

## **Genetic Phylogeography of Introduced *Oreochromis niloticus* (Pisces: Cichlidae) in Uganda**

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### **ABSTRACT**

Molecular genetic methods have been used to investigate the historical population patterns resulting from introduction of the Nile tilapia into the lakes of Uganda. Genetic polymorphisms were studied in populations of *Oreochromis niloticus* from the Lake Victoria basin and the Lake Kyoga Basin, in both of which it is not native. Genetic variation was also studied in populations from Lake Albert and the Lakes Edward-George system, from which transplanted Nile tilapia populations of Lakes Victoria and Kyoga are thought to have originated. Genetic variation was determined using the method of Randomly Amplified Polymorphic DNA (RAPD). Genetic differences between populations have been determined by measuring RAPD band sharing and calculating genetic similarity from the percent of band sharing. Populations within a lake basin (i.e., Lake Victoria or Lake Kyoga) were more similar to each other than to populations from other basins. Populations from the Lake Victoria basin were more similar to the putative source population of Lake Edward than to other possible sources, while the populations from Lake Kyoga were more similar to the putative source population sampled from Lake Albert. Populations from the Lake Kyoga basin were less diverse than those from the Lake Victoria basin, consistent with the hypothesis that introductions into Lake Kyoga are more recent and that a more diverse set of introductions may have contributed to the older populations of Lake Victoria.

we present molecular evidence concerning the genetic population structure of naturalized *O. niloticus* in the LVR.

In this study we employed the Random Amplified Polymorphic DNA (RAPD) technique (Hedrick, 1992; Dawson et al., 1992; Russell et al., 1993). Bardakci and Skibinski (1994) and Naish et al. (1995), have previously reported successful use of the RAPD technique in identifying subspecies and strains of *O. niloticus*.

## MATERIALS AND METHODS

**Sample locations:** Samples were obtained from several locations in East Africa, encompassing three lakes in the Lake Victoria basin and four from the adjacent Lake Kyoga basin. These two lake basins, plus Lakes Edward, George, Kivu, and scores of minor, satellite lakes, comprise the Lake Victoria Region (sensu Kaufman et al. 1997). Populations sampled in the Lake Kyoga basin include Lakes Kyoga, Muwuru, Nawampasa, and Nakuwa. Populations were obtained in the Lake Victoria basin from Lakes Victoria, Nabugabo, and Kachira. Samples were also obtained from Lake Edward and Lake George. In this study only those lakes containing *O. niloticus* are included. In addition, this study included Lake Albert so that we could examine the likely source populations of *O. niloticus* for the original introductions to the LVR. For the purpose of this study, each lake was assumed to contain a single panmictic population of *O. niloticus*.

**Tissue collection:** Fish samples were taken from the landings of commercial fishermen as well as from our own seine-net and gillnet sets. Tissue samples for DNA analysis were removed from the right epaxial musculature of each *O. niloticus* specimen. Tissue (2 g) was then placed in a vial containing 95% ethanol. After one hour the 95% ethanol was decanted and replaced, and the vial sealed and labeled for shipment to the laboratory for DNA extraction.

**Molecular methods:** DNA extraction was performed using the standard phenol/chloroform extraction method. RAPD bands were visualized after amplification by PCR. PCR reaction mixtures of 25  $\mu$ l final volume contained approximately 50 ng of genomic DNA, 25  $\mu$ M final concentration of each of the four nucleotide bases (dATP, dTTP, dCTP, dGTP), 2.5  $\mu$ l of 200 nM primer, 2.5  $\mu$ l of a reaction buffer from BRL technologies, and 0.25 units of 5  $\mu$ M Taq polymerase enzyme. RAPD decamer primers (Operon Technologies, Alameda, California) were used in a Perkin-Elmer thermocycler at the following sequence: a hot start for 3 min at 94°C, then 45 cycles for 30 seconds at 94°C, 1 min at 35°C, and 2 min at 72°C, with a ten minutes delay at 72°C at the end of the 45 cycles. Repeatability and potential contamination of reaction conditions was checked using both a positive and a negative control for every reaction set, for each primer. Amplifications were separated by 1.6% agarose synergel electrophoresis, and visualized under UV light after ethidium bromide staining. All DNA samples were subjected to at least duplicate amplification reactions to ensure repeatability of banding patterns.

## INTRODUCTION

Fish rank second only to plants as a protein source in many developing nations. Although Uganda has a high potential for aquaculture, nearly all of its native foodfish have been removed from the wild. Pressure on wild fisheries stocks in the Lake Victoria Region (LVR) by the early 1950's, prompted resource managers to begin widespread introduction of exotic fish species into Ugandan waters (Fryer and Iles, 1972). The most notorious exotic is the Nile perch (*Lates niloticus*), a voracious predator that contributed to the extinction of hundreds of indigenous species in all the lakes where it was introduced (Kaufman, 1992, Witte et al. 1992, Kaufman and Ochumba 1993). Less celebrated but potentially as important are several exotic tilapia cichlids, especially the Nile tilapia, *Oreochromis niloticus*. Today, the fisheries of Lake Victoria as well as several of the nearby, lesser lakes, are dominated by Nile perch and Nile tilapia, and the two endemic tilapiines of this region hover near extinction.

The idea of exotic fish introductions was initially opposed by some, but the prospect of a quick fix for wild stock declines held sway. The introduced food fishes eventually exploded in abundance, clearly an immediate benefit, but most of the original ecosystem were destroyed, with the loss of large numbers of indigenous species as well as unknown and largely unforeseeable long term consequences. Among the losses were several major native food fishes: the two endemic tilapiines *O. esculentus* and *O. variabilis*, the anadromous cyprinids *Labeo victorianus* and *Barbus altianalis*, several catfish species, chiefly *Bagrus docmac*, *Clarias gariepinus*, and *Schilbe intermedius*, and the lungfish, *Protopterus aethiopicus*. All of these taxa persist as small populations in minor lakes in the LVR (Kaufman and Ochumba 1993). The apparent mass extinction of hundreds of haplochromine taxa was also widely decried, not only because biodiversity is currently recognized as having intrinsic and opportunity values, but also because these fishes were most likely important in maintaining ecosystem resiliency and integrity prior to their loss (Kaufman 1992).

Trewavas (1983) reports that by 1924 *O. niloticus* had already been introduced in the LVR at Lake Bunyoni, a satellite lake in extreme southwestern Uganda, some distance from Lake Victoria, followed by subsequent plantings from either Lake Edward or Lake Albert, directly or via ponds, into many small lakes and dams throughout the LVR. By 1951 *O. niloticus* was present in Lake Victoria, probably unintentionally introduced along with deliberate introductions of *Tilapia zilli*. Intentional introductions in Lake Kyoga and surrounding minor lakes occurred in the late 1950s. *O. niloticus* is now the dominant tilapia in lacustrine habitats throughout the LVR (Ogutu-Ohwayo, 1990; Balirwa, 1992). The protracted frenzy of introductions, plus the transplantation of indigenous Lake Victoria species into minor lakes and reservoirs within the LVR, created a complex web of more or less isolated tilapia subpopulations. This tortured history greatly complicates the identification of genetically meaningful units for conservation or management. Local differentiation of tilapia phenotypes is sometimes apparent by eye, but there are few quantitative data- genetic, ecological, or morphological- to support this contention. Here

Eight RAPD primers from the M set of Operon Technologies were used for amplification. The products were scored relative to the position of a standard 123 bp DNA ladder. Bands were matched with corresponding standard band positions of migration, and were scored as '1' for present at that position, and '0' if absent from an individual lane. Each band position scored in any amplification using a primer was assumed to represent a single locus.

**Data analysis:** Band sharing proportions, similarity indices, and genetic distances were estimated following Nei and Li (1979). The genetic distances were used to determine population relationships using the Neighbor-joining method. RAPD band presence/absence in single individuals was used as character data to investigate the phylogeny of the populations by cladistic methods, using PAUP version 3.1 (Swofford, 1991). Gene frequencies and genetic statistics were estimated following Lynch and Milligan (1994). Number and proportion of polymorphic loci were determined as all loci that had equal to or less than 95% frequency in the case of a population sample of ten individuals.

## RESULTS

**Band sharing and similarity indices:** Eight RAPD primers produced a total of 177 reproducible RAPD markers (markers that could be scored on duplicate independent amplifications of the same DNA sample). Populations shared more bands within than between populations (Table 1). At a higher level, populations from the Kyoga basin (lakes Kyoga, Muwuru, Nawampasa, and Nakuwa) and Victoria basin (Lakes Victoria, Nabugabo, and Kachira) shared more bands among populations of the same basin, than between the two basins. In general, populations within the LVR had higher band sharing with Lake George and lake Edward populations than with the Lake Albert population (Table 1). The Lake Nakuwa and Muwuru populations had the highest band sharing and similarity index, and were also geographically very close to each other. The index of genetic similarity presented basically the same picture of relationship as bands sharing (Table 2). Populations from LVR had higher similarity indices to the Lake George population than to either Lake Edward or Lake Albert populations, and in many cases even when compared amongst themselves. There was also a greater similarity between populations of Lakes George and Albert than between populations of Lakes George and Edward. This was seen despite the fact that the latter two lakes are connected by an unobstructed 23km long dispersal corridor, the Kazinga Channel, and are closer to each other than either of them is to Lake Albert. Lake Edward is downstream from Lake Albert on the Semliki River, but the Semliki is very long and broken by strong rapids. There is no direct connection between the two more similar of the putative "source" populations, those from Lakes Albert and George.

**Table 1. Percent Band Sharing Between Samples**

	Nabug.	Kachira	Victoria	Kyoga	George	Albert	Nakuwa	Muwuru	Nawamp.	Edward
Nabugabo	0.491	0.326	0.315	0.209	0.274	0.233	0.258	0.253	0.198	0.258
Kachira		0.731	0.406	0.392	0.296	0.264	0.401	0.382	0.226	0.250
Victoria			0.722	0.333	0.352	0.265	0.396	0.368	0.319	0.247
Kyoga				0.667	0.357	0.259	0.430	0.402	0.364	0.309
George					0.683	0.400	0.421	0.369	0.409	0.271
Albert						0.664	0.301	0.387	0.233	0.176
Nakuwa							0.785	0.643	0.378	0.322
Muwuru								0.863	0.278	0.272
Nawampasa									0.710	0.232
Edward										0.613

**Table 2. Index of Genetic Similarity**

	Nabug.	Kachira	Victoria	Kyoga	George	Albert	Nakuwa	Muwuru	Nawamp.	Edward
Nabugabo	1.000	0.534	0.520	0.362	0.467	0.403	0.405	0.374	0.329	0.381
Kachira		1.000	0.559	0.560	0.419	0.379	0.530	0.479	0.313	0.372
Victoria			1.000	0.480	0.500	0.382	0.525	0.465	0.445	0.370
Kyoga				1.000	0.529	0.390	0.594	0.526	0.529	0.482
George					1.000	0.594	0.573	0.488	0.587	0.419
Albert						1.000	0.416	0.507	0.340	0.275
Nakuwa							1.000	0.780	0.506	0.460
Muwuru								1.000	0.353	0.369
Nawampasa									1.000	0.359
Edward										1.000

Of the nine populations, Lake Albert population exhibited the highest gene diversity, whereas Lake Edward population had the lowest level (results not shown). The Lake Albert population exhibited a relatively high number of unique alleles, compared to other populations. Lake Victoria basin populations had comparably lower levels of polymorphism than populations from Lake Kyoga basin, Lake Edward, or Lake Albert. The Lake Edward population shows much stronger similarity to the Lake Victoria basin populations in all attributes of genetic variability studied than to populations from the Lake Kyoga basin.

The degree of population structure was measured using Wright's  $F_{ST}$ , which measures the percent of genetic variability in the entire array of populations which exist as between population genetic differences. The degree of population differentiation was very high, with an estimated  $F_{ST}$  value of 0.7. Within basins, the Lake Victoria basin populations had less population substructure, while the Lake Kyoga populations showed comparably higher between population gene diversity estimates.

**Population differentiation:** Genetic distances were calculated from band sharing proportions according to Nei and Li (1979) and were used to determine relationships among the nine populations. Populations clustered by basin, comprising a metapopulation structure. A higher level of structure was evident among the basins. Lake Victoria and Lake Edward metapopulations are each others' closest relatives. The Lake Kyoga, George, and Albert metapopulations are apparently related. When individuals were analyzed cladistically using PAUP, there is a significant tendency for individuals to cluster by their respective lake populations. The Kyoga basin individuals remained a coherent group as in the population dendrograms, but the Lake Victoria basin individuals are more scattered across the cladograms.

## DISCUSSION

Even as compared to other tilapiine cichlids, *O. niloticus* is remarkable for its functional versatility and labile ecology (Fryer and Iles, 1972; Trewavas, 1983; Sanderson et al. 1996; Batjakas et al. 1997). This, together with its great adaptability to virtually all tropical and subtropical environments helps to explain its position among the top aquaculture species in the world. The successful evolution of the *O. niloticus* fishery in Lake Victoria, in the face of a voracious introduced predator - the Nile perch, is credited by Baliwra (1992) to several factors: a long history of co-existence with the Nile perch in its native range both in Lake Turkana (Rudolf) and Lake Albert (Mobutu); trophic virtuosity, allowing access to a wide range of ecological niches; and ability to breed under conditions that congeners and other species would not tolerate, such as high littoral turbidity due to deforestation. *O. niloticus* is also postulated to have the ability to swamp out closely related congeners through competition and introgressive hybridization (Fryer and Iles, 1972; Trewavas, 1983; Ogutu-Ohwayo, 1990 & 1988).

Several species were introduced to Lake Victoria together with *O. niloticus*, but none were as successful in establishing themselves as *O. niloticus*. Population structure can provide clues to help explain both their success and their durability in a rapidly fluctuating ecosystem. The *O. niloticus* populations we examined were well differentiated by basin, as indicated by the high within-basin bandsharing, smaller differences in within and between population gene diversity estimates, and difference in proportion of homozygous alternate alleles among basins. This could be due either to low levels of gene flow that exists among the respective basins, or to the uniformity of the origin of the seed used in the introductions both in source and time. Nonetheless, there was also considerable variation among populations within a basin.

The Lake Victoria metapopulation appears to have descended from Lake Edward stock, while the Lake Kyoga population appears more likely or more predominantly to have been founded by stock from Lake Albert. Results suggest that *O. niloticus* may have been established earlier in the Lake Victoria basin than in the Lake Kyoga basin. The coherent nature of the Lake Kyoga basin populations, both on the distance dendrograms and on cladograms constructed using individuals, as compared to the greater dispersal of Lake Victoria individuals on the cladograms, supports the hypothesis that the Lake Kyoga populations are the result of more recent introductions. According to Trewavas (1983), introductions of *O. niloticus* first occurred in the early 1920s in the Lake Victoria through the adjoining minor lakes, while the earliest report of introductions into Lake Kyoga are from the late 1950s and early 1960s (Fryer and Iles, 1972; EAFFRO, 1947-1967). Lake Victoria basin populations show a closer similarity to Lake Edward population than Lake Kyoga basin populations. This is certainly a difference in timing associated with the origin of the seed used in the introductions. Earlier introductions through Lake Bunyonyi and other surrounding minor water bodies into Lake Victoria basin were known to have been *O. niloticus* from Lake Edward (Trewavas, 1983), recognizable as the Lake Edward subspecies (*O. niloticus eduardianus*). Later introductions between 1950 and the mid 1960s involved a multitude of sources, including Lake Turkana, Lake Albert, and Lake George. This could explain the closer similarity between Lake Kyoga basin populations and those of Lakes George and Albert.

Greenwood (1981) noted that the Edward-George system was rife with biogeographical peculiarities. For example, Lake Edward has many endemic haplochromine species, even though the two lakes' share many other species that are found all through the Kazinga Channel. The closer similarity between *O. niloticus* populations of Lake George and Lake Albert, than between those of Lake George and Lake Edward, offers another such puzzle. The simplest explanation, however, troubling, is that *O. niloticus* populations in Lake George have been influenced by anthropogenic introductions of stock from other sources, even though the species already occurred there. Lake Albert *O. niloticus* (*O. niloticus niloticus*) could have been introduced to Lake George at the same time as the introductions into Lake Victoria. Morphologically, we have noted significant differences between Lake George and Lake Edward *O. niloticus* (Mwanja, unpublished data); and the Lake George type is known to grow faster and larger

(Kamanyi, per comm). Some may be skeptical that anthropogenic translocations could leave such a strong mark on such enormous lake systems. In response, we offer the astonishing discovery in Lake Edward, in 1996, of both the highly threatened *O. esculentus* (endemic to lakes Victoria and Kyoga), and *Tilapia rendalli*. Neither species had ever been recorded in Lake Edward before, and neither one occurs there naturally (Fryer and Iles, 1972; Trewavas, 1983). The historical biogeography of fishes in East Africa will always be of great interest due to its fascinating evolutionary implications (Greenwood 1981, Kaufman et al. 1997). This is all the more reason for practitioners to beware the oft-times unfortunate and confounding influence of humanity's fervor to reorganize the nature of Africa.

## SUMMARY

Genetic differences between populations of *Oreochromis niloticus* from various localities in Uganda have been determined using RAPDs. Populations within a lake basin (i.e., Lake Victoria or Lake Kyoga) were more similar to each other than to populations from other basins. Populations from the Lake Victoria basin were more similar to the putative source population of Lake Edward than to other possible sources, while the populations from Lake Kyoga were more similar to the putative source population sampled from Lake Albert. Populations from the Lake Kyoga basin were less diverse than those from the Lake Victoria basin, consistent with the hypothesis that introductions into Lake Kyoga are more recent and that a more diverse set of introductions may have contributed to the older populations of Lake Victoria.

## ACKNOWLEDGMENTS

We wish to thank our colleagues from the Ugandan Fisheries Research Institute for assisting in the collection of some of the samples used in this study. This research was funded by grants from the National Science Foundation (DEB-9300065 to PAF and LK and INT-9308276 to LK, L. Chapman and C. Chapman), a grant from the USDA North Central Regional Aquaculture Center to PAF, a grant from the Columbus Zoo (to PAF), a grant for subSaharan Africa Dissertation Research from the Rockefeller Foundation (to WM), and a Pew Scholars for Conservation and the Environmental Award (to LK) by the Pew Charitable Trusts.

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