GENETIC DIVERSITY AND POPULATION STRUCTURE OF *OREOCHROMIS MOSSAMBICUS* IN THE LOWER SHIRE RIVER SYSTEM OF MALAWI

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Abstract

The Lower Shire is renowned for large biomasses of *O. mossambicus*, *O. placidus*, *Clarias spp* and *Bargus spp*. The study investigated the genetic variability and population structure of *O. mossambicus* populations in three strata at six microsatellite loci. The populations exhibited considerable allelic variability with a mean of 21 alleles per locus, a total allele number range of 4-40 and allele size range of 68-103 base pairs. Three diversity indices (observed number of alleles (na), effective number of alleles (ne) and Shannon Information Index (I), revealed that Lisuli and Magamba were the least and most genetically diverse populations respectively. Based on the three diversity indices, the three strata exhibited similar allelic diversity. Most populations (71%) were not in HWE probably due to sampling error caused by Wahlund effect. This assertion is

supported by 73% homozygosity excess and high gene flow observed among the populations (*Nm*=3). Mantels test showed that genetic relationships among the populations are less influenced by geographical distance (r = 0.23; *p*=0.9410) implying that the populations do not fit into the isolation by distance model. Nevertheless, the populations are significantly differentiated

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($p \leq 0.05$) and moderately structured (F_{ST} = 0.08). Conservation efforts, therefore, need to concentrate on each population since the populations are still distinct though going toward homogeneity given the high gene flow.

Keywords: *O.mossambicus*, microsatellite loci, Stratum, Populations, Genetic diversity, Lower Shire, Conservation

Introduction

The fisheries sector is important in Malawi, contributing 4% of the GDP (GoM, 2010). Until 1980s, fish used to constitute 60-70% of all animal protein intake of Malawians (Tweddle, 1995) but recent estimates put the figure at 28% (Jamu and Chimatiro 2005) owing to overfishing resulting from high human population growth rate (CIA, 2010).

Over 70% of the country' fish is supplied by Lake Malawi alone (Turner 1995). Apart from Lake Malawi and other lakes, perennial rivers and streams form an important component in the Malawi fishery by providing fish breeding grounds and habiting large biomass that is exploited by communities living along the rivers (Tweddle, 1985). Hastings (1973) observed that in 1972, the Lower Shire recorded total fish production of 10,000 tonnes, of which 94% comprised *Oreochromis* and *Clarias* spp. Recent riverine fish biodiversity survey by Likongwe (2005) showed that 14 rivers of the central and southern Malawi including Shire river were inhabited by 199 fish taxa belonging to 13 families. Cyprinidae was the largest family constituting 36% of the riverine taxa followed by cichlidae (23%) and the upper Shire and lower Ruo registered the highest number (39) of species (Likongwe, 2005). However, Banda and Tomasson (1997) observed that human activities adversely affected fish production of commercially important lacustrine and fluviatile fishes in Malawi. These activities included the destruction of fish breeding grounds, overfishing and disturbance of fish migration. Chimatiro and Mwale (1998) reported that fish production in the Lower Shire dropped from a range of 5,000-10,000 tonnes to 1,000-2,000 tonnes between 1983 and 1996 respectively. The number of fishermen ranged between 2,000-3,000 while fishing gears ranged between 4,000-7,000 (Chimatiro and Mwale, 1998). During the same period, the National Statistical Office (NSO, 1998) recorded a 78% population increase (from 301,183 in 1977 to 537,145 in 1998) within two decades in the Lower Shire districts of Chikwawa and Nsanje and estimated that close to 75% of the population is

engaged in fishing. The Lower Shire, has a record of 61 fish species but *O. mossambicus, O. placidus, Clarias* spp and *Bargus spp*. have large biomasses and economic importance (EAD, 2010).

This study, therefore, endeavoured to determine the intra-stratum and inter-stratum genetic diversity and population structure of *O. mossambicus* populations in the lower Shire using microsatellite markers in order to provide information to fisheries and aquaculture sectors for developing strategic management protocols for the species. The study also used *O. mossambicus* populations as case study for assessing the power of DNA tools in resolving wildlife forensic issues.

Materials and methods

Description of the study area

The study was carried out in the Lower Shire which is the lower course of Shire river, the largest river in the country, characterised by wetlands because of the topography of the area (Figure 1). The Fisheries Department of Malawi subdivided the wetlands into eight minor strata for fisheries management purposes. These strata are, North West Elephant Marsh, South West Elephant Marsh, North East Elephant Marsh, South East Elephant Marsh, Bangula Lagoon, West Ndinde Marsh, East Ndinde Marsh and Shire River (Chikwawa-Nsanje). However, the study concentrated in the following three main areas (strata); Chikwawa (Gumbwa, Lisuli, Malemia), Elephant Marsh (Mwala, Kadamera, Maere) and Ndinde Marsh (Ngwangwa, Malandiro, Magamba, Bitilinyu, Marka) (Figure 1).

Figure 1: Map of the Lower Shire showing sample sites

Sample collection and tissue extraction

A minimum of 40 fish samples were collected in August 1999 from 11 sites from three main fishing areas, namely, Chikwawa lagoons in Chikwawa, Elephant Marsh at Chiromo and Ndinde Marsh in Nsanje (Figure 1, Table 1). Muscle tissue of 5-10mm^2 were removed from the left side of dead fish specimens and preserved in 95% ethanol in well labelled 2ml vials. Tissue samples were taken to the Molecular Biology and Ecology Research Unit (MBERU) laboratory, Department of Biological Sciences, Chancellor College for DNA analysis.

Table 1 Fish population names/sampling sites and sample sizes

Site	Population ID* number	Sample size

Chikwawa (*Stratum 1*)		
Gumbwa	1	50
Lisuli	2	50
Malemia	3	50
Elephant Marsh (*Stratum 2*)		
Mwala	4	50
Kadamera	5	50
Maere	6	50
Ndinde Marsh (*Stratum 3*)		
Ngwangwa	7	50
Malandiro	8	34
Magamba	9	50
Bitilinyu	10	50
Marka	11	50

*ID = identification. In some of the tables and figures below, population ID number has been used instead of site or population name.

DNA Extraction

Genomic DNA was extracted using a protocol outlined in Ambali (1996). Muscle tissue of 3mm^2 size was macerated and placed in a 2.0ml microfuge tube containing 1.0ml high TE (100mM Tris-Cl, 40mM EDTA). The mixture was vortexed for 30s and left to stand for 10min before aspirating off the high TE. Extraction buffer (250µl) (10mM Tris-Cl pH=8.3, 1mM EDTA, 200mM LiCl, 0.8% SDS) and 2.5µl Proteinase K were added to the microfuge tube and incubated at 50°C in an automated Advantec water bath for three hours with intermittent mixing until the tissue was completely digested. This was followed by vortexing the mixture and centrifugation at 15000rpm for 5min. The supernant was transferred into new 2.0ml microfuge tube, 500µl TE (10mM Tris-Cl, 1mM EDTA) was added and vortexed. The mixture was precipitated in 750µl cold Isopropanol placed under -20°C for one hour after addition of 35µl of

NaCl. The DNA pellet was separated from the suspension by centrifugation at10000 rpm for 10min and decanting the supernatant. The pellet was rinsed in 500µl 70% ethanol and centrifuged again at 10000 rpm for 1 min. The ethanol was decanted and the DNA air-dried for 15min and rehydrated in 100µl low TE buffer and stored at -20°C.

DNA amplification and PCR products detection

DNA was amplified in 12.5 µl final volume at six polymorphic microsatellite loci (Table 2). The template DNA (2µl) was placed in 0.2ml PCR tube to which 10.5µl of the PCR master mix cocktail was added. The PCR master mix consisted of 5.7µl PCR grade water (ddH_2O), 1µl of 10mM dNTP mix, 1.25 µl of 10 x PCR buffer, 1µl of 25mM Magnesium Chloride ($MgCl_2$), 0.2µM (0.75 µl) each of both forward and reverse primers, 0.06µl of 5U AmpliTaq-Gold. The amplification were carried out in a Perkin Elmer GeneAmp PCR system 9600 thermocycler with the following PCR conditions: one AmpliTaq-Gold activation cycle at 95°C for 12 min, followed by 10 amplification cycles, each consisting of a 30s denaturing step at 94°C, 15s annealing step at primer specific temperature, and a 30s extension step at 72°C. This was followed by another 25 cycles, each consisting of denaturing step at 89°C for 30s, annealing step at primer specific temperature for 15s and extension step at 72°C for 15s. The final extension was at 65°C for 20min followed by a soaking temperature of 4°C.

Amplified microsatellite bands were visualized using silver sequence staining procedure on 6% polyacrylamide gels (26 ml of ultra pure water, 7.5 ml of 40% acrylamide:bis (19:1) solution, 5.0 ml of 10 X TBE, 14µl of TEMED (N,N,N',N'-tetramethylethylenediamine), and 357µl of 10% Ammonium persulphate) which were poured in BIORAD Sequi-Gen® GT nucleic acid electrophoresis cell. Two band size standard markers {pGem DNA marker and X174 DNA *Hinf* 1(Promega, USA)} were used to score the bands over a light box.

Table 2 Microsatellite primer sequences used in this study and their annealing temperatures

Primer	Sequence	T_{ann}(°C)
Os-08 F	5`- AGAGGAAATGAGCAGCCTC -3`	54
Os-08 R	5`- GATGCGGCAACAGTTATGTC -3`	
Os-64 F	5`- CAGTGTCTTCAGTTCCTTGC -3`	54
Os-64 R	5`- CAGAAGCATCTTATTGATGAC -3`	
Os-75 F	5`- AGCCTAAAATAATGGAATCAC -3`	49
Os-75 R	5`- CCACAGAGTCATGGTTCAC -3`	
UNH-103 F	5`- CAATGTCCATCCTTCCT-3`	54
UNH-103 R	5`- CTGTCTGACTGCAAATGTAA -3`	
UNH-132 F	5`- ATATAAGAAACTGAGTCGGTGAG -3`	54
UNH-132 R	5`- TGGAAATAGAGGGTGGGTGAG -3`	
UNH-154 F	5`- ACGGAAACAGAAGTTACTT -3`	54
UNH-154 R	5`- TTCCTACTTGTCCACCT-3`	

Data analysis

POPGENE version 1.31 computer program (Yeh *et al*., 1999) was used to compute a number of measures of genetic variation within and between populations. The following variables were computed to determine the genetic diversity among populations: number of alleles per locus, total number of alleles in each population, mean observed number of alleles per population (na) and effective number of alleles per population (ne) (Crow and Kimura, 1970), heterozygosity, Shannon's (1949) Information Index (I) and Nei's (1978) unbiased genetic distance.

NTSYS pc version 2.1 (Rohlf, 2001) was used to generate symmetric pair wise dissimilarity matrix from Nei's (1978) unbiased genetic distance which was used to construct a dendrogram from Unweighted Neighbour-joining clustering method (Saitou and Nei, 1987)

Genepop (Raymond & Rousset 1995) was employed to conduct the following analyses: test for conformity to Hardy-Weinberg Equilibrium (Haldane, 1954; Weir, 1990; Guo & Thompson, 1992), test for genotyping linkage equilibrium, test of genic and genotypic differentiation, estimation of effective number of migrants using Slatkin's private allele method (Slatkin, 1985) and computation of Wrights statistics (Wright, 1969).

Mantel's test was done to determine correlations between geographical and genetic distance matrices among the *O. mossambicus* populations. The MXCOMP programme of NTSYS pc version 2.1 (Rohlf, 2001) was used to compute a product-moment correlation coefficient (i.e. normalized mantel's statistics Z) for the two matrices (Rohlf, 2001). In order to determine if the correlation was significant, actual coefficient was compared to the values produced by randomly permuting the matrix pair 5000 times.

WHICHRUN version 4.1 program (Banks and Eichert, 2000) was used to estimate allocation of individuals to their most likely source population or stratum.

Graph pad PRISM version 3.00 for Windows (GraphPad Software, San Diego, California, USA) www.graphpad.com.) was used to plot standard error graphs for mean observed number of alleles (na), effective number of alleles (ne) and Shannon's Information Index (I) per stratum.

Results and Discussion

Allelic variability

A high proportion (13 out 15; 87%) of locus pairs studied showed no significant linkage disequilibrium (non-random assortment of alleles between two loci) ($p \leq 0.05$) (data not shown). These loci were therefore not linked and thus a full array of genotypes reflected random combination for each locus (linkage equilibrium). The populations exhibited considerable allelic variability at the six loci with a mean of 21 alleles per locus and a total allele number range of 4 (Os 64)-40 (UNH 154) (Table 3). Allele sizes ranged from 68bp in Os 75 to 272bp in UNH 103 (Table 3). Ambali (1996) obtained similar allele size ranges using the same Os primers on domesticated *O. mossambicus* [Os-08 (100-140), Os-64 (123-133), Os-75 (84-126)] (Table 3).

Table 3 Total number of alleles per locus (A), and allele size range (SR) in base pairs (bp)

Locus	A	SR
Os-08	13	100-148

Os-64	4	122-138
Os-75	27	68-136
UNH-103	31	148-272
UNH-132	13	114-160
UNH-154	40	102-212

Ambali (1996) scored 6 alleles at locus Os-08, 3 alleles at locus Os-64, and 7 alleles at locus Os-75 in domesticated *O. mossambicus* whereas 13, 4, and 27 alleles were scored at loci Os-08, Os-64 and Os-75 in these wild populations of this species respectively. The general reduction in allelic diversity among domesticated *O. mossambicus* as reported by Ambali (1996) compared to the present study would be due to reduced population size and inbreeding that is usually observed when stocks are isolated from wild populations (Hedgecock and Sly, 1990). Significant reductions in heterozygosity have been found between progenitor populations and hatchery stocks of fish species (Ryman and Stahl, 1980). Wada (1986) observed a gradual reduction in number of alleles per locus and observed heterozygosity in pearl oysters, *Pinctada fucata martensii*, selected for six generations when compared with cultured stocks collected from the same location as the base population.

Genetic diversity indices

The study used three indices of genetic diversity; observed number of allele (na), effective number of alleles (ne) and Shannon Information Index (I). The observed number of alleles (na) ranged between 7.67±0.36-10.67±0.61 while the effective number of alleles (ne) ranged between 3.66±0.17-5.55±0.29 and Shannon's information index (I) ranged between 1.48±0.06-1.77±0.11 (Table 4). Observed heterozygosity ranged between 0.40-0.61 and expected heterozygosity ranged between 0.68-0.78 (Table 4). The allele number ranges among the three indices and the observed heterozygosity range are indicative of high levels of genetic variation in all populations. In general, the mean effective number of alleles (ne) was lower than observed number of alleles (na). This is so because of high number of alleles observed in microsatellites loci, some of which have low frequencies and contributed very little to the average genetic variance (Crow and Kimura, 1970). On the basis of the three indices, Lisuli (stratum 1) was the least genetically diverse among the *O. mossambicus* populations while Magamba (stratum 3) was

the most diverse. Generally, all the population in three strata, exhibited similar allelic diversity. This observation is supported by similar trends among the three genetic diversity indices as depicted in Figure 2. Mean of observed number of alleles was 9.06±0.45, 9.39±0.50 and 9.44±0.52 in strata 1, 2 and 3 respectively. Mean of effective number of alleles was 4.5±0.26, 4.87±0.25 and 5.08±0.27 in strata 1, 2 and 3 respectively. Mean of Shannon's information index was 1.63±0.07, 1.68±0.07 and 1.70±0.08 in strata 1, 2 and 3 respectively.

Table 4 Summary of genetic variation statistics for all populations

Population	na*	ne*	I*	Obs-Het	Exp-Het**
Gumbwa	9.17±0.47	4.46±0.20	1.65±0.06	0.57	0.73
Lisuli	7.67±0.36	3.66±0.17	1.48±0.06	0.50	0.68
Malemia	10.33±0.51	5.38±0.41	1.74±0.08	0.56	0.73
Mwala	8.00±0.40	4.47±0.22	1.58±0.07	0.40	0.71
Kadamera	9.50±0.49	4.75±0.18	1.72±0.06	0.49	0.75
Maere	10.67±0.61	5.38±0.34	1.75±0.08	0.52	0.73
Ngwangwa	10.50±0.59	5.35±0.36	1.74±0.09	0.58	0.73
Malandiro	9.17±0.58	5.06±0.28	1.69±0.07	0.56	0.75
Magamba	10.17±0.63	5.55±0.29	1.77±0.11	0.61	0.78
Bitilinyu	8.17±0.37	4.35±0.19	1.59±0.06	0.55	0.72
Marka	9.17±0.44	5.07±0.25	1.71±0.07	0.52	0.74

na* = observed number of alleles, ne* = effective number of alleles (Kimura and Crow, 1964); I* = Shannon's information index (Lewontin, 1972); ** Expected heterozygosity (Levene, 1949)

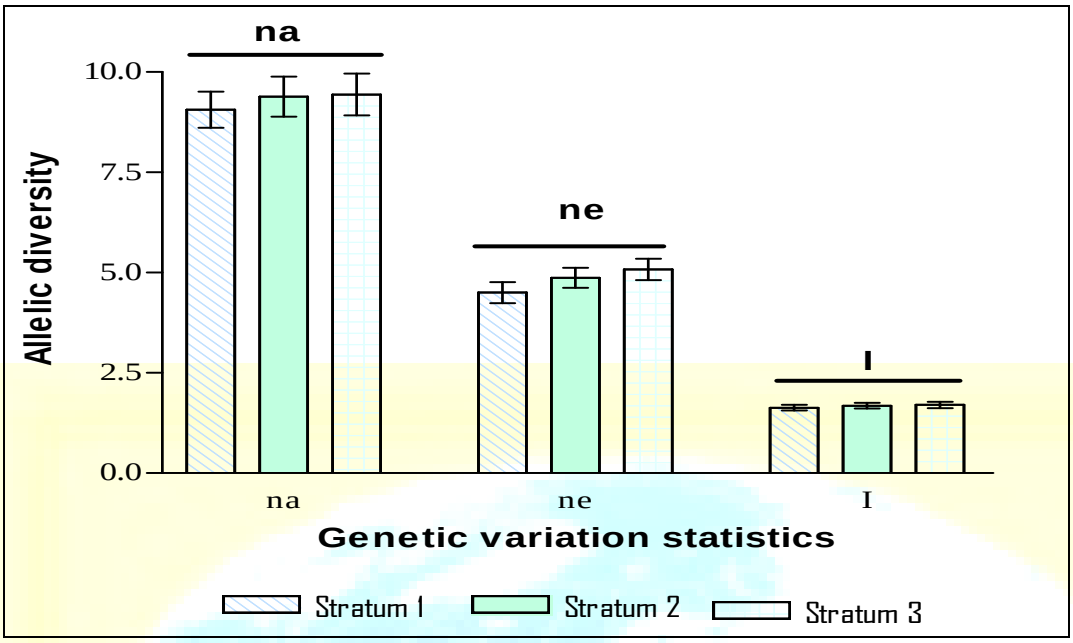

Figure 2: Summary of genetic variation statistics for all loci per stratum.

(na = observed number of alleles, ne = effective number of alleles, I = Shannon's Information Index)

Conformity to the Hardy-Weinberg Equilibrium and heterozygosity

Probability values for test for Hardy-Weinberg Equilibrium are presented in Table 5. The test showed that 19 (29%) of the 66 locus-population combinations were in no significant departure from HWE ($p > 0.05$) while 47 (71%) were in significant departure from HWE ($p < 0.05$). Highest conformities to HWE (91%) were observed in Os-64 and highest departures to HWE (100%) were observed in Os-75. With the exception of locus Os-64-population combinations, most populations were not in HWE probably due to sampling error caused by Wahlund effect (Hartl and Clark, 1989) which implies that the populations were a mixture of fish from more than one population. This assertion is supported by 73% homozygosity excess observed among the populations (Table 6) and high migration among populations of three individuals per generation (Table **7**).

Table 5 Probability values of Chi-square test for Hardy-Weinberg Equilibrium

Population	Os-08	Os-64	Os-75	UNH-154	UNH-103	UNH-132
Gumbwa	0.001	0.438	0.000	0.000	0.218	0.331

Lisuli	0.000	0.368	0.000	0.076	0.195	0.000
Malemia	0.000	0.438	0.000	0.000	0.002	0.000
Mwala	0.000	0.647	0.000	0.000	0.000	0.000
Kadamera	0.000	0.242	0.000	0.000	0.000	0.000
Maere	0.133	0.822	0.000	0.000	0.005	0.000
Ngwangwa	0.000	0.579	0.000	0.423	0.000	0.000
Malandiro	0.000	0.394	0.000	0.279	0.000	0.000
Magamba	0.000	0.000	0.000	0.000	0.292	0.000
Bitilinyu	0.011	0.647	0.001	0.000	0.000	0.000
Marka	0.000	0.579	0.000	0.000	0.098	0.000

Heterozygosity excess or deficiency values (F_{IS}) are presented in Table 6. Mean F_{IS} values were positive indicating heterozygosity deficiency in all populations. The inbreeding coefficient values (F_{IS}) showed heterozygote deficiency at loci Os-08, Os-75, UNH-132, UNH-154 and heterozygote excess was 100% at locus Os-64 followed by 64% at locus UNH-103. This observation shows that on average the populations experienced homozygosity excess which is evidenced by high mixing of populations as exhibited by high rates of migration (Table 7) within and among populations. Several studies that used microsatellite have reported similar significant departure from HWE (Bruford and Wayne, 1993) and in most cases there tend to be more heterozygote deficiencies than excess (Devlin *et al*., 1990). Durand *et al*. (1993) observed that in natural populations of oysters heterozygote deficiency was common and heterozygote excess was common in hatchery stocks due to selective breeding and change in selective constraints in an artificial environment.

Table 6 Heterozygosity excess and deficiency (F_{IS}) using Weir and Cockerham method*

Pop.\Locus	Os-08	Os-64	Os-75	UNH-103	UNH-132	UNH-154	Mean F_{IS}
Gumbwa	0.226	-0.203	0.089	0.042	0.890	0.059	0.184
Lisuli	0.068	-0.207	0.563	-0.163	0.775	0.355	0.232
Malemia	0.302	-0.222	0.431	-0.123	0.565	0.237	0.198

Mwala	0.397	-0.162	0.394	0.233	0.925	0.528	0.386
Kadamera	0.021	-0.313	0.578	0.127	0.806	0.508	0.288
Maere	0.215	-0.118	0.229	0.073	0.655	0.389	0.241
Ngwangwa	0.337	-0.199	0.420	-0.101	0.551	0.035	0.174
Malandiro	0.444	-0.249	0.401	-0.071	0.493	0.172	0.198
Magamba	0.574	-1.000	0.234	-0.092	0.698	0.379	0.132
Bitilinyu	0.156	-0.170	0.220	-0.059	0.725	0.268	0.190
Marka	0.447	-0.166	0.266	-0.120	0.580	0.532	0.257

* negative F_{IS} values indicate heterozygosity excess while positive values indicate heterozygosity deficiency. Heterozygosity excess was exhibited by 27% of the population-locus combinations while 73% showed heterozygosity deficiency.

Genetic structure and differentiation among populations

Tests for a genic and genotyping differentiation showed that all the populations were significantly differentiated ($p \leq 0.05$; data not shown). The estimate of population differentiation (F_{ST}) among the population generally showed moderate differentiation of 8% which is supported by high gene flow of three individuals per generation(Table 7). Wright (1978) considered F_{ST} values ranging from 5-15 as illustrating moderate differentiation. Migration rate was highest (Nm=4.63) within stratum 2 and lowest (Nm=2.74) within stratum 3 (Table 7). Generally, the rates of migration were high since studies have shown that $Nm \geq 1$ leads to considerable homogeneity among populations while population structuring and divergence can occur when $Nm \leq 1$ (Mills and Allendorf, 1996).

Table 7: Estimates of number of migrants per generation (Nm) and fixation index (F_{ST})

Populations/strata	Nm	F_{ST}
For all populations	2.82	0.081
Within stratum 1	4.21	0.056
Within stratum 2	4.63	0.051
Within stratum 3	3.27	0.071
Stratum 1-Stratum 2	3.11	0.074

Stratum 2- Stratum 3	3.25	0.072
Stratum 1-Stratum 3	2.74	0.084

Genetic relationships and cluster analysis of the populations

Figure 3 shows genetic relationships among the populations based on Nei's (1978) genetic distances. Population pairs (Bitilinyu and Marka) and (Malandiro and Magamba) of stratum 3 are both geographically and genetically close while Lisuli and Malemia of stratum 1 are genetically close but geographically separated. Kadamera and Maere (stratum2), Ngwangwa and Malandiro (stratum3) are both geographically close but isolated genetically. Mantel's test, which determined the correlation between geographical distance (data not shown) and genetic distance (data not shown) among the populations revealed positive but weak insignificant correlation ($r = 0.23$; $p=0.9410$). The low relationship between genetic and geographical distance suggest that these populations do not fit well with isolation by distance model (Wright 1943). Under the model gene flow is highest among close populations, therefore is expected that close populations should have similar genetic composition which is not widely the case in these populations. Therefore, moderate structuring ($F_{ST}=0.081$) observed among the in *O. mossambicus* populations is not necessarily due to isolation by geographical distances.

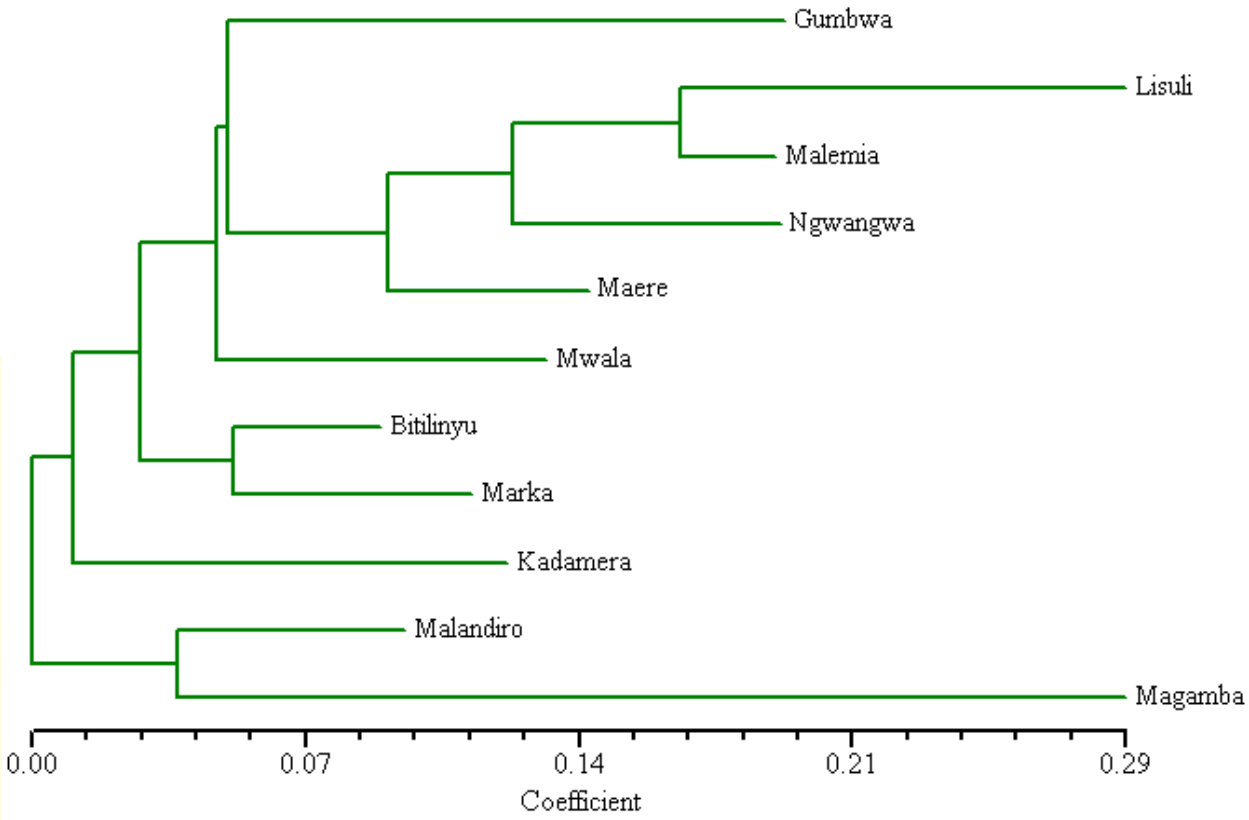

Figure 3: Dendrogram based on Nei's (1978) Genetic Distance using Neighbour-joining clustering method

The power of DNA in resolving wildlife forensic issues

Table 8 shows probability values (%) for assigning an individual from unknown population to the right population. P-values for making right identification ranged from 57-95% (bold). The study also showed there were high probabilities of assigning an individual fish from unknown population to the right river stratum (Table 9). The P-values for making right identification ranged from 52-100% (bold). The probabilities for assigning individuals to right sources were considerably high (52-100%) at population level and (57-95%) at stratum level. Individual fish that were purchased from markets in the area were assigned to other sources than those indicated by the fish traders. Such analysis would be useful in cases were fishing regulations are flouted by fishers especially where closed seasons are used as means for controlling exploitation of juvenile fish.

These results demonstrated the power of DNA tools in resolving wildlife forensic issues. Indeed, the high variability of microsatellites has enabled them to be important tools for wider applications.

Table 8: Probability (%) of assigning an individual from unknown population (*horizontal*) to the known population (*vertical*) based on WHICHRUN analysis (Banks and Eichert, 2000)

	1	2	3	4	5	6	7	8	9	10	11	%Total
1	**83**	3	2	2	2	0	3	0	0	0	5	100
2	0	**83**	8	5	0	2	0	0	0	0	2	100
3	2	11	**65**	2	0	2	8	0	0	2	8	100
4	2	8	0	**76**	2	5	0	0	0	2	5	100
5	5	2	2	2	**58**	2	0	8	2	11	8	100
6	0	0	3	2	2	**66**	12	8	0	5	5	100
7	8	2	8	12	0	8	**57**	5	0	0	0	100
8	9	0	0	0	6	0	6	**70**	0	9	0	100
9	0	0	0	0	0	0	0	0	**95**	5	0	100
10	5	2	5	8	5	0	0	2	0	**68**	5	100
11	0	5	0	8	5	2	0	0	0	18	**62**	100

* Refer to Table 1 for population identification number (ID).

Smouse and Chevillon (1998) observed that microsatellite DNA provides essentially, limitless, highly varied information within species and thus provide a means for distinguishing individuals and populations. Queller *et al*. (1993) reported a wide array of applications of microsatellites such as population structure analysis, systematics of closely related populations or species, studies of parentage and kinship, genetic mapping and forensics.

Table 9: Probability (%) of assigning unknown population to the known stratum based on WHICHRUN analysis (Banks and Eichert, 2000)

Unknown pop	Stratum 1	Stratum2	Stratum3	% Total
Gumbwa	**83**	10	7	100
Lisuli	**93**	7	0	100
Malemia	**78**	15	7	100
Mwala	28	**72**	0	100
Kadamera	13	**70**	17	100
Maere	5	**73**	22	100
Ngwangwa	30	18	**52**	100
Malandiro	11	9	**80**	100
Magamba	0	0	**100**	100

Conclusions

O. mossambicus populations in the study showed a considerable allelic variability at six loci with the three strata exhibiting similar genetic variability. In general, the degree of allelic variability in the wild populations of *O. mossambicus* was higher than the allelic variability observed in domesticated populations of this species. Further genetic analysis revealed separate populations of the species that are moderately structured and not related to each other by isolation by distance model. Conservation efforts, therefore, need to concentrate on each population since the populations are still distinct though going toward homogeneity given the high gene flow.

Microsatellite loci analysis demonstrated the power of DNA tools in resolving wildlife forensic issues. This revelation could be the basis for designing protocols for sustainable management and utilisation of both wild and domesticated populations of commercially important tilapias in Malawi.

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