Genetic diversity analysis of *Oreochromis shiranus* species in reservoirs in Malawi

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Introduction

It is estimated that there are more than 800 reservoirs in Malawi of size range of 0.4 - 7.7 ha, spread throughout the country (VINCKE, 1990). Most of them have capacity of less than 50,000 m^3 with catchment of less than 1-2 km^2. Artificial stocking of the reservoirs was carried out in early 1960s with *Oreochromis shiranus chilwae* and *Tilapia rendalli* especially those in the southern region. The reservoirs in general are rarely harvested and have not been managed for commercial fish production.

According to mode of ownership, reservoirs are classified into three categories namely; government owned; estate owned and communally owned. Government owned reservoirs are those that belong to government institutions like schools, the Forestry Departement and agricultural development projects, among others. These are located on public land. Estate owned reservoirs are located on leasehold land belonging to tea, tobacco and sugar estates. Communal reservoirs are located on customary land controlled by chiefs or heads of ethnic groups. Ownership of

communal reservoirs is unclear because they belong to every member of the ethnic group, although major activities carried out on the reservoirs by individuals have to be approved by the chief.

The government reservoirs were constructed between 1950 and later in the 1960s. They were mainly constructed for irrigation purposes, for instance those that belong to Forestry Department and Ministry of Agriculture were for supplying drinking water for livestock and irrigating tobacco seedlings. As a realized benefit, water from reservoirs is also used for domestic activities. Stocking of fish in the reservoirs was carried out during the colonial period and was done only once in almost all the reservoirs in the country.

Estate reservoirs were stocked by estate management (KANDOOLE and AMBALI, 1992). Limited investment is made in fishery management because most activities are concentrated on crop production from where estates derive almost all their gross margins.

Communal reservoirs were stocked by Fisheries Department during the colonial period but limited number of local community members recall when stocking took place. Some of them even believe that stocking was through streams that drain into the reservoirs. Fishing by village members is allowed although people still believe that the reservoirs belong to government.

The objective of the study was to determine genetic diversity of reservoir populations of *O. shiranus sp*; and determine the extent of genetic differentiation occurring in the reservoirs.

Materials and methods

Source of samples

Oreochromis shiranus sp samples were collected from seven reservoirs, namely Chilala (CHL), Bvumbwe (BVU), Mikolongwe (MKW), Mpemba (MPA), Mvonia (MVO), Bishop (BSR) and Bunda (BCR). Of these, five belong to government and private institutions and two are communal (Table 1).

Reservoir	Code	District/City	Type of ownership	n
Chilala	CHL	Blantyre	Government	40
Bvumbwe	BVU	Blantyre	Communal	42
Mikolongwe	MKW	Blantyre	Government	40
Mpemba	MPA	Blantyre	Government	32
Mvonia	MVO	Blantyre	Communal	40
Bunda	BCR	Lilongwe	Institutional	55
Bishop's	BSR	Mzuzu	Institutional	55

Table 1
Summary of the reservoirs studied and number of individuals analysed.

Species Composition

Sampling was carried out using a seine net cast across several areas of each reservoir. The net was pulled to the edge where fish caught were identified to the species they belonged. Random samples of *O. shiranus* were collected from catches and individual standard length was measured before blood or muscle were collected for DNA analysis.

DNA analysis

Blood samples were collected from BCR, BSR and MVO populations while muscle tissues were collected from BVU, CHL, MBA and MPA populations. The procedure outlined in BROOKER *et al.* (1994) was used to extract DNA from blood and a modification of the extraction procedure of KAMONRAT *et al.* (in prep) was used to extract DNA from muscle. Tissue of 3 mm^2 size was placed in a microtube and 1.0 mL high TE was added. The mixture was vortexed for 30 s and left to stand for 10 min before the supernantant was decanted. Two hundred and fifty µl MGPL lysis buffer and 2.5 µl Proteinase K were added to digest tissue in a 45°C waterbath for at least 3 h until the tissue had been completely

digested. The mixture was vortexed and spun in Eppendorf microcentrifuge for 5 min. The aqueous phase was transferred to new microtube where 500 µl TE was added before mixing by vortexing. Thirty five µl of 3 M NaCl and 750 µl of cold isopropanol were added to the mixture and vortexed until the solutions had completely mixed. The mixture was incubated at -80°C to precipitate DNA and then spun in the Eppendorf microcentrifuge for 10 min. The liquid was decanted and DNA pellete was washed with 500 µl of 70% cold ethanol by vortexing the mixture and then spinning for 5 min. Ethanol was decanted and the tube was spun again to remove residual ethanol. The pellete was air dried for 10-15 min before resuspending DNA in 100 µl TE.

Five polymorphic microsatellite loci were analyzed using primers developed by AMBALI (1996). Amplification was carried out in a Biocycler PCR machine where samples were at first cycled 7 times through the series: denaturation at 94°C for 1 min, annealing at primer specific temperature for 30 s and extension at 72°C for 1 s. These were followed by 28 cycles through the series: denaturation at 88°C for 30 s, annealing at primer specific temperature for 30 s and extension at 72°C for 1 s. PCR products were electrophoresed on a 8% denaturing acrylamide gel and sized using a M13 DNA ladder.

Data analysis

Mean and range for each population were calculated for the standard length measurements. These were compared to wild populations from lakes Chilwa, Chiuta, and Malombe. The exact Hardy-Weinberg test in GENEPOP version 1.2 (RAYMOND and ROUSSET, 1995) was used to compute observed and expected heterozygosity values, and to test for conformity to Hardy-Weinberg equilibrium (HWE) using the exact test by GUO and THOMPSON (1992).

The sequential Bonferroni correction was used to adjust significant level (LESSIOS, 1992). In the procedure, the tests were ordered from

the highest to the lowest according to their probability values. The highest probability, p_m was compared to the significant level α. If p_m was greater than α the comparison continued with the subsequent probabilities, each compared to $\alpha'_{i+1} = \alpha/(1+i)$ where i is the number of tests already performed.

BIOSYS-1 computer program (SWOFFORD and SELANDER, 1989) was used to compute a number of measures of genetic variation within and between sample populations. The following variables were computed to determine allelic diversity: number of alleles per locus, actual number of alleles per locus for each reservoir, mean number of alleles per locus per reservoir, effective number of alleles per locus per reservoir (CROW and KIMURA, 1970).

The DIPLOIDL program in GENEPOP was used to compute Wright's F-statistics (WRIGHT, 1978) according to WEIR and COCKERHAM (1984). The among-population component of genetic variance F_{ST} was computed to measure the proportion of total variation that could be ascribed to differences between population allele frequencies. F_{IS} values were also calculated to determine heterozygote deficiency and excess within populations.

The GENDIST program in PHYLIP version 3.4 (FELSENSTEIN, 1990) was used to compute CAVALLI-SFORZA and EDWARDS (1967) chord distance between species and subspecies. Mantel's test was carried to determine the correlation between geographic distance and CAVALLI-SFORZA and EDWARDS (1967) chord distance. The test was based on the null hypothesis that there was no correlation between genetic distance and the geographic distance between locations where the population samples were collected.

The MXCOMP program of NTSYS-pc was used to compute a product-moment correlation coefficient (*i.e.* normalized Mantel's statistic Z) for each pair of distance matrices (ROHLF, 1992). To determine if the correlations were significant, actual coefficients were compared to values produced by randomly permuting each matrix pair 1000 times.

Results

Fish species found in the reservoirs

Data on species caught in the reservoirs are provided in Table 2. The predominant species were *O. shiranus sp*, *T. rendalli*, *Barbus sp*, *Clarias gariepinus*, *Serranochromis robustus* and other species in the haplochromid family like *Pseudocrenilabrus philander* and *Astatotilapia calypterus*. *O. shiranus sp*, *T. rendalli* and *S. robustus* were artificially stocked while the other species were stocked through natural streams. With the exception of the MVO reservoir, *O. shiranus sp* was the most predominant species in the catches in all the reservoirs. *T. rendalli* was observed in five of the reservoirs but not in MPA and MVO. *C. gariepinus* was found in CHL, MKW and MVO reservoirs; the species was mostly abundant in MKW reservoir. *S. robustus* was only found in BVU reservoir while *Barbus sp* was found in CHL, MKW and MPA reservoirs.

Reservoir	O. sh.	T. rend	C. gar.	S. rob	Barbus
Chilala	+	+	+	-	+
Bvumbwe	+	+	-	+	-
Mikolongwe	+	+	+	-	+
Mpemba	+	-	-	-	+
Mvonia	+	-	+	-	-
Bishop's	+	+	-	-	-
Bunda	+	+	-	-	-

Table 2
Species composition of the catch in various reservoirs at the time of sampling. O. sh. (*O. shiranus sp*), T. rend (*T. rendalli*), C. gar (*C. gariepinus*), S. rob (*S. robustus*), Barbus (*Barbus sp*): (+) species present, (-) species not observed.

Mean and range standard length (SL) of *O. shiranus sp* found in the reservoirs and lakes are presented in Table 3.

Although differences in mean SL of the various populations did not necessarily imply that reservoir populations grew faster than those in lakes, they generally shed some light on individual size distribution found in the two types of waterbodies. *O. shiranus* populations in the reservoirs had higher mean SL than those in the lakes. The range suggests that reservoir populations were shifted towards large size distribution compared to lake populations. In the reservoirs where there were large predators like *C. gariepinus* and *S. robustus*, *O. shiranus* caught were of large size, implying that predators reduced the population of small tilapia individuals. *O. shiranus* samples analyzed for MVO reservoir were collected in March 1995 when the species was most abundant in the catch. Ten months later, in December 1995, when the water volume had declined due to drought, the species was less than 1% of the total catch, and the predominant species became *C. gariepinus*.

Reservoir/Lake	Mean SL (cm)	Range (cm)
Chilala	19.9	14.5 - 26.0
Bvumbwe	20.5	15.0 - 25.5
Mikolongwe	13.9	10.5 - 17.0
Mpemba	17.7	10.0 - 17.0
Mvonia	13.6	13.8 - 24.7
Bishop	-	-
Bunda	14.1	11.3 - 20.1
Lake Chilwa	11.4	9.9 - 14.5
Lake Chiuta	8.1	6.6 - 13.5
Lake Malombe	9.3	7.7 - 10.7

Table 3
Mean and range standard length (SL) of *O. shiranus sp* in the reservoirs and lakes.

Conformity to Hardy-Weinberg Equilibrium

Tests for conformity to Hardy-Weinberg Equilibrium (HWE) are presented in Table 4. According to the exact test, there were more locus-population combinations (77.1%) that showed no significant departure from the HWE equilibrium than those that showed significant departure from HWE (22.9%).

Populations	Os-7	Os-25	Os-7R	Os-64	Os-75
CHL	0.240	0.094	0.034	0.737	0.242
BVU	0.007	0.228	0.001	0.737	<0.001
MKW	0.910	<0.001	0.016	0.762	0.009
MPA	0.399	1.000	0.527	1.000	0.067
MVO	0.096	0.141	<0. 00 1	0.515	0.357
BCR	0.162	0.006	0.012	0.004	<0.001
BSR	0.471	0.893	0.197	0.001	0.021

Table 4
Level of significance of departure from HWE using the exact test.

Genetic diversity

Summary of observed and expected heterozygosity values is presented in Table 5. All loci were polymorphic in all the reservoir populations. Mean observed heterozygosity ranged from 0.537 ± 0.076 to 0.713 ± 0.034. The highest heterozygosity values were observed in BSR and BCR populations and in their decreasing order MKW, BVU, CHL, MPA and MVO, although their 95% confidence intervals (mean ± 2SE) suggest that heterozygosity values between populations were not significantly different.

Pop		Os-7	Os-25	Os-7R	Os-64	Os-75	Mean ± SE
CHL	Observed	0.676	0.667	0.724	0.650	0.450	0.633 ± 0.047
	Expected	0.789	0.708	0.838	0.707	0.421	0.693 ± 0.072
BVU	Observed	0.632	0.462	0.769	0.487	0.925	0.655 ± 0.087
	Expected	0.555	0.585	0.563	0.459	0.596	0.552 ± 0.024
MKW	Observed	0.571	0.306	0.974	0.667	0.789	0.661 ± 0.112
	Expected	0.594	0.585	0.829	0.644	0.819	0.694 ± 0.054
MPA	Observed	0.542	0.390	0.854	0.467	0.780	0.607 ± 0.090
	Expected	0.494	0.342	0.836	0.437	0.811	0.584 ± 0.101
MVO	Observed	0.353	0.575	0.795	0.425	0.538	0.537 ± 0.076
	Expected	0.500	0.642	0.767	0.481	0.711	0.620 ± 0.057
BCR	Observed	0.571	0.769	0.750	0.540	0.766	0.679 ± 0.051
	Expected	0.553	0.855	0.914	0.657	0.891	0.774 ± 0.071
BSR	Observed	0.783	0.760	0.750	0.600	0.674	0.713 ± 0.034
	Expected	0.827	0.792	0.861	0.683	0.871	0.807 ± 0.034

Table 5
Observed and expected heterozygosity at five microsatellite loci.

Measures of allelic variability are presented in Table 6. The average number of alleles per population ranged from 3.2 ± 0.37 to 10.2 ± 2.15. The effective number of alleles was high in more recently stocked reservoirs (BSR and BCR) than those which were stocked between 1950 and 1960 (*i.e.* reservoirs in Blantyre district) and where carnivorous species were observed.

Population structure

Levels of intra- and interpopulation variation are presented in Table 7. Inbreeding coefficient (F_{IS}) ranged from 0.040 to 0.143, with mean of 0.090; implying that there was heterozygosity deficiency at all loci in the populations. The F_{ST} values ranged from 0.147 to 0.370, with mean of 0.248.

Populations	Os-7	Os-25	Os-7R	Os-64	Os-75	Total	A
CHL	7	8	10	7	5	37	7.4±0.81
	(4.5)	(3.31)	(5.66)	(3.30)	(1.71)	(18.5)	(3.7±0.66)
BVU	4	3	3	2	4	16	3.2±0.37
	(2.21)	(2.36)	(2.25)	(1.82)	(2.43)	(11.1)	(2.2±0.10)
MKW	4	7	8	5	11	35	7.2±1.28
	(2.40)	(2.26)	(5.49)	(2.73)	(5.20)	(18.2)	(3.6±0.70)
MPA	4	5	11	3	9	32	6.4+-0.09
	(1.93)	(1.51)	(5.72)	(1.75)	(5.04)	(16.0)	(3.2±0.90)
MVO	2	3	7	2	4	18	3.6±0.93
	(1.97)	(2.73)	(4.11)	(1.90)	(3.16)	(13.9)	(2.8±0.41)
BCR	8	10	14	6	14	52	10.2+9.15
	(2.21)	(6.50)	(10.53)	(2.86)	(8.44)	(30.6)	(6.1±1.59)
BSR	8	10	13	4	11	46	9.6±1.63
	(5.50)	(4.62)	(6.79)	(3.09)	(7.19)	(27.2)	(5.4±0.74)

Table 6
Measures of allelic variability at five loci in seven reservoir populations of *O. shiranus sp* (number of alleles per locus per population, total number of alleles per population, effective number of alleles per locus (in parentheses) mean ±SE number of alleles (A) and mean ± effective number of alleles (in parentheses).

Locus	F_{IS}	F_{ST}
Os-7	0.076	0.268
Os-25	0.078	0.305
Os-7R	0.040	0.147
Os-64	0.143	0.370
Os-75	0.130	0.175
All loci	0.090	0.248

Table 7
Levels of intra- and interpopulation variation at five loci in seven reservoir populations of *O. shiranus sp.*

Correlation between genetic distance and geographic distance

Matrices of CAVALLI-SFORZA and EDWARDS (1967) chord distance are presented in Table 8. Mantel's correlation coefficient between genetic distance and geographic distance (data not shown) was 0.265 suggesting that there was poor correlation between population genetic distance and geographic distance between reservoirs.

Populations	CHL	BVU	MKW	MPA	MVO	BCR
CHL						
BVU	0.145					
MKW	0.155	0.180				
MPA	0.161	0.164	0.024			
MVO	0.197	0.204	0.120	0.129		
BCR	0.148	0.135	0.122	0.114	0.157	
BSR	0.101	0.156	0.153	0.130	0.156	0.090

Table 8
CAVALLI-SFORZA and EDWARDS (1967)
genetic chord distance between reservoir populations.

Discussion

Species composition

The most dominant species in the reservoirs was *Oreochromis shiranus sp* which was stocked between 1955 and 1960 but there were no commercial fish harvesting operations in the reservoirs. It is speculated that with proper management an additional 80-140 tonnes per annum could be produced from the reservoirs in

Malawi (VINCKE, 1990). The occurrence of carnivorous species like *S. robustus* and *C. gariepinus* had considerable effect on the size distribution of tilapias in the reservoirs. The major problem was that there was no specific predator/prey ratio used in the reservoirs which was detrimental to the tilapia populations as observed in the MVO population during periods of recession. MATHOTHO (1975) recommends a stocking of no more than 6% predators. The proportion of *C. gariepinus* and *S. robustus* was on average above this rate. Production of large tilapia by using predators to control recruitment has been practiced in ponds. OFORI (1988) observed that a predator/prey ratio combination of 1:80 produced the largest average individual size tilapia compared to lower predator/prey ratios of 1/250 and 0. The total biomass was however highest in the treatments where there were no predators. Determination of optimum predator/prey ratio and the size of predator to stock is still problematic in tilapia culture. Lack of management of the reservoirs in Malawi exacerbated the predation problem because there was no culling carried out to reduce the predator population which kept growing through natural recruitment. It was therefore observed that the reservoir fishery was gradually being dominated by *C. gariepinus*.

Genetic diversity

Genetic variability was high in those relatively more recently stocked and managed reservoirs (BCR and BSR) where there was considerably low abundance of carnivorous species. The carnivorous species preyed upon tilapia to the extent that the effective population size of the prey population was reduced. Although the actual numbers of tilapia that remain in the reservoirs is not known, there is evidence showing that their recruitment is continuously controlled by predators. Population size is the most important factor in maintaining a high level of genetic variation in a stock (MEFFE, 1986). Population decline results in genetic variation for future generations being preserved in a relatively small number of individuals. The effective population size (N_e), which is equal to the harmonic mean $1/N_e = 1/[t(N_1 + N_2 + ... + N_t)]$ where t is the number of generations (FRANKLIN, 1990), becomes reduced. The

low Ne, subjects populations to dispersive processes of allele frequencies like genetic drift, bottlenecking and inbreeding.

AMBALI (1996) determined effective number of alleles in lake, reservoir and farm populations. The effective number of alleles in the reservoir populations of Blantyre district which were stocked between 1955 and 1960 were similar to the farm populations that were stocked from the National Aquaculture Centre (NAC) in the early and mid 1980s. The fact that the genetic variability in the reservoirs was close to that of recently domesticated populations implies that the rate of decline of allelic diversity might have been lower in the reservoirs than in the farm populations. For instance, none of the loci were monomorphic in the reservoir populations while there were monomorphic loci in the farm populations.

Despite the predators, the history of stocking in the reservoirs also points to the fact that government and communal reservoir populations were founded on a narrow genetic base. The sizes of founder populations were generally low. ICLARM and GTZ (1991) quote stocking rates in government owned reservoirs ranging from 34 tilapias, 2 haplochromids and 19 *S. robustus* per reservoir to 173 tilapias, 20 haplochromids and 26 *S. robustus*. The tilapias were usually a mixture of *O. shiranus sp* and *T. rendalli*, although there was more shiranus than rendalli.

Genetic distance

Mantel's correlation coefficient between genetic distance and geographic distance was low and not significantly different from zero. Lack of strong correlation was due to the fact that unlike BCR and BSR populations which were founded on single populations of pure strains, the reservoir populations in Blantyre were composite. Despite this, their genetic variation was low. MATHOTHO (1975) indicate that reservoirs in the southern region of Malawi were stocked with mixtures of *O. sh. shiranus* and *O. sh. chilwae*, although the proportions of the two species are not indentified and the actual reservoirs are not indicated. Although Mvonia and Chilala reservoirs were only 3.2 km apart, the alleles observed at locus Os-7 in the two populations (MVO and CHL) were different.

In the MVO population, two alleles observed at the locus were not observed in the CHL population. Alleles in MVO were of larger size than those in CHL. Possibility of contamination with other species of tilapia could not be ruled out, as MATHOTHO (1975) indicates that *O. mossambicus* and *O. placidus* were found in some of the reservoirs in the southern region for instance Lujeri Estate in Mulanje district.

Conclusion

The BSR and BCR reservoirs have demonstrated that genetic diversity can be maintained in the reservoirs over a long period of time. The major factors affecting the biodiversity in the Malawian reservoirs are management, predator/prey ratios and genetic diversity of the founder population. The BCR is the most productive and best managed reservoir in the country (ICLARM and GTZ, 1991) and the BSR in the north is also well managed where feeding is done on regular basis, harvesting is done twice a year and only about 200 kg of large fish is removed *per* harvest. There were no carnivorous species observed during sampling in the two reservoirs. A different situation in the communal and government reservoirs is observed. There is no management being carried out and there is no control in the population of carnivorous species and the effective population sizes of the founder populations were low.

The present setup shows that BCR and BSR can be used for *in situ* conservation but a lot of improvements need to be made on the communal and government reservoirs for them to be used for conservation. There is need to control continuous recruitment of the carnivorous species by carrying out scheduled culling operations. The Fisheries Department of Malawi is currently carrying out stock assessment and limnological experiments in the reservoirs in order to develop management procedures for enhancing productivity. This should be complemented with restocking programs utilizing pure strains of tilapia. The major social issue that needs to be resolved in

reservoir management is that of tenure. Like many other African countries, communal or state-owned reservoirs have presented problems of ownership and fishing rights to the effect that interest in fisheries management in reservoirs has declined in the recent years (ICLARM and GTZ, 1991).

References

AMBALI (A.J.D.), 1996 — *The relationship between domestication and genetic diversity of* Oreochromis species *in Malawi:* Oreochromis shiranus shiranus *(Boulenger) and* Oreochromis shiranus chilwae *(Trewavas).* Ph.D. thesis, Dalhousie University, 204 p.

BROOKER (A.L.), COOK (D.), BENTZEN (P.), WRIGHT (J.M.), DOYLE (R.W.), 1994 — Organization of microsatellites differs between mammals and cold-water teleost fishes. *Can. J. Fish. Aquat. Sci.*, 51: 1959-1966.

CARVALLI-SFORZA (L.L.), EDWARDS (A.W.F.), 1967 — Phylogenetic analysis: models and estimation procedures. *Evol.*, 32: 550-570.

CROW (J. F.), KIMURA (M.), 1970 — *An Introduction to Population Genetics Theory.* Harper & Row, Publishers, 591 p.

FELSENSTEIN (J.), 1990 — *PHYLIP (Phylogeny Inference Package) Version 3.3.* University of Washington, Seatle.

FRANKLIN (I. R.), 1990 — Evolutionary change in small populations. *In* M.E. SOULE and B.A. WILCOX (eds): *Conservation Biology: An Evolutionary-Ecological Perspective.* Sinaeuer Associates, Sunderland, MA. p 135-139.

GUO (S. W.), THOMPSON (E. A.), 1992 — Perfoming the exact test for Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48: 361-372.

Iclarm, GTZ, 1991 — *The Context of Small-scale Integrated Agriculture-Aquaculture Systems in Africa: a Case Study of Malawi.* Iclarm studies and Reviews 18. Iclarm, Manila, Philippines, and GTZ, Germany, 302p.

KANDOOLE (B. F.), AMBALI (A. J. D.), 1992 — *Socio-economic study of fish farming in the CNRFF Project.* Consultancy report submitted to EC Delegation, Malawi, 90p.

LESSIOS (H.A.), 1992 — Testing electrophoretic data for agreement with Hardy-Weinberg expectations. *Mar. Biol.*, 112: 517-523.

MATHOTHO (A.J.), 1975 — *A simple guide to rearing fish in small dams in Malawi.* Fisheries Bulletin, Malawi Department of Fisheries, 8 p.

MEFFE (G.K.), 1986 — Conservation genetics

and the management of endangered fishes. *Fisheries,* 11(1): 14-23.

OFORI (J.K.), 1988 —
The effect of predation by Lates niloticus on overpopulation and stunting in mixed sex culture of tilapia species in ponds, p. 69-73. In PULLIN (R.S.V.), BHUKASWAN (T.), THONGTHAI (K.), MACLEAN (J.L.), (eds): *The Second International Symposium on Tilapia in Aquaculture.* Iclarm Conference Proceedings 15, 623 p.

ROHLF (F.J.), 1992 —
NTSYS-pc. Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, NY.

SWOFFORD (D.L.), SELANDER (R.B.), 1989 —
BIOSYS-1: a computer programme for the analysis of allelic variation in population genetics and biochemical systematics; release 1.2. Illinois Natural History Survey, 43 p.

RAYMOND (M.), ROUSSET (F.), in press —
GENEPOP version 1.2: a population genetics software for exact test and ecumenism. *J. Hered.*

VINCKE (M.M.), 1990 —
Field verification of water bodies inventoried for aquaculture development inCentral and Northern Regions of Malawi. FAO. Rome, Italy, 46p.

WEIR (B. S.), COCKERHAM (C. C.), 1984 —
Estimating F-statistics for the analysis of population structure. *Evol.* 38: 1358-1370.

WRIGHT (S.), 1978 —
Chicago: University of Chicago Press. *Evolution and Genetics of populations. Vol 2. Theory of Gene Frequencies.* Chicago : University of Chicago press.