecific status of this population (*O. n. niloticus* instead of *O. n. cancellatus*). Another difference between our results and

TREWAVAS (1983) nomenclature is the genetic differentiation observed in *O. n. niloticus*. All West African populations (Senegal, Niger, Volta, Chad basins) are closely related whereas populations from Nile are closer to East African populations (Lake Edward, Turkana, Baringo and River Suguta). Morphological differentiation on which the subspecific nomenclature is based, is then different of genetical differentiation. For the genetic point of view, natural populations of *O. niloticus* are clustered in three groups: 1) the West African populations (Senegal, Niger, Volta, Chad drainages), 2) the Ethiopian Rift Valley populations (Lake Ziway, Awasa, Koka and Sodore hot springs in the Awash River), 3) The Nile drainage populations (Nile, Lake Tana, Edward) and the Kenyan Rift Valley populations (lake Turkana, Baringo and River Suguta).

These results and a better knowledge of the morphological differentiation of *O. niloticus* populations will be essential to modify the subspecific taxonomy of natural populations of this species.

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References

AGNESE (J.-F.), 1989 — *Différenciation génétique de plusieurs espèces de Siluriformes Ouest-Africains ayant un intérêt pour l'aquaculture*. Ph.D. Thesis, university of Montpellier, France.

BARDAKCI (F.), SKIBINSKI (D.O.F.), 1994 — Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity*, 73: 117-123.

BASIO (Z.U.), TANIGUCHI (N.) 1983 — An investigation of enzyme and other protein polymorphism in Japanese stocks of the tilapias *Oreochromis niloticus* and *Tilapia zillii*. *Aquaculture*, 38: 335-345.

BERNATCHEZ (L.), DANZMANN (R.G.) 1993 —
Congruence in Control-Region Sequence and Restriction-site variation in Mitochondrial DNA of Brook Charr (*Salvelinus fontinalis* Mitchill). *Mol. Biol. Evol.* 10(5): 1002-1014.

CRAPON DE CRAPONA (M.D.), FRITZSCH (B.), 1984 —
Interspecific fertile hybrids of haplochromine Cichlidae (teleostei) and their possible importance for speciation. *Neth. J. Zool.*, 34: 503-538.

DAGET (J.), MOREAU (J.), 1963 —
Hybridation introgressive entre deux espèces de *Sarotherodon* (Pisces, Cichlidae) dans un lac de Madagascar. *Bull. nat. hist. nat. Paris* (4) 3A(2): 689-703.

DUVERNELL (D.D.), ASPINWALL (N.) 1995 —
Introgression of *Luxilus cornutus* mtDNA into allopatric populations of *Luxilus chrysocephalus* (Teleostei: Cyprinidae) in Missouri and Arkansas. *Molecular Ecology*, 4: 173-181.

ELDER (H.Y.), GARROD (D.J.), WHITEHEAD (P.J.P.), 1971 —
Natural hybrids of the African Cichlid fishes *Tilapia spilurus* and *T. leucosticta* : a case of hybrid introgression. *Biol. J. Linn. Soc. Lond.* 3: 103-146.

FRYER (G.), ILES (T.D.) 1972 —
The Cichlid fishes of the great lakes of Africa: their biology and evolution. Oliver and Boyd, Edimburgh.

HAFFER (J.), 1982 —
General aspects of the refuge theory. *In Biological diversification in the tropics.* Edited by G. T. Prance New York, 6-24.

KAUFMAN (L.), 1992 —
Catastrophic change in species-rich freshwater ecosystems. The lessons of Lake Victoria. *Bioscience* 42 (11): 846-858.

LIVINGSTONE (D.A.), 1982 —
Quaternary geography of Africa and the refuge theory. *In Biological diversification in the tropics.* Edited by G. T. Prance New York, 523-536.

MALEY (J.), 1991 —
The African rain forest vegetation and paleoenvironments during late quaternary. *Clim. Change*, 19: 79-98

MARACANAS (J.M.), AGUSTIN (L.Q.), ABLAN (C.K.), PANTE (J.R.), EKNATH (A.A.), PULLIN (R.S.V.), 1995 —
Genetic improvement of farmed tilapias: biochemical characterization of strain differences in Nile tilapia. *Aquaculture International*, 3: 43-54.

MC ANDREW (B.J.), MAJUMDAR (K.C.), 1983 —
Tilapia stock identification using electrophoretic markers. *Aquaculture*, 30: 249-261.

NAISH (K.A.), WARREN (M.), BARDAKCI (F.), SKIBINSKI (D.O.F.), CARVALHO (G.R.), MAIR (G.C.) 1995 —
Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). *Molecular Ecology*, 4: 271-274.

NEI (M.), 1972 —
Genetic distances between populations. *Am. Nat.* 106: 283-292.

OGUTU-OHWAYO (R.), 1990 —
The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environ. Biol. Fish.* 27: 81-96.

PASTEUR (N.), PASTEUR (G.), BONHOMME (F.), CATALAN (J.), BRITTON-DAVIDIAN (J.), 1987 —
Practical Isosyme genetics. Hellis Horwood Ltd., Chichester, UK, 215 p.

POUYAUD (L.), 1994 —
Génétique des populations de tilapias d'intérêt aquacole en Afrique de l'Ouest. Relations phylogénétiques et structuration populationnelle. Ph.D.

Thesis Université de Montpellier-II, France.

POUYAUD (L.), AGNESE (J.F.), 1995 — Phylogenetic relationships between 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *J. Fish Biol.*, 47: 26-38.

ROGNON (X.), 1993 — *Diversité génétique et relations phylogénétiques chez les tilapias (Pisces, Cichlidae). Comparaison des données du polymorphisme enzymatique et mitochondrial.* Ph.D. Thesis université de Paris-Sud Orsay, France.

ROGNON (X.), ANDRIAMANGA (M.), MCANDREW (B.), GUYOMARD (R.), 1996 — Allozyme variation in natural and cultured populations in two Tilapia species: *Oreochromis niloticus* and *Tilapia zillii*. *Heredity*. 76: 640-650.

SHAKLEE (J.B.), ALLENDORF (F.W.), MORIZOT (D.C.), WHITT (G.S.), 1990 Gene nomenclature for protein coding loci in fish. *Transaction of the American Fisheries Society, 119*: 2-15.

SEYOUM (S.), KORNFIELD (I.), 1992a — Taxonomic notes on the *Oreochromis niloticus* subspecies-complex (Pisces:Cichlidae), with a description of a new subspecies. *Canadian Journal of Zoology*, 70: 2161-2165.

SEYOUM (S.), KORNFIELD (I.), 1992b — Identification of the subspecies of *Oreochromis niloticus* (Pisces:Cichlidae) using restriction endonuclease analysis of mitochondrial DNA. *Aquaculture*, 102: 29-42.

TREWAVAS (E.), 1983 — *Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia*. British Museum (Natural History), London.