The role of the Yala swamp lakes in conservation of Lake Victoria region haplochromine cichlids: evidence from molecular genetic and trophic ecology studies

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Abstract

Lake Kanyaboli, a satellite lake of Lake Victoria, has been suggested as a potential refugium for haplochromine cichlids that have gone extinct in Lake Victoria.

We employed mitochondrial DNA and microsatellite DNA molecular markers as well as feeding ecology studies to re- evaluate the evolutionary and ecological significance of Lake Kanyaboli haplochromines. The mitochondrial DNA and microsatellite markers revealed high genetic diversity in the endangered *Xystichromis phytophagus* and also the presence of mtDNA haplotypes that may have either gone extinct in Lake Victoria or have arisen *in situ*. Lake Kanyaboli thus acts as a 'genetic reservoir' for the Lake Victoria species flock.

Gut content analysis revealed six trophic groups among the six haplochromine species. The haplochromine community in Lake Kanyaboli therefore exhibits trophic specializations. The relatively high trophic diversity in this cichlid community contrasts with the currently simplified trophic relationships of Lake Victoria. This high trophic diversity contributes to high energy flow and overall ecological efficiency of the lake.

Lake Kanyaboli and similar satellite lakes therefore provide an opportunity for conservation of both genetic and trophic diversity threatened by introduction of exotics in the Lake Victoria basin. Lake Kanyaboli should thus be recognized as an important Evolutionary Signficant Unit (ESU) for Lake Victoria region haplochromine species. Basin wide molecular genetic characterization of the other tilapiine cichlid species as a basis of identifying genetically robust stocks that can be used in aquaculture or to restock Lake Victoria should be undertaken.

Key words: cichlids, conservation, trophic ecology, genetics, Victoria, Yala swamp.

Introduction

The haplochromine cichlids of Lake Victoria have been noted to have one of the highest rates of speciation and adaptive radiation among living vertebtates (Seehausen, 2002). This extraordinary adaptive radiation has been attributed to sexual selection as well as feeding specializations. Feeding specializations of the haplochromine cichlids have been instrumental in resource partitioning and therefore in shaping the cichlid community structure (Seehausen and Bouton, 1997) and maintaining the high diversity (Bouton *et al*., 1999). This trophic differentiation contributed to the evolution and adaptive radiation of the cichlid flock of Lake Victoria.

The cichlid fauna of Lake Victoria formed an important component of the fisheries of the lake and therefore played a critical role in provision of protein requirements to the riparian communities (Sumaila, 2000). However, in the 1980's the haplochromine cichlid fauna of Lake Victoria experienced unprecedented rate of extinction. Loss of the haplochromines have been attributed to pollution that hinders sexual selection (Seehausen *et al*., 1997) as well as predation from the exotic Nile perch (*Lates niloticus*, L) (Ogutu – Ohwayo, 1990).

The conservation of the remaining cichlid species is therefore of utmost importance. In order to protect the remaining populations it is important to be able to characterize them genetically. Such genetic information can be used as basis for future re-stocking or aquaculture. Some of the extinct cichlid species still thrive in small isolated water bodies (commonly referred to as satellite lakes) scattered around the Lake Victoria basin (Loiselle, 1996, Aloo, 2003). These range from small lakes to dams and reservoirs. These water bodies have been recognized to have special significance in the conservation and the future survival of these cichlids since they act as 'refugia' (Kaufman and Ochumba, 1993; Maithya, 1998).

The aim of this work was to evaluate the conservation significance of Lake Kanyaboli, the largest Yala wetland lake by studying trophic ecology of the six common haplochromine cichlid species as well as population genetics of the endangered *Xystichromis phytophagus* (Greenwood, 1965) using neutral molecular markers.

Materials and methods

The study area

The study was carried out in Lake Kanyaboli, a small (10.5 km^2) and shallow freshwater lake (average depth: 2.5 m; maximum depth: 4.5 m) situated in the Yala wetlands in Western Kenya (Fig. 1). The Yala swamp is Kenya's largest freshwater wetland and covers about 175 km^2 along the northern shores of Lake Victoria. It is bordered to the North by the Nzoia River and to the South by the Yala River. Three main lakes exist in the Yala wetlands (Kanyaboli, Namboyo, Sare), of which Lake Kanyaboli is the largest and most remote from Lake Victoria. Lake Kanyaboli is separated from Lake

Victoria by massive papyrus swamps that presently inhibit faunal exchanges between the two lakes. No Nile Perch has ever been observed in Lake Kanyaboli, corroborating that it has been isolated from Lake Victoria at least since the 1950's. The fish fauna of Lake Kanyaboli is dominated by cichlids – three species of tilapia (*Oreochromis esculentus,* (Graham, 1929) *Oreochromis variabilis* (Boulenger, 1904), and *Oreochromis leucostictus* (Trewavas, 1983) and haplochromine cichlid species (Kaufman & Ochumba 1993; Aloo, 2003). Besides its cichlid fauna, the Yala swamp is home to a rich and complex community of animals including the endangered Sitatunga antelope (*Tragecephalus spekei*) as well as papyrus endemic birds.

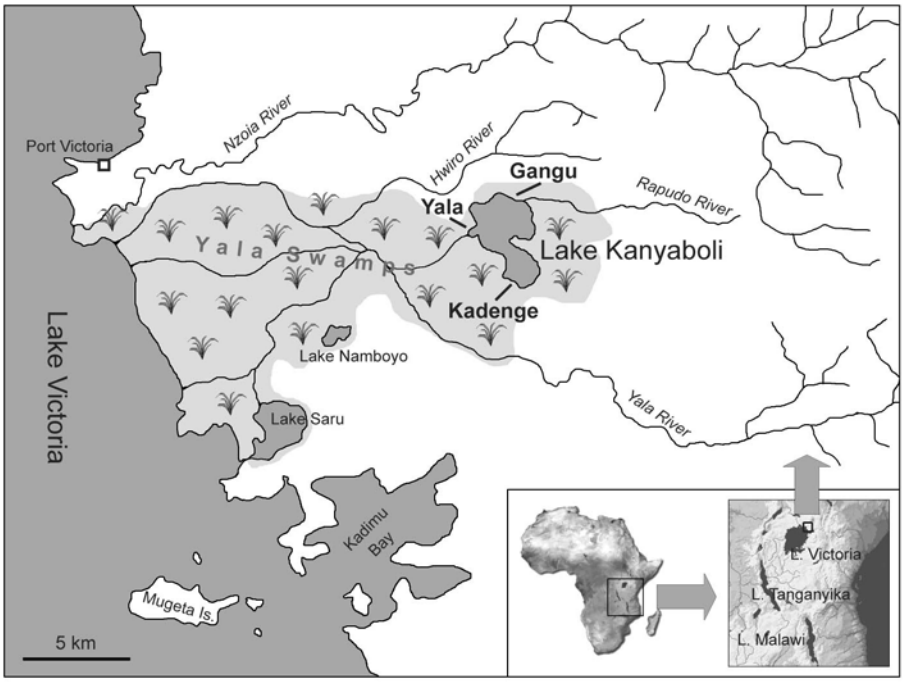

Figure 1. Map of yala swamp showing the position of Lake Kanyaboli and other associated lakes. (Re-drawn fro Crafter *et al*., 1992).

Fish sampling and trophic relationships of the haplochromine cichlids

Six species of haplochromines namely *Astatoreochromis alluaudi* (Pellegrin, 1903), *Lipochromis maxillaris* (Greenwood, 1965), *Astatotilapia nubila* (Boulenger, 1906), *Xystichromis phytophagus* (Greenwood, 1965), *Pseudocranilabrus multicolor victoriae*

(Seegers, 1990) and *Astatotilapia* 'big eye' (Kaufman, 1996) were collected from Lake Kanyaboli using a 3.81 cm multifilament gill net. Due to its small size, *P.m. victoriae* samples were obtained by angling along the papyrus fringing swamps. *X. phytophagus* specimens were grouped into three distinct populations – Kadenge, Gangu and Yala for population genetic analysis. The fish were gutted and preserved in 90% ethanol. Contribution of each food item to the diet was determined by determining the relative abundance, percentage occurrence and prominence values as described in Hyslop (1980).

DNA extraction, amplification via PCR, purification and sequencing

Muscle tissue from 90 % ethanol preserved specimens was used as source of DNA.

Total DNA was extracted by sodium chloride extraction and ethanol precipitation after initial proteinase K digestion (Bruford *et al.,* 1998).

For polymerase chain reaction (PCR) amplification of the first section of the mitochondrial control region, the fastest evolving segment of the mitochondrial genome, the published primers L-Pro-F and TDK-D were used. PCR amplification was performed in a reaction volume of 21.1 µL (9.9 µL HPLC water, 2 µL buffer, 1.6 µL 10mM dNTPs, 1.4 µL 10 mM $MgCl_2$, 2 µL of each primer/2nM, 0.2 µL TAQ DNA polymerase and 2 µL of diluted DNA) under the following conditions: 35 cycles with a denaturation phase at 94 °C for 30 s, an annealing phase at 52 °C for 30 s, and an extension phase at 72 °C for 90 s. PCR products were visualized by mini-gel electrophoresis using Ethidium-Bromide staining and 1 % agarose gels.

Two micro-liters of purified PCR product were used as template in the cycle sequencing reaction. The reaction mixture for cycle sequencing was made up of 1 µL of 10 µM L-Pro-F primer, 1.5 µL of the Big Dye termination reaction mix (Applied Biosystems) and 5.5 µL of HPLC water. The annealing temperature for cycle sequencing was adjusted to 50 °C. The cycle-sequenced products were purified

with an ethanol – sodium acetate precipitation, re-suspended in 15 µL of HPLC water and analyzed on an ABI 3100 capillary DNA sequencer (Applied Biosystems).

Microsatellite survey

Six microsatellite loci, which were developed for the cichlids *Copadichromis cylicos* (Kellog *et al*., 1995), *Tropheus moori* (Zardoya *et al.,* 1996) and the East African mollusc crusher *Astatoreochromis alluaudi* (Wu *et al*., 1999) were initially tested by sequencing to assess their utility for population studies in the haplochromine cichlids. The six microsattelites loci tested were chosen for population analyses on the basis of the repeat number in the sequenced alleles. The selected loci show atleast 10 uninteruppted CA or AC dinucleotide repeats and were, therefore, considered to exhibit sufficient potential for polymorphism for population analysis. The selected loci were TMOM11, TMOM5, UNH001, UNH002, OSU20D and TMOM27A. For sequencing, Polymerase Chain Reaction (PCR) were carried out in 21.1µl volumes (9.9 µl HPLC water, 2.0 µl buffer, 1.6 µl dNTP, 1.4 µl 10mm MgCl2, 2.0µl of each locus specific primer, 2.0 µl of diluted DNA and 0.2 µl Taq DNA Polymerase) using the above PCR conditions. The PCR product was diluted 1:10. 1 µl of the diluted PCR was added to 0.125 µl 500bp Size Standard and 0.9 µl HPLC water. Denaturation was performed for 4 minutes at 94^{o}C and the samples immediately placed on ice. Direct sequencing was carried out on an ABI PRISM^{R} 3100 automatic sequencer. Detection of microsatellite alleles in genomic DNA was achieved by end – labeling the forward primer of the pair with FAM or HEX dyes. The sequencer output was automatically analysed with an adapted GENESCAN® ANALYSIS programme (Applied Biosystems) and the fragment size (genetic loci polymorphism) determined (scored) using GENOTYPER® software.

Data analysis I: Mitochondrial control region

For the mtDNA data genetic variability was estimated by calculating the haplotype diversity in each population. The genetic differences between the sampled populations of the *Xystichromis phytophagus* were tested using F – statistics (The fixation index) (Weir and Cockerham, 1984) as calculated by ARLEQUIN^{R} 2.000 sofftware (Schneider *et al*., 2000). The fixation index, F, serves as a convenient and widely used measure of genetic differences between populations (Wright, 1978). Theoretically F_{ST} has a minimum of 0, indicating no genetic difference and a theoretical maximum of 1, indicating fixation for alternative alleles/ haplotypes in the sub – populations.

Data analysis II: Microsatellite markers

For the microsatellite data the genetic polymorphism was estimated for each population with GENEPOP 3.1d (Raymond and Rousset, 1995) as implemented in the ARLEQUIN^{R} 2.000 sofftware (Schneider *et al*., 2000) as the number of alleles per locus (N_A), the observed (H_0) and the expected heterozygosity (H_E). Departure from Hardy – Weinberg expectation for every locus was calculated in each population and between populations using a test analogous to Fisher's exact tests (Guo and Thompson, 1992) estimated with a 100 000 step, 1000 iteration, Monte Carlo Chain series of permutation, as implemented in the software ARLEQUIN^{R} 2.000 (Schneider *et al.*, 2000). Genotypic linkage disequilibrium was evaluated with GENEPOP 3.1d as implemented in the ARLEQUIN^{R} 2.000 software (Schneider *et al*., 2000). Differences between populations in their haplotypic (mtDNA sequences) and allelic (microsatellites) distributions was tested with an exact test of population differentiation (Raymond and Rousset, 1995) using the software ARLEQUIN^{R} 2.000 (Schneider *et al.,* 2000) as F_{ST}, the pairwise fixation indices, based on haplotype or allele frequency variations. The significance of genetic subdivision was assessed using 1000 permutations.

Results

Feeding and trophic relationships of the haplochromine cichlids

Gut content analysis revealed that eight food items comprised the diet of the six haplochromine species. These food items are algae (both blue green algae and diatoms), chironomid and *Chaoborus* larvae, other unidentified insects, mollusks, fish embryos, fish eggs, plant remains and detritus. Based on frequency of occurrence, chironomid and chaobarus larvae was the main food taken, occurring in 64.3 % of the guts examined, followed by plant remains (43.8%), detritus (37.5%) and other insects (33.5%). All the six haplochromine species examined fed on chironomid/*Chaoborus* larvae. Molluscivory and paedophagy (egg and embryo feeding) were restricted to *Astatorechromis alluaudi* (Pellegrin, 1903) and *Lipochromis maxillaris* (Greenwood, 1980) respectively. The two food items therefore contributed less to the total amount of food items taken by the haplochromines. Only 15.2% of the guts examined contained mollusks and 16.1% and 6.25% contained fish embryos and eggs respectively.

Based of relative abundance chironomid and chaobarus larvae constitutes the highest amount of food taken (29.3%). The low values of relative abundance shows that each type of food is taken in low quantities. The overall diets of the six haplochromine species expressed as frequency of occurrence and relative abundance are given in table 1. *Astatotilapia nubila* (Boulenger, 1906) can be classified as an insectivore, *Astatotilapia* 'big eye' (Kaufman, 1996) as an algivore, *Lipochromis maxillaris* as a paedophage, *Xystichromis phytophagus* (Greenwood, 1965) as a plant feeder, *Astatoreochromis alluaudi* as a molluscivore and *Pseudocranilabrus multicolor victoriae* (Seegers, 1990) as an algivore.

Population genetic structure inferred from the mitochondrial DNA sequences

DNA sequence of the 400 base pair segment of the mtDNA revealed 11 distinct haplotypes present in the 205 specimens of *Xystichromis phytophagus* from Lake Kanyaboli. Mitochondrial DNA thus revealed high genetic diversity within this species. Private haplotypes were only found in two populations, Gangu and Kadenge, however in very low abundance. The haplotype frequency especially of the three main haplotypes occurring in 83.9% of all specimens was similar in the three populations (Table 2).

Population genetic structure inferred from the microsatellites

The six microsatellite markers exhibited high polymorphism in *X. phytophagus* characterized by multiple numbers alleles. A total of 152 alleles were found in 191 individuals. At each of the six loci the total number of alleles observed ranged from 12 to 20 in TMOM5, 13 to 19 in TMOM11, 21 to 24 in UNH001, 11 to 14 in UNH002, 30 to 34 in OSU20D and 10 to 12 in TMOM27A. Except for the locus TMOM27A, the allele frequencies for the most frequent alleles were low (less than 0. 3). The microsatellite alleles exhibit similarities across the three populations although some alleles were restricted to only one of the three populations (Tables 25 - 30). Levels of allelic diversity were consistently high in each of the three populations (19.2 $\pm$9.6). For all the loci, the same alleles were the common alleles in all populations.

Genetic diversity as measured by expected heterozygosity was high (0.89$\pm$0.00025) (Table 3). The observed heterozygosity was high and ranged from 0.83 to 0.96 in TMOM5, 0.84 to 0.85 in TMOM11, 0.89 to 1.00 in UNH001, 0.83 to 0.97 in UNH002, 0.85 to 0.93 in OSU20D and 0.48 to 0.79 in TMOM27A. The genotype frequency at locus TMOM27A showed relatively high deficiency of heterozygotes in the three populations. This locus thus probably has null alles. The locus OSU20D exhibited the highest variability (i.e. most polymorphic) with 30 to 40 alleles per population while the locus TMOM27A with 10 to 12 alleles per population was the least variable. Genotype frequencies at the loci UNH002 and TMOM27A showed deviations from Hardy – Weinberg expectations in the Yala population while the loci TMOM11, UNH002, OSU20D and TMOM27A showed deviations in the Kadenge populations. All loci were in accordance with the Hardy – Weinberg expectation in the Gangu population ($P < 0.05$). There was also no evidence of linkage disequilibrium between any pair of loci in any of the three populations.

Discussion

A relatively high number of trophic groups was observed in this study compared to the lower number of trophic groups in the Nile perch impacted lakes of Victoria region. The absence of Nile perch in Lake Kanyaboli can be attributed to the presence of the massive swamp separating Lake Kanyaboli and Lake Victoria. Similar higher trophic diversities have also been made in the satellite lakes Nawampasa, Gigati and Agu in Uganda that have been impacted by the Nile perch (Mbabazi *et al*., 2004). Greenwood (1981) and van Oijen (1990) showed that the pre Nile perch Lake Victoria haplochromines exhibited diverse feeding habits including detritivory, insectivory, higher plant feeding, zooplanktivory, molluscivory, paedophagy and piscivory. The presence of Nile perch has however simplified the trophic structure of the haplochromines from the above diverse trophic groups to only two belonging to a single trophic level (Namulemo, 1998). Most of the pre Nile perch trophic groups are however still represented among the six Lake Kanyaboli haplochromines and here they occupy three trophic levels i.e primary, secondary and tertiary levels. Lake Kanyaboli therefore has a more direct flow of energy from primary to tertiary consumers through the haplochromines and as such the haplochromines play an important role in energy flow and overall ecological efficiency of the lake system.

Understanding the spatial and temporal dynamics of endangered species and populations are critical to effective conservation and management (Soule, 1987). One goal of conservation biology is to conserve genetic diversity and evolutionary processes. Quantification of levels of genetic biodiversity in extant endangered species is being recognized as important in the recognition of taxonomic units in need of protection (Avise, 2000, Moritz, 1994). Most endangered species exhibit very low genetic variabilities probably due to genetic drift and historical bottlenecks in population size. In some case studies, plausible arguments have been advanced for a direct association between observed molecular variability and the long – term viability of an endangered taxon (Soule, 1987). An understanding of the genetic structure of a population is also key to our understanding of the importance of genetic resources and the importance of genes for the conservation of species and biodiversity. In the broadest sense, conservation of the genetic diversity and intergrity of a species relies on identifying the critical genetic units and then managing these units in a co-ordinated manner (Lesica and Allendorf, 1995).

Both molecular markers employed in this study have revealed high genetic variability within *Xystichromis phytophagus*. The high genetic variability exhibited in this species augurs well for the species and implies that genetic variability has been conserved in this species. This reflects a historically large effective population size and lack of population bottlenecks in the past. The theory of genetic drift predicts that bottlenecked populations should exhibit very low heterozygosities. Large effective population

sizes are important in minimizing the effects of drift which may lead to population differentiation even in the presence of gene flow. The species is thus not in immediate danger of extinction in Lake Kanyaboli.

One strategy that has been proposed to restore the cichlid populations in the Lake Victoria basin has been aquaculture and captive breeding (Maithya and Okeyo-Owuor, undated abstract). The success of such aquaculture ventures rely on identifying genetically pure and robust populations as a source. The finding of a genetically robust population of *X. phytophagus* in Lake Kanyaboli indicates that this population can be used as source to re-stock other genetically depauparate lakes of the Lake Victoria region (Loiselle, 1996). The high genetic diversity occurring in the *X. phytophagus* population can be used as a strong case to support this. There is therefore need to genetically characterize other cichlid populations within the Lake Victoria satellite lakes. Among the tilapiines, populations of *Oreochromis esculentus* (Graham, 1929) and *Oreochromis variabilis* (Boulenger, 1904) still thrive in Lake Kanyaboli and other Yala swamp lakes. Molecular genetic characterization of such populations can form the basis of 'fingerponds' environmentally sustainable aquaculture along the fringes of the lakes.

This study has shown that Lake Kanyaboli provides an opportunity for conservation of not only species but also of trophic and genetic diversity threatened by introductions of exotics and other anthropogenic impacts in Lake Victoria. Lake Kanyaboli is therefore an important refugium to Lake Victoria haplochromines. There is an urgent need to spearhead the conservation of this lake. Because of the critical socio – economic role Lake Kanyaboli plays in the lives of the local community (Abila, 2002, 2005) it is strongly recommended that any conservation initiatives be community based. Unfortunately, ongoing land use changes ostensibly to improve food security in this area have greatly altered the wetland and is major threat to the future survival of its biodiversity.

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