THE RELATIONSHIP BETWEEN DOMESTICATION AND GENETIC DIVERSITY OF *OREOCHROMIS* SPECIES IN MALAWI: *OREOCHROMIS SHIRANUS SHIRANUS* (BOULENGER) AND *OREOCHROMIS SHIRANUS CHILWAE* (TREWAVAS)

by

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Submitted in partial fulfillment of the requirements for
the degree of Doctor of Philosophy
at
Dalhousie University
Halifax, Nova Scotia
February, 1996

ISBN 0-612-15812-8

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "The Relationship Between Domestication and Genetic Diversity of *Orechromis* SP in Malawi: *Oreochromis Shiranus Shiranus* (Boulenger) and *Oreochromis Shiranus Chilwae* (Trewavas)"

by Aggrey J.D. Ambali

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated March 15, 1996

External Examiner [redacted]

Research Supervisor

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DALHOUSIE UNIVERSITY

DATE: 15 FEBRUARY, 1996

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TITLE: THE RELATIONSHIP BETWEEN DOMESTICATION AND GENETIC DIVERSITY OF *OREOCHROMIS* SPECIES IN MALAWI: *OREOCHRMIS SHIRANUS SHIRANUS* (BOULENGER) AND *OREOCHROMIS SHIRANUS CHILWAE* (TREWAVAS)

DEPARTMENT OR SCHOOL: BIOLOGY

DEGREE: PH. D. CONVOCATION: MAY YEAR: 1996

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DEDICATION

To my father **JUSTICE B. AMBALI** who missed the materialization of his dream by nine months.

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ABSTRACT:

The fish species domesticated in most African aquaculture activities have not been genetically identified and characterized; and the distribution of their genetic diversity is not known. As a result there is continuous mixing of otherwise genetically differentiated strains leading to loss of between-population genetic variation. In this study, microsatellite DNA markers for tilapia were developed to analyze genetic diversity of wild and domesticated populations of *O. shiranus* species in Malawi.

The primers developed with *O. shiranus* DNA amplified microsatellites in other species of the tilapiine fishes. Genetic relationships among populations of *O. shiranus* in Malawi were determined and it was observed that the populations in Lakes Chilwa and Chiuta belonged to *O. shiranus chilwae* while the population in Lake Malombe belonged to *O. shiranus shiranus*.

Measures of genetic diversity declined in the domesticated populations compared to wild populations. The loss of diversity was correlated with the time elapsed since the founding of farm stock and population genetic differentiation was also strongly influenced by the pattern of known exchange of germplasm among farms. Social factors, as measured by transfer proximity, are the key determinants of genetic distance, not geographic distance. Genetic diversity in the reservoir populations declined with increase in predator populations which were stocked either artificially to control tilapia populations, or naturally through streams. Uncontrolled transfer of germplasm between reservoirs resulted in genetic contamination of the populations.

Socio-economic analysis of small scale farms in Malawi showed that growout operations were economically viable primarily because pond inputs comprised recycled on-farm and household waste. The predominance of integrated crop/livestock/fish farming system was a favorable indicator of the possibility of involving farmers in community-based aquaculture biodiversity conservation programs at farm level. The programs would benefit from the knowledge and experience farmers had already acquired in conserving indigenous breeds of livestock and crops.

LIST OF ABREVIATIONS AND SYMBOLS

BAP	Bacterial alkaline phosphate
bp	Base pair
BP	Before Present
CNRFFP	Central and Northern Regions Fish Farming Project
ddH_2O	Deionized distilled water
EDTA	Ethylenediaminetetraacetic acid disodium salt
FD	Fisheries Department of Malawi Government
GIFT	Genetic Improvement of Farmed Tilapias
GTZ	Deutsche Gasellschaft fur Technische Zusammenarbeit (German Agency for Technical Cooperation)
h	hour
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite Allele Model
ICLARM	International Centre for Living Aquatic Resources Management
IDRC	International Development Research Centre, Canada
m	Minute
MAGFAD	Malawi Germany Fisheries and Aquaculture Development Project (of the GTZ)
MGPL	Marine Gene Probe Laboratory of Dalhousie University
MFF	Mapendo Fish Farm
MK	Malawi Kwacha (unit of currency)
NAC	National Aquaculture Centre of the Department of Fisheries of Malawi Government
NDF	Naming'azi Demonstration Farm
OXFAM	Oxford Committee for Famine Relief
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism

RAPD	Randomly Amplified Polymorphic DNA
SADC	Southern African Development Community
SDS	Sodium deudecyl sulphate
SMM	Stepwise Mutation Model
SSC	Salt Sodium citrate
TE	Tris EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Averaging
VNTR	Variable Number Tandem Repeats
YT	Yeast Extract Tryptone

ACKNOWLEDGEMENTS

I am grateful to the International Development Research Centre (IDRC) of Canada for providing me a scholarship to pursue the Ph. D. program. The research for this thesis work was supported by funds from IDRC, the Sub-Saharan Africa Dissertation Internship Award Program of the Rockefeller Foundation and the Marine Gene Probe Laboratory.

I would like to express my appreciation to my supervisor, Dr. Roger W. Doyle for supporting my application for financial assistance from IDRC and the Rockefeller Foundation, for inviting me to Canada, for the guidance and continued support he has provided during the entire period of my program at Dalhousie. Thanks to him also for the critical comments he made on the thesis. Gratitudes are also extended to members of my supervisory committee, Drs. Tony Charles, David Patriquin and Jonathan Wright (in alphabetical order), for supporting my application for research grant from the Rockefeller Foundation. My research also benefited from their advice provided during the Acceptance to Candidacy and Preliminary Exams. I am also grateful to my co-supervisor Dr. Leonard Kamwanja for supervising my field research and the critical contributions he made in redesigning the research protocols in Malawi.

Invaluable technical expertise and guidance in the Marine Gene Probe Laboratory was provided by Doug Cook. Thanks to him for all the support he has provided from the time I had my first exposure to microsatellite DNA analysis to present. I am grateful to Mick O'Connell, Doug Cook, Tony Charles, Janet Doyle and Lawrence Malekano for the valuable comments they made on most chapters of my thesis. To my fellow graduate students, Greg Hammond, Wongpathom Kamonrat, Shelley Lang and Sudhindra Gadagkar, I express my gratitude for their support and provision of a friendly work environment.

The following organisations provided support during sample collection in Malawi: the National Aquaculture Centre, the Malawi German Fisheries and Aquaculture Development Project and the Central and Northern Regions Fish Farming Project (CNRFFP). To them I would like to express my thanks. The CNRFFP allowed me to have access to their data base on small scale farmers in the northern region.

I am grateful to my parents, wife, daughter, son and family friends for the sacrifice they have gone through during the period I have been away. It is through their continued moral support that I have been able to put this work together.

CHAPTER 1

GENERAL INTRODUCTION

1.1 *Introduction*:

The Malawi government's commitment to aquaculture is stipulated in the national development policy. The policy on aquaculture development aims at optimizing self sufficiency in food fish in rural and urban areas by increasing sustainable productivity; optimizing use of rural resources in terms of land, water and farm by-products and improving technology transfer in terms of control of fingerling production, species selection and control of production factors. The policy also strongly prohibits the introduction into Malawi of live exotic fish species. In Malawian households, fish forms the most affordable form of animal protein where it contributes about 70% of total animal protein consumed (Balarin, 1987). The per capita fish consumption has however declined from 14.7 kg/annum in 1970 to less than 7.0 kg/annum in the 1990s. This is due to the general growth in human population and stagnation in total landings from capture fisheries (Fisheries Department, 1995). The government is promoting aquaculture to supplement fish production from capture fisheries and increase availability of fish to the protein-deficient population in rural upland areas.

Several species of fish have been domesticated in the aquaculture industry in Malawi. These include tilapia, cyprinids and catfish. The tilapia and catfish that have been cultured so far are all indigenous. An exotic cyprinid, common carp (*Cyprinus carpio*) was introduced into the country from Israel in the 1980s, but the government banned the culture of this species in fear that it might have detrimental effects on the indigenous

species if it accidentally got into natural water bodies. The exotic common carp was a fast-growing species and performed well in most small scale farms. Since the ban was imposed in 1991, the aquaculture industry has been faced with the problem of identifying indigenous species that have fast growth under various levels of management.

In Malawi, the genus *Tilapia* is represented by *T. rendalli* (Boulenger) while the genus *Oreochromis* is divided into two sub-genera: *O.* (*Oreochromis*) and *O.* (*Nyasalapia*). The *O.* (*Oreochromis*) flock comprises *O.shiranus* (subdivided into *O. sh. shiranus* (Boulenger) and *O. sh. chilwae* (Trewavas)), *O. mossambicus* (Peters), and *O. placidus* (Trewavas). The *O.* (*Nyasalapia*) species flock is composed of *O. karongae* (Trewavas), *O. lidole* (Trewavas), *O. saka* (Trewavas) and *O. squamipinnis* (Gunther). *O. shiranus* sp is the most widely cultured species in the country.

1.2 *Management capabilities of fish genetic resources in Malawi and SADC*[1] *region*:

Most of the domestication programs in the SADC region and perhaps in the whole African continent have been constrained by the fact that they work on species that have not been properly identified or characterized. As a result, information has not been reproducible, consistent or easily transferred among programs. In Malawi, the domestication efforts of the nyasalapia species flock have been carried out without sorting out the confusion

[1] Southern African Development Community. Member states are Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, Swaziland, South Africa, Tanzania, Zambia and Zimbabwe.

surrounding the identity of the various species of *Oreochromis* (*Nyasalapia*) sp. In Zambia, it has been observed that some of the cichlids that are being used in aquaculture have been wrongly identified. For example, *O. mortimeri* (Trewavas) population at Chilanga in the southern province of Zambia has been wrongly identified, by fisheries personnel, as *O. mossambicus* and most of the species available in the wild have not been described (Thys van den Audenarde, 1994). Lack of documentation of the available genetic material is due not merely to lack of facilities and trained personnel to identify the species, it is also due to a fundamental lack of knowledge of the structure of the gene pool of species relevant to aquaculture.

Small scale farmers and government stations do not practice deliberate and effective genetic selection. They reserve a portion of fingerlings from growout ponds at time of harvest for the next grow-out cycle. Although no deliberate criteria are known to be used in selecting the fingerlings, strong indirect or domestication selection can nevertheless occur, e.g. if the fingerlings come from very few parents or from particularly early or late maturing parental stock (Doyle *et al.* 1995). Not much is known at present if such a system depletes genetic variation or not, but DNA analysis of farm populations indicates that depletion is actually rather rapid.

The increasing pressure from human population growth and activities associated with the lakes on aquatic biodiversity in Malawi is of particular concern. Cyprininds like *Opsaridium microleps* and *Labeo mesops* have disappeared from their traditional natural

habits where they used to occur in abundance. *O. Nyasalapia* sp catches have declined considerably in the wild. The country is currently faced with the challenge of conserving the aquatic biodiversity in the lakes. *Ex situ* conservation is not economically feasible in such a country where financial resources are limited; the economic feasibility of *in situ* conservation offers achievable promise as an alternative means of preserving the otherwise threatened biodiversity. Several reservoirs do exist in the country but most of them are underutilized for fishery production.

1.3 *Objectives of the thesis*:

In this thesis, molecular genetic techniques are used (1) to characterize *O. shiranus* subspecies in the wild and on farms, (2) to determine the population structure of the species and (3) to assess the distribution of its genetic diversity in the wild and farms. In view of the fact that the causes of loss of genetic diversity are social and economic, a study was carried out to determine the socio-economic status of small scale fish farming in Malawi with the objective of identifying factors that could be optimized in order to promote sustainable genetic diversity conservation in aquaculture. The thesis therefore deals with (1) molecular procedures for the development of microsatellite DNA markers, (2) genetic relationships among cultured mouth brooding tilapia in Malawi, (3) historical events in aquaculture development and their impact on genetic changes of *O. shiranus* sp populations, (4) an assessment of genetic diversity distribution in the reservoir populations and (5) socio-economic characterization of small scale aquaculture in Malawi. *O.*

shiranus sp was an appropriate species for the study because it is the species most widely cultured by small scale farmers using simple methods and it is an indigenous species so that direct genetic variation comparison can be made between the wild and domesticated stock.

1.4 *Genetic variation in populations*:

The total genetic variance of a species consists of a within-population genetic diversity and variance among populations. Loss of within-population variation reduces the basis for future genetic adaptation while loss of variation among populations results in replacement of many populations by one (Meffe, 1986). Within-population variation is reduced by three main processes: genetic drift, genetic bottlenecks and inbreeding through their effect on effective population size. Between-population variation is reduced by outright extinction of populations or by replacement of some populations by others.

1.4.1 *Genetic drift*:

Genetic drift is the random change in gene frequency due to sampling error in small populations (Hartl and Clark, 1988). It occurs whenever the population is less than infinite in size and of course reaches its extreme in the very small populations discussed under bottlenecking. Genetic drift results in two main changes in the genetic characteristics of domesticated populations: 1)

reduction in the proportion of polymorphic loci by fixing an allele at a locus. This results in loss of genetic variation and the locus remains monomorphic unless variation is introduced through mutation or immigration. Allendorf and Phelps (1980) reported allele variation at five loci of a wild population of 85 cutthroat trout (*Salmo clarki*) which could not be observed in over 300 hatchery stocks of the species. 2) The number of alleles per locus declines due to genetic drift; this may eventually result in total loss of rare alleles at loci. A reduction of 29% of genetic variability has been reported in the domesticated stocks of cutthroat trout (Allendorf and Phelps, 1980).

1.4.2 *Genetic bottleneck*:

A bottleneck is a sudden and drastic reduction in size of a population. Change in genetic variation due to bottlenecking depends on the genetic diversity of the original population, size and duration of the bottleneck and the degree of randomness of surviving individuals (Frankel and Soule, 1981). Bottlenecks reduce the additive variance[2] but may increase genetic variation through conversion of epistatic variation[3] into additive variance. It is suggested that with high epistatic variance in the ancestral population, bottlenecking can increase total additive (quantitative) genetic variance within populations (Goodnight, 1987; 1988). Due to founder events there tends to be partial fixation of alleles at each locus which leads to reduction in the

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[2] Fitness of two or more gene loci when their joint effect is equal to the sum of the effects of individual loci.

[3] Fitness of two or more gene loci when their joint effect differs from the sum of the effects of the loci taken separately.

number of genetic environments an allele experiences. This has been shown to convert epistatic interaction into additive variance.

Leberg (1992) reported significant differences in allele frequencies at three of the seven loci examined between bottlenecked and founder populations of mosquitofish (*Gambusia holbrooki*). The difference was attributed to loss of rare alleles during the bottleneck. The common alleles were even more prevalent in the bottlenecked population than in the wild stock.

Although bottlenecks may have severe effects on genetic variation, most studies have not been able to detect such effects because they have measured allozyme heterozygosity which is not a very sensitive variable (Gosling, 1982; Hedgecock and Sly, 1990; Durand *et al.* 1993). In this study, average heterozygosity, proportion of polymorphic loci, number of alleles per locus, the various F-statistics of Sewall Wright and genetic distance within and between species were determined by using microsatellite DNA markers. The polymorphism at microsatellite loci is generally very high, which greatly increases the likelihood that temporal changes can be observed (Wright, 1993).

1.4.3 ***Inbreeding***:

Inbreeding is defined as the mating of individuals related by common ancestry (Meffe, 1986). Inbreeding does not necessarily reduce the overall genetic variation in a population (if the

population is large) but results in an increase in homozygous individuals. Homozygosity affects fitness characters leading to "inbreeding depression" especially of the traits that have low heritability like fecundity, fertility, age at maturity, growth and survivorship, although the actual mechanism is not known (Meffe, 1986). Kincaid (1976) has shown that certain level of inbreeding can adversely affect survival and weight in rainbow trout (*Oncorhynchus mykiss*).

Inbreeding in domesticated stocks of fish can occur for two reasons: (1) small population size, where chance mating of relatives is a frequent occurrence, and (2) large populations where systematic mating of relatives is practiced, either deliberately or inadvertently as an incidental part of the culture regime.

1.5 *Socio-economics*:

The high-yielding plant and animal materials being cultivated by farmers at present are those that have lost their ancestral traits due to intensive selection. Agricultural technology has advanced substantially, especially in the developed countries, but many farmers in developing countries are still practicing traditional farming systems. The majority of farming systems in the world are characterized by low- to medium inputs and will still dominate in a large proportion of the next century. The consequences are that the intention to increase production by promoting the universal use of "superior breeds" developed through centralized genetic evaluation programs will not result in sustainable agriculture for a large part of the next century. The "superior breeds" would not be sustainable in the low-input agriculture with high

stress environments. There is therefore need to develop environment-specific strains which would be adapted to low-input and otherwise high stress environments (Doyle *et al.* 1991; Hammond, 1994).

Rege (1994) argues that extinction is not a major concern among domesticated populations at species level because the "protective custody of the society" ensures that large numbers are maintained. The major issue of concern is to prevent the loss of breeds (strains) due to continuous reduction of among-population variation; implying that community-based conservation involving small scale farmers and the local leadership at village level may be the most sustainable mode. There are several socio-economic factors that are likely to influence genetic biodiversity conservation and these include: education, size of the household, level of income, cost effectiveness of the management systems, poverty status, division of labor between sexes and power structure at household and village levels and social relationship among farmers. A socio-economic survey was carried out as a component of the thesis research in order to understand the socio-economic setup of the community at village level.

1.6 *Thesis outline*:

Chapter 1: General introduction

Chapter 2: Development of microsatellite DNA markers for tilapia

Chapter 3: Genetic relationships of *Oreochromis shiranus* sp in Malawi

Chapter 4: Genetic changes in *Oreochromis shiranus* sp associated with the early stages of national aquaculture development

Chapter 5: The potential of small water bodies as *in situ* gene banks for aquaculture: a genetic assessment of the Malawian reservoirs

Chapter 6: Socio-economic status of small scale fish farming in Malawi

Chapter 7: General conclusions.

CHAPTER 2

DEVELOPMENT OF MICROSATELLITE DNA MARKERS FOR TILAPIA

2.1 ABSTRACT:

Genetic markers are those that represent the genetic profile of an animal or plant based on biochemical tests like electrophoresis of enzymes and DNA fingerprinting to identify and characterize the animal or plant. In this study genetic markers based on microsatellite or simple sequence repeats were developed and tested for polymorphism in various species of tilapia. The objective was to develop markers that could be used for identifying tilapia and determining their genetic diversity. Eight sets of microsatellite probes were made based on *O. shiranus* DNA in the Marine Gene Probe Laboratory (MGPL). Considerable variation in ease of scorability of alleles on the autorads and allelic variability among the loci was observed. Five sets of primers which were easy to score and polymorphic were used for further analysis of populations; these were Os-7, Os-25, Os-7R, Os-64 and Os-75.

The five sets of primers were tested on other species of tilapia of the genera *Oreochromis*, *Sarotherodon* and *Tilapia*. With a few exceptions, the loci analyzed were polymorphic in most of the species. The total number of alleles varied among loci, ranging from 26 to 35 alleles per locus in five species. The number of alleles per locus per species ranged from three to 33, and allele frequency distribution varied from weak unimodal to multimodal. As in other organisms, this work has demonstrated that microsatellite primers based on *O. shiranus* DNA amplifies DNA in other species of the tilapiine fishes. This is an important contribution to genetic analysis studies of the genus (*Oreochromis*) which has been globally distributed for aquaculture.

2.2 INTRODUCTION

2.2.1 *Genetic markers*

Genetic markers are those that represent the genetic profile of an organism based on biochemical tests such as electrophoresis of enzymes and DNA fingerprinting (Nielsen, 1992) to identify and characterize the organism. This definition excludes natural markers which includes morphometric, meristic and parasitic markers. The natural markers are affected by genetic, environmental and physiological factors and as such they are not consistent throughout the life of an organism. Genetic markers are inherited in a simple Mendelian fashion and are assumed to be selectively neutral, i.e. the marker used have no effect on the fitness of an individual fish (Nielsen, 1992; Park and Moran, 1994). The polymorphism observed in organisms is maintained by forces such as mutation, migration and genetic drift (Park and Moran, 1994). In this study, microsatellite DNA markers were developed to analyze the various species and subspecies of tilapia.

2.2.2 *Multi-locus and Single-locus DNA fingerprinting*

Multi-locus and single-locus DNA fingerprinting involves the use of simple sequences, minisatellites or microsatellites, which consist of tandem arrays of up to several hundred base pairs, widely scattered throughout the chromosomes of animals and plants (Bruford *et al.* 1991; Wright, 1993).

2.2.2.1 ***Minisatellites:***

Minisatellites are tandemly-arrayed sequences of 9-65 base pairs in length, frequently GC-rich and their alleles may vary in size by 25 kbp at some loci (Wright, 1993). An increase or decrease in the size of repeat array results from changes in the repeat copy number thought to arise from high rates of unequal crossing-over during meiosis (Avise, 1994). These repeat regions are thought to be hotspots for recombination (Jerman and Wells, 1989 cited in Avise, 1994). The original use of minisatellite as genetic markers was by Jeffreys *et al.* (1985) who developed probes that were used to hybridize to conserved core sequences (10-15 bp long) scattered in "numerous arrays about the human genome as part of a system of dispersed tandem repeats" (Avise, 1994).

The original Jeffreys' probes were isolated from a myoglobin intron in humans and applied to forensic studies in humans (Avise, 1994). The 33-bp sequence of the first intron of human myoglobin gene was used as a probe to identify several minisatellite clones, for instance 33.5, 33.6 and 33.15 in the human genomic library (Jeffreys *et al.* 1985; Zhang and Tang, 1993). In the procedure, digested genomic DNA is cross-hybridized with the probe under low stringency conditions using the Southern blotting procedure to produce multilocus bands of 4-25 kbp size range (Bentzen *et al.* 1991, Zhang and Tang, 1993). The band pattern produced is specific for each individual and is inherited in a Mendelian fashion. These characteristic of minisatellites have allowed their application in pedigree construction (Wright, 1993).

The probes developed from human myoglobin genes by Jeffreys, others developed from gene III of bacteriophage M13 and synthetic probes have been used to DNA fingerprint several other plant and animal species (see Avise, 1994 and references therein for examples). The disadvantage of DNA fingerprinting using non-specific minisatellite probes is that a complex multilocus pattern (about 20 or more bands per individual) is produced. It is difficult to determine which band belongs to a specific locus and therefore allelism can not be established (Avise, 1994). Thus, heterozygosity and homozygosity cannot be determined and genotype frequencies cannot be calculated hence the application of population genetic analysis to determine gene flow and other population variables cannot be done (Avise, 1994).

2.2.2.1 ***Microsatellites***:

Microsatellites or simple sequence repeats consist of short repeated nucleotide motifs spaced at 7-10 kilobase pair intervals in the eukaryotic genome (Tautz, 1989; Wright, 1993). These repetitive sequences are believed to play a role in recombination, replication and the modulation of transcriptional activity (Purdue *et al.* 1987). Microsatellites are five times more abundant in mammals than in plants (Lagercrantz *et al.* 1993). The most common repeats are the dinucleotides $(AC)_n$, $(AG)_n$ and $(AT)_n$ (Rafalski and Tingey, 1993), although trimeric and tetrameric tandem repeats have also been isolated in humans (Edwards *et al.* 1992) and fish (Wright, 1993). While in vertebrates the $(CA)_n$ /$(GT)_n$ repeats are the most abundant and informative, in plants it is the $(AT)_n$ repeats (Rafalski

and Tingey, 1993 and references therein). These observations in plants however contradict the database search results by Lagercrantz *et al.* (1993) who observed that the AA/TT were the most common repeat motifs followed by AT/TA and CT/GA. The high abundance of GT repeats has been taken as a direct evidence for the hypothesis that the repeats are maintained by some special mechanism. The alternating purine/pyrimidine sequences like GT and AT repeats both have a potential for forming Z-DNA, implying that AT repeats in plants could serve a similar function as GT repeats might do in mammals. If so, the exchange of AT for GT as the most common dinucleotide repeat in plants is believed to be a chance event early in evolution of the sequences (Lagercrantz *et al.* 1993).

The variability in the pattern of allele frequency distribution at microsatellites has obscured a proper understanding of the mechanisms underlying the change in their allele frequency in populations (Bruford and Wayne, 1993). Microsatellites have very high mutation rates, estimated to as high as 10^{-3} - 10^{-4} (Dietrich *et al.* 1992; Weissenbach *et al.* 1992). Inter-allelic polymerase slippage mutation during replication has been considered to be the predominant means by which new length alleles are generated (Schlotterer and Tautz, 1991). These observations have therefore prompted investigations into whether the allele frequencies are consistent with a stepwise mutation model (SMM) in which mutation events occur as a loss or gain of a single repeat unit or infinite allele model (IAM) where new alleles are created. Simulations by Shriver *et al.* (1993) show that the allele distribution is consistent with stepwise mutation if the product of effective population size

and mutation rate is greater than one, but there is no correlation between mean allele size and mutation rate. Deka *et al.* (1995) have observed that both IAM and SMM can explain variation in human populations. The authors observed that 79% of the locus-population combinations of allele frequency distributions conformed to the expectations of IAM, while 75% conformed to the SMM. Although rare, there were other locus-population combinations that did not conform to either of the two models. These observations therefore suggest that further work needs to be done before one can confidently determine the type of mutation occurring in a particular set of microsatellite markers.

Microsatellites are abundant, widely spread throughout the chromosome and are highly polymorphic in eukaryotic genomes (Tautz, 1989). A very small quantity of DNA is used in the analysis of microsatellites. Development of hypervariable single-locus probes is described below under **Materials and Methods**. Microsatellites are scored as single locus markers and variability is based on changes in the length of product, i.e. a change in the number of repeats. They are codominant such that heterozygotes can be distinguished from homozygotes. These characteristics make microsatellites an important molecular marker for the future (Wright and Bentzen, 1994), although it is not known why they occur so frequently in the genome (Tautz, 1989; Queller *et al.* 1993).

2.2.3 *Objectives*

The objectives of this component of the study were:

(i) to develop microsatellite DNA markers for tilapia,

(ii) to determine levels of cross-hybridization of the primer sets developed based on *O. shiranus* sequences across the various species of the genera *Oreochromis*, *Sarotherodon* and *Tilapia*,

(iii) to determine the number of alleles per locus per species for the species analyzed,

(iv) to determine the allele frequency distribution of the various loci in five tilapia species.

2.3 MATERIALS AND METHODS:

The following strategy was used to find and characterize microsatellite loci in *O. shiranus*. About 0.3-0.5 mL blood was drawn along the lateral line of fish using a syringe rinsed with 0.5 M EDTA to prevent instant clotting and preserved in 0.5 mL of 100% ethanol. A size selected genomic library was made using the plasmid pUC18, where short DNA fragments of 300-700 base length were cloned. The library was then screened with radiolabelled $(GT)_{15}$ probe to identify clones that contained microsatellite sequences. Clones that hybridized to the $(GT)_{15}$ probe were picked and sequenced in order to develop primers. Eight PCR primer pairs were designed, made and optimized in the Marine Gene Probe Laboratory. Standard protocols outlined in Sambrook *et al.* (1987) were used with some modifications based on procedures published in more recent literature. Detailed procedures followed for each of the steps are described below.

2.3.1 *Constructing the genomic library*:

DNA was extracted from blood using the phenol extraction procedure (Sambrook *et al.* 1987). Blood/ethanol suspension of 250 µL was washed twice with 1 mL high TE buffer (100 mM Tris-Cl, 40 mM EDTA) by spinning for 10 s in Eppendorf microcentrifuge and decanting the supernatant. The cell pellet was resuspended in 500 µL extraction buffer (0.1 M Tris-Cl pH 8.0, 0.1 M EDTA, 0.25 M NaCl, 1% SDS) and 100 µg/mL proteinase K (ICN Biomedicals)

and incubated for 18 h at 55°C. Proteins were removed from the cell lysate by extracting with buffer saturated phenol and chloroform. Five hundred µL of buffer saturated phenol was added to the cell lysate and mixed for 10 m before spinning in Eppendorf microcentrifuge for 5 m. The aqueous phase was transferred to a clean tube and re-extracted with buffer saturated phenol. The aqueous phase was transferred to a clean tube and extracted with 500 µL chloroform. The aqueous phase was transfered to a clean tube where 2 volumes of ethanol were added and mixed. The mixture was incubated at -20°C for at least 2 h to precipitate DNA. DNA was recovered by spinning in the Eppendorf microcentrifuge for 5 m and the supernatant was discarded. One mL cold 70% ethanol was added to the DNA pellet, spun for 2 m and the supernatant was discarded. DNA was then dried in speedvac for 5 m and dissolved in 50 µL TE (10 mM Tris-Cl, 1 mM EDTA).

Fifty µg of genomic DNA from *O. shiranus* was digested with 100 units of each of the following enzymes; *Pst*I, *Rsa*I, *Hinc*II and *Alu*I (Pharmacia) according to the manufacturer's instructions. Digested DNA was subjected to electrophoresis in a 1% low melting point agarose gel and fragments from 300 to 700 bp were removed from the gel using a phenol freeze fracture procedure. A segment of the gel containing these fragments was chopped, placed in corex tubes and incubated at -80°C to solidify. Three volumes of buffer saturated phenol were added and the tube sealed with parafilm, and vortexed until the chopped gel fragments had completely dissolved. The mixture was spun in a Beckman J2-21 cetrifuge at 10,000 g for 20 m. The upper aqueous phase was transferred to a clean corex tube and

extracted with buffer saturated phenol. The aqueous phase was transferred to a clean tube and re-extracted with an equal volume of chloroform. The aqueous phase was transferred to a clean tube where 20 μg/mL glycogen, NaCl to a final concentration of 0.2 M and 2 volumes of ethanol were added. The mixture was incubated at -80°C for 30 m to precipitate DNA before spinning in the Beckman J2-21 centrifuge at 30,000 g for 30 m. The supernatant was decanted and DNA in tube was resuspended in 0.5 mL TE (10 mM Tris-Cl, 1 mM EDTA), vortexed and trasferred to a 1.5 mL microfuge tube. NaCl to a final concentration of 0.2 M and two volumes of ethanol were added and the mixture was incubated at -80°C for 15 m before spinning in the Eppendorf microcentrifuge for 20 m. The supernatant was decanted and DNA was dried in the speedvac and resuspended in 25 μL TE. The efficiency of the extraction was examined by electrophoresing a 5 μl aliquot of the sample in a 1% agarose minigel before ligation.

2.3.2 *Cloning*

The DNA ligation mix contained 40 ng of vector pUC 18 *Sma*I/BAP (Pharmacia), 500 ng of the digested insert DNA, 1X ligation buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$), and 2 U of T4 DNA ligase (Pharmacia) in a total volume of 25 μL. The reaction was incubated at 16°C for 20 h. Five μL of the ligated DNA was used to transform 100 μL of MAX efficiency DH5α competent cells (Bethesda Research Laboratories) according to manufacturer's recommendation. The final mixture was shaken at 225 rpm at 37 °C for 1 h

and spread in volumes of 50 μL on 2X YT medium (1.6% Bacto tryptone, 1% yeast extract, 0.1 M NaCl) with 100 μg/mL ampicilin.

2.3.3 *Colony screening*

Following transformation, colonies were lifted onto Hybond-N (Amersham) filters and filters were processed according to manufacturer's recommendation.

A synthetic oligonucleotide $(GT)_{15}$, synthesized on an Applied Biosystems 390 DNA synthesizer, was used as a probe. Eighty ng of the oligonucleotide was end-labeled with 0.5 μL 3000 Ci/mmol [γ ^{32}P] ATP (ICN), 5 U of T4 polynucleotide kinase (Pharmacia), 0.5 μL of T4 kinase buffer (Pharmarcia) and 2.5 μL of sterile ddH_2O. The labelled probe was incubated at 37°C for 30 m, then heated to 70°C for 10 m to denature the enzyme.

Filters were incubated in pre-hybridization solution [6.25 mL 20X SSPE pH 7.6 (174 g NaCl, 27.6 g $NaH_2PO_4.H_2O$, 7.4 g EDTA to a total volume of 500 mL), 2.5 mL 50X Denhardt's (5 g Ficoll-Type 400, 5 g Polyvinyl pyroline, 5 g BSA (pentax fraction) to total volume of 500 mL), 10% (w/v) SDS to a total volume of 100 mL containing 100 μg/mL tRNA] at 65°C for 1 h before addition of heat denatured (70°C for 5 m) radio-labeled probe. The hybridization reaction was performed in a Hybaid hybridization oven at 60°C overnight. Filters were washed three times; first with 2X SSC, 0.1% [w/v] SDS at room temperature, followed by a second

wash with 1X SSC, 0.1% SDS for 15 m at room temperature and finally with 1X SSC, 0.1% SDS at 60°C for 15 m. Filters were wrapped in Saran Wrap and subjected to autoradiography (Kodak XAR5 film) at -80°C with one intensifying screen overnight.

Eighty five individual putative positive colonies were picked as agar plugs using sterile tooth picks and grown overnight in YT broth containing 100 mg/mL ampicillin. Speedpreps of plasmid DNA were made from positive clones using the procedure of Goode and Feinstein (1992).

2.3.4 *Sequencing of clones*

DNA templates from eighty five positive clones were sequenced using the T7Sequencing™ kit (Pharmacia LKB Biotechnology) following the manufacturer's instructions. Sequencing products were separated in a 8% denaturing polyacrylamide gel containing 7.8 M urea using 1X Tris-borate-EDTA buffer (10X TBE: 0.9 M Tris base, 0.9 M Boric acid, 20 mM EDTA pH 8.3) at 1600 V for 2.5 h. Sequencing gels were fixed in 10% methanol and 10% acetic acid for 30 m and dried onto Watman 3MM paper under vacuum for 2 h. Dried gels were subjected to autoradiography overnight at room temperature.

2.3.5 *PCR amplification of microsatellite loci*:

PCR primers for *O. shiranus* complementary to the unique sequences flanking the microsatellite were designed. Primers were placed as close to the repeat array as possible, aiming for equivalent GC content for both forward and reverse primers and close to 50% GC content overall. The primer sequences were designed to contain at least one C or G at the 3' end, where possible, and 19 to 23 bp in length. A total of eight primer pairs were designed.

Primers were tested using a variety of annealing temperatures with template DNA from *O. shiranus*. An initial annealing temperature (T_{ann}) was estimated for each primer based on the following calculation: 4 x (sum of G and C) + 2 x (sum of A and T) - 6 = T_{ann}. For amplification; 1X PCR buffer (10 mM Tris pH 8.3, 50 mM KCl, 1 mM $MgCl_2$, 0.01% gelatin), 400 μM each dNTP, 200 μM each primer, 1μM of one primer γ-^{32}P-labelled primer, 0.25 U T*aq* polymerase and 25 ng of genomic DNA were used. Total volume of the PCR cocktail was 10 μL overlayed with 1 drop of mineral oil. Amplification was carried out in a Biocycler™ oven (Bios Corporation) where samples were at first cycled 7 times through the series: denaturation at 94 °C for 1 m, annealing at primer specific temperature for 30 s and extension at 72 °C for 1 s. These were followed by 28 to 35 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. The products were analyzed by electrophoresis through 8% denaturing polyacrylamide gel fixed and sized relative to a M13 sequencing ladder.

2.4 RESULTS

2.4.1 *Microsatellite sequences*

The eight microsatellite sequences obtained from putative clones of *O. shiranus* are presented in Table 2.1. The repeat units observed were all dimeric. The microsatellite repeats were classified according to Weber (1990) and the various categories identified are presented in Table 2.2. At least 87.5% of the dinucleotide repeat sequences were perfect without interruptions and without adjacent repeats of another sequence. The rest (about 12.5%) were compound perfect repeat sequences. The GT/CA repeat sequence length ranged from 12 to 33 repeats while the GA repeat sequence was 7 repeats long.

Primer sequences designed from the regions flanking the tandem repeats are presented in Table 2.3. Most of the primers were designed based on a single clone sequence but in one of the clones, two repeat sequences were flanked by a long sequence in which two sets of primers were developed from the same clone sequence (Os-7 and Os-7R). All the eight sets of primers varied considerably in the quality of product amplified. The products amplified by primers Os-7, Os-7R, Os-25, Os-64 and Os-75 produced sharp bands on the autorads which could be unambigously scored. Those five primer sets were therefore used throughout the analysis of the various populations that were studied. The annealing temperature at which signal-to-noise ratio was optimized varied between primers (Table 2.3).

Table 2.1: *O. shiranus* sp microsatellite sequences used to design primers

1. Os - 3

5'TATCGCGTTACAGTCACGGCGCCTCGGCTCCGCCCCCTCCCGTCGGTCGCCGGT
TATAGGAGGT(**G**)$_5$TGCACGCTCTCC(**CT**)$_5$TCACTCACACACATAAAACAGATGCA
TCCTATTTCAAAAGATACCACAGATATTAACTCTTCACAGATTGAGACCATTCTC
AG-3'

2. Os - 7 & Os-7R

5'TGTCATAAAAACACTTATTTTTACTCCAGCAGAGAAGAAGAAGCAGCGCTCTCT
TTGTCTGCTGCCTCGGCCTGTAATGTGTGTGCTGGTGCATGAAAGTGCACATATC
(**TG**)$_{12}$TAGATATTAAACTGGCGATGCGGCAACAGTTATGTCAAG(**AC**)N_XAGATAT
TAAACTGGCGATGCGGCAACAGTTATGTCAAG(**AC**)$_{33}$TGTTTCATGTTCAATTACA
CAGAGGCTGCTCATTTCCTCACCCTGGTTTATTTCCT-3'

3. Os - 25

5'ACTTGGGTTGTGAAATTGCATTGCACTCTCGCGCG(**CA**)$_{16}$CTTGCAGAGGATCAA
AGGGAGTTGGAGCCCTGCACAGCAGCCCTTCTGCCTCCTGCCCTTGGCTAAATG
GACGACCAAACATTCCAGGCTGTCCAGCCCAGTAATATCTGGC-3'

4. Os - 64

5'CTAGACAACAGGGGGCAGTAGAGATCGCTCCAGGTGTTTTCTACAGTCATCGG
TCTGAATGAGTAATGTCACATTAACAGATAGACTCGCGCAAGATGAGATTTGTG
CACGCCCCTGCCCAGTGTCTTCAGTTCCTTGCCTTATACGAAATTACTGTTTTGG
AGAATGC(**TG**)$_{12}$(**GA**)$_7$AGGCTTTCTTATATTTAGGTTATTAGTCATCAAN_XTTTCTT
ATATTGAGTTTATTAGTCATCAATAAGATGCTTCTGTGA-3'

5. Os-74

5'AAGATATATATTCTTTTGCCTTAAAAATATACAGAACCGATTTAACTAGAACCA
TTCATTGCATATAT(**GT**)$_X$$N_X$TATACAGAACCGATTTAACTAGAACCATTCATTGCA
TATAT(**TG**)$_{32}$TAAGTGAGTGTGTTTTGCAGTGGTAGGGCACTAGTTCAAAGCACT
GCTTTTTAGTGTGATAGGCAGTCAGTGGAGAGTTA-3'

Table 2.1: continued

6. Os - 75

5'TGGAGGGGTTTGTGAGCATTTTAAAGCCTAAAATAATGGAATCAC(**TG**)$_{31}$CTGA
TGGCAGGTGAACCATGACTCTGTGGGGTGCAGGTGATGTTCAGCGGTGGATTCA
GACAACAT-3'

7. Os -81

5'TGGAGTTTCCTGTCGGGTGGTTGGATTGTGTAGCATTTGTTTGGACTTTACAGA
GAATATCTACATGATCTTACAGTCTTCAGTAGAGACTTGCTGCTGACAGTC(**GT**)$_{18}$
N_XTTCAGACACATCATGACTTTAGATGTAGTAAAACGGAAAGTTGGTGTTATAAA
CACTTACATCAACTCAAATTGCACTAAAACTCAGGCAAAGTCAGCCTTCAGATTA
TATGCTACACGTTGTAGCATCCACATTGGCATGAAAAGGGGGTCTCAGGGGTTT
CTGCAGTCCTGTGCAAA-3'

8. Os - 213

5'TAACATCTGGAAGTGAGCCGTTCTTTGGTGGTAATTAACAGTTTATTCTTGTTG
GTAATTATGTAATGGAAGGTAGCAGTAAAAAT(**CA**)$_{15}$GACACACTTAGCCGCTGC
TCTGAGTTTGTTCCAGACTCAAACAAAAATAATCCATTTAAAGAATAAAAACATG
-3'

Table 2.2: Types of microsatellite sequences

Sequence	Sequence of the cloned microsatellite	Type of sequence
Os-7	$(TG)_{12}$	Perfect
Os-7R	$(GT)_{33}$	Perfect
Os-25	$(CA)_{16}$	Perfect
Os-64	$(TG)_{12}(GA)_7$	Compound (perfect)
Os-74	$(CA)_{32}$	Perfect
Os-75	$(TG)_{31}$	Perfect
Os-81	$(GT)_{18}$	Perfect
Os-213	$(CA)_{15}$	Perfect

Table 2.3: Primers designed from the flanking regions of the microsatellites and optimum annealing temperatures (T_{ann}).

	T_{ann} (°C)
Primers Os-7	
Forward sequence: 5'-TGTCTGCTGCCTCGGCCTG-3'	58
Reverse sequence: 5'-ACTGTGCCGCATCGCCAG-3'	
Primers Os-7R	
Forward sequence: 5'-AGAGGAAATGAGCAGCCTC-3'	55
Reverse sequence: 5'-GATGCGGCAACAGTTATGTC-3'	
Primers Os-25	
Forward sequence: 5'-TTGTGAAATTGCATTGCACTC-3'	54
Reverse sequence: 5'-AACTCCCTTTGATCCTCTGC-3'	
Primers Os-64	
Forward sequence: 5'-CAGTGTCTTCAGTTCCTTGC-3'	54
Reverse sequence: 5'-CAGAAGCATCTTATTGATGAC-3'	
Primers Os-74	
Forward sequence: 5'-GAACCGATTTAACTAGAACC-3'	49
Reverse sequence: 5'-GAGTGCTTTGAACTAGTGC-3'	
Primers Os-75	
Forward sequence: 5'-AGCCTAAAATAATGGAATCAC-3'	49
Reverse sequence: 5'-CCACAGAGTCATGGTTCAC-3'	
Primers Os-81	
Forward sequence: 5'-GATCTTACAGTCTTCAGTAG-3'	51
Reverse sequence: 5'-GATGTAAGTGTTTATAACACC-3'	
Primers Os-213	
Forward sequence: 5'-AACATCTGAAGTGAGCCG-3'	56
Reverse sequence: 5'-AACAAACTCAGAGCAGCGG-3'	

2.4.2 *Cross hybridization with other species*

The level of variability of PCR products in the various tilapia species is presented in Table 2.4. The five *O. shiranus* primers were cross hybridized with DNA from other tilapia species of three genera, namely *Oreochromis*, *Sarotherodon* and *Tilapia*. A notable distinction among the three genera is that *Oreochromis* are maternal mouth brooders, *Sarotherodon* are bi-parental or paternal mouth brooders and *Tilapia* are substrate brooders (Trewavas, 1982). According to the above category; *O. shiranus* sp, *O. mossambicus*, *O. Nyasalapia* sp, *O. placidus*, *O. niloticus* (Linnaeus), *O. aureus* (Steindachner) and *O. hornorum* (Trewavas) are maternal mouth brooders; *S. galilaeus* (Hasselquist) is a bi-parental mouth brooder (Fishelson and Heinrich, 1963); *T. rendalli* and *T. zilli* (Gervais) are substrate brooders. All the five primers amplified PCR products that could be scored easily in all the mouth brooders (i.e genera *Oreochromis* and *Sarotherodon*). In the two substrate brooding tilapia species, all the primer sets except Os-75, produced a visible PCR product on the autorad.

All the five loci were polymorphic in the representatives of *Oreochromis* species available in Malawi, namely *O. shiranus* sp, *O. mossambicus*, *O.* (*Nyasalapia*) sp and *O. placidus*. Four of the five loci scored were polymorphic in the only substrate brooding tilapia available in Malawi (*T. rendalli*) but no product was observed at locus Os-75. Polymorphism was also observed in other *Oreochromis* species that are not available in Malawi (*O. aureus*, *O. hornorum* and *O. niloticus)* with the exception of *O. aureus* where

loci Os-7R and Os-75 were monomorphic. *S. galilaeus* showed polymorphism at loci Os-25 and Os-75 and monomorphism at the other three loci. Both substrate brooders, *T. rendalli* and *T. zilli* were polymorphic at four loci. While *T. zilli* was monomorphic at locus Os-75, there was no visible product at the locus in *T. rendalli*.

The numbers of alleles scored at each locus in each population are presented in Table 2.5. Due to limited number of samples available ($n < 5$) for *O. placidus*, *O. aureus*, *O. hornorum*, *S. galilaeus* and *T. zilli*, no further analysis was carried out on the species. The analyses presented below were carried out on *O. shiranus* sp, *O. mossambicus*, *O. Nyasalapia* sp, *O. niloticus* and *T. rendalli*. The total number of alleles scored varied between loci and between species. The highest number of alleles was scored at locus Os-75 (35 alleles) and in their decreasing order Os-7R (32 alleles), Os-25 (29 alleles), Os-64 (27 alleles) and Os-7 (26 alleles). The number of alleles at locus Os-64 in *T. rendalli* was higher than in the *Oreochromis* sp by nine alleles.

Table 2.4: Locus variability in the various species of tilapia

Species	Locus Os-7	Locus Os-7R	Locus Os-25	Locus Os-64	Locus Os-75
O. shiranus sp	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Polymorphic
O. mossambicus	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Polymorphic
O. Nyasalapia sp	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Polymorphic
O. placidus	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Polymorphic
O. aureus	Polymorphic	Monomorphic	Polymorphic	Polymorphic	Monomorphic
O. hornorum	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Polymorphic
O. niloticus	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Polymorphic
S. galilaeus	Monomorphic	Monomorphic	Polymorphic	Monomorphic	Polymorphic
T. rendalli	Polymorphic	Polymorphic	Polymorphic	Polymorphic	No product
T. zilli	Polymorphic	Polymorphic	Polymorphic	Polymorphic	Monomorphic

Table 2.5: Total number of alleles (A) and allele size range (SR) in bp in tilapia species

Species	Locus Os-7		Locus Os-7R		Locus Os-25		Locus Os-64		Locus Os-75	
	A	SR	A	SR	A	SR	A	SR	A	SR
O. shiranus sp	16	94-146	28	80-162	20	77-125	13	97-141	33	70-144
O. mossambicus	8	96-140	6	100-140	3	79-89	3	123-133	7	84-126
O. Nyasalapia sp	13	110-146	21	100-162	9	67-107	7	129-161	10	74-102
O. niloticus	7	104-134	7	76-116	7	83-117	5	133-163	8	86-108
T. rendalli	5	120-162	6	82-130	11	71-139	15	81-159	-	
All *Oreochromis*	23	94-146	31	76-162	22	77-125	18	97-163	35	70-144
All 5 species	26	94-162	32	76-162	29	71-139	27	81-163	35	70-144

2.4.3 *Allele frequency distribution*

Locus-specific allele size distributions between species and genera are presented in Figs 2.1 to 2.5. The plots of allele frequency distribution at each locus are as follows: (a) *O. shiranus* sp; (b) *O. mossambicus* (c) *O. Nyasalapia* sp (d) *O. niloticus* and (e) *T. rendalli.*

2.4.3.1 ***Locus Os-7***:

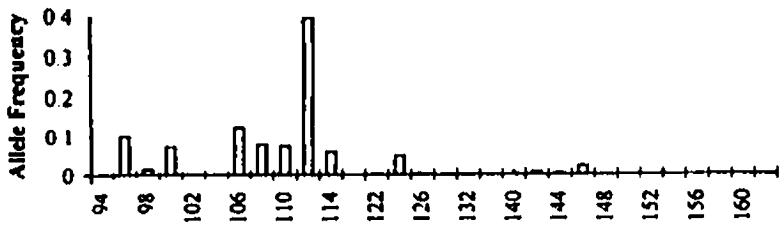
Allele frequency plots for locus Os-7 are presented in Fig. 2.1(a-e) and data on allele size range are presented in Table 2.5. In *O. shiranus* sp, 16 alleles were scored and their size ranged from 94 to 146 bp (Table 2.5; Fig. 2.1a). The most common allele was 112 bp long with a frequency of 40%. The distribution plot shows that most alleles of less than 112 bp length had a higher frequency than those that were larger than 112 bp.

In *O. mossambicus*, the number of alleles scored was reduced to about half the number in *O. shiranus* sp but the two species had a common allele of similar size (112 bp). The frequency of the most common allele was higher in *O. mossambicus* than in *O. shiranus* sp. Although the allele size range in *O. mossambicus* was 96-140 bp, more than 95% of the alleles were distributed within a narrower range; between 106 and 120 bp (Fig.2.1b).

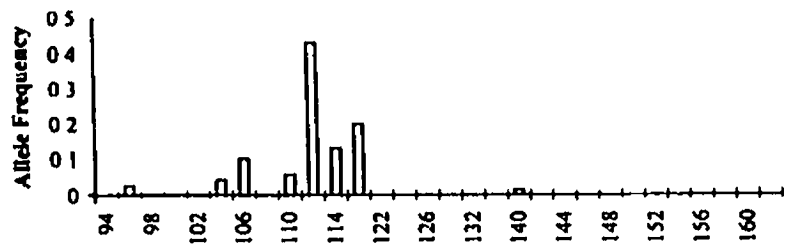
In *O. Nyasalapia* sp, three fewer alleles were observed than in *O. shiranus* sp (Fig 2.1c). The most common allele in *O. Nyasalapia* was 12 bp longer than in *O. shiranus* and *O. mossambicus*. The allele size range in *O. Nyasalapia* was 110-146 bp and more than 85% of the alleles were larger than 112 bp. The most common allele in the species was similar to that of *O. niloticus* (124 bp) (Figs. 2.1c and 2.1d). In *O. niloticus*, a close to normal allele frequency distribution was observed with a general shift of alleles towards large size range.

The distribution of allele frequencies in the substrate brooder is plotted in Fig 2.1e. Alleles of larger size were observed in the substrate brooder than in the mouth brooders. The alleles size range in *T. rendalli* was 120-162 bp and the three largest alleles scored at the locus were not observed in any on the *Oreochromis* species. The most common allele was 128 bp long with frequency of 68%.

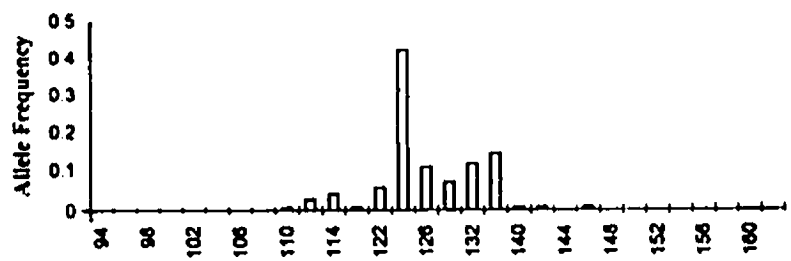
(a) *O. shiranus* sp



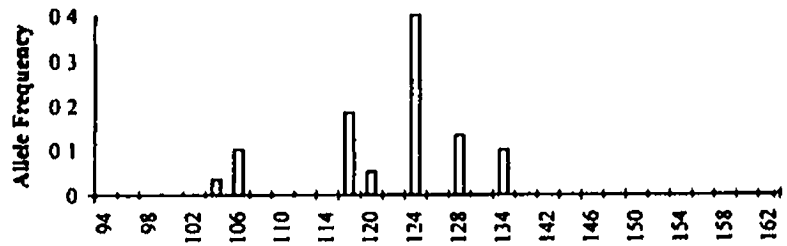
(b) *O. mossambicus*



(c) *O. Nyasalapia* sp



(d) *O. niloticus*



(e) *T. rendalli*

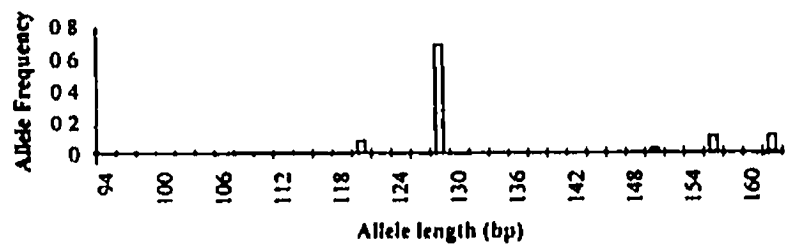


Figure 2.1(a-e): Allele frequency distribution at locus Os-7. Refer to the text for details.

2.4.3.2 ***Locus Os-7R:***

Plots of allele frequency distribution at locus Os-7R are presented in Figs.2.2 (a-e). In *O. shiranus* sp allele frequency distribution was multimodal with no alleles of a frequency of more than 11%. The highest variability at the locus was observed in this species where 28 alleles were scored. In *O. mossambicus*, six alleles were scored at the locus and there was a general shift in allele distribution towards a larger size. The largest allele was the most common with frequency of 39%. The locus was second most variable in *O. Nyasalapia* sp where 21 alleles were observed. The largest allele was the most common with frequency of 13.6%. The frequency distribution was close to multimodal. In *O. niloticus*, the allele frequency distribution was close to unimodal and the most common allele had frequency of 53.8%. Compared to the other three mouth brooding tilapia, there was a general shift in allele distribution towards small size in *O. niloticus*. The smallest allele in *T. rendalli* was the most common with frequency of 81%. The other five alleles had individual frequencies of less than 10%.

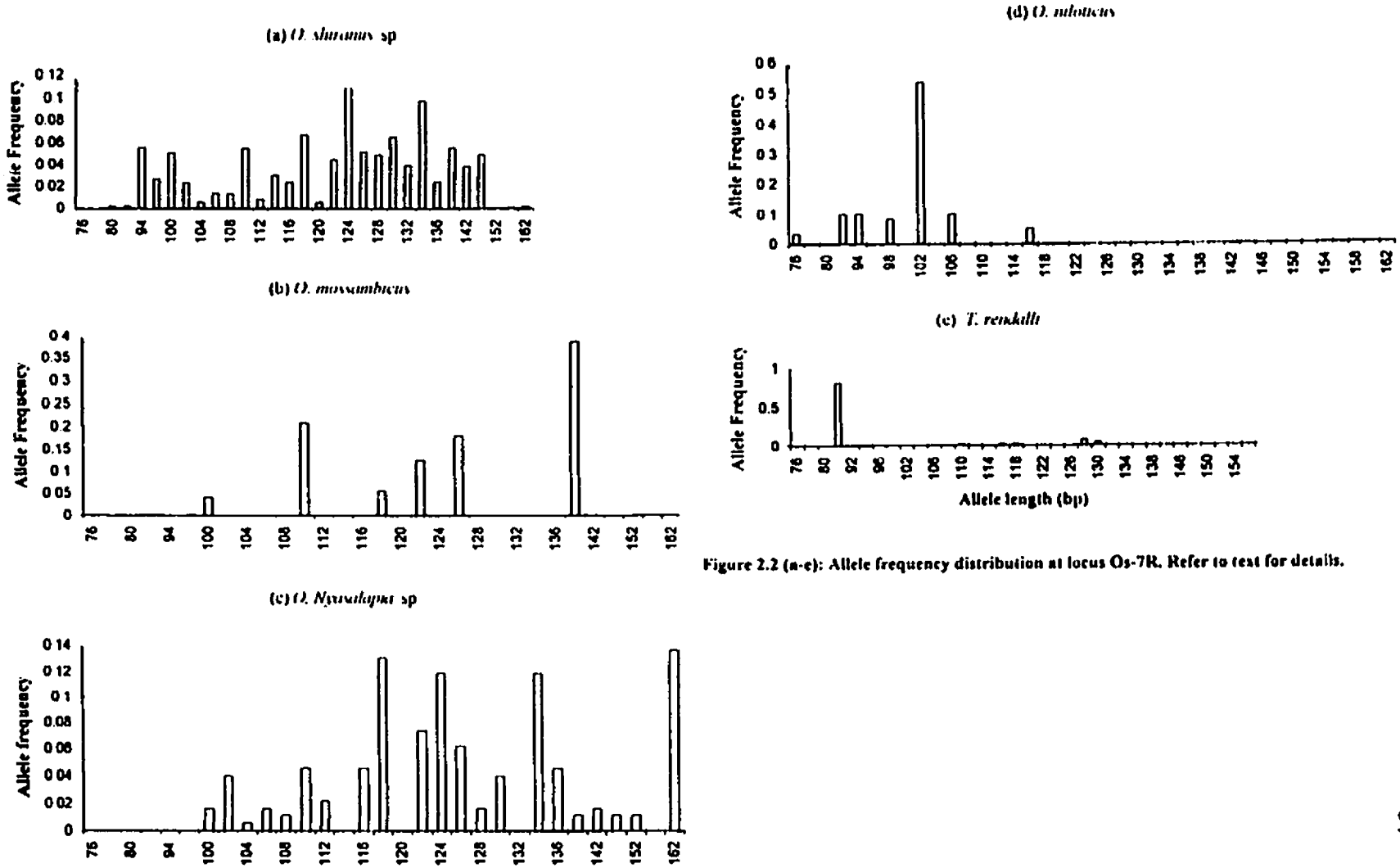


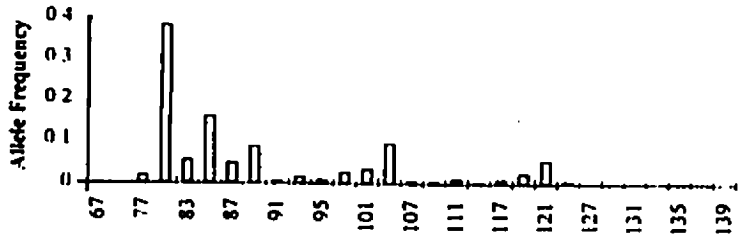
Figure 2.2 (a-e): Allele frequency distribution at locus Os-7R. Refer to text for details.

2.4.3.3 ***Locus Os-25***:

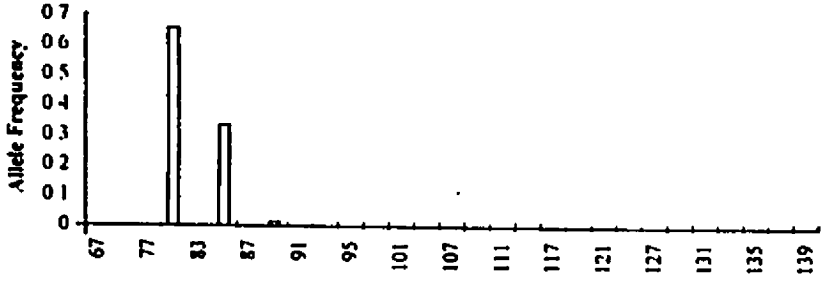
Allele frequency plots for locus Os-25 are presented in Fig. 2.3 (a-e). In *O. shiranus* sp, the most common allele at the locus was 79 bp long and more than 50% of the other alleles were of larger size. The allele frequency distribution was close to multimodal (Fig. 2.3a). The number of alleles in *O. shiranus* sp at the locus was more than six-fold the number in *O. mossambicus* and two-fold in *O. Nyasalapia* sp (Table 2.5). *O. shiranus* sp and *O. mossambicus* shared a similar common allele at the locus; the frequency was however higher in *O. mossambicus* (65.4%) than in *O. shiranus* sp [(38.2%); Figs. 2.3a and 2.3b]. The allele size range in *O. Nyasalapia* sp was 67-107 bp and more than 85% of the alleles were distributed between 79 and 89 bp length. The most common allele in the species (frequency 68.8%) was two bp longer than that of *O. shiranus* sp and *O. mossambicus* (Fig. 2.3c). *O. Nyasalapia* sp and *O. niloticus* had the most common allele of the same size (83 bp). The frequency of the allele was higher in *O. Nyasalapia* sp than in *O. niloticus*. All the alleles observed in *O. niloticus* were also observed in the mouth brooding tilapia from Malawi, there were therefore no private alleles in *O. niloticus*.

Although the allele size range in *T. rendalli* was 71-139 bp, about 80% of the alleles were longer than 100 bp (Fig. 2.3e). The smallest allele scored in the species was of similar length to the most common allele in *O. Nyasalapia* sp and *O. niloticus*.

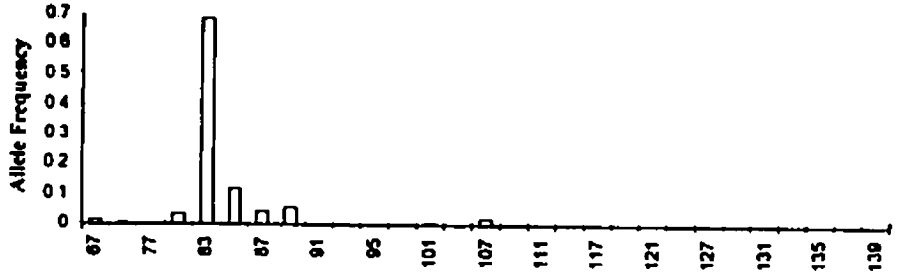
(a) *O. shiranus* sp



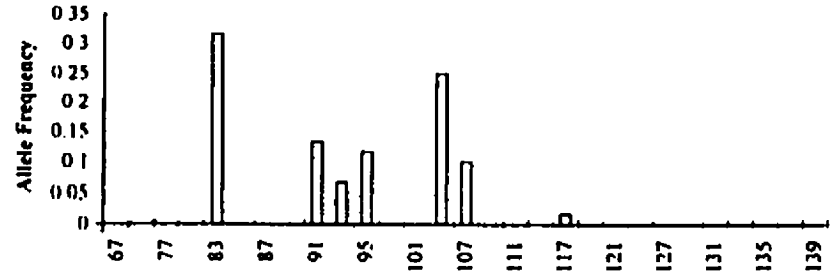
(b) *O. mossambicus*



(c) *O. Nyasalapia* sp



(d) *O. niloticus*



(e) *T. rendalli*

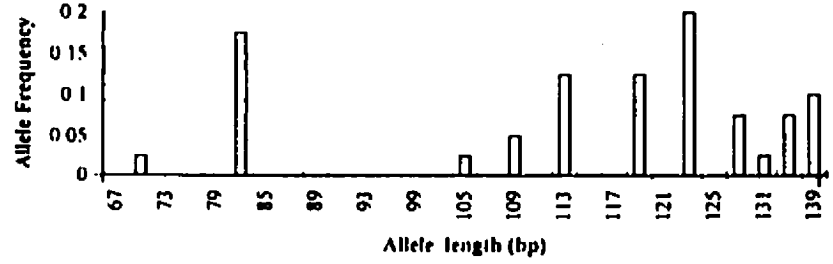


Figure 2.3 (a-e): Allele frequency distribution at locus Os-25. Refer to the text for details.

2.4.3.4 ***Locus Os-64***:

Allele frequency plots for locus Os-64 are presented in Fig. 2.4 (a-e). In *O. shiranus* sp allele frequency distribution was close to unimodal but skewed to the left. The four smallest and the three largest alleles had frequency of less than 5%. The most common allele in the species had frequency of 32.3%. In *O. mossambicus*, three alleles were observed and the most common allele had frequency of 76.4%. While allele size range in *O. shiranus* sp was 97-141 bp, a narrower range was observed in *O. mossambicus*, 123-133. In *O. Nyasalapia* sp, the smallest allele was 129 bp long and was the most common with frequency of 33.0%. The other six alleles were distributed towards large allele size observed at the locus. In *O. niloticus*, the largest allele (163 bp) was the most common with frequency of 43.4%. The allele distribution at the locus was bimodal. It was observed that locus Os-64 showed higher polymorphism in the substrate brooding tilapia, *T. rendalli*, than in the mouth brooding tilapia. The most common allele in the species was two bp smaller and two bp larger than the most common alleles in *O. Nyasalapia* sp and *O. shiranus* sp, respectively. The allele frequency distribution in *T. rendalli* was multimodal.

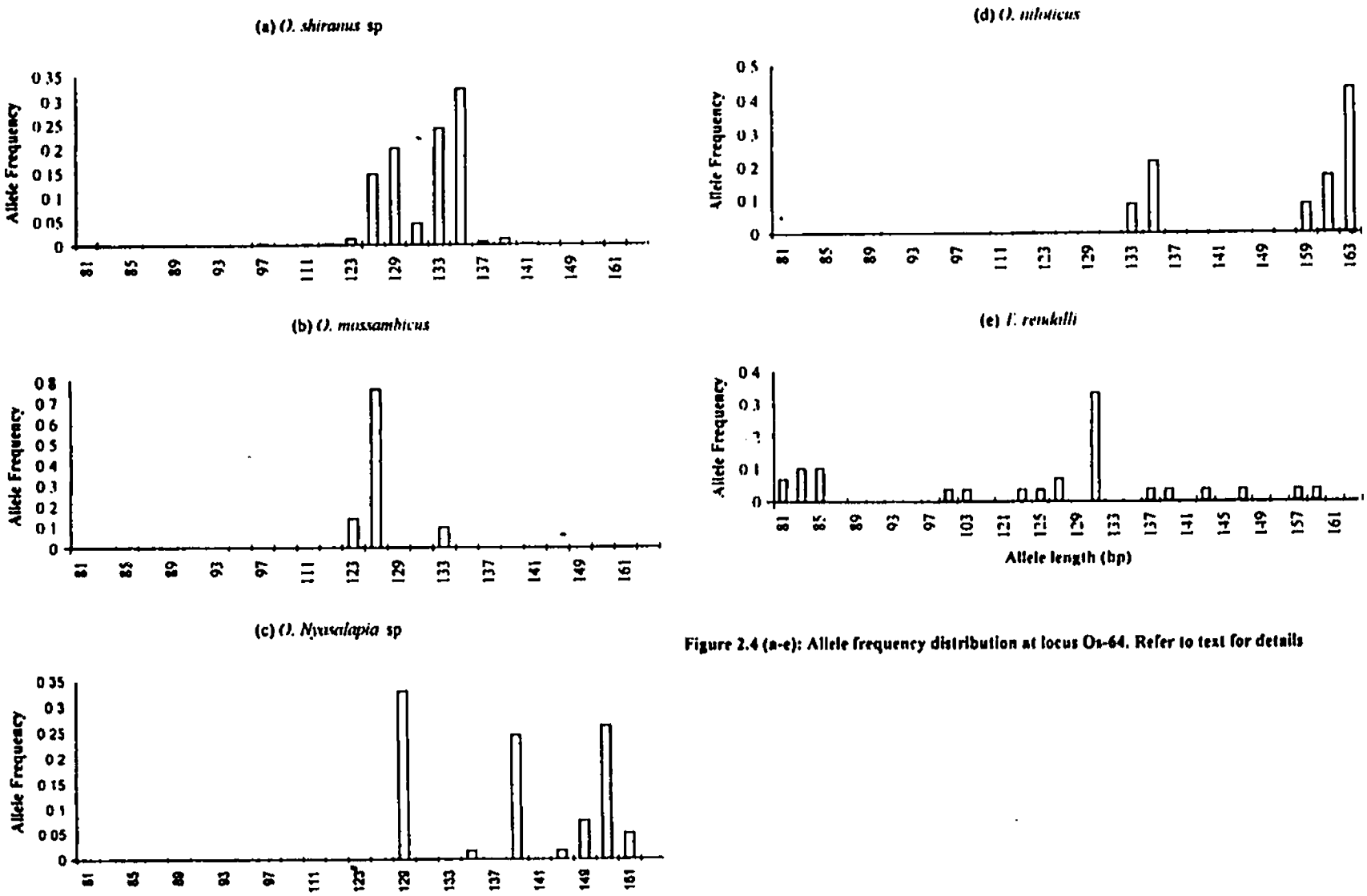
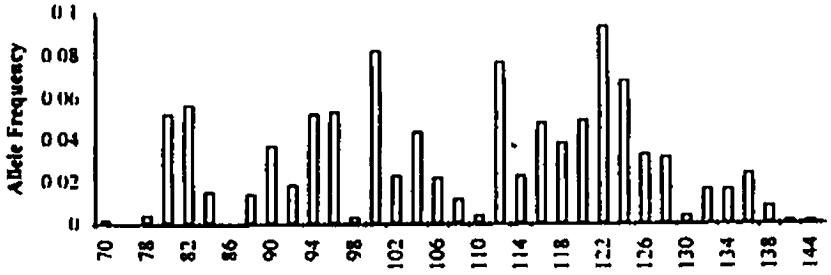


Figure 2.4 (a-e): Allele frequency distribution at locus On-64. Refer to text for details

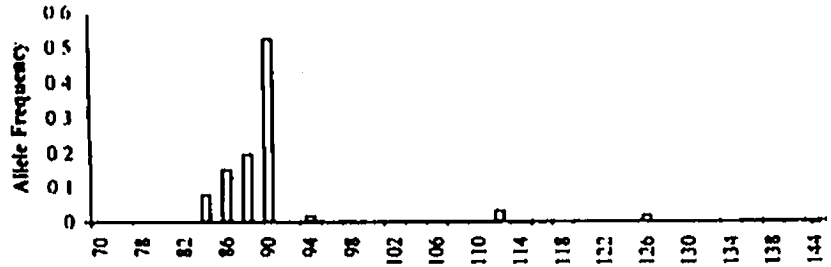
2.4.3.5 ***Locus Os-75***:

Allele frequency plots for locus Os-75 are presented in Fig.2.5 (a-d). This locus showed the highest polymorphism among all the five loci. In Fig. 2.5a, it was observed that allele frequency distribution was multimodal in *O. shiranus* sp and that none of the alleles had frequency of more than 10%. The allele size ranged from 70 to 144 bp and 33 alleles were observed in the species. In *O. mossambicus*, seven alleles were observed at the locus. Alleles with high frequencies ranged from 84 to 90 bp length; the frequency of the most common allele was 52.2%. The three large size alleles had individual frequencies of less than 5%. In *O. Nyasalapia* sp, the allele frequency distribution shifted towards small size. The smallest allele was the most common with frequency of 36.5%. In *O. niloticus*, the alleles were of intermediate size with range of 74 to 102 bp and the most common allele had frequency of 36.5%. Primer set Os-75 did not amplify visible product in *T. rendalli*.

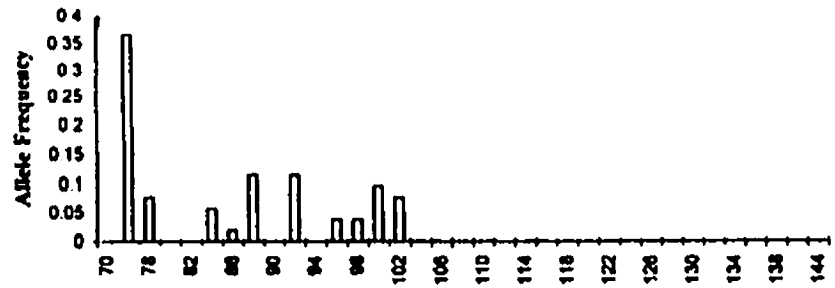
(a) *O. shiranus* sp



(b) *O. mossambicus*



(c) *O. Nyasalapia* sp



(d) *O. niloticus*

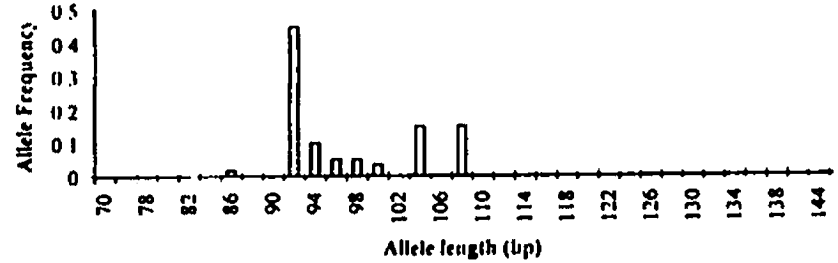


Figure 2.5 (a-d): Allele frequency distribution at locus Os-75. Refer to text for details.

2.5 DISCUSSION

2.5.1 *Cross hybridization*:

Cross-hybridization of primers to other species has been shown to work in other species, for example cattle (*Bos taurus*) primers amplify microsatellite loci in goats (*Capra hircus*; Pepin *et al.* 1995), brown trout (*Salmo trutta*) primers amplify rainbow trout (*Onchorynchus mykiss*) DNA (Estoup *et al.* 1993), Atlantic cod (*Gardus morhua*) primers amplify microsatellites loci in haddock (*Malanogrammus aeglefinus*) and pollock [(*Pollachius virens*); Brooker *et al.* 1994], and human (*Homo sapiens*) microsatellite primers amplify chimpanzee (*Pan troglodytes*) microsatellite loci (Garza *et al.* 1995). This study demonstrated that *Oreochromis* primers amplify DNA of other tilapia genera; *Sarotherodon* and *Tilapia*. The variability of the loci however varied from species to species.

2.5.2. *Variability*:

The five loci studied in detail in tilapia showed that the most variable loci were those that had long microsatellite repeat arrays. For instance the microsatellites flanked by primer sets Os-75 and Os-7R were 31 and 33 repeats long in their original isolates, respectively and showed the highest variability. A direct relationship between the variability of the marker and number of repeats has been reported in humans (Weber, 1990). This

relationship tend to be more obvious in sequences of 12 to 20 repeats and weakens in sequences of more than 21 repeats (Weber, 1990). Loci Os-25, Os-7 and Os-64 were relatively less variable than loci Os-7R and Os-75. The low variability could be due to the low number of repeats in the array of the loci especially Os-7 and Os-25. The non-perfect array at locus Os-64 might have attributed to the low variability. The correlation between repeat length and variability suggests that mutation rate may be correlated with repeat size, although the structure of the repeat may also be important. In the sequence of the original isolate, there was a change in dinucleotide repeats motifs from (TG) to (GA). The fact that interruptions in repeat sequences reduces variability at a locus has been observed in humans (Weber, 1990). The observed increase in the number of alleles in *T. rendalli* at the locus could probably be due to the fact that array in *T. rendalli* genome was probably not interrupted.

The monomorphism observed in the other species listed in Table 2.4 is not claimed to be definitive because of the limited number of samples that were analyzed. Inferences based on inter-locus comparison would not be appropriate because it was not unusual for an individual to be homozygous at one locus and heterozygous at another.

2.5.3. *Allele frequency distributions*:

Allele frequency distribution varied considerably at the five loci in the five species of tilapia studied. Almost all the loci showed a multimodal distribution in all the species with

a few exceptions where weak unimodal distribution was observed. This has been attributed to a stepwise mutation pattern as the mechanism for generating new alleles in humans (Valdes *et al.* 1993) and the same observation in this study may reflect the same mechanism in tilapia. This is still speculative because studies have recently demonstrated that generation of alleles in microsatellites cannot be attributed to step-wise mutation only, the infinite allele mutation also plays an equally significant role (Deka *et al.* 1995).

2.6 CONCLUSION

The microsatellite markers developed in this study showed polymorphism across several genera of tilapia. The variability profile produced at the five loci varied both within individuals and within species. The general trend observed was that loci developed from sequences flanking long tandem repeats showed higher variability than those made from sequences flanking shorter repeats. The allele frequency distribution varied from unimodal to multi-modal but the latter distribution was more common than the former.

CHAPTER 3:

GENETIC RELATIONSHIPS OF *O. SHIRANUS* SP. IN MALAWI

3.1 ABSTRACT

Classification of tilapia has mainly been based on morphological characteristics and breeding behavior. Allozyme electrophoresis has been successfully applied in identifying tilapia at genus and species level but not at subspecies level. In Malawi, *O. shiranus* sp, an indigenous mouth brooding tilapia has been widely distributed in fish farms and reservoirs. Subspecies of *O. shiranus* have been difficult to distinguish morphologically. Microsatellite DNA analysis was carried out to provide data for postulating the genetic relationships of *O. shiranus* sp populations found in the natural water bodies in the country and to determine genetic distance between *O. shiranus* sp and other tilapia species, namely *O. mossambicus*, *O. Ny. karongae* and *O. niloticus*.

Calculation of Cavalli-Sforza and Edwards (1967) chord distance showed that Lake Chiuta population was closer to Lake Chilwa population (D = 0.034) than to Lake Malombe population (D =0.070). A dendogram constructed using the UPGMA method produced two major groups; the mossambicus group tilapia (*O. shiranus* sp and *O. mossambicus*) clustered together while the second cluster was that of non-mossambicus tilapia (*O. karongae* and *O. niloticus*). Among the mossambicus tilapia, Lakes Chilwa and Chiuta populations clustered together as *O. sh. chilwae* while the Lake Malombe population formed a second cluster as *O. sh. shiranus*. The results of heirarchical clustering were similar to species and subspecies grouping done by using principal

components analysis and multidimensional scaling. The ordination analysis, especially principal components analysis suggested that although *O. karongae* and *O. niloticus* formed a clade in the UPGMA dendogram, the two species were genetically very different.

The classification of populations into *O. sh. chilwae* and *O. sh. shiranus* was supported by known history in the geological events associated with the lakes. Lakes Chilwa and Chiuta constituted a single open lake in the past which became partitioned by a sand bar during the early Holocene humid phase (8,000 - 9,000 B.P). There was no connection between the two lakes and Lakes Malawi-Malombe drainage system where *O. sh. shiranus* was found. The waterfalls on the Shire River form a natural barrier preventing interspecific hybridization between *O. mossambicus* and *O. sh. shiranus*. The former species occurs in the lower course of the river while the latter is distributed in the upper course.

3.2 INTRODUCTION:

3.2.1 *Evolutionary relationships among the tilapia*

Tilapia are widely distributed in Africa and have been globally distributed for aquaculture (Welcomme, 1981). The classification of tilapia has mainly been based on morphological characteristics and breeding behavior. The most recent classification by Trewavas (1983) has not been universally accepted (Sodsuk and McAndrew, 1991; Franck *et al.* 1994). Trewavas (1983) categorized tilapia into three genera, namely *Oreochromis*, *Sarotherodon* and *Tilapia*. This grouping is based on reproductive behavior, in which the genus *Oreochromis* consists of maternal mouth brooding tilapia, the genus *Sarotherodon* consists of paternal and biparental mouth brooding tilapia and the genus *Tilapia* consists of substrate brooding tilapia (Trewavas, 1982; 1983). The belief in this classification is that mouth brooders evolved from substrate brooders; *Oreochromis* being the most recent genus to evolve while the genus *Sarotherodon* has remained relatively unchanged. Peters and Berns (1982, cited in Franck *et al.* 1994) who believe that all the tilapia should be in one genus *Tilapia*, argue that the genus *Oreochromis* is conserved while the paternal and biparental mouth brooders are the most recent. Both groups of authors, however, believe that the mouth brooders evolved from substrate brooders (McAndrew and Majumdar, 1984; Sodsuk and McAndrew, 1991; Franck *et al.* 1994). Most of the recent studies of evolutionary relationships within the tilapiine fishes support the model of evolution

proposed by Trewavas (McAndrew and Majumdar, 1984; Abban, 1988 cited in Pouyaud and Agnese, 1995; Sodsuk and McAndrew, 1991; Franck *et al.* 1994).

Classification to species and subspecies levels is still a central issue of controversy in the phylogenetic studies of the tilapia because the species show little morphological diversity and most characters overlap among subspecies (McAndrew and Majumdar, 1983; 1984; Trewavas, 1983; Seyoum and Kornfield, 1992; Bardakci and Skibinski, 1994; Pouyaud and Agnese, 1995). The power of morphological classification is further reduced by the potential of environmental influences on meristic traits and morphometric characters (Seyoum and Kornfield, 1992).

3.2.2 *Classification of tilapia in Malawi and implications for aquaculture genetics*

The cichlid flock of Lake Malawi is believed to have evolved recently (less than 1-2 million years ago) and to have originated from more than one species but mtDNA sequences suggest that the flock is monophyletic (Meyer *et al.* 1990). They differ substantially in their ecology, but only moderately in their external morphology and very little in their characteristics at the mtDNA level. The genus *Tilapia* is represented by *T. rendalli* while the genus *Oreochromis* is divided into two sub-genera; *O.* (*Oreochromis*) and *O.* (*Nyasalapia*) (Trewavas, 1983). The *O.* (*Oreochromis*) subspecies comprises *O. shiranus* sp (subdivided into *O. sh. shiranus* and *O. sh. chilwae*), *O. mossambicus* and *O.*

placidus. The *O.* (*Nyasalapia*) species flock is composed of *O. Ny. karongae*, *O. Ny. lidole*, *O. Ny. squamipinnis* and *O. Ny. saka*. The genus is endemic to Lakes Malawi and Malombe. The taxonomy of *O. Nyasalapia* sp is based almost entirely on morphometric data, some of which is extremely preliminary. Contrary to the species division listed above, multivariate morphometric analysis carried out recently suggests that there are only three species of *O. (Nyasalapia)*. These are: *O. karongae*, *O. lidole* and *O. squamipinnis*. *O. saka* is classified as a subspecies of *O. karongae* (Turner and Robinson, 1990). Difficulties in identifying the *O. Nyasalapia* species has deterred proper broodstock handling procedures and a development of improved strains for aquaculture. The *O. Nyasalapia* species flock appear morphologically to be the derivatives of *S. galilaeus* group (Fryer and Iles, 1972) and they have affinities with *O. machrochir* and *O. rukwaensis*. Thys van Audernaerde (1968 cited in Sodsuk *et al.* 1995) proposed a separate subgenus *Nyasalapia* as part of the genus *Tilapia* which would be only restricted to the Lake Malawi species and sub-genus *Lowuwiala* to include all other taselled male species, for instance *O. (Ny) machrochir*.

O. shiranus sp has been divided into two subspecies based on morphological and meristic characteristics (Trewavas, 1983); *O. shiranus chilwae* and *O. sh. shiranus*. The species is believed to have evolved from about 9000 years B.P (Furse *et al.* 1979). The subspecies are from a common ancestor *O. shiranus* which existed in Rovuma River into which the ancient Lakes Chilwa-Chiuta drained to the Indian Ocean via Lujenda River. The most

nearly related species is *O. rovumae* and together with *O. mossambicus* and *O. placidus* they belong to the mossambicus group of tilapia (Fryer and Iles, 1972).

On geographical distribution, Trewavas (1983) reports that *O. sh. shiranus* is found in Lake Malawi and its tributary rivers, streams, lagoons and upper Shire River including Lake Malombe while *O. sh chilwae* is confined to Lake Chilwa (Fig. 3.1). The Lake Chiuta population of *O. shiranus* sp cannot up to present be ascribed with certainty to either of the subspecies because of its intermediate morphological characteristics. Questions have therefore been raised as to whether the differences between the two subspecies are genetically fixed. For instance Trewavas (1983, page 356) concludes "... I therefore retain the subspecific names as a record of the present state of our understanding of these related populations, but note that there is less reason for recognizing the Lake Chilwa population than that of Lake Chiuta as distinct from the nominate subspecies". The doubt is based on the fact that there was an opportunity for the Lakes Chiuta and Chilwa populations to have diverged from a common ancestor prior to ten thousand years ago when the ancient Chilwa-Chiuta was an open water lake (Furse *et al.* 1979; refer to section 3.4.5 below). There have been no follow up studies of the systematics of *O. shiranus* sp to address most of the phylogenetic questions that Trewavas (1983) raises in her monograph. All these tilapia species have retained their species and subspecies names throughout all the debate. However the Malawian aquaculture industry has the dilemma of identifying the populations and designing genetic management procedures for the various

species and subspecies populations that have been domesticated and are being domesticated. The management consequencies are that strains that have desirable production characteristics can not be identified; there is production of unplanned hybrids due to mixing of species and/or strains which may in the future destroy the genetic differentiation in the wild.

O. niloticus is usually confused with *O. aureus* (Trewavas, 1965; Pullin, 1983). The two species are distinguished based on live coloration where *O. aureus* has a caudal with less regular markings and the dorsal and caudal fins in males have red margins. The caudal fin in *O. niloticus* has regular vertical stripes throughout its depth and the margin of dorsal fin is grey or black. The two species are not native to southern Africa; *O. aureus* is found in the Jordan Valley, Nile Delta, Chad, Niger and Senegal. The three subspecies of *O. niloticus* which are of importance to aquaculture are distributed as follows (Lowe-McConnell, 1988; Pullin, 1983): *O. n. niloticus* is widely distributed from west Africa to the Nile and Yokon Rivers; Lake Chad basin and river Niger, Benoue, Volta, Gambia and Senegal. *O. n. eduardianus* is distributed from Lakes Edward and George basins, Lake Albert, Lake Kivu, Ruziz River and Lake Tanganyika and has been artficially stoked in Lakes Victoria and Kyoga. *O. n. vulcani* is distributed from Lake Turkana and Crater lakes on Central Island. In this thesis, *O. shiranus* sp and *O. mossambicus* are referred to as mossambicus group tilapia as distinguished from *O. karongae* and *O. niloticus* which are non-mossambicus tilapia.

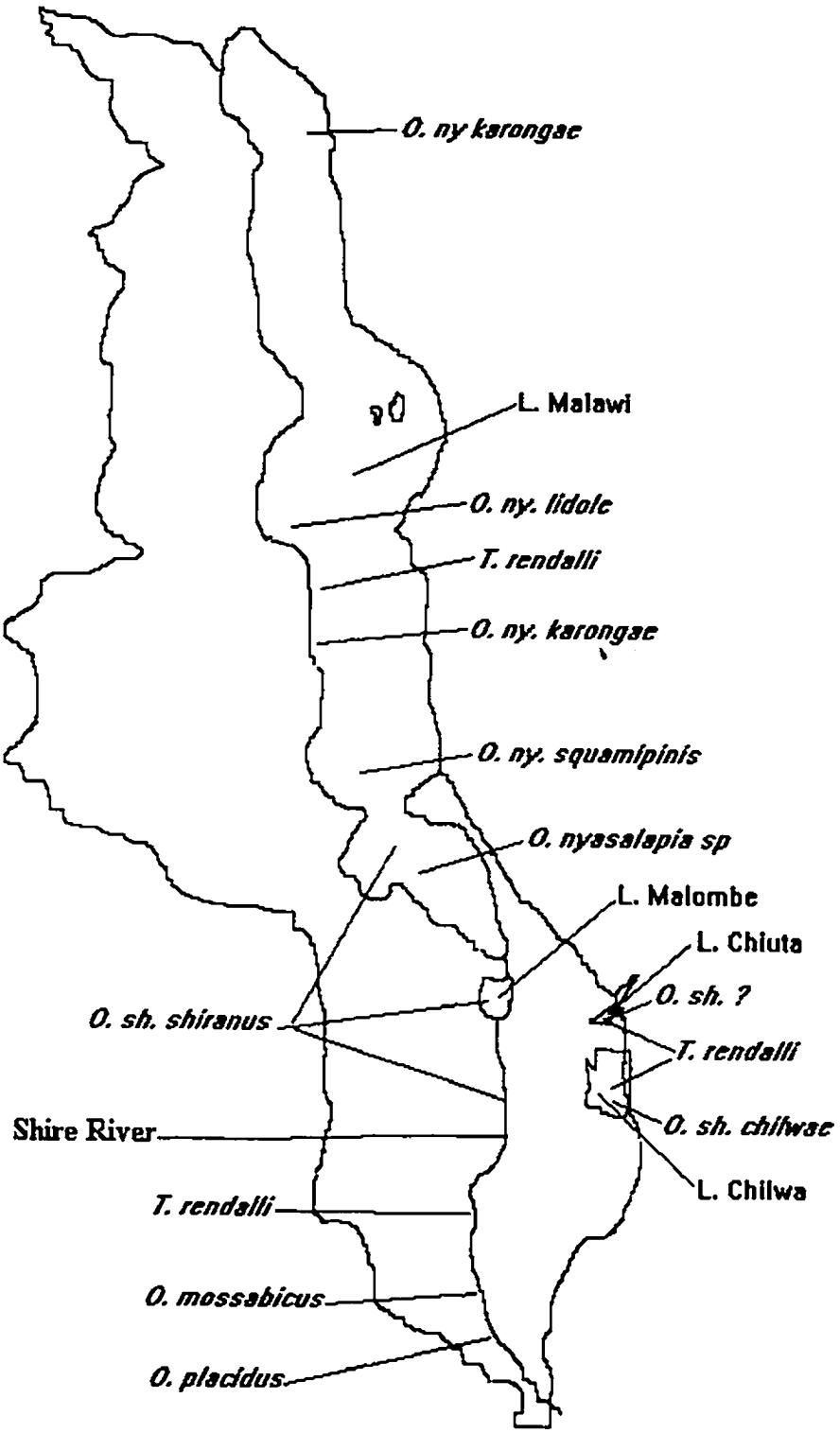


Fig. 3.1: Map of Malawi showing the distribution of tilapia; adapted from Msiska, 1988.

3.2.3. *Use of molecular genetic markers in phylogenetic studies of tilapia*

Genetic characterization, an essential component of aquaculture management, is carried out in order to identify the various strains of a particular species. The most common biochemical technique that has been used to identify tilapia is allozyme electrophoresis (Kornfield *et al.* 1979; Cruz *et al.* 1982; McAndrew and Majumdar, 1983; Basiao and Taniguchi, 1984; Brummett *et al.* 1988; Golman *et al.* 1988; Van Der Bank *et al.* 1989, Sodsuk and McAndrew, 1991; Pouyaud and Agnese, 1995; Sodsuk *et al.* 1995). Some of these applications have successfully discriminated among species. The major weakness of the technique is that it has a low resolution and is not sufficiently sensitive to reveal discriminatory genetic characteristics in these recently evolved cichlids. For instance, 32 enzyme loci scored in five subspecies of *O. niloticus* did not show any diagnostic alleles that could be used to discriminate among the subspecies. More than 87% of the loci scored were monomorphic (Seyoum, 1990). Lack of enzyme variation has also been reported in *T. zilli* (Cruz. *et al.* 1982) and in cichlids from the sea of Galilee (Kornfield *et al.* 1979). The low resolution power of enzyme electrophoresis has prompted investigations into alternative biochemical markers. Most of these analyses have been carried out at DNA level. Seyoum and Kornfield (1992) used 42 restriction enzymes to discriminate among seven subspecies of *O. niloticus* based on RFLPs of mtDNA. The seven subspecies were unambiguously discriminated and dichotomous keys for identifying them were developed based on the consensus between the RFLPs and morphological

characters. Reid *et al.* (1990) used RFLP analysis to identify *O.* (*Nyasalapia*) species from Lake Malawi. Of the eight restriction endonucleases used to digest mtDNA, one endonuclease *Ava*II was found to be a useful marker for characterizing three subspecies of *O.* (*Nyasalapia*) flock. Comparatively less effort has been devoted to analyzing nuclear DNA than mtDNA for phylogenetic studies of tilapia (Bardakci and Skibinski, 1994). Franck *et al.* (1994) used the SATA satellite DNA of 237 bp (Type I), 230 bp (Type II) and 209 bp (Type III) to infer phylogenetic relationship among the tilapiine fishes. They demonstrate a closer relationship between genera *Oreochromis* and *Sarotherodon* than between either of the two mouth brooding genera and *Tilapia*. Bardakci and Skibinski (1994) have successfully used RAPD markers to distinguish between *O. aureus*, *O. mossambicus* and *O. niloticus* and between four subspecies of *O. niloticus*.

The variability of microsatellite markers has stimulated interest amongst scientists in exploring their use in phylogenetic studies of tilapia and other cichlids. Several laboratories have initiated studies to isolate tilapia microsatellites (T.D. Kocher, *pers. comm*). There is therefore potential for microsatellite markers to find a wide application in evolutionary studies of tilapia in the near future. Contrary to this view, Wright and Bentzen (1994) speculate that microsatellites have less utility in phylogenetic and systematic studies because very little is understood about their mutation.

Microsatellite regions are conserved in position and sequence between species (Schotterer *et al.* 1991; Stallings *et al.* 1991; Garza *et al.* 1995) and are therefore being used in phylogenetic studies to construct phylogenies (e.g. Bowcock *et al.* 1994). Several distance measures are being used; these include those that are calculated based on allele frequencies and differences between allele sizes. These distance measures are based on either the infinite allele model or stepwise mutation model assumptions (Garza *et al.* 1995).

Repeat number in the VNTR is generated by three main mechanisms; gene conversion, mutation and selection on the loci (Garza *et al.* 1995). Gene conversion is especially important in the minisatellites where it has been demonstrated to generate new alleles (Jeffreys *et al.* 1994). Its importance in the microsatellites is not yet known, and may probably not have a significant impact because gene conversion only involves replacement of one copy of the locus by another but does not hinder the overall drift of the average repeat number (Garza *et al.* 1995). Mutation plays the most significant role in regulating the repeat numbers in the microsatellites. It has been observed that most of the mutations are predominantly biased towards an increase in size. The bias being more pronounced in repeats which are substantially different in size than in repeats that are similar (Garza *et al.* 1995). The large mutations may be due to such processes as unequal crossing-over as opposed to strand slippage by DNA polymerase which is thought to bethe major mechanism of microsatellite evolution (Levison and Gutman, 1987 cited in Garza *et al.* 1995).

The observation that microsatellites may be associated with some cellular functions provides the basis for speculating that selection may play an important role in regulating repeat number (Garza *et al.* 1995). Dinucleotide repeats are thought to possibly affect regulation of transcription of at least one gene in mammalian cells (Hamada *et al.* 1984). Stallings *et al.* (1991) have observed that microsatellites are evenly distributed in the genome but they tend not to be concentrated in traditional areas of transcription regulators. Microsatellite primers (Mfd 5) which successfully amplify a locus which lies within the gene regulating transcription in humans (*Homo sapiens*) does not amplify in chimpanzees (*Pan troglodytes*; Garza *et al.* 1995). CA repeats have the potential of forming Z-DNA which probably facilitates packaging during chromosomal condensation in meiosis (Hamada *et al.* 1982).

Microsatellites are conserved in closely related species. Stallings *et al.* (1991) found 100% homology in the positions of two loci in humans (*H. sapiens*) and chimpanzees (*P. troglodytes*). Bowcock *et al.* (1994) report that many human microsatellite loci are also present in the great apes (Pongidae). The GT repeats are present and conserved in similar positions in species that are distantly related like humans and rats (Stallings *et al.* 1991). These observations imply that some of the microsatellite loci are ancient in origin and the homology that they manifest provides a basis for utilizing microsatellites in phylogenetic studies (Garza *et al.* 1995). The authors support the fact that caution should be exercised in phylogenetic interpretation when microsatellites are used especially at interspecific level;

otherwise microsatellites are useful for tree building at species level and in closely related species (Goldstein *et al.* 1995). The argument is based on the fact that alleles of the same size within a species may be identical by descent and might have evolved through the same mutational events. These properties do not however apply to similar size alleles in different species.

Deka *et al.* (1995) observe that microsatellite loci provide resolution beyond the power of traditional blood-group and protein loci in evolutionary studies and should therefore be used in phylogenetic studies. The exceptionally high mutation rates of microsatellites may prove more informative for working out relationships among closely related species as well as among subpopulations of a single species (Goldstein *et al.* 1995). In this study microsatellite DNA markers were used to examine the genetic relationships of *O. shiranus* sp in Malawi.

3.2.4 **Objectives**

The objectives of this component of the study were:

i. to determine the genetic relationships between *O. shiranus* sp found in Lakes Chiuta, Chilwa and Malombe (Fig 3.1). Identification of these wild populations was important in order to characterize the various semi-wild and domesticated populations of the species that were analyzed in **Chapters 4 and 5**,

ii. to determine the relationship between genetic relationship of *O. shiranus* sp and the known events in the geological history of Lakes Chilwa, Chiuta and Malombe,

iii. to determine the phylogenetic relationship between *O. shiranus* sp and other species of the genus *Oreochromis*.

3.3 MATERIALS AND METHODS:

3.3.1 *Sources of samples*

A summary of sources of the samples for the species and the number of individuals analyzed is provided in Table 3.1 and Fig 3.2

3.3.1.1 *O. shiranus* sp.

O. shiranus sp samples were collected from Lakes Chilwa, Chiuta, and Malombe (Fig 3.2). According to the existing classification, the Lake Chilwa population is a definite representative of *O. sh. chilwae* while the Lake Malombe population is a representative of *O. sh. shiranus* (Trewavas, 1983). Lake Chiuta population is intermediate between the two subspecies and is therefore problematic to assign to a particular subspecies by conventional means.

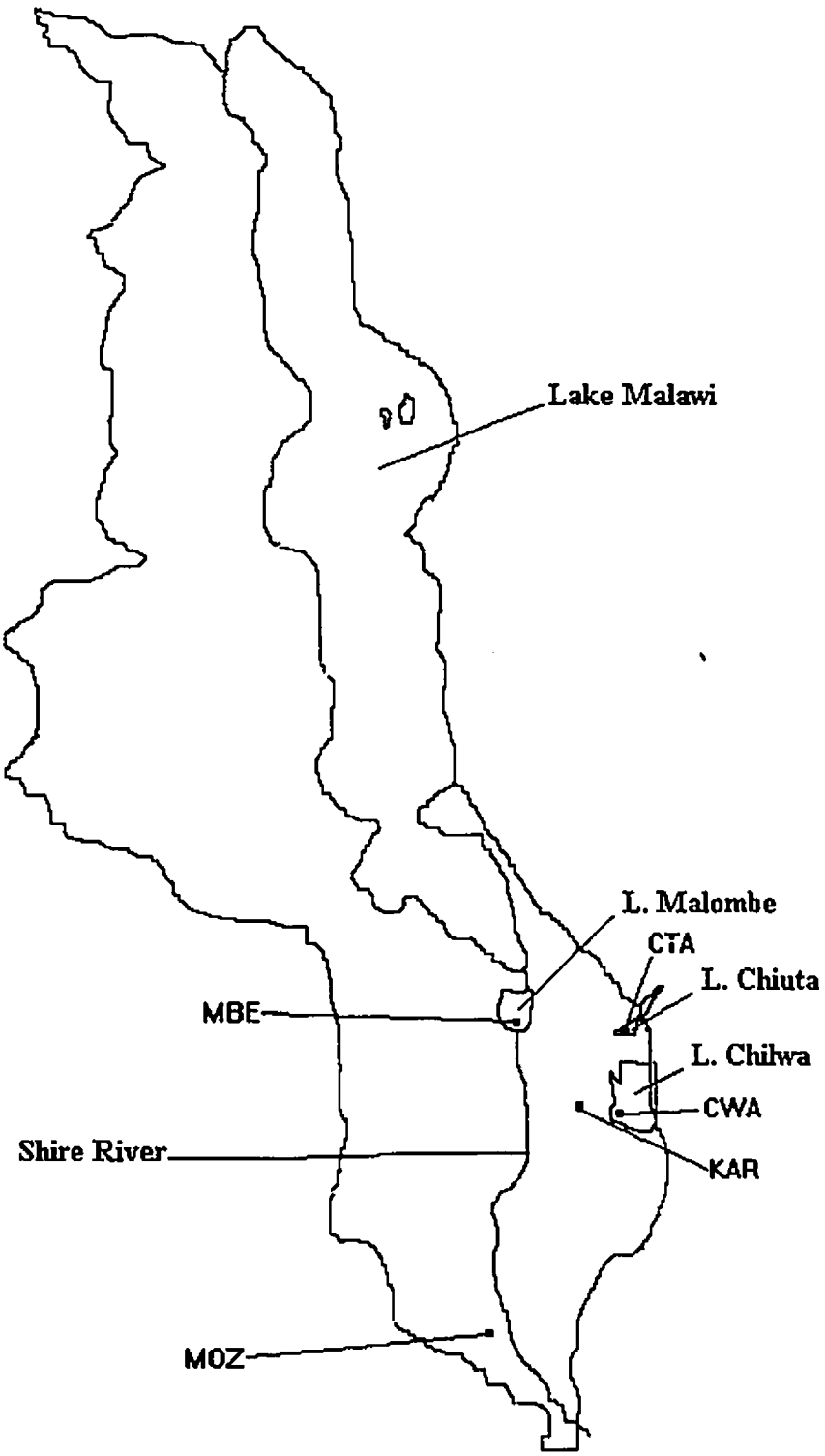


Figure 3.2: Map of Malawi showing locations where samples of *Oreochromis* sp. were collected. The populations are presented in abbreviations as defined in Table 3.1.

3.3.1.2 *O. karongae*

The wild population of *O. karongae* was collected from Lake Malawi and identified while in ponds at NAC (G.F. Turner, *pers. comm*). All the individuals analyzed were in their breeding stages so they were identified to species level (Lowe, 1959; McKaye and Stauffer, 1988; Turner *et al.* 1991).

3.3.1.3 *O. mossambicus*

The culture of *O. mossambicus* is restricted to the southern region of Malawi, especially in the Lower Shire hence samples for this study were collected from Kasinthula Pilot Fish Farm (KPFF), a government station and distribution center for the species. The progenitors of the population came from Shire River (Fig 3.2).

3.3.1.4 *O. niloticus*

The *O. niloticus* samples were obtained from Thailand by the Marine Gene Probe Laboratory (MGPL). The species has been introduced widely in southeast Asia from Africa.

Table 3.1: Summary of sources of samples analyzed

Species	Abbreviation	n	Source	Country
O. sh. chilwae	CWA	53	L. Chilwa	Malawi
O. shiranus sp.	CTA	50	L. Chiuta	Malawi
O. sh. shiranus	MBE	50	L. Malombe	Malawi
O. mossambicus	MOZ	40	KPFF	Malawi
O. karongae	KAR	40	NAC	Malawi
O. niloticus	NIL	30	?	Thailand

3.3.2. ***Blood sample collection and DNA analysis***:

Blood samples were collected from all the species. Blood collection, extraction and DNA analysis were carried out according to the procedure outlined in **Chapter 2**.

3.3.3. ***Geological history of the lakes in Malawi***:

Data and information on the geological events in the history of lakes in Malawi was obtained through desk study of the relevant literature.

3.3.4. ***Data Analysis***:

3.3.4.1 *Test for Hardy-Weinberg Equilibrium and population structure*

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. The DIPLOIDL program in GENEPOP was used to compute F-statistics according to Weir and Cockerham (1984).

3.3.4.2 *Genetic relationship*

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. Patterns of genetic relatedness between species and subspecies were examined using cluster and ordination analyses in NTSYS-pc program Version 1.80 (Rohlf, 1992). A phylogenetic tree was constructed using the unweighted pair-group method with arithmetic averaging (UPGMA) in the SAHN program. The goodness of fit of the cluster analysis was determined by computing cophenetic correlation between the cophenetic value matrix and the original distance matrix using the MXCOMP program (Rohlf, 1992).

Non-hierarchical patterns among the species and subspecies were analyzed using principal components analysis and multidimensional scaling. The principal component analysis was carried out using the arcsine transformation of the gene frequencies to determine the clusters in plots of the first two dimensions (Sokal and Rohlf, 1994; Rohlf, 1992; Phelps *et al.* 1994). Mantel's test was carried out to assess the goodness of fit of the principal components analysis (Rohlf, 1992). Multidimensional scaling of the Cavalli-Sforza and Edwards (1967) chord distance was carried out to examine the relationship among species and subspecies. This methods of ordination differs from principal components analysis in that it does not assume linearity or the complete absence of hybridization among OTUs (Lessa, 1990). Multidimensional scaling was carried out using the MDSCALE program where a statistic called stress was also computed to measure the goodness of fit of the distances in the configuration space to the monotone function of the original distance (Rohlf, 1992).

3.4. **RESULTS**:

3.4.1 *Allelic variation*

A summary of number of alleles is presented in Table 3.2. The number of alleles varied within species at different loci. Among the three *O. shiranus* sp (CWA, CTA and MBE); the highest locus variability was observed at loci Os-7R and Os-75 and lowest variability at loci Os-7 and Os-64. In MOZ the highest number of alleles was observed at loci Os-75 and Os-7. While locus Os-25 was the third most variable in *O. shiranus* sp populations, it was the least variable in MOZ with three alleles. The order of locus variability in KAR was similar to that observed in CWA, CTA and MBE. In the NIL population all the loci were equally variable. The number of alleles scored per locus ranged from five to eight; the lowest variability was observed at locus Os-64 and the highest at locus Os-75. Loci Os-25, Os-7R and Os-64 showed similar degree of variability; seven alleles were scored at each of the loci.

Table 3.2 : Levels of interlocus variation in five *Oreochromis* sp.

	Number of alleles					
Locus	CWA	CTA	MBE	MOZ	KAR	NIL
Os-7	5	6	6	8	10	7
Os-25	6	16	11	3	8	7
Os-7R	14	21	22	6	17	7
Os-64	4	5	6	3	6	5
Os-75	14	17	17	7	10	8

3.4.2. ***Heterozygosity and test for conformity to HWE***:

Observed and expected heterozygosity values have been summarized in Table 3.3. The lowest expected heterozygosity values were in CWA, 0.585 with range of 0.292 to 0.902. The CTA population showed the highest heterozygosity; 0.791 with range of 0.602 - 0.942. The results of tests for conformity to HWE are shown in Table 3.4. The exact test shows that when each locus-population combination was treated individually at 5% level of significance several species showed significant departure from HWE. With correction for Bonferroni's multiple testing, most of the locus-population combinations showed no significant departure from HWE. About 24 (80%) of the 30 population-locus combinations showed no significant deviation from HWE while 6 (20%) showed significant deviation ($p<0.05$).

Table 3.3: Observed and expected heterozygosity at five microsatellite loci

Locus	CWA	CTA	MBE	MOZ	KAR	NIL
Os-7						
Observed	.265	.469	.489	.706	.649	.833
Expected	.292	.602	.709	.754	.789	.778
Os-25						
Observed	.577	.808	.771	.278	.447	.900
Expected	.495	.863	.752	.439	.469	.805
Os-7R						
Observed	.673	.880	.837	.667	.842	.767
Expected	.810	.942	.925	.763	.914	.687
Os-64						
Observed	.216	.429	.604	.333	.559	.826
Expected	.434	.649	.566	.393	.706	.734
Os-75						
Observed	.826	.755	.644	.667	.600	.767
Expected	.902	.899	.926	.642	.842	.753

Table 3.4 : Level of significance of departure from HWE using the exact test.

Locus	CWA	CTA	MBE	MOZ	KAR	NIL
Os-7	0.05	0.03	<0.01*	0.31	0.30	0.92
Os-25	0.01	0.24	0.81	0.04	0.37	0.73
Os-7R	<0.01*	0.22	0.13	0.06	0.19	0.92
Os-64	<0.01*	<0.01*	0.97	0.18	0.04	0.72
Os-75	0.13	0.01	<0.01*	0.10	<0.01*	0.24

3.4.3. ***Population structure***:

Table 3.5 shows the level of intra- and interpopulation variation at the five loci. The inbreeding coefficient values (F_{IS}) represent overall deviation from HWE. Locus Os-64 showed the highest deviation from HWE with 18.6% more homozygotes than expected. At loci Os-75 and Os-7 there were 14.6 and 13.2%, respectively, more homozygotes than expected. The deviation from HWE was low at loci Os-25 and Os-7R where there were 0.9 and 8.6%, respectively, more homozygotes than expected. At all loci combined, there were 11.0% more homozygotes than expected. The F_{ST} (θ) values show the proportion of the total variation that can be ascribed to differences between population allele frequencies (Deka *et al.* 1995). The F_{ST} ranged from 10.8 to 29.0% and the average F_{ST} was 22.0%. All the loci showed high amount of variability with heterozygosity range of 0.611 to 0.830 and overall mean of 0.705.

Table 3.5: Level of intra- and interpopulation variation at five loci in *Oreochromis* sp

Locus	F_{IS}	F_{ST}	H
Os-7	0.132	0.283	0.646
Os-25	0.009	0.273	0.629
Os-7R	0.086	0.108	0.830
Os-64	0.186	0.290	0.611
Os-75	0.146	0.146	0.808
Average	0.110	0.220	0.705

3.4.4. *Genetic distance and phylogeny*:

Cavalli-Sforza and Edwards (1967) chord distance measures are presented in Table 3.6. The Lake Chiuta population (CTA) was closer *O. sh. chilwae* (CWA) than to *O. sh. shiranus* (MBE; D = 0.034 vs D=0.095). The CTA population was also closer *O. sh. shiranus* than to *O. mossambicus* (MOZ; D=0.070 vs D=0.098). *O. karongae* (KAR) was closest to MBE (D=0.089) and *O. niloticus* (D=0.111). A dendogram of Cavalli-Sforza and Edwards (1967) chord distance using UPGMA clustering is presented in Fig 3.3. The two major clusters were (1) the mossambicus group tilapia (CWA, CTA, MBE and MOZ) and (2) the non-mossambicus tilapia (KAR and NIL). Among the mossambicus tilapia, Lakes Chilwa and Chiuta populations clustered together representing *O. sh. chilwae* and the next closest population was MBE representing *O. sh. shiranus*. The cophenetic correlation was 0.86 and is considered a good fit (Rohlf, 1992).

Table 3.6: Cavalli-Sforza and Edwards (1967) chord distance between *Oreochromis* species

	CWA	CTA	MBE	MOZ	KAR
CTA	0.034				
MBE	0.095	0.070			
MOZ	0.091	0.098	0.107		
KAR	0.125	0.112	0.089	0.122	
NIL	0.137	0.117	0.104	0.148	0.1107

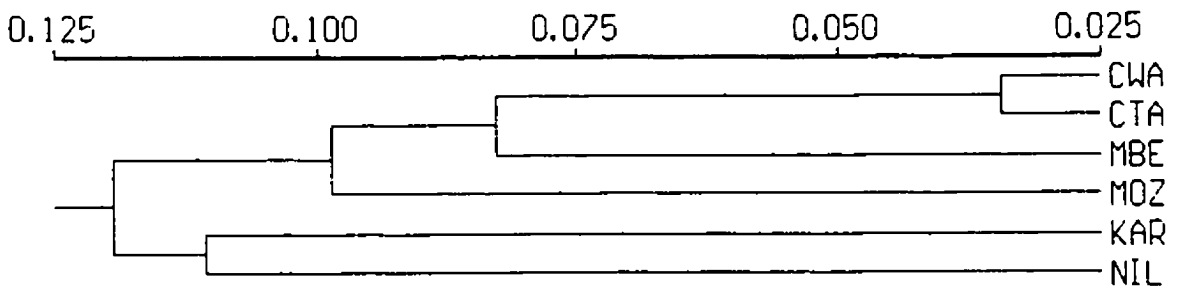


Figure 3.3: Dendogram constructed by the UPGMA method of the Cavalli-Sforza and Edwards (1967) chord distance.

A plot of principal components analysis is presented in Fig 3.4. The mossambicus tilapia were grouped with high values of component 1 (PC1) while the non-mossambicus tilapia were grouped with low values of component 1. *O. niloticus* was separated from the Malawian tilapia with low value of component 2 (PC2). The cluster also shows that although in the phylogenetic tree presented above KAR and NIL form a clade, they were genetically distant species. The correlation between the matrix of distance among all pairs of points and the matrix of distance among species was 0.88 and is considered a good fit (Rohlf, 1992).

A plot of the first two dimensions of multidimensional scaling analysis is presented in Fig 3.5. The clustering of the mossambicus tilapia was similar to that shown by the UPGMA method above where mossambicus group tilapia were separated from non-mossambicus species. Stress (a measure of goodness of fit) was 0.00 and is considered perfect (Rohlf, 1992).

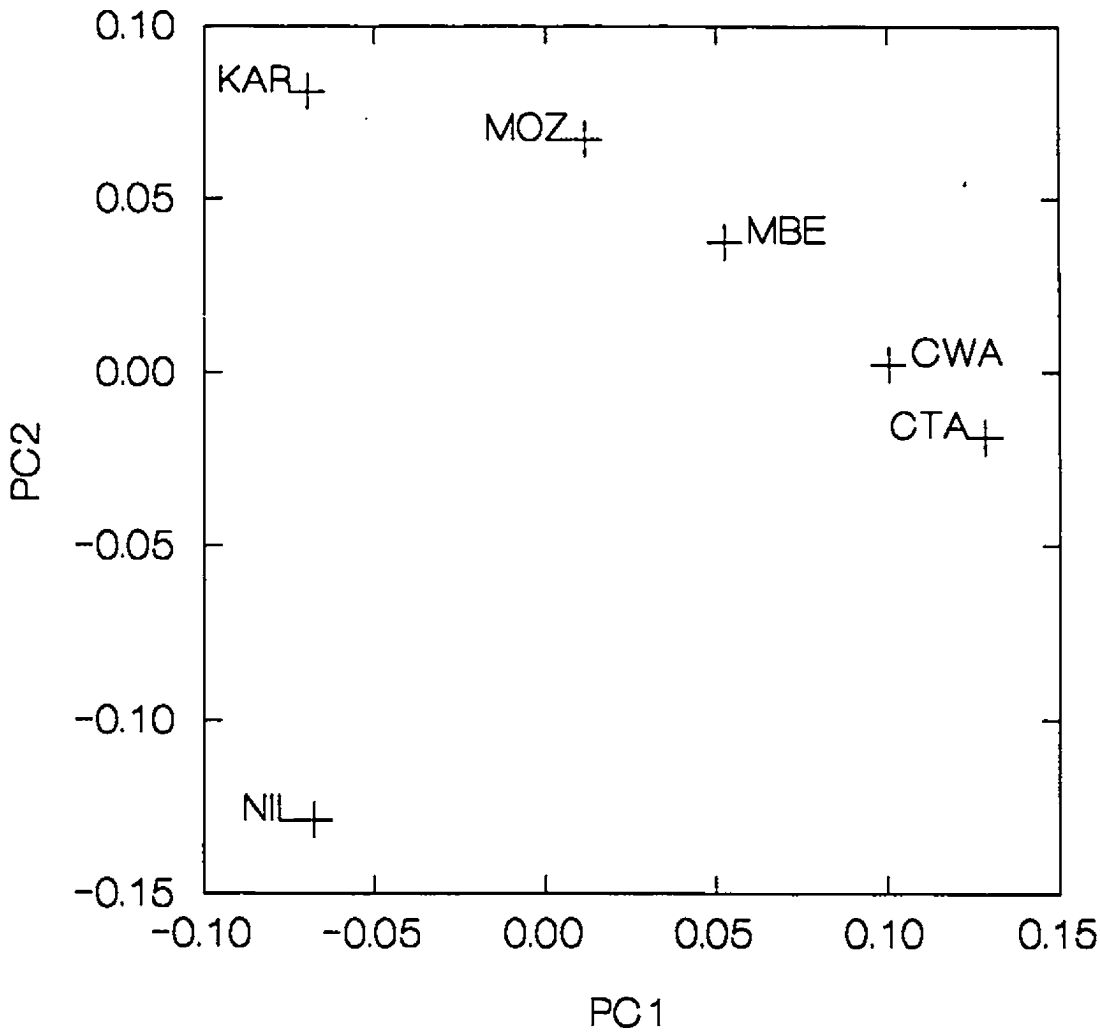


Figure 3.4: Plot of tilapia species against values for the first two principal components

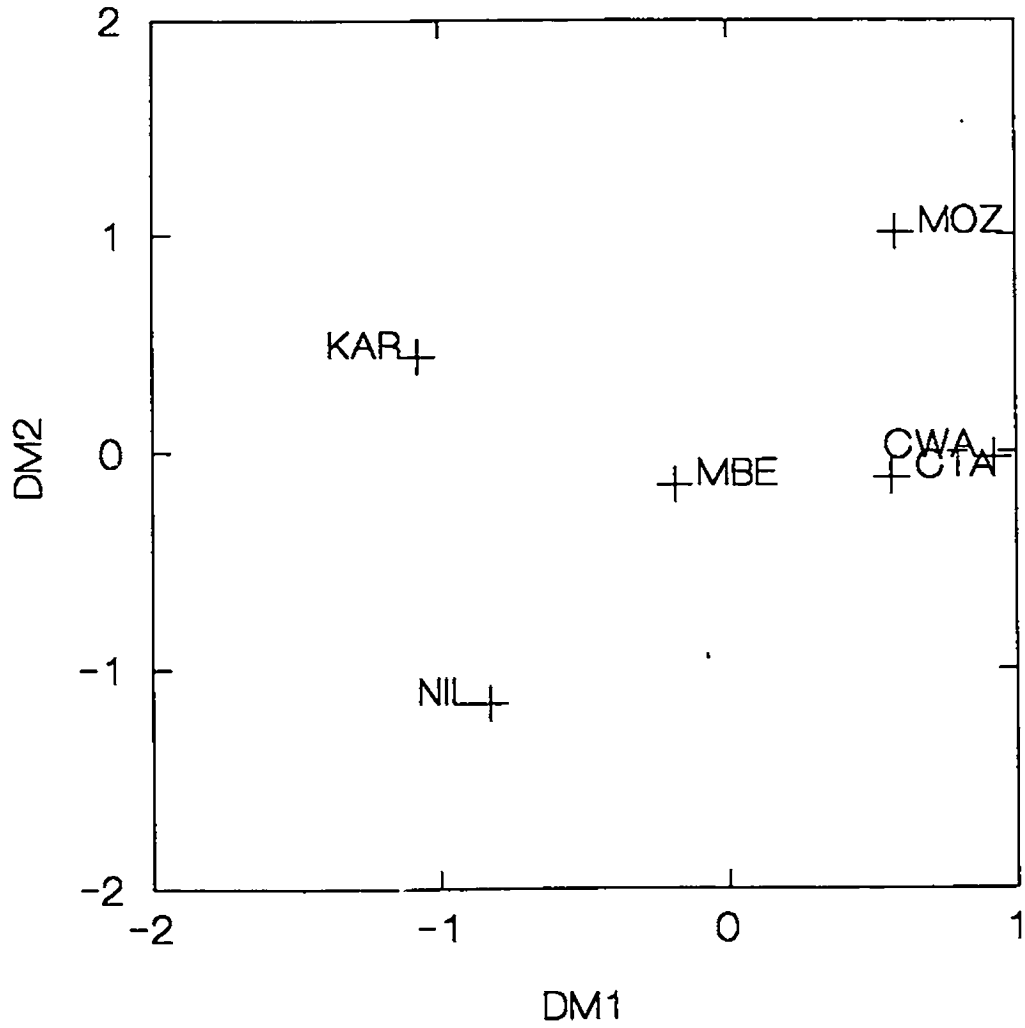


Figure 3.5: Plot of tilapia species against the first two dimensions of the configuration produced by multidimensional scaling of Cavalli-Sforza and Edwards (1967) chord distance

3.4.5. *Geological relationship between Lakes Chiuta, Chilwa and Malombe*

3.4.5.1 *Lake Chilwa*

Lake Chilwa is the twelfth largest lake in Africa and the second largest in Malawi. It is centered on 15° 30'S latitude and 35°30'E longitude, being the most southerly major African lake (Lancaster, 1979). The lake is 100 km to the southeast of Lake Malawi, 50 km to the southeast of Lake Malombe and 85 km to the south of Lake Chiuta. The total area of the lake is 1,836 km^2 of which 678 km^2 is open water, 578 km^2 is surrounded by swamps and marshes and 580 km^2 is grassland which becomes inundated seasonally (Lacanster, 1979).

The Chilwa-Chiuta basin is estimated to have been formed during the Cretaceous Period, 65-70 million years ago (Lancaster, 1979). This was a result of downwarping of the central part of the Chilwa-Phalombe Plain; with the consequent termination of the course of the ancestral Phalombe-Lugenda River (Fig. 3.6), resulting in ponding which formed the ancestral Lake Chilwa. The lake is *endorheic*, that is it has no outlet (Lancaster, 1979). It is separated from Lakes Malawi, Malombe and Shire River by a narrow watershed. There is no evidence to support earlier speculations that Lake Malawi ever had an outlet via the Chilwa area to the Ruo River (Dixey, 1926 cited in Lancaster, 1979) or that the Malawi-Chilwa system drained southeast to the Indian Ocean near Quelimane. It is only Chilwa and Chiuta that were a single open lake with an area of about 5,500 km^2 and

drained to the Indian Ocean by the Lugenda River in Mozambique; and the two lakes are now separated by a sand bar (Fig. 3.6).

The sand bar between Chiuta and Chilwa was formed probably during the early Holocene phase (8000-9000 B.P) or earlier, by "an easterly movement of beach sand across the northern end of Lake Chilwa" (Lancaster, 1979). Initially it started as a growth of a series of recurred spits from the western shore of the lake; then the wind action on the exposed beach sand resulted in the formation of dunes on the crests of the spits. The sand was mainly washed down into the lake through bed loads of Domasi and Sumulu Rivers. The sand bar is about 1-1.5 km across, and in places reaches a height of 25 m above the north Chilwa plains. It is narrow towards the east and reaches a height of 12 m above the plain (Lancaster, 1979).

Five major rivers drain into Lake Chilwa from the Shire Highlands and Zomba mountain; these are: Domasi, Likangala, Thondwe, Namadzi and Phalombe (Fig. 3.6). In total they all contribute 70% of the total inflow to the lake. Other influent rivers include Sumulu and Lingoni from Chikala Hills.

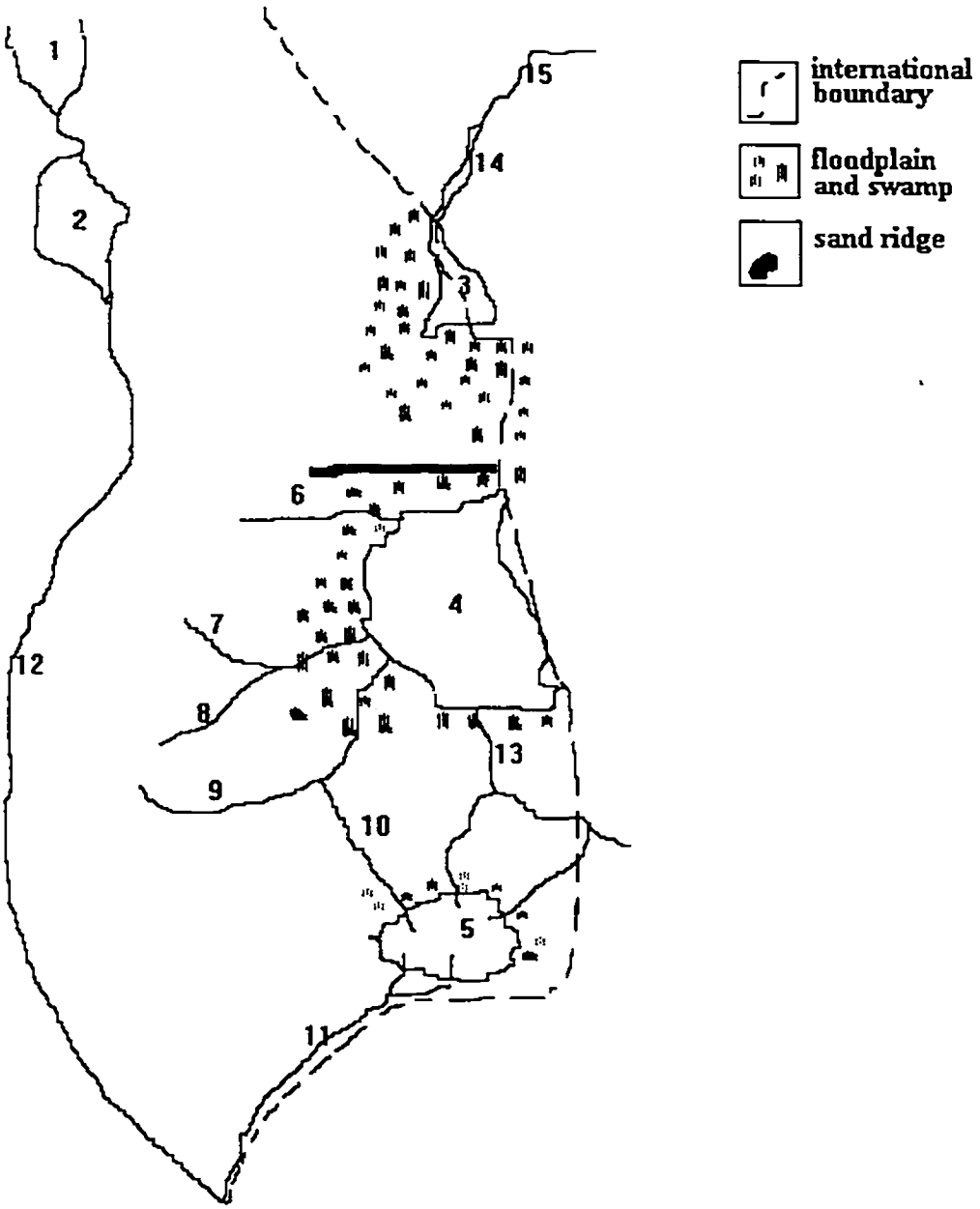


Figure 3.6: Map of the river and lake systems of the Chilwa-Chiuta drainage system. 1. Lake Malawi; 2. Lake Malombe; 3. Lake Chiuta; 4. Lake Chilwa; 5. Mulanje Mountain; 6. Domasi River; 7. Mulunguzi River; 8. Thondwe River; 9. Namadzi River; 10. Phalombe River; 11. Ruo River; 12. Shire River; 13. Sombani River; 14. Lake Amaramba; 15. Lugenda River.

3.4.5.2 ***Lake Chiuta***

Lake Chiuta has a total area of 113 km^2 of which only 50 km^2 is on the Malawian side and the rest is on the Mozambican side (ICLARM and GTZ; 1991). It is linked to Lake Amaramba and drains to the Indian Ocean by Lugenda River (Fig.3.6)

3.4.5.3 ***Lake Malombe***

Lake Malombe is shallow and has an area of 390 km^2. Its maximum depth is about 6 m. It is a flood plain lake in the course of Shire River. It receives its waters from Lake Malawi through the upper course of the Shire River and drains to the middle course of the river.

3.5 DISCUSSION:

3.5.1 *Conformity to Hardy-Weinberg Equilibrium*:

The level of heterozygosity observed in the microsatellite loci analyzed (overall mean = 0.705) was much higher than those observed in allozymes e.g. Sodsuk *et al.* (1995) observed heterozygosity values of 0.08 for *O. sh. shiranus* and 0.108 for *O. karongae*. This likely reflects the higher mutation rates at microsatellite loci (Terauchi and Konuma, 1994; Watkins, *et al.* 1995). Since expected heterozygosity is a function of number of

alleles and allele frequencies at a locus, it was observed that the number of heterozygotes was much higher than that reported in allozymes. Several studies in microsatellites report departure from HWE (see Bruford and Wayne, 1993) and in most cases there tend to be more heterozygote deficiencies (homozygote excess) than excess (Devlin *et al.* 1990). In this study, heterozygote deficiency was observed at all the loci. The test for HWE which is based on the difference between the observed and expected number of each distinct genotype in population is more complicated in the VNTR because of the considerably high error in the estimation of fragment lengths. The power of the test is also weakened by the high number of alleles and genotypes at microsatellite loci. The other possible source of error may be the fact that homozygosity or heterozygosity in the microsatellites is determined based on the length of alleles and not on whether the PCR products have identical sequences or not. There are also cases of null alleles which are not amplified. These could result in pseudohomozygotes whereby heterozygotes are indistinguishable. It is therefore questionable as to whether the inconsistencies observed regarding departure from HWE were chance daparture or not.

Deviation from HWE is brought about by several forces like selection, inbreeding, phenotypic assortive mating and population subdivision (Devlin *et al.* 1990). These forces are generally supposed to be more pronounced in populations which have been subjected to human intervention than in wild populations. The results in Table 3.5 show that there were incidences of significant deviation from HWE in populations which had been

subjected to human intervention (CWA and KAR) as well as those that had not (MBE and CTA). These departures from HWE could therefore be by chance due to multiple testing and the large number of alleles and genotypes in the microsatellites all of which compromise the power of the test.

3.5.2 *Relationship between geological history of the lakes and genetic distance*

The results show that there were genetic differences between the Lake Malombe population and the Chilwa-Chiuta populations of *O. shiranus* sp. Msiska (1988) speculates that the population in Lake Chiuta is *O. sh. shiranus* but this study shows that it was closer to *O. sh. chilwae* than to *O. sh. shiranus*. This supports an earlier hypothesis by Trewavas (1983, page 356) that there is no genetic difference between Lakes Chilwa and Chiuta populations hence the two populations belong to the subspecies *O. sh. chilwae*.

The DNA analysis was supported by geological evidence that the two lakes were originally open waters and provided normal habitat for *O. sh. chilwae* (Fig. 3.6; Trewavas, 1983) and that Lakes Chilwa and Chiuta did not harbor any of the endemic species of Lake Malawi other than *O. shiranus* and *Astotilapia callipterus* (Gunther; Lancaster, 1979). The observations that *O. sh. shiranus* and *O. sh. chilwae* were closer to *C. mossambicus* than to *O. karongae* support the theory that *O. shiranus* sp unlike *O.*

Nyasalapia species flock entered Lake Malawi from the south (Trewavas, 1983; page 356).

3.5.3 *Application of the molecular sytematics evidence to aquaculture in Malawi*:

The molecular systematics results reported in this study will provide useful information for identifying the various populations that have been domesticated in Malawi. The close relationship between *O. mossambicus* and *O. sh. chilwae* posed problems in distinguishing the various species and populations within species in aquaculture which were reported to be mainly hybrids (Msiska, unpublished). The two species were the first to be domesticated at the National Aquaculture Center and distributed to small scale farmers as discussed in detail in **Chapter 4**.

3.6 **CONCLUSION**:

Lack of reliable procedures for identifying cultured species in Malawi has deterred proper broodstock handling procedures and development of improved strains. The microsatellite DNA markers used in this study, distinguished among the various species of tilapia, and subspecies of *O. shiranus* sp were identified.

The molecular systematics results confirmed the previous speculation that there was no genetic difference between Lakes Chilwa and Chiuta populations, hence they were not distinct subspecies and that *O. shiranus* sp was genetically close to *O. mossambicus*, a species which is distributed to the south of Malawi. The discrimination of the subspecies was also supported by the known events in geological history of the lakes whereby Lakes Chilwa and Chiuta were an open water lake.

CHAPTER 4

GENETIC CHANGES IN *OREOCHROMIS SHIRANUS* SP. ASSOCIATED WITH THE EARLY STAGES OF NATIONAL AQUACULTURE DEVELOPMENT

4.1 ABSTRACT:

Malawi has experienced rapid aquacultural development since the mid 1980s. The most widely cultured species is *O. shiranus* sp, a mouthbrooding tilapia which has been recruited into aquaculture from wild populations less that 40 years ago. Microsatellite DNA probes were used to investigate the genetic diversity during domestication and population structure of *O. shiranus* sp. The objective was to estimate the loss of genetic diversity and to see if it could be associated with events in the known history of aquaculture development. Five polymorphic microsatellite loci were scored in 14 populations. The mean number of alleles per locus range from 4.4 ± 1.17 to 13.2 ± 3.31; the number of alleles was higher in the wild populations than in the domesticated populations. Other measures of genetic diversity were also lower in the domesticated compared to wild populations, and the decline of diversity was correlated with the time elapsed since the founding of the farm stock. Cluster analysis grouped domesticated populations into three; (1) those that trace their genealogy from Lakes Chiuta and Chilwa populations and are now spread all over the country, (2) those that come from Lakes Malawi and Malombe and (3) hybrids between *O. shiranus* sp and *O. mossambicus*. A Monte Carlo technique (Mantel's test) demonstrated a correspondence between matrices of genetic distance and known stock transfers between farms, fisheries stations and natural populations but no significant relationship to geographical distance *per se*. Mantel's correlation coefficients of 0.33 and -0.66 were observed between genetic distance and geographic distance and between genetic distance and transfer proximity, respectively.

Genetic differentiation among farms was strongly influenced by the pattern of known exchanges among the farmers and introgressive hybridization which had occurred between *O. shiranus* sp. and *O. mossambicus* in the farmers' ponds. It is concluded that the process of genetic changes in the species subsequent to domestication are best explained and predicted by socio-economic factors that influence the behavior of farmers, rather than the time-and-distance models of standard population genetics.

4.2 INTRODUCTION:

4.2.1. *General*

Improved aquaculture breeds and increased aquaculture production are urgently needed in Malawi as natural fish stocks become exploited to the point of collapse. The government, recognising that the genetic improvement should be accomplished by active conservation of genetic biodiversity, has banned the importation of species and breeds (e.g. *O. niloticus*) from outside the country. The situation in Malawi thus offers an opportunity to study the genetic consequences of the early stages of domestication of species new to aquaculture. In particular, it permits investigations into the trade-off betweeen breed improvement (or possibly, deterioration) and the loss of genetic diversity in a setting which is typical of those developing countries that possess useful aquaculture genetic resources. These countries are faced with the challenge of reconciling breed conservation programs with yield improvement. Experience in livestock management shows that although production can be achieved by improved management of indigenous breeds, the common tendency is to import exotic breeds (Hall and Bradley, 1995). The consequences are that the developing countries are continously dependent on foreign aid to meet the high nutrition and veterinary care requirements of the exotic breeds. Diversion of attention to maintenance of exotic breeds has been attained at the expense of indigenous breeds which disappear even before they are described and documented (Rege, 1994; Hall and Bradley, 1995). The Malawi government policy on aquaculture development prohibits

importation of exotic species of fish; this offers opportunity to concentrate on the development of indigenous strains.

The dominant group of fish cultured in the Malawian aquaculture industry are the tilapia, of which the most widely cultured species is *O. shiranus* sp. Several government and non-governmental organisations have been involved in the domestication of the species, notably, the Fisheries Department of Malawi, the University of Malawi, the Ministry of Agriculture, donor-funded aquaculture development projects and churches like the Roman Catholic Church in northern Malawi (Kandoole and Ambali, 1992) and the Church of Central African Presbyterian in Zomba district. The industry has experienced rapid expansion since the mid-1980s through increased support for aquaculture development from external aid (Refer to **Chapter 6** for details). The number of fish farmers has almost doubled since then (ICLARM and GTZ; 1991).

The increase in the number of farmers has translated into increased demand for tilapia fingerlings and broodstock. The situation was aggravated in the 1990s when the government of Malawi banned culture of common carp (*C. carpio*), an exotic species. Several complaints about a supposed continuous deterioration in the growth rate of *O. shiranus* sp. have been brought to the attention of the Fisheries Department and its collaborators in the country, which have conducted various feeding and water quality trials in an attempt to improve the situation (Costa-Pierce and Pullin, 1992).

4.2.2. *History of domestication and stock transfer of* O. shiranus *sp.*

The domestication of *O. shiranus* sp. for small scale aquaculture has its roots in the establishment of the National Aquaculture Centre (Formerly known as Domasi Experimental Fish Farm) in 1959. The fish ponds at NAC were first stocked in May 1960 with 19 *T. rendalli* and 42 *O. shiranus* sp. from a reservoir in Blantyre. In July 1960 an additional 142 *O. sh. shiranus* from Shire River was added to the ponds (Fig. 4.1). The next major stocking of *O. shiranus* sp at NAC took place in 1967 for conservation purposes when Lake Chilwa dried in 1966/67 and there were large mortalities of the species in the lake. After the lake reflooded in 1969, the lake was restocked with progeny of the broodstock that was maintained at NAC and supplemented with fingerlings from the reservoirs in the southern region (Morgan, 1971; Mathotho, 1975; ICLARM and GTZ; 1991).

Apart from NAC other non-governmental organisations were also involved in the domestication of *O. sh. shiranus*. The Roman Catholic church in northern Malawi constructed a reservoir at the Bishop's residence which was stocked in 1964 with fingerlings from Lake Malawi. Although there are no records of the exact place where the fish were collected, it is believed that the collection was done in the northern part of the lake (Bishop's residence management, *pers. comm*). The Fisheries Department has collected fingerlings and broodstock from the Bishop Reservoir for distribution to small scale farmers in the northern region.

Although NAC was established in the early 1960s as a centre for aquaculture research and development, its activities in promoting small scale aquaculture during the period 1960-70 have not been documented in the Fisheries Department reports (Kalinga, 1990). The thrust of small scale aquaculture extension started in 1970 in the upland areas of Zomba district at Chingale (Fig. 4.2) by the Oxford Committee for Famine Relief (OXFAM). OXFAM distributed *O. mossambicus* to the farmers; the choice of species was probably based on the earlier research reports which indicated that the species was more tolerant to lower temperatures than *O. shiranus* sp. *O. mossambicus* was later introduced to NAC where various research trials were carried out on the species (ICLARM and GTZ; 1991). Farmers in the lowland areas of Zomba started fish farming later after the OXFAM project had been established at Chingale. After 1985 there has been such a rapid increase in the number of fish farmers that NAC has not been able to cope with the fingerling demand. In order to alleviate the fingerling shortage, the Fisheries Department has encouraged progressive fish farmers to provide fingerlings to the new entrants. A "seed loan" system has been instituted whereby the FD provide fingerlings to "nucleus farmers" who pay the "fingerling debt" to the FD by stocking a new farmer's pond (ICLARM and GTZ; 1991). Although this system seems to work, the department has lost close control over stock movement among the small scale farms and this study has attempted to trace the stock transfers between the government stations, small scale farms and the wild through DNA analysis and farmer follow-up interviews.

The domestication phenomena observed in aquaculture are also experienced in crops and livestock. In Africa over 95% of the domesticated ruminants are indigenous. Although these indigenous breeds have become adapted to the continent's pests, parasite, diseases and climate, very little is known about them in terms of how many breeds there are, the population sizes and their degree of genetic adaptation (Rege, 1994).

4.2.3 *Objectives*:

The objectives of this component of the study were:

i. to determine the distribution of genetic diversity in the wild and domesticated populations of *O. shiranus* sp,

ii. to determine the relationship between genetic diversity and the known events in the cultural practices in the farms,

iii. to determine the relationship between genetic distance of populations of *O. shiranus* sp and other distance variables like geographic and stock transfer distance,

iv. to identify the subspecies of *O. shiranus* sp to which the various domesticated populations belonged.

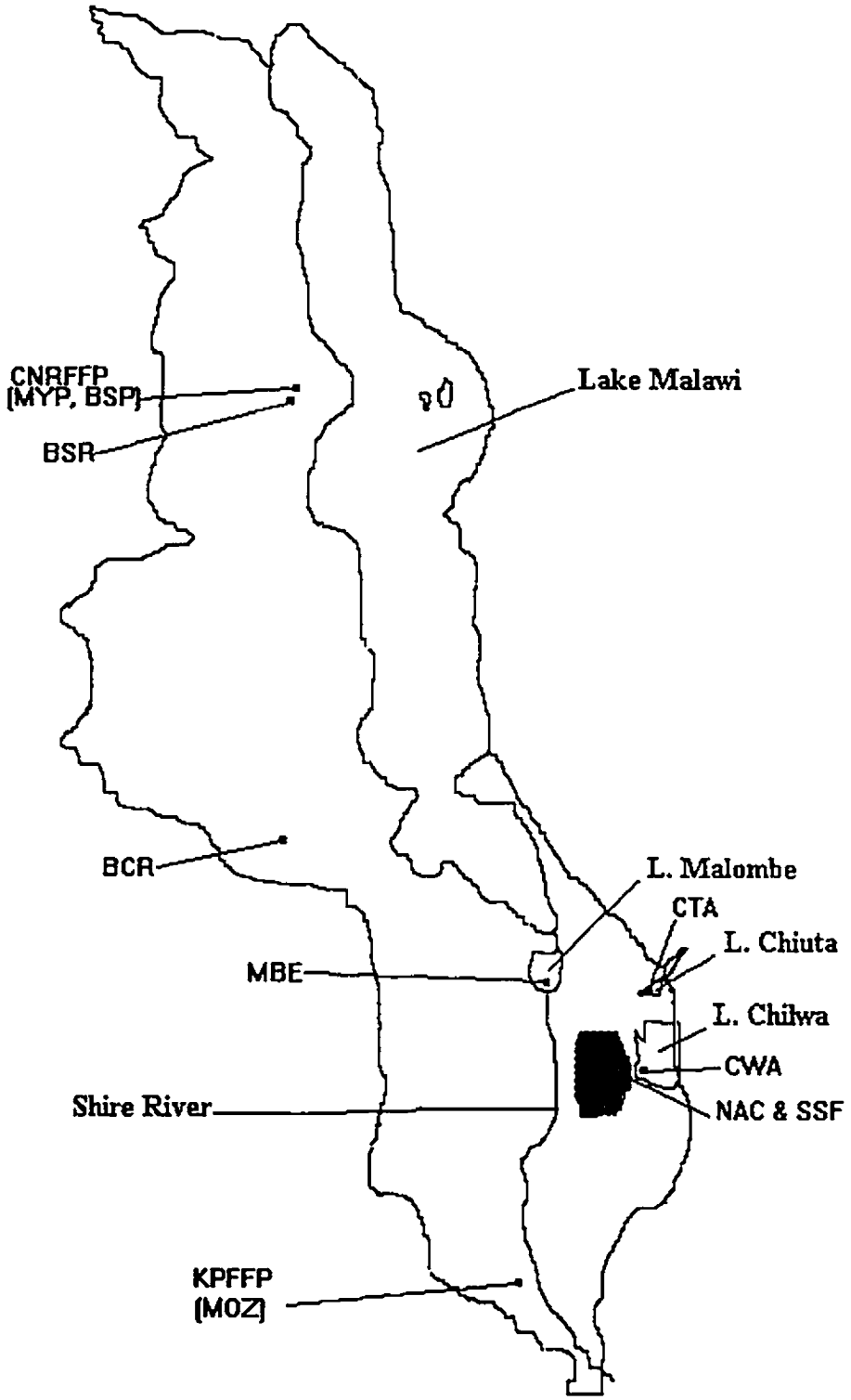


Figure 4.1: Map of Malawi showing areas where the populations analyzed were collected. Refer to text for the abbreviations and to figure 4.2 for the location of NAC and small scale farms in Zomba district.

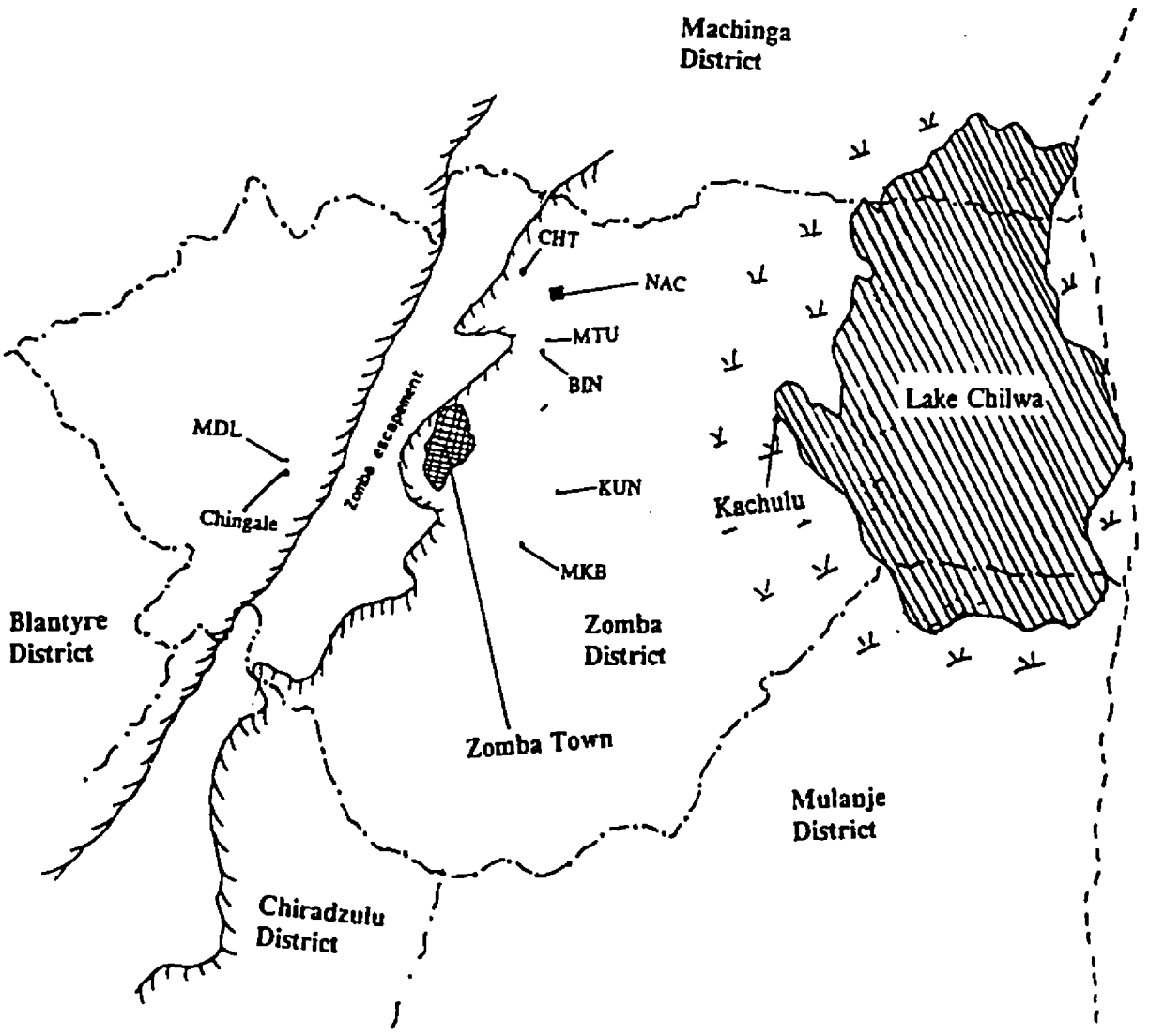


Figure 4.2: Map of Zomba district showing Lake Chilwa, NAC and small scale farms where populations were collected.

4.3 MATERIALS AND METHODS:

4.3.1 *Sample collection and DNA analysis:*

A summary of sources of samples analyzed is presented in Table 4.1 and the locations are shown in Figs.4.1 and 4.2. Samples from domesticated populations were collected from six small farms in Zomba district and two government stations. Five known sources of progenitor populations were also included; namely, Bishop Reservoir, Bunda Reservoir, Lakes Chilwa, Chiuta and Malombe. Blood was collected in all the populations and DNA was extracted and analyzed as outlined in **Chapter 2**.

4.3.2 *Farmer interviews and transfer proximity*:

During the sample collection period in 1993, an unstructured questionnaire was administered to fish farmers and government stations to find out when they started fish farming and where their first batch of fingerlings came from. The DNA analysis however showed that most of the government stations and small fish farms might have had secondary sources in addition to the primary source they had indicated earlier in 1993. A second follow-up questionnaire was therefore administered in January 1994 to the farmers and NAC through NAC to find out if the hypothesized transfers between farmers as indicated by the DNA analysis were true. The results of the two interviews were coded on a three point transfer proximity scale: 0 = no transfer of stocks between farms, 1 = indirect

transfer (by way of an intermediate) and 2 = direct transfer (Table 4.1 and Fig. 4.5). The coding is somewhat defective in at least two ways: (1) it is a symmetrical scalar (an exchange) while stock transfer is directional (a vector) and (2) the numerical coding is arbitrary and is in fact a ranking, not a continuous variable. This is the best we can do with the available information, however, and the coding follows the practice established by Waddle (1994).

Table 4.1: Summary of sources of *O. shiranus* sp population analyzed [government fish farms (GFS), small scale fish farms (SSF)]

Population	Abbrev	District/City	Status of population
NAC , GFS	NAC	Zomba	Domesticated since 1992
CNRFFP-1*, GFS	BSP	Mzuzu	Domesticated since 1990
CNRFFP-2*, GFS	MYP	Mzuzu	Domesticated since 1990
Malikebu, SSF	MKB	Zomba	Domesticated since 1979
Binali, SSF	BIN	Zomba	Domesticated since 1989
Kuntumanji, SSF	KUN	Zomba	Domesticated since 1983
Matululu, SSF	MTU	Zomba	Domesticated since 1990
Chitonya, SSF	CHT	Zomba	Domesticated since 1984
Maundala, SSF	MDL	Zomba	Domesticated since 1989
Bunda Reservoir	BCR	Lilongwe	Semi-wild, stocked in 1968
Bishop Reservoir	BSR	Mzuzu	Semi-wild, stocked in 1964
Lake Chilwa	CWA	Zomba/Machinga	Wild, restocked in 1969
Lake Chiuta	CTA	Machinga	Wild, no human intervention
Lake Malombe	MBE	Machinga/Mangochi	Wild, no human intervention

4.3.3 ***Data analysis***:

4.4.3.1 *Genetic variation*

Five polymorphic microsatellite loci were analyzed in 14 populations of *O. shiranus* sp. 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 the allelic diversity associated with transfer among water bodies: number of alleles per locus, actual number of alleles per locus on each farm, mean number of alleles per locus per farm and effective number of alleles per locus per farm (Crow and Kimura, 1970).

Test for conformity to Hardy-Weinberg equilibrium (HWE) was carried out as described in **Chapter 3** (3.3.4.1). Direct count heterozygosity was compared among the various populations to determine the amount of genetic variance in populations which is an indicator of potential response to selection.

4.3.3.2 *Population structure*

The DIPLOIDL program in GENEPOP was used to compute Wright's (Wright, 1978) F-statistics 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. Hypothetical gene flow (migration) between populations based on stepping-stone or island models were not estimated because the history of transfers (Table 4.7) was to a considerable extent known. The assumptions (e.g. equilibrium, uni-directional gene flow) underlying such estimates of gene flow were clearly inappropriate in this situation.

4.3.3.3 *Genetic relationship*

Genetic distance between populations, heirarchical clustering and ordination analysis were carried out as outlined in **Chapter 3** (section 3.3.4.2). Mantel's test was carried to determine the correlation between geographic distance and Cavalli-Sforza and Edwards (1967) chord distance, and between genetic distance and coded transfer proximity. The test was based on the null hypotheses that there was no correlation (i) between genetic distance and the geographic distance between locations where the population samples were collected and (ii) between genetic distance and transfer proximity of fingerlings/broodstock from one water body to another (Fig. 4.3). First-order partial

correlation was estimated to determine the correlation between transfer proximity and genetic distance when geographic distance was held constant ($r_{13.2}$; Sokal and Rohlf, 1994). Partial correlations indicates the degree to which some of the original association between two matrices is attribute to or explained by a third matrix. (Hubert, 1985; Sokal and Rohlf, 1994).

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 1 000 times. The significance of the partial correlation cannot be directly estimated using Mantel's test. However, Hubert (1985) has proposed another test to estimate the significance of the effect of one matrix (geographic distance) on the relationship between others (transfer proximity and genetic distance). In this test, a matrix with elements $a_{ij}b_{ij}$ was estimated by multiplying the elements of transfer proximity and genetic distance. The matrix of the products was compared with a matrix of geographic distance using Mantel's test. A significant Mantel's test indicates that the control matrix has an effect on the interaction (Hubert, 1985; Manly, 1991).

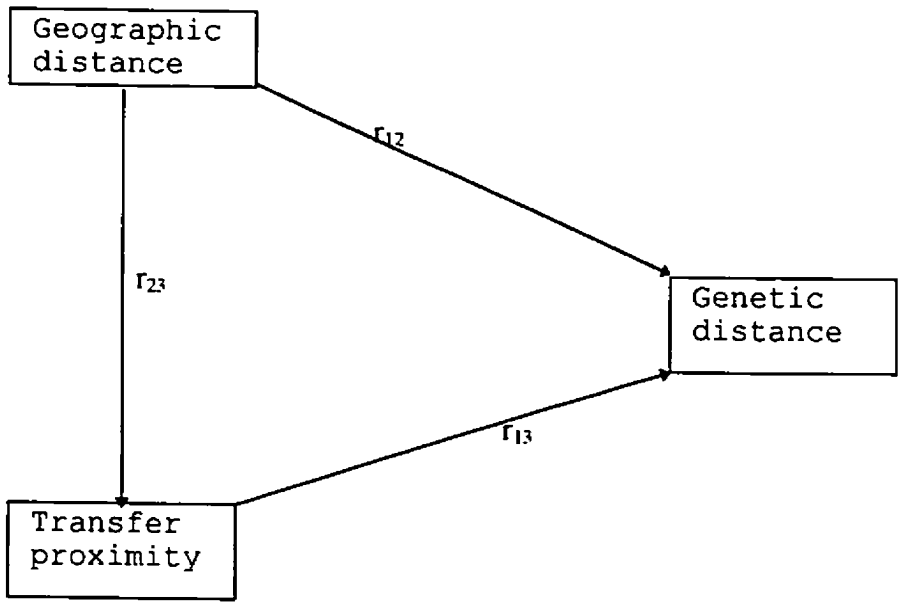


Figure 4.3: Structural explanations for the correlations calculated r_{12} between genetic distance and geographic distance; r_{13} between genetic distance and transfer proximity and r_{23} between geographic distance and transfer proximity.

4.4 RESULTS:

4.4.1 *Genetic diversity*:

Indices of genetic diversity are presented in Table 4.2 and a plot of mean effective number of alleles is presented in Fig. 4.4. In Fig 4.4 the CWA? and NAC? populations (open circles) are assumed ancestral populations which existed and have been lost. The CWA? is the ancestral populations of Lake Chilwa prior to 1966/67 when the lake dried. A population sample of CWA? was stocked at the National Aquaculture Centre in 1966/67, that is NAC?, but the pure strain of the population has disappeared. The NAC? population was widely distributed among farmers in Zomba district and was also the progenitor of the BCR and CWA (filled rectangle) populations. Since the CWA? and NAC? came from a lake which was at one point continuous with CTA and gave rise to BCR, their location on the vertical axis of Fig. 4.4 can be confidently placed between the CTA and BCR populations. The NAC population (filled rectangle) that has been recently domesticated at the station is from Lake Malombe. Most of the alleles observed in the wild populations were also observed in the domesticated populations except at locus OS-64, where three alleles were observed in the domesticated populations that were absent from samples in any of the wild and semi-wild (progenitor) populations. The mean number of alleles in the samples ranged from 4.40 to 13.2. With the exception of NAC and CWA populations; wild and semi-wild populations had a higher mean effective number of alleles than domesticated populations. Considerable reduction in the effective number of alleles was

observed in the CWA population, 3.87 alleles, compared to the other two wild populations, 7.22 and 6.65 for CTA and MBE, respectively. Among the domesticated populations, an inverse relationship was observed between the mean effective number of alleles and the length of time the population had been domesticated on a particular farm (Fig 4.4). Mean effective numbers of alleles of 2.38, 3.03 and 3.49 were observed in the MKB, KUN and CHT populations, respectively, where fish had been domesticated since 1979, 1983 and 1985 and was higher in the most recently domesticated populations, NAC, BIN, MYP, BSP and MTU. Farm populations which were established from progenitors that had low genetic variation showed low number of alleles despite having been recently recruited into ponds; e.g. MDL.

Table 4.2: Measures of genetic variation at 5 loci in 14 *O. shiranus* sp populations (sample size (n), 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).

Pop	n	Os-7	Os-25	Os-7R	Os-64	Os-75	Total	A
NAC	40	8	7	15	7	16	53	10.60±2.01
		(3.56)	(4.32)	(10.05)	(4.67)	(8.24)	(30.84)	(6.17±1.41)
MYP	40	4	8	9	8	7	36	7.20±0.86
		(3.35)	(5.93)	(6.20)	(3.15)	(4.26)	(22.89)	(4.58±0.71)
BSP	40	4	8	8	3	9	32	6.60±1.33
		(3.62)	(3.63)	(5.18)	(2.24)	(6.47)	(21.14)	(4.23±0.81)
MKB	40	6	1*	7	3	4	21	4.40±1.03
		(2.51)	(1.00)	(3.26)	(1.78)	(3.34)	(11.89)	(2.38±0.50)
BIN	40	7	7	14	5	11	44	8.80±1.62
		(4.45)	(1.68)	(8.13)	(3.24)	(5.83)	(23.44)	(4.67±1.23)
KUN	30	3	5	5	1*	8	22	4.40±1.17
		(1.85)	(2.56)	(3.37)	(1.00)	(6.39)	(15.17)	(3.03±1.03)
MTU	40	7	8	9	3	8	35	7.00±1.05
		(3.42)	(2.67)	(4.55)	(2.39)	(5.65)	(18.68)	(3.74±0.68)
CHT	35	4	6	6	1*	9	26	5.20±1.32
		(2.89)	(2.21)	(5.60)	(1.00)	(5.77)	(17.46)	(3.49±1.06)
MDL	31	5	5	7	3	5	25	5.00±0.63
		(1.47)	(1.26)	(1.95)	(1.63)	(2.65)	(8.97)	(1.79±0.27)
BCR	55	8	10	14	6	14	52	10.20±2.15
		(2.21)	(6.50)	(10.53)	(2.86)	(8.44)	(30.54)	(6.11±1.78)
BSR	55	8	10	13	4	11	46	9.60±1.630
		(5.51)	(4.62)	(6.80)	(3.09)	(7.19)	(27.21)	(5.44±0.83)
CWA	55	5	6	14	4	14	46	8.60±2.23
		(1.41)	(1.96)	(5.05)	(1.73)	(9.23)	(19.37)	(3.87±1.67)
CTA	55	6	16	21	5	17	65	13.20±3.31
		(2.47)	(6.89)	(14.84)	(2.80)	(9.09)	(36.08)	(7.22±2.55)
MBE	50	6	11	22	6	17	62	12.40±3.14
		(3.35)	(3.91)	(11.91)	(2.27)	(11.83)	(33.27)	(6.65±2.40)

* the population is monomorphic at the locus

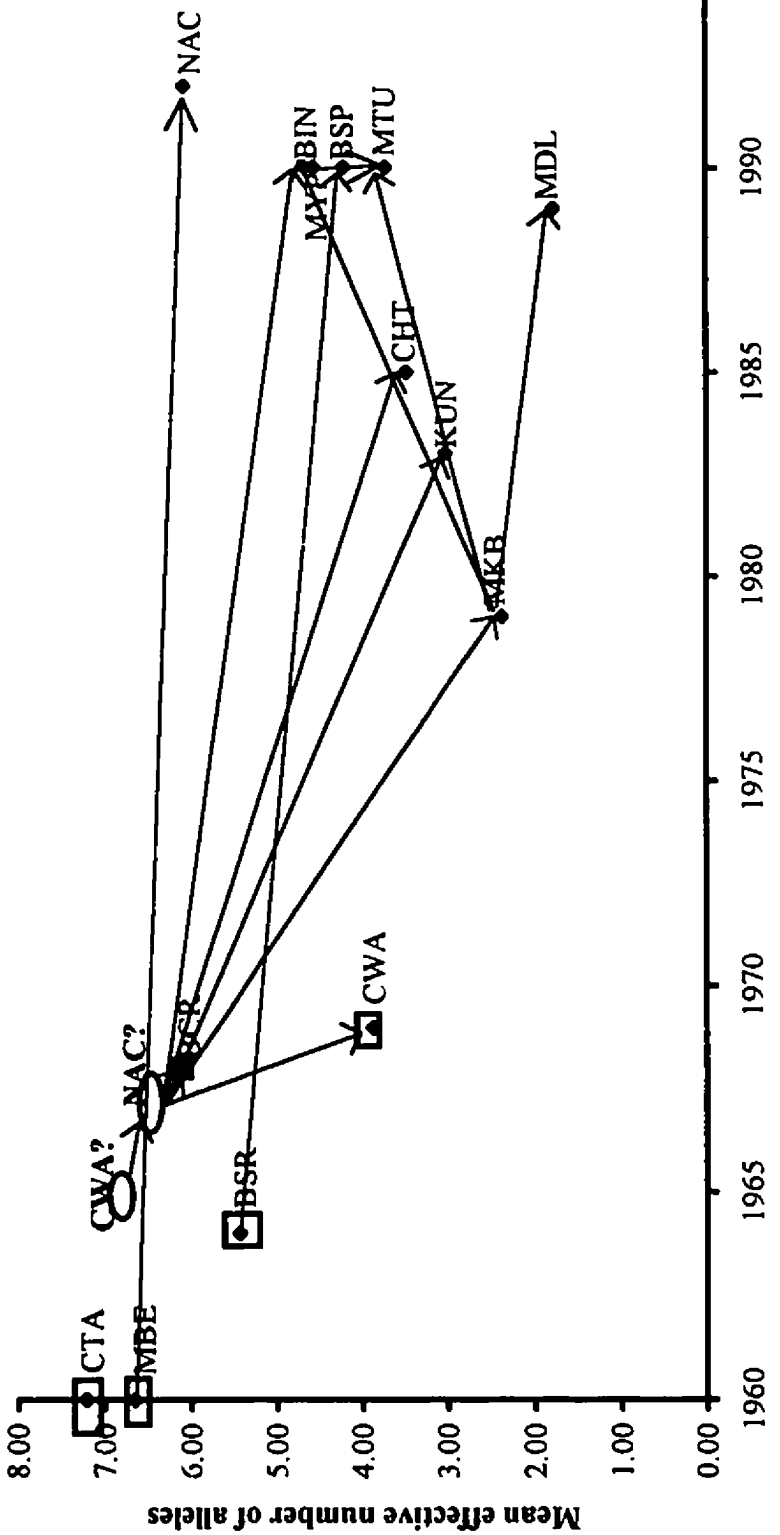


Figure 4.4: Plot of mean effective number of alleles for wild, semi-wild and domesticated populations of *O. shiranus* sp against year of stocking. Filled squares enclosed in open rectangles are wild and semi-wild populations, open circles are assumed CWA? and NAC? populations which occurred in 1960s and filled squares are farm populations. Arrows indicate population transfers in the direction of the arrow.

All the loci were polymorphic in the wild and semi-wild populations but in the domesticated populations, OS-25 was monomorphic in MKB and OS-64 was monomorphic in CHT and KUN populations.

Observed and expected heterozygosity values are presented in Table 4.3. Mean heterozygosity ranged from 0.299±0.055 to 0.759±0.041. Among the domesticated populations, the lowest heterozygosity was observed in the MDL population and the highest was in the NAC population. The lowest heterozygosity values in the wild and semi-wild populations were observed in the Bishop reservoir population. With the exception of the MKB and KUN populations, the expected heterozygosity values in the other populations were higher than observed heterozygosity.

Tests for conformity to Hardy-Weinberg equilibrium are presented in Table 4.4. According to the exact test, most locus-population combinations did not show significant departure from HWE (74.3%) while 25.7% showed significant departure.

Table 4.3: Observed and expected heterozygosity at five microsatellite loci

Pop		Os-7	Os-25	Os-7R	Os-64	Os-75	Mean±SE
NAC	Observed	0.600	0.794	0.781	0.839	0.781	0.759±0.041
	Expected	0.730	0.780	0.915	0.799	0.893	0.823±0.035
BSP	Observed	0.758	0.697	0.743	0.486	0.515	0.640±0.058
	Expected	0.712	0.844	0.851	0.684	0.777	0.774±0.034
MYP	Observed	0.657	0.686	0.706	0.529	0.656	0.647±0.031
	Expected	0.734	0.735	0.819	0.561	0.859	0.742±0.051
MKB	Observed	0.595	0.000	0.800	0.400	0.676	0.494±0.140
	Expected	0.610	0.000	0.702	0.443	0.710	0.493±0.132
BIN	Observed	0.667	0.368	0.946	0.686	0.727	0.679±0.092
	Expected	0.791	0.410	0.889	0.702	0.848	0.728±0.085
KUN	Observed	0.593	0.733	0.667	0.000	0.696	0.538±0.136
	Expected	0.470	0.619	0.716	0.000	0.862	0.533±0.148
MTU	Observed	0.760	0.500	0.897	0.483	0.476	0.623±0.087
	Expected	0.722	0.634	0.794	0.592	0.843	0.717±0.047
CHT	Observed	0.704	0.594	0.813	0.000	0.643	0.551±0.142
	Expected	0.666	0.557	0.834	0.000	0.857	0.583±0.156
MDL	Observed	0.200	0.161	0.423	0.280	0.429	0.299±0.055
	Expected	0.327	0.213	0.496	0.394	0.670	0.420±0.078
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
CWA	Observed	0.265	0.577	0.673	0.212	0.826	0.511±0.118
	Expected	0.292	0.495	0.810	0.426	0.902	0.585±0.116
CTA	Observed	0.531	0.808	0.880	0.429	0.755	0.680±0.086
	Expected	0.602	0.863	0.942	0.649	0.899	0.791±0.069
MBE	Observed	0.489	0.771	0.837	0.604	0.644	0.669±0.061
	Expected	0.709	0.752	0.925	0.566	0.926	0.776±0.069

Table 4.4: Level of significance of departure from HWE using the exact test

Pop	Os-7	Os-25	Os-7R	Os-64	Os-75
NAC	0.023	0.772	0.019	0.127	0.006
BSP	0.002	0.043	0.258	0.116	0.007
MYP	0.684	0.634	0.189	0.005	0.001
MKB	0.042	-	0.194	0.385	0.461
BIN	0.073	0.128	0.012	0.317	0.021
KUN	0.252	0.377	0.386	-	0.024
MTU	0.004	0.011	0.392	0.028	0.001
CHT	0.675	0.325	0.038	-	0.012
MDL	0.001	0.230	0.193	0.148	0.117
BCR	0.158	0.007	0.010	0.005	<0.001
BSR	0.459	0.905	0.203	0.001	0.018
CWA	0.053	0.010	0.007	<0.001	0.170
CTA	0.028	0.269	0.166	0.002	0.005
MBE	<0.001	0.857	0.194	0.967	<0.001

4.4.2 *Population structure*:

Levels of intra- and interpopulation variation at five loci are shown in Table 4.5 and pairwise F_{ST} values are presented in Table 4.6. The inbreeding coefficient values (F_{IS}) show that there was heterozygosity deficiency at all loci. F_{IS} values ranged from 0.039 at locus Os-25 to 0.20 at locus Os-75.

The F_{ST} values ranged from 0.100 to 0.336 and the mean F_{ST} at all loci was 0.198±0.043. Although the overall F_{ST} values were high, pairwise F_{ST} values of as low as 0.029 were observed (Table 4.6). The general trend was that the proportion of total variation that could be ascribed to differences between population allele frequencies (F_{ST}) was low in populations that were known to have had the same progenitor populations and high in those that did not have the same progenitor populations.

Table 4.5: Levels of intra- and interpopulation variation at five loci in fourteen *O. shiranus* sp populations

Locus	F_{IS}	F_{ST} (θ)
Os-7	0.079	0.230
Os-25	0.039	0.234
Os-7R	0.079	0.123
Os-64	0.161	0.336
Os-75	0.200	0.100
Total	0.115±0.033	0.198±0.043

Table 4. 6: Matrix of pairwise F_{ST} (θ) values

	NAC	BSP	MYP	MKB	BIN	KUN	MTU	CHT	MDL	BCR	BSR	CWA	CTA
BSP	0.078												
MYP	0.098	0.060											
MKB	0.313	0.360	0.352										
BIN	0.164	0.210	0.211	0.105									
KUN	0.279	0.310	0.305	0.348	0.262								
MTU	0.182	0.229	0.245	0.142	0.036	0.271							
CHT	0.256	0.284	0.282	0.334	0.235	0.066	0.242						
MDL	0.350	0.382	0.372	0.154	0.173	0.392	0.230	0.381					
BCR	0.148	0.155	0.135	0.265	0.173	0.114	0.197	0.126	0.281				
BSR	0.075	0.063	0.050	0.300	0.184	0.267	0.205	0.253	0.321	0.110			
CWA	0.261	0.279	0.259	0.299	0.229	0.061	0.260	0.088	0.320	0.077	0.230		
CTA	0.138	0.138	0.116	0.266	0.169	0.117	0.188	0.093	0.287	0.041	0.113	0.082	
MBE	0.029	0.076	0.086	0.339	0.192	0.316	0.214	0.299	0.365	0.160	0.062	0.282	0.156

4.4.3 *Transfer of fingerlings/Broodstock between water bodies*

Figure 4.5 shows transfers that occurred between the populations sampled. Lakes Chilwa and Chiuta were one open water in the past; hence it is not surprising that the strains in the two lakes were similar. MKB, CHT and KUN populations were distributed from NAC and the fish were ultimately derived from progeny of the Lake Chilwa population (NAC?). In addition to the NAC? stock, MKB obtained fingerlings from a farmer in Chingale area in 1979. BIN and MTU received fingerlings from NAC? and supplemented the stock with fingerlings from MKB in the early 1990s. MDL received fingerlings from MKB through the MAGFAD project which used to carry out extension activities in the upland areas of Zomba. MKB was recruited by MAGFAD as an extension agent to provide farmer-experience extension advice to new entrants into fish farming. He advised farmers on pond construction and also provided fingerlings from his farm to the new farmers. Some of such farmers were MDL, BIN and MTU. In 1992, BIN sold fingerlings to MTU when the latter did not have sufficient seed to restock.

In 1968, BSR (Fig. 4.1) was stocked with *O. sh. chilwae* fingerlings from NAC? (L. Kamwanja, *pers. comm*). The BSR populations came from Lake Malawi in 1964. In 1990, the Fisheries Department collected broodstock from the Bishop's reservoir for stocking ponds at its regional fish farming headquarters in Mzuzu (Fig. 4.1). Some of the ponds at the headquarters have been stocked with *O. shiranus* sp. from Moyale Reservoir which belonged to the Malawi Army and was about 1 Km away from the station. The progenitor

population in the reservoir came from Lake Malawi during the colonial period when several reservoirs were constructed and stocked with tilapia in the northern region by the then Ministry of Agriculture (Kandoole and Ambali, 1992).

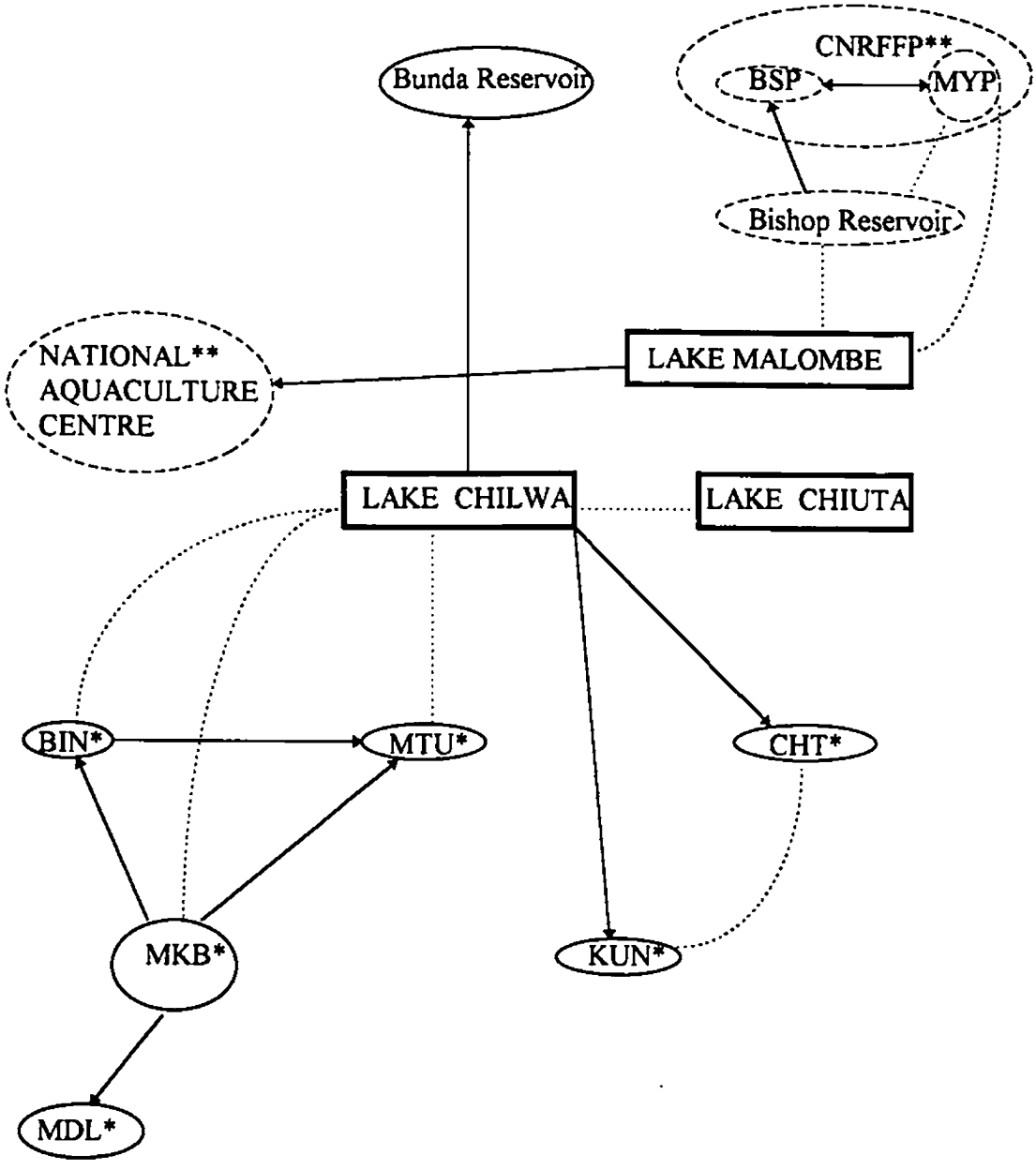


Figure 4.5. Transfer of broodstock/fingerlings between water bodies in Malawi: no transfer (0) no line connection; indirect transfer (1) connected by broken line; direct transfer (2) connected by solid arrow in the direction of transfer, ** government fish farms, * small scale fish farms.

4.4.4 *Genetic distance*:

Matrices of Cavalli-Sforza and Edwards (1967) chord distance, transfer proximity and geographic distance are presented in Tables 4.7 and 4.8. Based on the known events in the transfer of stocks between farms, *O. mossambicus* was included in the genetic distance analysis. Genetic distance values ranged from 0.029 to 0.144. The UPGMA tree constructed from the genetic distance values is presented in Fig. 4.6. The dendogram showed three major divisions between populations; from top to bottom these are (1) those that traced geneology from *O. sh. shiranus*, (2) from *O. sh. chilwae* and (3) populations in small farms which were introgressive hybrids between *O. shiranus chilwae* and *O. mossambicus*. The populations in Mzuzu (BSR, BSP and MYP) and NAC belonged to *O. sh. shiranus* and clustered with the Lake Malombe (MBE) population. The CTA, CWA, BCR, CHT and KUN populations belonged to *O. sh. chilwae* while BIN, MTU, MKB and MDL were hybrids between *O. shiranus* sp and *O. mossambicus*. Among the hybrid farm populations, BIN clustered closely with MTU and MKB with MDL. The cophenetic correlation for the dendogram was 0.87 and is considered a good fit (Rohlf, 1992).

Plots of principal components analysis and multidimensional scaling are presented in Fig 4.7 and 4.8, respectively. In Fig 4.7, the hybrid populations on small farms and *O. mossambicus* (L to O) were grouped together with low values of principal component 2 and were separated from *O. sh. shiranus* and *O. sh. chilwae*. Principal component 1 separated *O. sh. shiranus* populations from *O. sh. chilwae* where the former were

grouped with low values of PC1 and the latter with high values. The normalized Mantel's correlation between the matrix of distance among all pairs of points and the matrix of distance among populations was 0.86 and is considered a good fit (Rohlf, 1992). In the plot of dimensions 1 and 2 of multidimensional scaling analysis (Fig. 4.8), the grouping of populations was similar to that of hierarchical clustering (Fig. 4.6). The three major clusters were (1) *O. mossambicus* (J) and hybrid populations (F, G, H and I), (2) *O. sh. shiranus* (A to E) and (3) *O. sh. chilwae* (K to O) populations. Stress was 0.19 and considered fair (Rohlf, 1992).

Table 4.7: Distance matrices; lower triangle is Cavalli-Sforza and Edwards (1967) chord distance matrix and upper triangle is a matrix of transfer proximity

	NAC	MYP	BSP	MKB	BIN	KUN	MTU	CHT	MDL	BCR	BSR	CWA	CTA	MBE	MOZ
NAC		1	1	0	0	0	0	0	0	0	0	0	0	2	0
MYP	0.061		1	0	0	0	0	0	0	0	0	0	0	1	0
BSP	0.073	0.042		0	0	0	0	0	0	0	0	0	0	1	0
MKB	0.114	0.144	0.134		2	0	2	0	0	0	0	1	0	0	1
BIN	0.076	0.112	0.105	0.044		0	2	0	1	0	0	1	0	0	1
KUN	0.114	0.131	0.117	0.099	0.093		0	2	0	1	0	2	1	0	0
MTU	0.085	0.125	0.123	0.049	0.031	0.088		0	0	0	0	1	0	0	1
CHT	0.111	0.127	0.110	0.104	0.096	0.033	0.088		0	1	0	2	1	0	1
MDL	0.114	0.133	0.128	0.029	0.045	0.108	0.060	0.114		0	0	1	0	0	1
BCR	0.092	0.095	0.076	0.110	0.091	0.054	0.101	0.064	0.108		0	2	1	0	0
BSR	0.059	0.034	0.033	0.118	0.101	0.113	0.113	0.110	0.114	0.067		0	0	0	0
CWA	0.100	0.105	0.086	0.099	0.086	0.035	0.092	0.038	0.100	0.035	0.082		1	0	0
CTA	0.083	0.086	0.063	0.105	0.084	0.056	0.093	0.043	0.110	0.038	0.064	0.034		0	0
MBE	0.029	0.056	0.058	0.127	0.091	0.120	0.101	0.121	0.118	0.079	0.038	0.095	0.070		0
MOZ	0.098	0.122	0.109	0.074	0.073	0.116	0.078	0.089	0.076	0.105	0.104	0.091	0.098	0.107	

Table 4.8: Matrix of geographic distance

	NAC	MYP	BSP	MKB	BIN	KUN	MTU	CHT	MDL	BCR	BSR	CWA	CTA	MBE
MYP	917													
BSP	917	0												
MKB	30	947	947											
BIN	5	922	922	25										
KUN	25	942	942	5	20									
MTU	3	920	920	27	2	22								
CHT	10	907	907	40	15	30	17							
MDL	40	957	957	32	35	37	37	52						
BCR	310	607	607	340	315	335	300	303	350					
BSR	910	7	7	940	915	935	900	900	957	600				
CWA	3	1007	1007	18	41	20	39	51	60	350	950			
CTA	300	1100	1100	330	315	175	313	295	340	400	1000	50		
MBE	185	807	807	205	190	210	188	171	225	300	900	227	100	
MOZ	121	1128	1128	91	116	96	118	131	123	421	1031	109	421	306

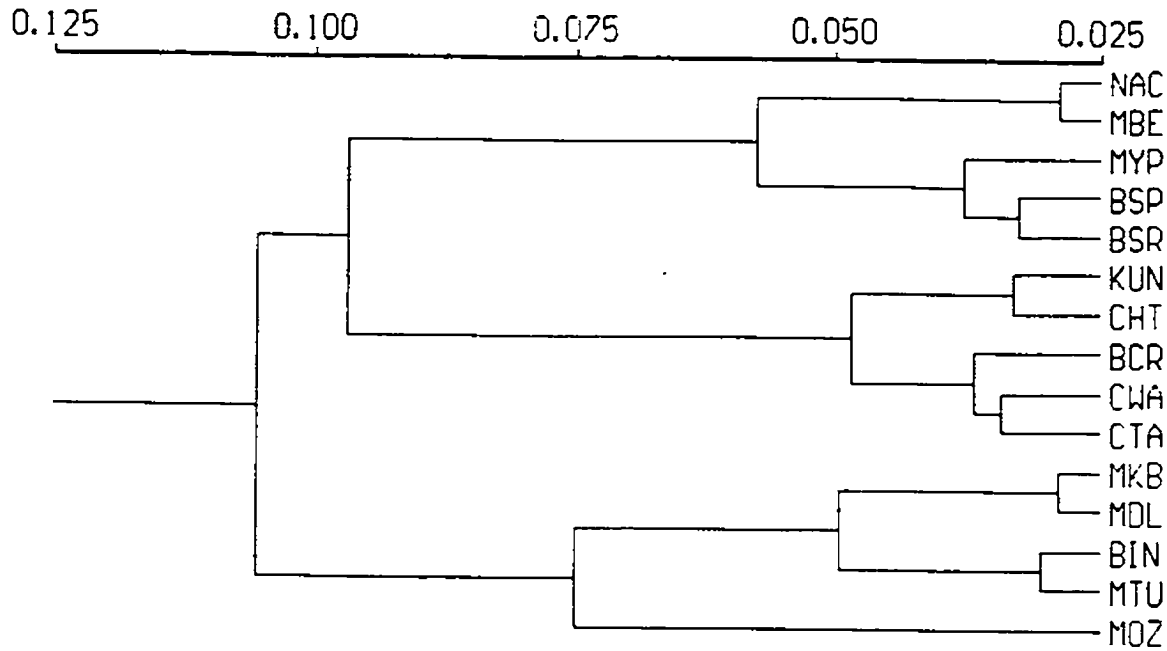


Figure 4.6: Dendogram constructed by the UPGMA method constructed from Cavalli-Sforza and Edwards (1967) chord distance.

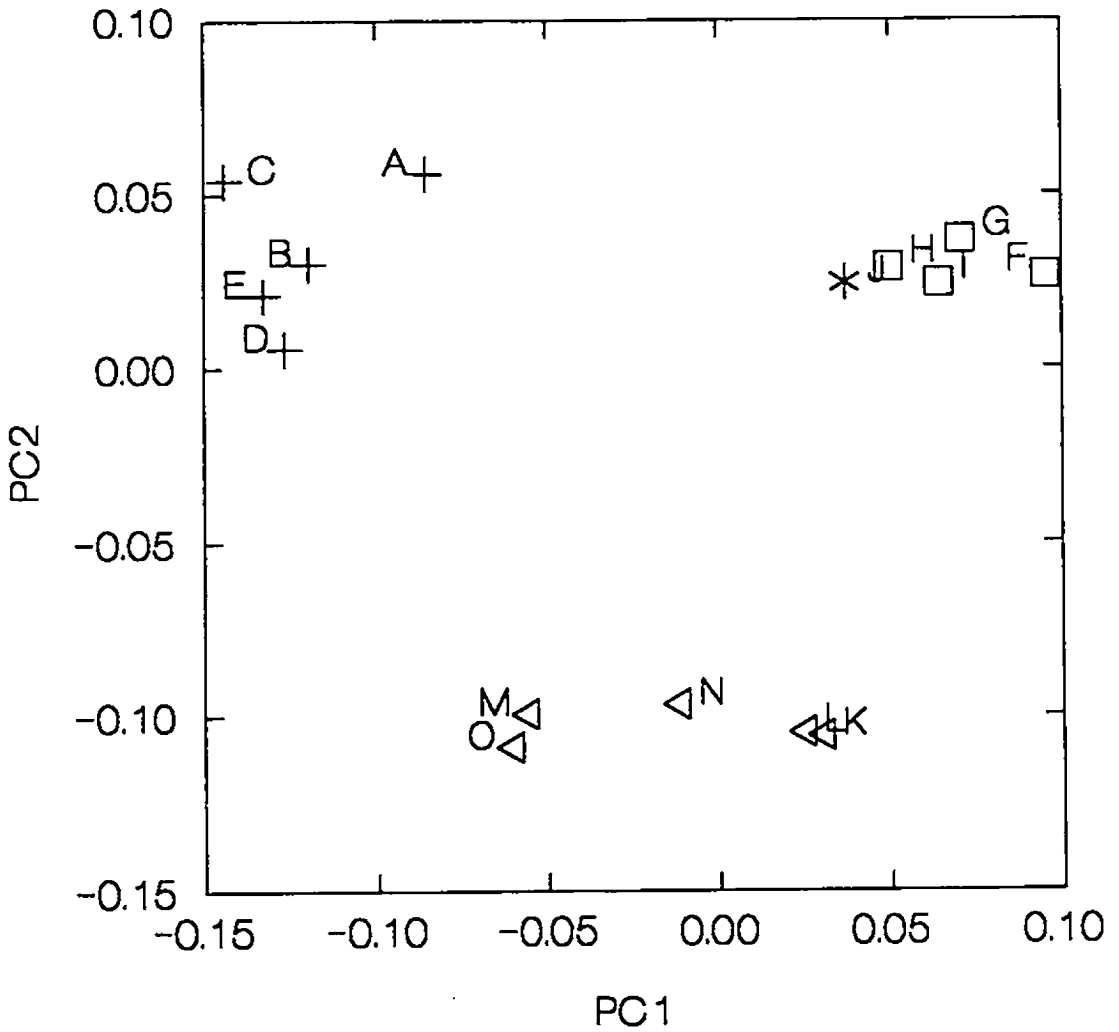


Figure 4.7. Plot of *O. shiranus* sp and *O. mossambicus* species against values for the first two principal components. For abbreviations in the plot, populations represented by crosses are NAC (A), MBE (B), MYP (C), BSP (D), BSR (E); populations represented by open rectangles are MKB (F), MDL (G), BIN (H), MTU (I), the population represented by a star is MOZ (J); and open triangles are KUN (K), CHT (L), BCR (M), CWA (N) and CTA(O).

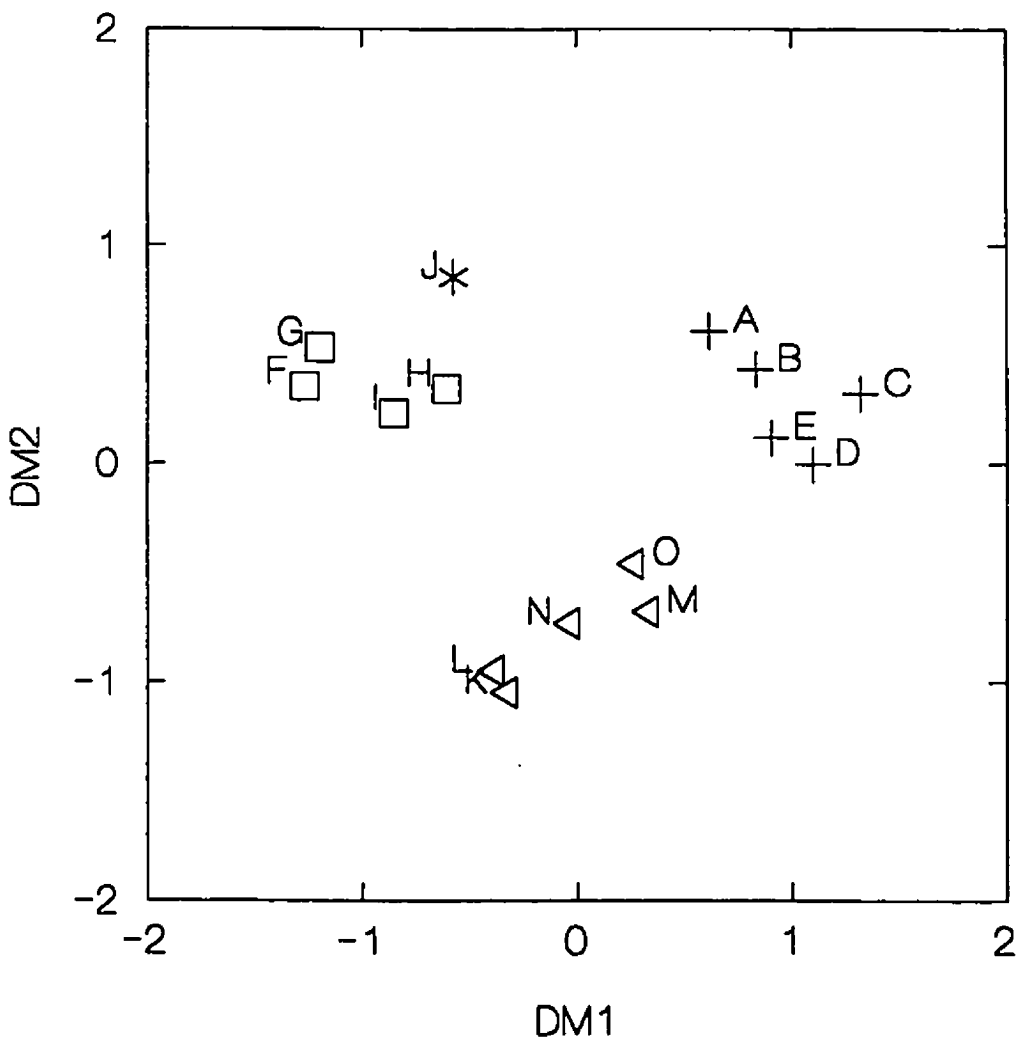


Figure 4.8: Plot of tilapia species against the first two dimensions of the configuration produced by multidimensional scaling of Cavalli-Sforza and Edwards (1967) chord distance. For abbreviations in the plot, populations represented by crosses are NAC (A), MBE (B), MYP (C), BSP (D), BSR (E); populations represented by open rectangles are MKB (F), MDL (G), BIN (H), MTU (I), the population represented by a star is MOZ (J); and open triangles are KUN (K), CHT (L), BCR (M), CWA (N) and CTA(O).

4.4.5 *Relationship between genetic distance and other distance measures*:

The normalized Mantel coefficients are presented in Table 4.9. The simple correlation between transfer proximity and genetic distance was strong and negative (-0.66) while the correlation between genetic distance and geographic distance was weak and positive but still significant by Mantel's test. The partial correlation of transfer proximity and genetic distance holding geographic distance constant ($r_{13.2}$) was -0.62. However, suggesting that geographic distance influenced genetic distance solely through its effect on transfer proximity (such transfers being negatively correlated with geographic separation). The significance of the partial correlation cannot be directly estimated using Mantel's test. Hubert's test showed that the effect of geographic distance on the relationship between genetic distance and transfer proximity was significant (p=0.006) although less significant than Mantel's test of the simple relationship between genetic distance and transfer proximity (p=0.001) or genetic distance and geographic distance (p=0.004).

Table 4.9: Normalized Mantel's coefficients and partial correlation between distance matrices

Matrices compared	r	P
Simple correlations		
Geographic distance and genetic distance (r_{12})	0.33	0.004
Transfer proximity and genetic distance (r_{13})	-0.66	0.001
Transfer proximity and geographic distance (r_{23})	-0.32	0.005
Partial correlation		
$r_{13.2}$	-0.62	

4.5 DISCUSSION:

4.5.1. *Genetic diversity*

There was a considerable reduction in the number of alleles per locus in domesticated populations. The most notable reduction was observed in the samples collected from farmers who had domesticated fish on their farms for at least seven years. In some of those farms there were loci which were almost fixed, for instance loci Os-25 in MKB and Os-64 in CHT and KUN populations. The decline in average heterozygosity followed similar trends in the average number of alleles per locus. The reduction in genetic variation in these populations is attributable to genetic drift, that is the random change in allele frequencies by mating (sampling) error in the production of new generation. Most of the farms sampled were relatively small in size and the cultural practices on those farms unintentionally minimized the number of contributing parents. All the farms had at least two ponds, one big growout pond of at least 200 m^2 and a small pond of less than 50 m^2. At the end of each growout cycle, farmers chose a few small fish which they stocked in the small pond for three months before the beginning of the next grow-out cycle. This system reduced the effective population size and consequently reduced the genetic variation of the stocks.

The effective number of alleles was on average 3.33 alleles less than the actual number of alleles per population. This was due to the large number of alleles of which some had low frequency and contributed very little to the average heterozygosity or genetic variance (Crow and Kimura, 1970). Although *O. shiranus* sp. had relatively recently been domesticated in some farms like MDL, the source of the founder stock had an impact on the genetic diversity of the populations (a secondary source in MDL's case, Fig 4.4). Some small-scale farmers assisted with extension services, notably MKB who played a role in advising on pond construction to new entrants into fish farming and selling fingerlings to those farmers. The analysis shows that farmers such as MDL who were provided fingerlings by MKB had populations of low genetic variation in their recently established fish farms. Low genetic variation in Lake Chilwa (CWA) population was due to an historical bottleneck.

4.5.2 ***Interpopulation variation***:

Pairwise interpopulation variation was low between populations that were known to have had a common source of progenitor populations or between populations where transfers were known to have occurred. This was demonstrated by low Mantel's correlation between geographic distance and genetic distance. Hubert's test of significance supports the conclusion from the observations on the Pearson correlations in which the geographic effect reduces the correlation only from -0.66 to -0.62; meaning that social transfer influences the genetic distance directly, not through correlation with geographic distance

per se. Transfer of broodstock and fingerlings between small scale farms was determined by the socio-economic relationship between the farmers. Some of the farmers reciprocated labor, e.g. BIN and MTU while others were being served by the same fish farming development project, e.g. the transfer of fingerlings from MKB and MDL was organised by the MAGFAD project. Social status of the farm also played an important role; for instance MKB population was regarded as a superior strain by other farmers because MKB was considered to be a progressive farm.

Genetic heterogeneity observed in Zomba district was attributed to the sources of progenitor populations; the NAC sample came from Lake Malombe while those of small farmers came from Lake Chilwa and some were hybrids between *O. shiranus* sp and *O. mossambicus*. The "foreign" alleles (not found in the natural populations) scored in domesticated populations of *O. shiranus* sp. contributed to the high genetic differentiation observed in domesticated populations of Zomba district. The "foreign" alleles were observed in *O. mossambicus*, a species which was distributed to farmers in Chingale area and also stocked at NAC. The results support earlier speculations that some of the domesticated population might have introgressively hybridized in ponds with *O. mossambicus*, especially in Zomba district (Msiska, unpublished).

4.5.3 *Genetic distance between populations and known history of genetic material transfer*:

The use of microsatellites to trace the history of domesticated breeds of livestock and plants have, among other organisms, been reported in cattle (*Bos taurus*, MacHugh *et al.* 1994), Soay sheep (*Ovis aries*, Bancroft *et al.* 1995) and *Arabidopsis thaliana* (Todokoro *et al.* 1995). In this study the resultant dendogram from cluster analysis of genetic distance showed concordance with the known history of the domesticated and semi-wild populations of fish in Malawi. The domesticated populations in Zomba district were directly or indirectly distributed from NAC where the largest wild broodstock recruitment was from Lake Chilwa in 1966/67. The populations in Mzuzu (BSR, BSP, MYP) clustered with Lake Malombe; an outlet from Lake Malawi where the progenitor populations were collected. The latest NAC population was collected from Lake Malombe in 1991 and the MBE population was collected from the same site of the lake in 1993.

4.6 *CONCLUSION*:

The Malawi case is typical of most aquaculture in developing countries where little thought has been given to genetic considerations during the domestication processes. These are characterized by uncontrolled transfer of stocks among farms and lack of selective breeding. We have demonstrated that genetic diversity in such culture systems is

influenced by social and economic relationships among farmers and domestication practices e.g. size of farm. The populations in the farms are small and over a period of time their genetic diversity has declined. Farmers who considered themselves progressive or who had been culturing fish for a relatively long time identified their strains as superior while in reality most such populations had low genetic diversity. Their yield performance is unknown. The major potential problem is that the strains are widely distributed in the farming community because most of the new entrants into fish farming bought fingerlings from the progressive farmers.

The lack of genetics planning in the domestication programs resulted in unnoticed and irreversible contamination of species and subspecies. Introgressive hybridization which occurred between *O. shiranus* sp and *O. mossambicus* has been unnoticed and its effects on production traits were unknown. The consequences were that there had been considerable variation in the performance of the stocks which were believed to be pure *O. shiranus* sp in the various culture systems even within the same farm.

Although NAC has continuously been recruiting wild stocks, there has generally been lack of ability to maintain the purity of the stocks for more than one generation (E. Kaunda, pers. comm.).

The government has lost control of the movement and quality of strains in small farms. The DNA analysis demonstrated that stock transfers can easily be traced by establishing

the social and economic relationships among farmers. There is a tendency for farmers who share common social characteristics, such as BIN and MTU reciprocate labor during harvesting, to exchange genetic materials. This also provides basis for community involvement is strain conservation. The existing socio-economic groups can be engaged in the conservation and yield improvement of the strain that they share among themselves.

CHAPTER 5

THE POTENTIAL OF SMALL WATER BODIES AS *IN SITU* GENE BANKS FOR AQUACULTURE: A GENETIC ASSESSMENT OF THE MALAWIAN RESERVOIRS

5.1 ABSTRACT:

The water bodies of Malawi have internationally been considered to be the home of valuable aquatic biodiversity, especially the fish species found in the lakes. Although the water bodies are rich in fish species, the current rapid increase in human activity associated with the lakes poses a serious threat to conservation of the aquatic biodiversity; and there is an apparent need for devising alternative *in situ* approaches to conservation.

Studies were carried out to assess the genetic diversity of reservoir populations of *O. shiranus* sp. It was observed that reservoirs harbored several species including *O. shiranus* sp, *T. rendalli*, *Clarius gariepinus*, *Serranochromis robustus*, *Barbus* sp and haplochromids. *O. shiranus* was found in all the reservoirs where the species were stocked between 1955 and 1968. Carnivorous species like *C. gariepinus* and *S. robustus* were found in some of the reservoirs; the former was stocked naturally through streams while the latter was artificially stocked to control recruitment of tilapia. Mean standard length of *O. shiranus* sp in the reservoirs ranged from 13.6 to 20.5 cm which was higher than the average size in the wild populations. The general trend observed was that in those reservoirs where predator:prey ratio was high, the size range of tilapia was larger than where the ratio was low.

Microsatellite probes were used to study allelic diversity of *O. shiranus* sp. The mean number of alleles ranged from 3.6 ± 0.93 to 10.2 ± 2.15. Allelic diversity was reduced in

reservoirs with high predator:prey ratios. Cavalli-Sforza and Edwards (1967) chord distance between reservoir populations ranged from 0.017 to 0.146. Mantel's normalized correlation coefficient between genetic distance and geographic distance was low (0.265) and not significant (p=0.8516). The microsatellite data showed that genetic hybridization occurred between *O. sh. shiranus* and *O. sh. chilwae* in most of the reservoirs in the southern region of Malawi.

These observations indicate that although the reservoirs have received little management attention in the country, some of them contain isolated populations which have high genetic diversity which could be conserved.

5.2 INTRODUCTION

5.2.1 *In situ* conservation

The water bodies of Malawi have internationally been considered to be the home of valuable biodiversity especially the fish species found in the lakes. Although the water bodies are rich in fish species, the rapid increase in human activity associated with lakes poses serious threat to the conservation of their biodiversity. The continuous decline of the *O. Nyasalapia* sp flock and disappearance of the cyprinids (e.g. *Opsaridium microleps* and *Labeo mesops*) are manifestations of the decline of biodiversity at the species level. It has also been demonstrated that aquaculture practices in Malawi have contributed to the deterioration of genetic biodiversity in *O. shiranus* sp (**Chapter 4**). There is therefore need to devise *in situ* conservation mechanisms by which the genetic biodiversity of cultured fish species and their wild relatives can be conserved during the anticipated increase in human populations and activities associated with the lakes.

In situ conservation is the maintenance of live animals and plants in their adaptive environment or environments as close to it as is practically possible. Conservation of live animals and plants in domesticated conditions is also considered *in situ* conservation. It differs from *ex situ* conservation, which is conservation of animals in environments removed from their normal habitats, and which may involve preservation of semen or embryos, in liquid nitrogen or live animals in zoos (Henson, 1992). In the developing

countries, *in situ* conservation has several advantages over *ex situ* conservation and should therefore be promoted in the conservation of aquatic biodiversity. *In situ* conservation requires less initial setup and maintenance costs, affords high local access to the genetic resources, preserves and promotes continuous adaptation to the local conditions (Henson, 1992).

In modern agriculture, the high yielding plant and animal materials being cultivated are those that have lost their ancestral traits due to drift and intensive selection. Agricultural technology has advanced substantially especially in developed countries but the majority of farmers in the developing countries are still practicing traditional farming. The farmers have limited funding to afford advanced technology; hence the highly selected, modern uniform varieties (breeds) are less reliable than the local breeds under traditional farming systems where there is limited control of environmental conditions (Brush, 1991; Altieri and Merrick, 1987). *In situ* conservation has therefore been carried out in several countries to preserve local breeds of crops and livestock. This has been achieved through conservative approaches taken by some farmers to save the local breeds from being genetically diluted or completely replaced. Studies of crops have shown that farmers are custodians of the local genetic material at village level. In northern Thailand, farmers did not grow any modern varieties of rice before 1969 and by 1984 only 20% of the rice planted in the region was of modern varieties (Brush, 1991).

In southern Ontario, pig farmers maintain and continuously improve a number of local races of pigs and resist the importation of the so-called "super swine" developed elsewhere. This conservation success is attributed to genetic advice provided to local farmers on a long-term basis by the University of Guelph (J. Gibson, per. comm to R. Doyle).

There are a number of instances of genetic conservation of animal breeds through the efforts of individual farmers in developing countries. In Brazil, the Crioulo Lageano strain of cattle has been conserved in this way (Henson, 1992). The breed has been developed for over 300 years and is well adapted to the rocky acid soils, high altitude and cold winter. Recently it has been gradually replaced by cross breeding with imported Indian and European breeds. For the past 40 years a farmer has collected individuals of true Crioulo Legeano strain from the southern region of Brazil and breeds them on unimproved pastures of his farm. The animal genetic resources program of the Brazilian Agriculture Cooperation and the Federal University of Santa Catarina have engaged in collaborative research with the farmer to evaluate and develop conservation strategies for the 150 cows and 10-15 bulls of the breed on the farm. A similar instance in the Hissar district of India is mentioned by Henson (1992). The Sahiwal and Hariana breeds of cattle have been maintained by a farmer who maintains 300 Sahiwal and 300 Hariana cattle in the belief that they have many adaptive characteristics which makes them ideal for the local farmers (Henson, 1992).

The concept of *in situ* genetic diversity conservation in aquaculture is relatively new compared to agriculture, and its promotion in small scale aquaculture has received mixed views. Some scientists believe that through fragmented farming systems at different scales, *in situ* conservation can be increased as experience has shown in crops in Latin America and Turkey (S. Brush; pers. comm to R. Doyle). On the contrary some scientists believe that the system cannot work because it has never been tried before (M. Prein, pers. comm to R. Doyle); or because there are unlimited fish genetic resources in the wild which constitute an everlasting source for aquaculture (T. Gjedrem, pers. comm to R. Doyle) or because superior strains such as the GIFT tilapia will eventually sweep away the less productive strains through economic competition (R. Pullin, pers. comm to R. Doyle). The argument by Gjedrem is no longer valid especially when one considers the collapse of the fishery even in relatively well managed systems; for instance the collapse of the Atlantic groundfishery in the early 1990s primarily due to overfishing (Charles, 1995). The aquatic ecosystems of the tropical developing countries are rich in biodiversity but they are poorly understood scientifically, relatively unmanaged and highly vulnerable to climatic change and human intervention (Pullin, 1992). *In situ* conservation in these habitats may have great potential for preservation of biodiversity.

Success of the conservation programs in developing countries depends on their cost effectiveness. The strong argument for *in situ* conservation lies in the fact that it is less costly than *ex situ* conservation. The agriculture-aquaculture systems in the developing countries are dominated by farmers who have limited capital to invest in farming hence

activities with long term benefits like conservation would not be among the priority areas of investment in competition with more basic needs.

In Malawi, several reservoirs were constructed during the colonial periods and stocked with fish. In the late 1960s the reservoirs in the southern region were a useful source of fingerlings and broodstock for restocking Lake Chilwa (Mathotho, 1975). This study was carried out to determine the genetic diversity of *O. shiranus* sp in reservoirs in the country.

5.2.2 *Small water bodies*

Small water bodies are defined as man made or natural water bodies that are smaller than lakes but larger than an average pond (Vincke, 1990). Reservoirs are a group of small water bodies. It is estimated that there are more than 800 small water bodies of size range of 0.4 - 7.7 ha in Malawi spread throughout the country (Vincke, 1990). According to mode of ownership, the 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 Department 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. Generally proper ownership of communal reservoirs is unclear because they belong to every member of the ethnic group, although major activities carried out in the reservoirs by individuals have to be approved by the chief.

5.2.3. ***Management of small water bodies***:

The reservoirs in Malawi were constructed between 1955 and late 1960s (ICLARM and GTZ; 1991). Most of them have capacity of less than 50,000 m^3 with catchment of less than 1-2 km^2. Artificial stocking in the reservoirs was carried out in the early 1960s with *O. sh. chilwae* and *T. rendalli* especially those in the southern region. The reservoirs are rarely harvested and have not been managed for commercial fish production.

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

Estate reservoirs were stocked by either the estate management or spouses of the estate managers (Kandoole and Ambali, 1992). Fish harvested from the reservoirs were sold to

the estate employees at a subsidized price and provided to the estate laborers for their daily ration. Limited investment was made in reservoir fishery management because most of the activities were concentrated on crop production from which the estates derived almost all its gross margins.

The communal reservoirs were stocked by the Fisheries Department during the colonial period but few of the members of the local community could recall when this occurred. Some of them even believed that stocking was through streams that drained into the reservoirs. Fishing by the village members is allowed although people still believe that the reservoirs belong to the government.

5.2.4 *Objective*:

The objective of the study was to determine genetic diversity of the reservoir populations of *O. shiranus* sp. This would provide basis for recommending whether they could be a possible alternative for *in situ* conservation of biodiversity in Malawi at relatively low maintenance costs.

5.3. MATERIALS AND METHODS:

5.3.1 *Source of samples*:

O. 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 or private institutions and two were communal (Table 5.1). BVU, MKW and MPA belongs to the Ministry of Agriculture in Blantyre district, BCR belongs to the University of Malawi in the central region, BSR belongs to catholic church in the northern region, MVO and CHL are both in Blantyre district and are communal. Blood samples were collected from BCR, BSR and MVO populations while muscle tissue was collected from BVU, CHL, MBA and MPA populations. The procedure outlined in **Chapter 2** was used to extract DNA from blood, and the following procedure was used to extract DNA from muscle tissue:

A modification of the extraction procedure of Kamonrat *et al.* (*in prep*) was used. 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 m before the liquid was decanted. Two hundred and fifty µL MGPL lysis buffer and 2.5 µL Proteinase K were added to digest the tissue in a 45 °C water bath for at least 3 h until the tissue had been completely digested. The mixture was vortexed and spun in the Eppendorf microcentrifuge for 5 m. 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 the DNA and then spun in the Eppendorf microcentrifuge for 10 m. The liquid was decanted and the DNA pellete was washed with 500 µl of 70% cold ethanol by vortexing the mixture and then spinning for 5 m. Ethanol was decanted and the tube was spun again to remove the residual ethanol. The pellete was air dried for 10-15 m before resuspending in 100 µL TE.

The procedures outlined in **Chapter 2** were used for carrying out PCR, electrophoresis of the PCR products and scoring the alleles.

5.3.2 ***Data analysis***:

Five polymorphic microsatellite loci were analyzed in seven reservoir populations. Data analysis was carried out according to the details provided in **Chapter 4** (sections 4.3.3.1 - 4.3.3.3). Mantel's test was carried out between genetic distance and geographic distance matrices only and not between genetic distance and transfer distance due to lack of records of stocking carried out in five of the reservoirs. The genetic diversity and patterns of genetic relationship in the reservoir populations were compared to those of wild and farm populations.

Table 5.1. Summary of the reservoirs studied

Reservoir	Code	District/City	Type of ownership	n
Chilala	CHL	Blantyre	Communal	40
Bvumbwe	BVU	Blantyre	Government	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

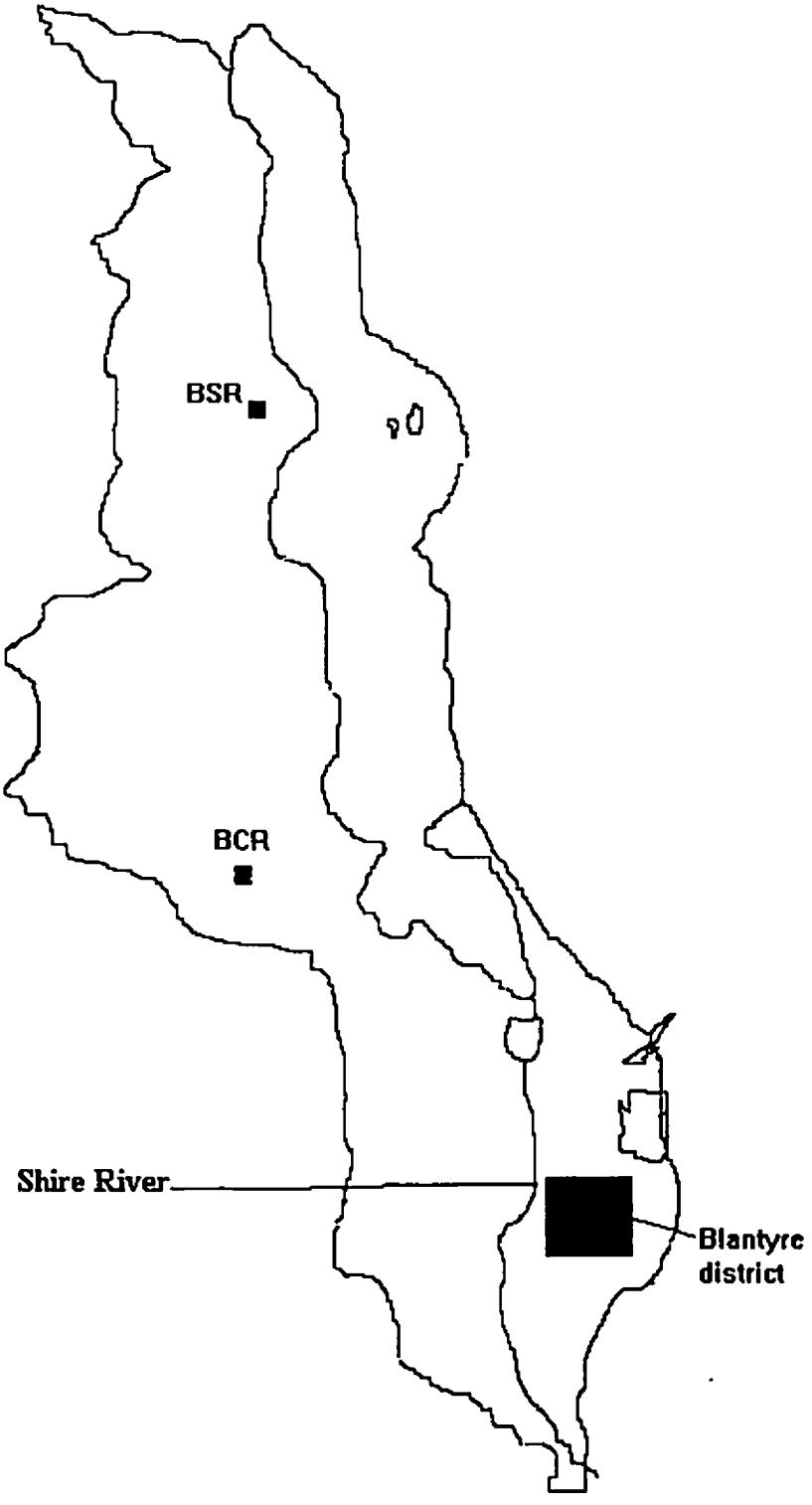


Figure 5.1: Map of Malawi showing areas where reservoir populations were collected. Populations CHL, BVU, MKW, MPA and MVO were collected in Blantyre district. Refer to Table 5.1 for the abbreviations and details.

5.4 *RESULTS*:

5.4.1 *Fish species found in the reservoirs*:

Data on species caught in the reservoirs are provided in Table 5.2. The predominant species were *O. shiranus* sp, *T. rendalli*, *Barbus* sp, *C. gariepinus*, *Serranochromis robustus* and other species in the Haplochromid family like *Pseudocrenilabrus philander* and *Atotilapia calypterus*. *O. shiranus* sp, *T. rendalli* and *S. robustus* and the haplochromids 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 the MKW reservoir. *S. robustus* was only found in BVU reservoir while *Barbus* sp was found in CHL, MKW and MPA reservoirs.

The mean and range standard length (SL) of *O. shiranus* sp found in the reservoirs and lakes are presented in Table 5.3. Although the differences in the mean standard length of the various populations did not necessarily imply that reservoir populations had faster growth than populations in the lakes, they generally shed some light on the species size distribution found in the two types of water bodies. *O. shiranus* populations in the reservoirs had higher mean SL than those in the lakes. The range shows 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*, the *O. shiranus* caught were of large size, implying that the predators reduced the population of small tilapia individuals. The *O. shiranus* samples analyzed for MVO reservoir were collected in March 1995 when the species was the 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, the predominant species became *C. gariepinus*.

Table 5.2. Species composition of the catch in various reservoirs at the time of sampling

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

O. sh. (*O. shiranus* sp), **T. rend** (*T. rendalli*), **C. gar** (*C. gariepinus*), **S. rob** (*S. robustus*), **Barbus** (*Barbus* sp)

Table 5.3: Mean and range standard length (SL) of *O. shiranus* sp in the reservoirs and lakes

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	13.8 - 24.7
Mvonia	13.6	10.0 - 17.0
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

5.4.2: ***Genetic diversity***:

Measures of genetic variability are presented in Table 5.4. The average number of alleles per population ranged from 3.2 ± 0.66 to 10.2 ± 2.15. A plot of mean effective number of alleles for wild, farm and reservoir populations is presented in Fig. 5.2. The populations plotted include all the farm, lake, BCR and BSR populations plotted in Fig 4.4 and those of reservoirs in Blantyre (CHL, BVU, MKW, MPA and MVO). The effective number of alleles in the reservoirs which were stocked between 1955 and 1960 ranged from 2.2 ± 0.10 to 3.7 ± 0.66. It was observed that the effective number of alleles declined in reservoirs where carnivorous fish species were observed. On the basis of mean ± 2SE, the effective number of alleles in those reservoirs were similar to those of MKB, KUN, CHT and MDL on the farms. The allelic diversity in BCR and BSR populations which were stocked after 1960 was similar to the recently domesticated populations like BSP, MYP, MTU and BIN.

Table 5.4 Measures of genetic variability at five loci in seven reservoir populations of *O. shiranus* sp (sample size (n), 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.

Pop	n	Os-7	Os-25	Os-7R	Os-64	Os-75	Total	A
CHL	40	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	40	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	38	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	41	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	40	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	55	8	10	14	6	14	52	10.2±2.15
		(2.21)	(6.50)	(10.53)	(2.86)	(8.44)	(30 6)	(6.1±1.59)
BSR	55	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)

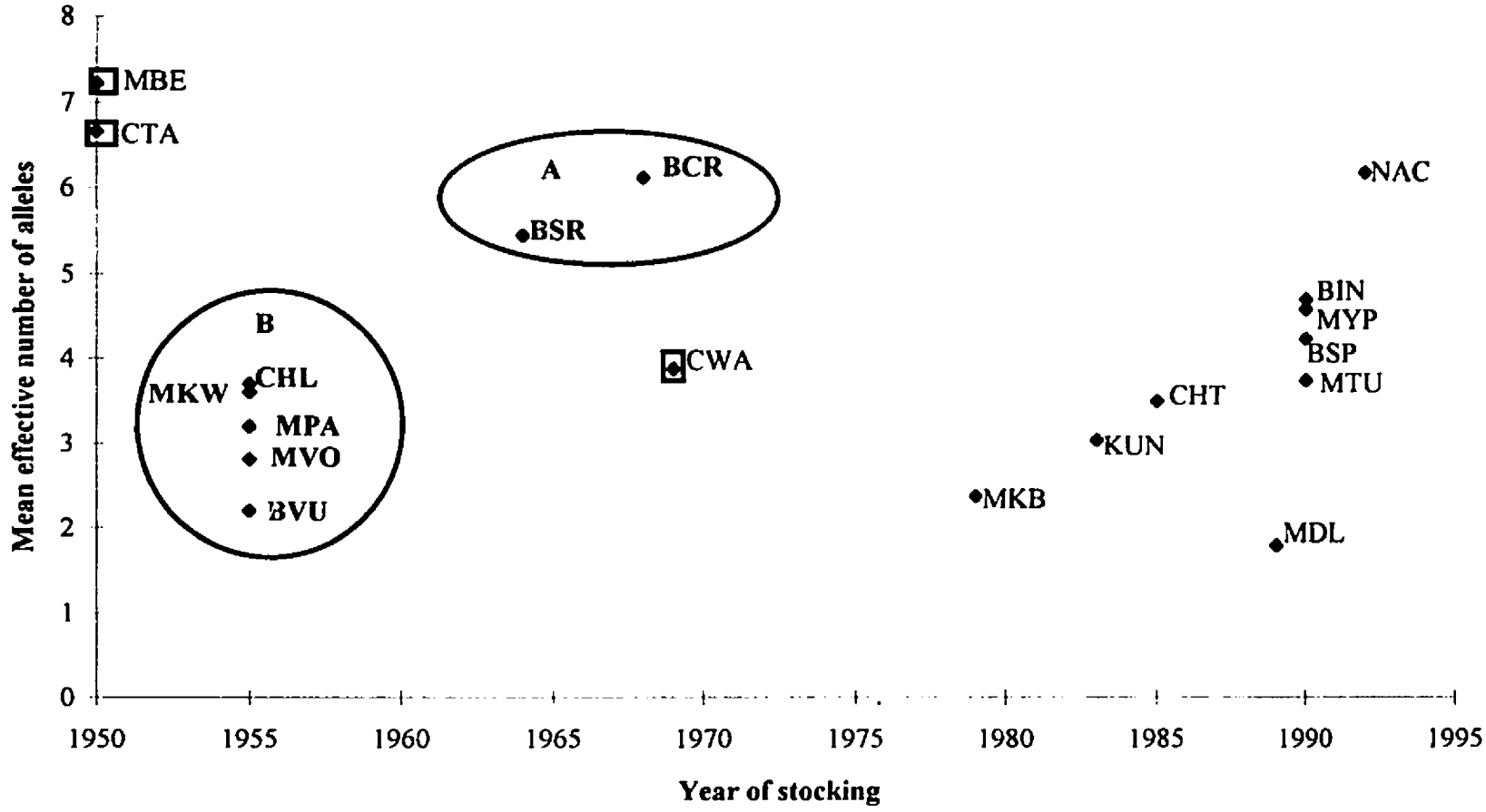


Figure 5.2: Plot of mean effective number of alleles for wild, semi-wild (all reservoirs) and domesticated populations of *O. shiranus* sp against year of stocking. Reservoir populations are in ellipses A are BCR and BSR and all the reservoirs in Blantyre are in ellipse B. Squares enclosed in circles are wild populations.

Summary of observed and expected heterozygosity values is presented in Table 5.5. All loci were polymorphic in all the reservoir populations. Mean 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.

Tests for conformity to HWE are presented in Table 5.6. 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%).

Table 5.5 Observed and expected heterozygosity at five microsatellite loci

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 5.6: Level of significance of departure from HWE using the exact test

Pop	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	<0.001	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

5.4.3 *Population structure*:

Levels of intra- and interpopulation variation are presented in Table 5.7. Inbreeding coefficient 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.

Table 5.7: Levels of intra- and interpopulation variation at five loci in seven reservoir populations of *O. shiranus* sp.

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

5.4.4 *Correlation between genetic distance and geographic distance*

Matrices of Cavalli-Sforza and Edwards (1967) chord distance for all *O. shiranus* sp populations, *O. mossambicus* and *O. karongae* are presented in Table 5.8 and the dendogram showing pattern of genetic relationship is presented in Fig. 5.3. In the cluster analysis four major groups were observed; (1) *O. sh. shiranus* sp; (2) *O. sh. chilwae*; (3) hybrids between *O. shiranus* sp and *O. mossambicus* and (4) three reservoir populations. Among the reservoir populations, BCR clustered with *O. sh. chilwae*; BSR and CHL clustered with *O. sh. shiranus* and the other three populations (MKW, MPA and MVO) clustered together without obvious identity of their putative progenitors among the wild populations. Cophenetic correlation for the cluster analysis was 0.84 and is considered a good fit.

A plot of the first two components of principal component analysis is presented in Fig 5.4. *O. karongae* is separated from the rest of the populations with high value of component 2 (PC2). The rest of the populations are separated by principal component 1 (PC1) where *O. sh. shiranus* populations are grouped with low values of PC1 while the other populations are grouped with high values. The plot seem to show a strong genetic contribution of *O. sh. chilwae* to the gene pool of MKW, MVO, MPA and BVU reservoir populations. The correlation between the matrix of distance among all pairs of points and the matrix of distance among populations was 0.68 and is considered a poor fit.

Mantel's correlation coefficient between genetic distance and geographic distance was 0.265 suggesting that there was a poor correlation between population genetic distance and geographic distance between reservoirs. A plot of the first two dimensions of multidimensional scaling is presented in Fig. 5.5. The pattern of relationship among species observed in the analysis was similar to that of the principal components analysis. Stress was 0.43 and is considered a poor fit (Rohlf, 1992).

Table 5.8: Cavalli-Sforza and Edwards (1967) chord distance between populations of *O. shiranus* sp. *O. mossambicus* and *O. karongae*

	NAC	MYP	BSP	MKB	BIN	KUN	MTU	CHT	MDL	BCR	BSR	CWA	CTA	MBE	MOZ	KAR	CHL	BVU	MKW	MPA
MYP	0.06																			
BSP	0.07	0.04																		
MKB	0.11	0.14	0.13																	
BIN	0.08	0.11	0.11	0.04																
KUN	0.11	0.13	0.12	0.10	0.09															
MTU	0.09	0.13	0.12	0.05	0.03	0.09														
CHT	0.11	0.13	0.11	0.10	0.10	0.03	0.09													
MDL	0.11	0.13	0.13	0.03	0.05	0.11	0.06	0.11												
BCR	0.09	0.10	0.08	0.11	0.09	0.05	0.10	0.06	0.11											
BSR	0.06	0.03	0.03	0.12	0.10	0.11	0.11	0.11	0.11	0.07										
CWA	0.10	0.11	0.09	0.10	0.09	0.04	0.09	0.04	0.10	0.04	0.08									
CTA	0.08	0.09	0.06	0.11	0.08	0.06	0.09	0.04	0.11	0.04	0.06	0.03								
MBE	0.03	0.06	0.06	0.13	0.09	0.12	0.10	0.12	0.12	0.08	0.04	0.10	0.07							
MOZ	0.10	0.12	0.11	0.07	0.07	0.12	0.08	0.09	0.08	0.11	0.10	0.09	0.10	0.11						
KAR	0.09	0.11	0.12	0.12	0.10	0.12	0.09	0.13	0.11	0.12	0.10	0.13	0.11	0.09	0.12					
CHL	0.06	0.07	0.09	0.12	0.10	0.11	0.10	0.11	0.10	0.11	0.07	0.10	0.09	0.06	0.12	0.11				
BVU	0.11	0.11	0.11	0.14	0.12	0.09	0.11	0.09	0.14	0.10	0.12	0.08	0.08	0.12	0.13	0.14	0.11			
MKW	0.10	0.10	0.12	0.13	0.10	0.10	0.12	0.09	0.11	0.09	0.11	0.09	0.09	0.11	0.11	0.11	0.11	0.13		
MPA	0.10	0.11	0.12	0.12	0.11	0.09	0.12	0.08	0.12	0.09	0.12	0.08	0.09	0.12	0.11	0.12	0.12	0.12	0.02	
MVO	0.12	0.12	0.12	0.13	0.09	0.13	0.12	0.11	0.12	0.10	0.12	0.11	0.11	0.11	0.13	0.12	0.15	0.15	0.09	0.10

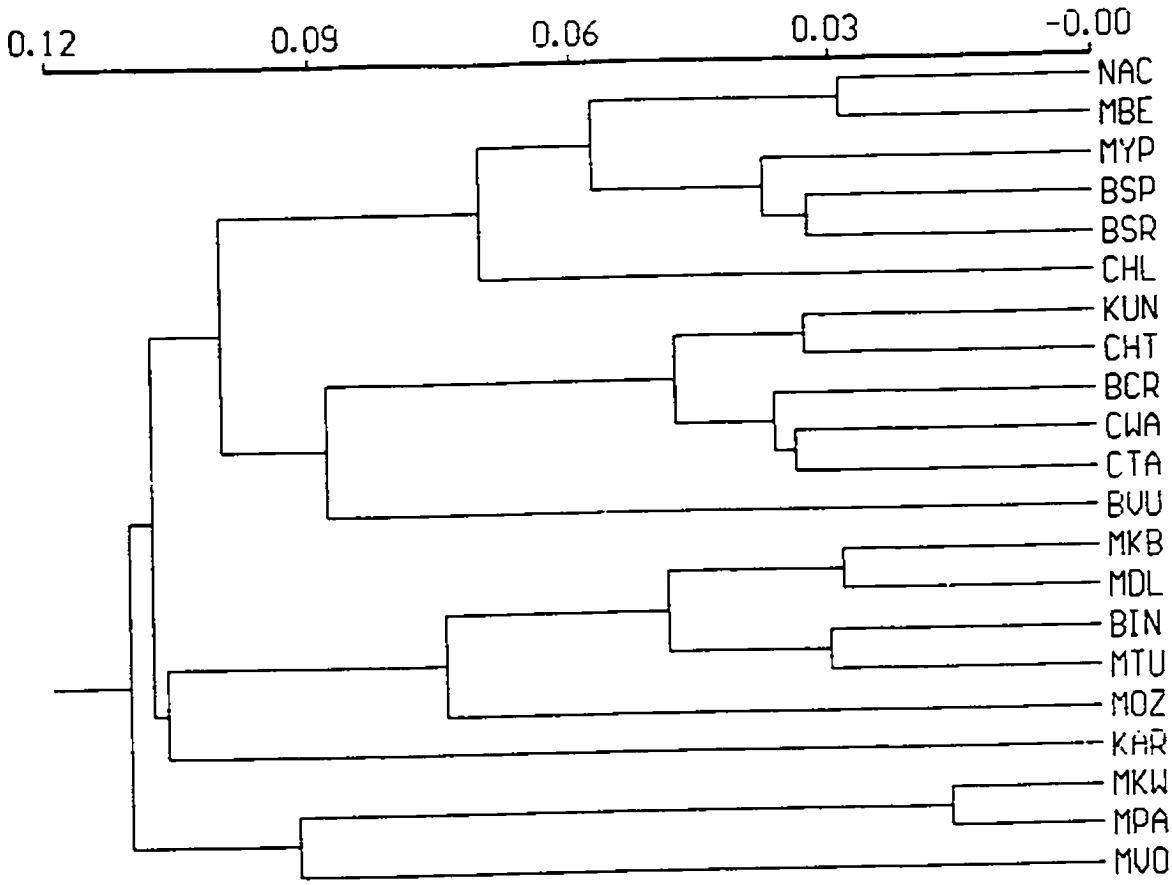


Figure 5.3: Dendogram constructed by the UPGMA method from Cavalli-Sforza and Edwards (1967) chord distance.

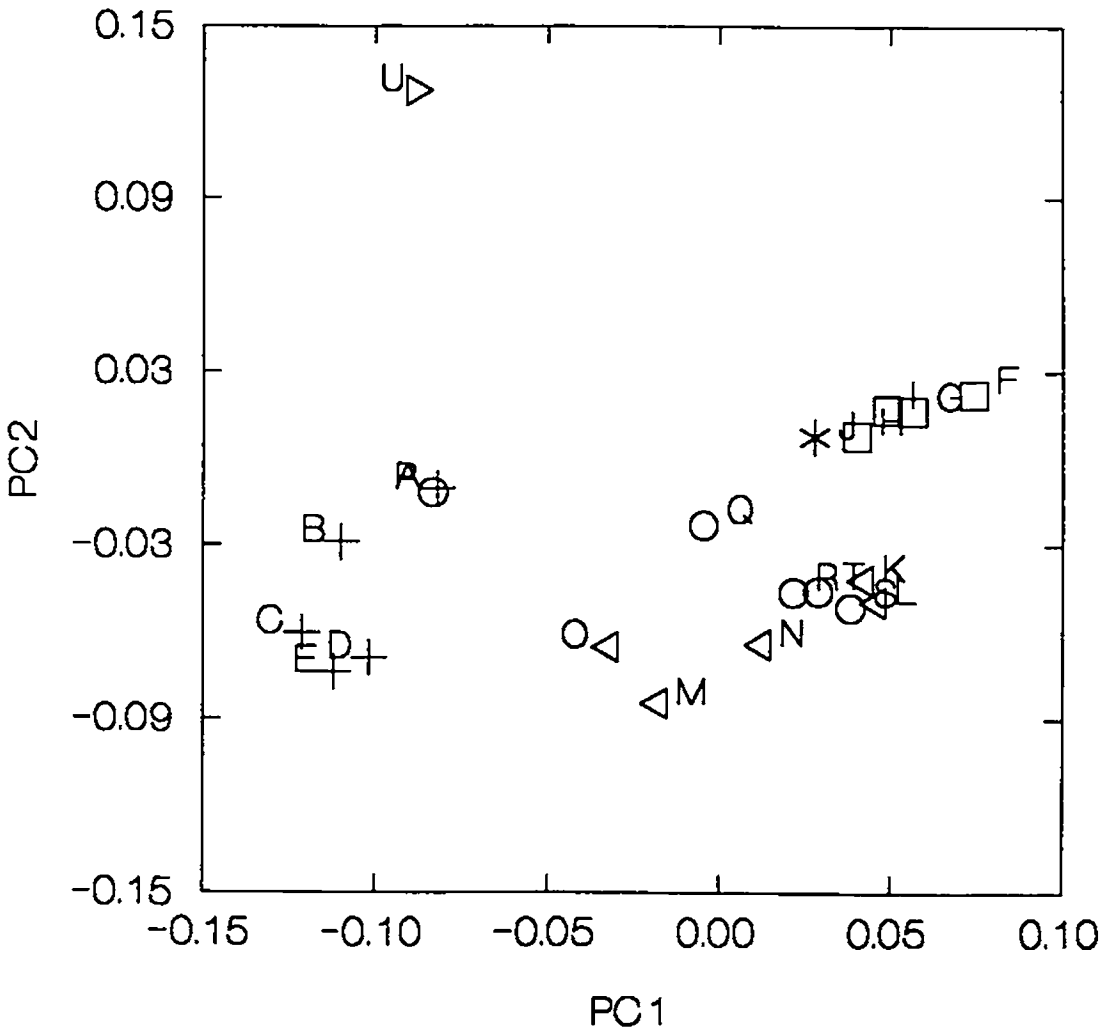


Figure 5.4: Plot of *O. shiranus* sp, *O. mossambicus* and *O. Ny. karongae* populations against values for the first two principal components. For abbreviations in the plot, populations represented by crosses are NAC (A), MBE (B), MYP (C), BSP (D), BSR (E); populations represented by open rectangles are MKB (F), MDL (G), BIN (H), MTU (I), the population represented by a star is MOZ (J); open triangles are KUN (K), CHT (L), BCR (M), CWA (N), CTA(O) and KAR (U), open circles are reservoir populations in Blantyre, CHL (P), BVU (Q), MKW (R), MPA (S) and MVO (T).

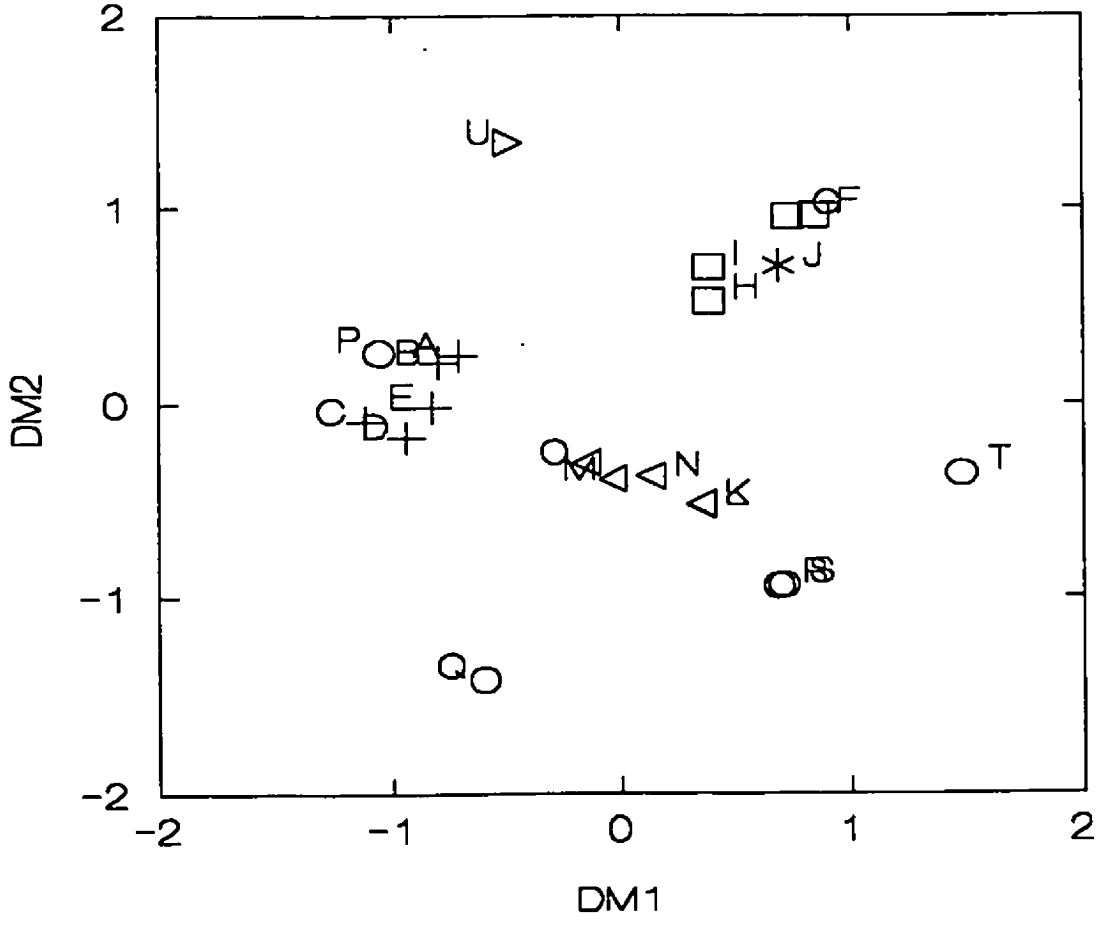


Figure 5.5: Plot of *O. shiranus* sp, *O. mossambicus* and *O. Ny. karongae* populations against the first two dimensions of the configuration produced by multidimensional scaling of Cavalli-Sforza and Edwards (1967) chord distance. For abbreviations in the plot, populations represented by crosses are NAC (A), MBE (B), MYP (C), BSP (D), BSR (E); populations represented by open rectangles are MKB (F), MDL (G), BIN (H), MTU (I), the population represented by a star is MOZ (J); open triangles are KUN (K), CHT (L), BCR (M), CWA (N), CTA(O) and KAR (U), open circles are reservoir populations in Blantyre, CHL (P), BVU (Q), MKW (R), MPA (S) and MVO (T).

5.5. **DISCUSSION**:

5.5.1 ***Species composition***:

The predominant species in the reservoirs was *O. shiranus* sp which was stocked between 1955 and 1960 but there were no commercial fish harvesting operations in the reservoirs. It is estimated 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 the tilapia 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 was observed in the MVO population. Mathotho (1975) recommends a stocking of no more than 6% of predators. The proportion of *C. gariepinus* and *S. robustus* were 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 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. The 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 has 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*.

5.5.2 *Genetic diversity*:

Genetic variability was high in those managed reservoirs (BCR and BSR) where there were considerably lower abundance of carnivorous species than tilapia. The carnivorous species preyed upon the 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 N_e subjects populations to dispersive processes of allele frequencies like genetic drift, bottlenecking and inbreeding.

The effective number of alleles in lake, reservoir and farm populations presented in Fig 5.2 shows that the lake populations (MBE and CTA) had the highest genetic diversity followed by reservoirs (BCR and BSR) and recently domesticated populations in the farms. 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 NAC in the early and mid 1980s. The fact the genetic variability was

comparable to that of the recently domesticated populations implies that the rate of decline of diversity may have been lower in the reservoirs than in the farm populations. None of the loci were monomorphic in the reservoir populations while there were monomorphic loci in the farm populations (**Chapter 4**). However the communal reservoirs had lower diversity than the source populations which suggests that they are not appropriate for *in situ* conservation of diversity over the long term.

The genetic variability of Lake Chilwa (CWA) was intermediate between BCR population and the reservoir populations in the southern region. Mathotho (1975) reports that *O. sh. chilwae* fingerlings were collected from reservoirs in the southern region of Malawi to supplement the genetic material maintained at NAC for restocking Lake Chilwa in 1969. The low genetic diversity in the reservoirs in Blantyre might have contributed to the dilution of the population that was maintained at NAC for restocking Lake Chilwa.

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 the founder populations were generally low. ICLARM and GTZ (1991) quote stocking rates in government owned reservoirs ranging from 34 tilapia, 2 haplochromids and 19 *S. robustus* per reservoir to 173 tilapia, 20 haplochromid and 26 *S. robustus*. The tilapia were usually a mixture of *O. shiranus* sp and *T. rendalli*, although there was more shiranus than rendalli.

5.5.3 *Genetic distance*:

Mantel's correlation coefficient between genetic distance and geographic distance is low and not significantly different from zero. Lack of correlation was due to the fact that unlike populations BCR and BSR which were founded on single populations, the reservoir populations in Blantyre were composite. Despite this their genetic variation was lower. Mathotho (1975) indicate that the 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 indicated 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, the two alleles observed at the locus were not observed in the CHL population. The alleles in the MVO were of larger size than those in CHL. The 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.

5.6 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 the 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 the 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).

CHAPTER 6:

SOCIO-ECONOMIC STATUS OF SMALL SCALE FISH FARMING IN MALAWI

6.1 ABSTRACT:

Malawi has experienced rapid development in small scale aquaculture since the mid-1980s. The number of fish farmers increased from 918 in 1988, to 2,104 in 1993. During the same period, aquaculture production from small scale farmers increased from 30.1 tones to 134.0 tones.

A study was carried out to determine the socio-economic status of small scale aquaculture in the country. It was observed that most of the small scale farmers were in the economically active age group (20-50 years). About 81.6% of the farmers had attained primary school education and most of the farmers (71.8%) did not hold leadership positions in their communities. The majority of the farmers (72.8%) worked as full time farmers in integrated crop/livestock/aquaculture farming systems. Pond sizes ranged from 45 to 1,650 m^2 with mean of 204 m^2. Most of the ponds (65.1%) were initially stocked with fingerlings provided by the Fisheries Department and there was gradual decline in the number of ponds that were provided fingerlings by the government in subsequent stocking. The predominant cultural practices in fish farming included stocking, feeding, manure application and pond maintenance. Most of the operations were carried out by farmers and their family members.

Case studies on economic assessment of fish farming showed that growout operations were economically viable. Operational profits of MK666.40 and MK205.32 were obtained

at Mapendo Fish Farm (MFF) and Naming'azi Demonstration Farm (NDF), respectively. Return to land did not differ considerably between the farms; MK0.98/m^2 on MFF and MK1.01/m^2 on NDF; however return to labor was higher on MFF than on NDF, MK63.86/man-day and MK25.44/man-day, respectively. The economic viability of fish farming was mainly due to use of on-farm and household resources as feeds and fertilizer.

6.2 INTRODUCTION:

6.2.1 *Small scale fish farming in Malawi prior to mid-1980s*:

6.2.1.1 *Northern region*:

Fish farming in the northern region started after 1950 when Messrs. Dunlop and Gifkin were appointed as Fish Ranger and Trout Warden, respectively, during the colonial period (Kalinga, 1990). Mr. Dunlop operated in the southern region while Mr. Gifkin was based in the northern region at Nchenachena in Rumphi District (Fig. 6.1). With the positioning of the latter, active aquaculture started in the northern region and Nchenachena became the main fish breeding center (Kalinga, 1990).

Mr. Gifkin worked with trout only for two years and later tried to stock tilapia from Lake Malawi into a pool above a trout hatchery house. He experienced high mortality of tilapia and instead constructed ponds specifically for culturing the species. The setup attracted a lot of local people and extension activities spread to Henga Valley and the adjoining region between Nchenachena and Livingstonia Mission and Chikwina-Kavuzi in Nkhata-Bay district (Kandoole and Ambali, 1992). The first small scale ponds were constructed in 1958 and by the end of the year, there were eight school and mission ponds and sixty-two

ponds belonging to the local people. In 1962, the dissemination of aquaculture extended to Misuku and Chisenga area of Chitipa (Fig. 6.1; Kalinga, 1990).

6.2.1.2 *Central region*:

The beginning of fish farming in the central region dates back to 1968 when a dam was constructed at Bunda College of Agriculture and fingerlings were distributed to farmers in the region. Within the central region, aquaculture has developed most rapidly in Dedza district with some notable pockets of fish farmers in Lilongwe, Dowa, Ntchisi and Ntcheu districts (Fig. 6.1). Small scale fish farming in Dedza developed in the mid 1980s without the involvement of the Fisheries Department of Malawi; and there was generally lack of records of fish farming operations in the region prior to that period (Kalinga, 1990).

6.2.1.3 *Southern region*:

Small scale aquaculture in the southern region is mainly concentrated in Zomba, Mulanje and Mwanza districts and is associated with the establishment of the National Aquaculture Center in 1959 (Fig. 6.1). The thrust of small scale aquaculture extension started in 1970 in the upland areas of Zomba district at Chingale by the Oxford Committee for Famine Relief (OXFAM). Farmers in the lowland areas of Zomba started fish farming later after the OXFAM project had been established at Chingale (Fig. 4.2; ICLARM and GTZ; 1991).

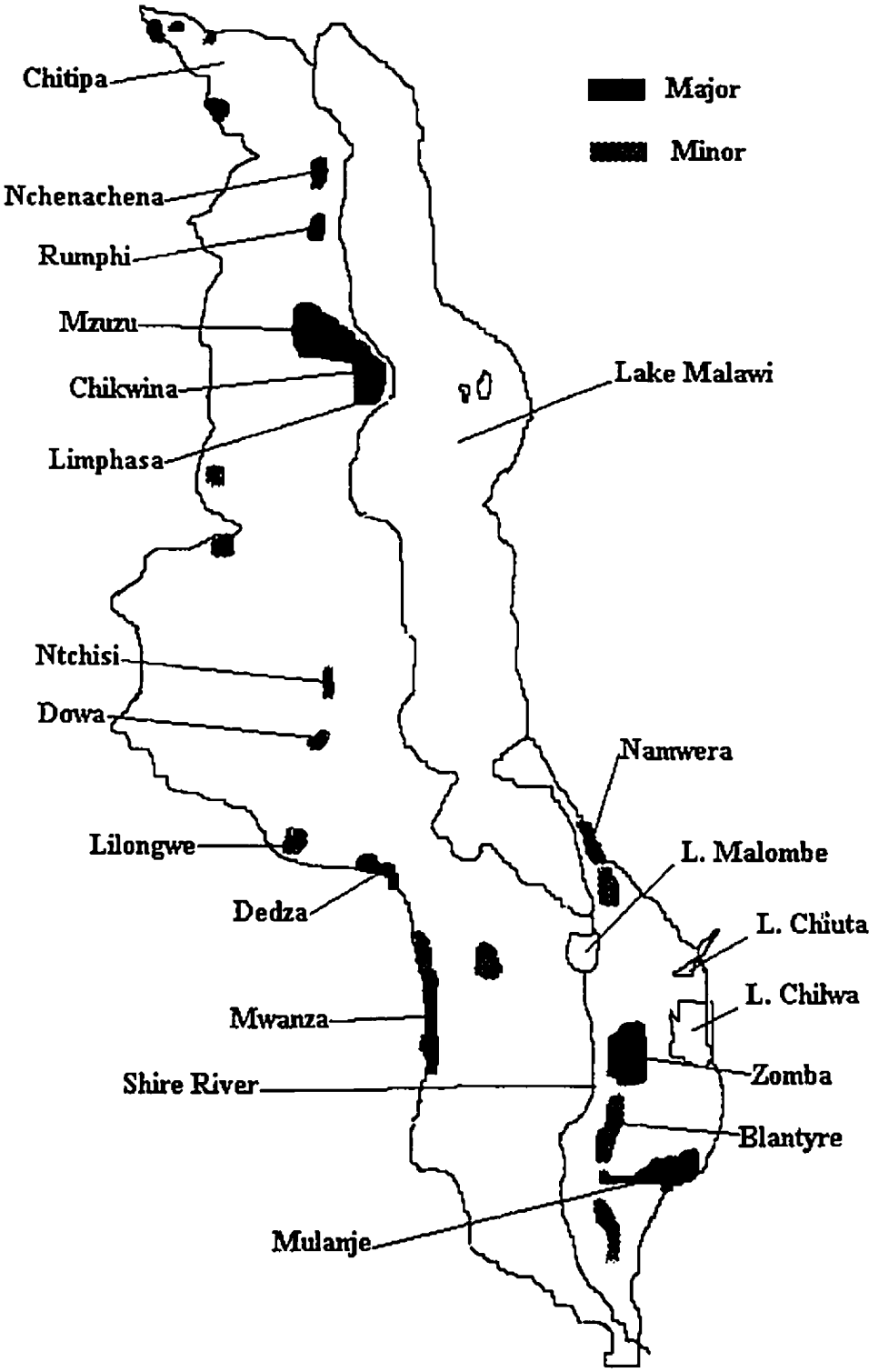


Figure 6.1:Map of Malawi showing areas of major and minor fish farming activities with potential for expansion.

Aquaculture in Mulanje district started in 1974 in Mulanje south and in 1975 in Mulanje west. Most of the fish farming activities in the district have developed through exchange of information among the farmers themselves (ICLARM and GTZ; 1991). In Mwanza district small scale aquaculture is mainly concentrated around Kunenekude where the number of fish ponds was estimated around 400-500 in 1987. Most of the ponds were constructed after 1981.

6.2.2. *Development of aquaculture from mid-1980s to present*:

Major developments in small scale aquaculture in Malawi took place from about 1986 through the assistance of development projects. The three main projects, among others, are (1) the International Center for Living Aquatic Resources Management (ICLARM), the Malawi Germany Fisheries and Aquaculture Development Project (MAGFAD) and Central and Northern Regions Fish Farming Project (CNRFFP). The three projects have been working in different parts of the country with different objectives as outlined below.

6.2.2.1 *ICLARM*

The project, started in 1986, is based at the National Aquaculture Center and is implemented by ICLARM staff. The broad objective is to develop appropriate technology

for small scale farmers in rural Africa. The project activities include on-station research in aquaculture pond dynamics and associated fish yield characteristics using agricultural crop and wild plant residues available on small scale farms in Malawi. The project includes extending the successful on-station results in integrated aquaculture-agriculture to small scale farmers in Malawi, and monitoring the development of introduced innovations through involvement of farmers in participatory research using a farming systems research approach.

6.2.2.2 *MAGFAD*

The project started in 1988 and is implemented by the aquaculture and capture fisheries units of the Malawi Fisheries Department. The broad objective of the project is to disseminate extension messages of working technologies to small scale fish farmers and fishermen in the southern region. In the aquaculture component, the project activities include: conducting in-service training for extension technical assistants, development of information kits of cultural practices for fish farmers and actual dissemination of the research results. The capture fisheries component has been involved in tree planting programs for supply of fuel wood for fish processing. Currently the project's major activity is development of new fisheries management strategies for Lake Malombe with the main objective of reducing fishing pressure in the lake.

6.2.2.3 ***CNRFFP***:

The project started in 1989 and is funded by the European Community with the primary objective of increasing fish production in the central and northern regions through aquaculture. This is achieved by diversifying small scale aquaculture production and expanding the base for income generating activities through research, extension and training. The project activities include: dissemination of aquaculture husbandry techniques, supplying fingerlings and carrying out on-station and on-farm research which is coordinated at the project's headquarters in Mzuzu. The farmer-technical staff contact is carried out in nine satellite stations of the project. The project has operated for six years and is working with more than 1,200 farmers who get an average fish yield of more than 1.4t/ha/yr (Fisheries Department, 1994).

6.2.3 ***Current aquaculture production***:

Through combined efforts of all these development projects and the Department of Fisheries, the number of fish farmers has increased. In the northern region the number of fish farmers increased from 121 in 1990 to 723 in 1994. During the same period, the total area under fish farming increased from 6.6 to 22.0 ha (Fisheries Department, 1994). In Dedza district where there were no fish farmers by 1987, there were 450 fish farmers in 1992; in Mulanje-Phalombe area, there were 74 farmers in 1988 before the government

got involved and when extension services began the number of fish farmers increased to 146 by 1992 (Kaunda, 1994). Total fish production from small scale farmers increased from 30.1 tones in 1991 to 134.0 tones in 1993 (Kaunda, 1994). The 1994 production estimates could be lower because most ponds were not operational due to drought (pers. observation).

6.2.4. *Objectives*:

The study was carried out with the following objectives:

i. to determine the socio-economic background of small scale farmers in Malawi

ii. to determine the history of transfers of fingerlings between small scale farms and government stations

iii. to identify major cultural practices carried out in small scale aquaculture in Malawi

iv. to determine the productivity of small scale fish farms

6.3 MATERIALS AND METHODS:

6.3.1. *Survey*

The survey was carried out in three satellite areas of the CNRFFP, namely Mzuzu, Limphasa and Chikwina (Fig. 6.1). A structured questionnaire was administered to a total of 103 farmers comprising; 43, 25 and 35 farmers from Mzuzu, Limphasa and Chikwina, respectively. These represented 35, 37 and 38%, of the total number of farmers in Mzuzu, Limphasa and Chikwina, respectively. The survey covered 151 ponds in total, representing 54% of the total number of ponds in the three areas (Fisheries Department, 1994). The following aspects were covered in the questionnaire: background of the fish farmers, household and wage labor participation in pond construction, stocking rate and transfer of fingerlings.

6.3.2 *Case Studies*

In-depth quantitative information about cultural practices, pond productivity and economic assessment was obtained through two case studies carried out at Mapendo Fish Farm (MFF) and Naming'azi Demonstration Farm (NDF) in Zomba district. Fingerlings from NAC were provided to stock 682 m^2 and 204 m^2 ponds at MFF and NDF, respectively. The operators carried out regular cultural practices throughout the growout period using their own inputs and rates advised to them by extension officers. Records of the operations were kept on daily basis.

6.3.3 **Data analysis**

The MICROSOFT EXCEL version 5 program was used to calculate mean, standard error, range and percentages. The following economic indicators were calculated to assess the economic viability of growout operations in the two case studies (Shang, 1990):

Operational profits = Total revenues and benefits - Operational costs

Return to land = Operational profits ÷Total pond area

Return to labor = (Operational profits + Cost of labor) ÷ Total man-days

Net profits and return to capital were not calculated due to lack of reliable data for valuating capital assets.

6.4 RESULTS AND DISCUSSION:

6.4.1 *Socio-economic background of fish farmers*:

Summary of background and characteristics of small scale farmers is presented in Table 6.1. The age bracket shows that 72% of the farmers were less than 51 years old while 28% were more than 50 years old. There were more male fish farmers (95.2%) than female fish farmers (4.8%). Only 6.8% of the fish farmers had not been married while 89.3% were married and 3.9% were divorced or widowed. The demographic pattern of fish farmers observed has significant implications on the success of fish farming in Malawi. Usually pond owners who are less than 20 years of age rely on other family members to operate their ponds and it was observed in Dedza district that such ponds are the least well managed (Kandoole and Ambali, 1992). The results showed that 68.9% of the farmers were within the 21-50 year age bracket which is a physically most able age group to operate agricultural activities. Ponds which belonged to farmers of more than 50 years of age were usually at risk of being abandoned as the owners become less capable of carrying out the activities due to waning of their physical strength and lack of successors to take over the operations. The majority of the fish farmers were married, a characteristic which contributes to successful fish farming. In labor intensive activities like fish farming, married families are usually more successful than single families because of the advantage of pooled labor force contributed by husbands, wives and children.

A large proportion of the farmers (71.8%) did not hold leadership positions in their community while 28% held positions like village headmanship (3.9%), church and political leadership, 9.71 and 14.8%, respectively. About 81.5% of the farmers had attained primary education while 9.7% had attained secondary education and 8.7% had not attended education at all. The low frequency of farmers who had leadership positions in their communities and those who were educated were favorable indications of the fact that one did not require those characteristics to become a fish farmer. This was contrary to earlier beliefs by some of the aquaculture development projects (CNRFFP management, pers. comm) that aquaculture in the country would only be adopted by small holder farmers who had leadership positions in their communities and those who were educated. In this study it was observed that availability of water supply seem to have been the main factor determining adoption of fish farming. This was reflected by pond size distribution; there were farmers who were still interested in raising fish although they did not have adequate land to construct a standard pond. A notable implication of the low literacy rate observed in the study is that the provision of extension services should take into account the farmers who have low education. Programs for disseminating technical advice should be designed in such a way that they are understood by those who are illiterate.

According to main occupation, 4.8% of the farmers were still in school, 72.8% were involved in full time farming, 13.6% worked in non agro-industries and 8.7% owned private business. Traditionally, Malawian small holder farmers are crop and livestock

growers and they have recently adopted fish farming as an additional enterprise. The Ministry of Agriculture in Malawi has succeeded to some extent in advising these farmers to reserve healthy looking crops of local varieties like corn and legume seed for subsequent growing season. The farmers have also maintained their own locally-adapted breeds of livestock like ruminants (e.g. goats and cattle) and pigs. The *in situ* conservation experience that they have gained over a long period of time from other commodities could be utilized by aquaculture genetics programs for sustainable conservation of local strains of fish.

Table 6.1: Background characteristics of the fish farmers

Characteristic	Number	Percentage
Age distribution (years)		
<20	3	2.9
21 - 30	21	20.4
31 - 40	26	25.2
41 - 50	24	23.3
>50	29	28.2
Sex		
Male	98	95.2
Female	5	4.8
Marital status		
Single	7	6.8
Married	92	89.3
Divorced/widowed	4	3.9
Social Status		
Village headman	4	3.9
Church leader	10	9.7
Party official	15	14.6
None	74	71.8
Education		
No education	9	8.7
Primary 1-4	25	24.3
Primary 5-8	59	57.3
Secondary +	10	9.7
Occupation		
Student	5	4.9
Farmer	75	72.8
Non-agricultural wage	14	13.6
Own business	9	8.7

6.4.2 ***Pond construction***:

A summary of data on pond history and construction is presented in Table 6.2. The three oldest ponds were constructed in 1961 and 1965, the rest of the ponds were constructed between 1989 and 1992. Pond area ranged from 45 m^2 to 1,650 m^2 with mean of 204 m^2. A large proportion of the ponds (66.9%) were of 101-299 m^2 area while 21.2% were of less than 100 m^2 and 11.9% were larger than 299 m^2 in area. The observation that mean pond size was 204 m^2 is a positive indication of the farmers' willingness to adopt extension advice disseminated by technical staff in aquaculture. The standard pond size recommended by ICLARM and other development projects for small scale farmers in Malawi was 200 m^2. This was based on results of the work carried at the Asian Institute of Technology, where it was observed that a 200 m^2 pond was suitable for supplying animal protein to an average family in Thailand (Edwards, *et al.* 1988).

Most of the ponds were constructed by using family labor; 47.7% by the farmers themselves and 27.2% by the farmer and members of the family. Hired labor was involved in the construction of 25.2% of the ponds. Of these 6.0% involved the farmer and hired labor, family members and hired labor (2.0%), hired labor only (16.6%) and reciprocal labor (0.7%). Payment for constructing a 204 m^2 equivalent pond varied considerably depending on the involvement of hired labor and the year the pond was constructed. It ranged from K15.00 to K280.00 with mean of K94.00 per pond. The number of days it took to construct an average 204 m^2 pond varied considerably among the farmers. The

period depended on the number of people involved, their experience and the type of instruments available for digging.

Table 6.2: History of fish farming and pond construction

Pond characteristic	Number	Percentage
Year the pond was constructed		
Before 1989	3	1.99
1989	4	2.65
1990	41	27.15
1991	76	50.33
1992	27	17.88
Pond size distribution (m^2)		
45 - 100	32	21.19
101- 199	70	46.36
200 - 299	31	20.53
300 - 399	7	4.64
400 - 499	5	3.31
500+	6	3.97
Person who constructed the pond		
Farmer alone	72	47.68
Farmer and the family	41	27.15
Farmer and hired labor	9	5.96
Family and hired labor	3	1.99
Hired labor	25	16.56
Communal group	1	0.66

6.4.3 *Stocking and fingerling distribution*:

Stocking carried out between 1991 and 1992 is summarized in Table 6.3. The species cultured in the ponds were *O. shiranus* sp and *T. rendalli*; none of the other species were reported. Of the 151 ponds, 123 were stocked during the early stages of the CNRFF project establishment as reported in the Fisheries data base. The mean stocking density was 1.90 fingerlings per m^2 with a range of 0.2 to 5.11/m^2. The correlation coefficient between total number of fingerlings stocked and pond area was not perfect (r = 0.57, not shown), suggesting that stocking density was not consistent among ponds. The mean stocking size was 5.0 ± 0.24 g and ranged from 1.1 to 16.2 g in the various ponds.

There were three sources of fingerlings identified, 6.5% of ponds were stocked with fingerlings produced by farmers as by-product of culture, 28% were bought from fellow farmers and 65.05% were provided from government stations. The stocking density was was higher in ponds that were stocked with fingerlings from government stations than those stocked with fingerlings from the other two sources.

While 59.4% of the ponds were stocked with fingerlings obtained in kind, fingerlings stocked in 40.6% were bought in cash; 19.5% from government stations and 21.1% from small scale farmers. The average price of fingerlings in government stations was 1.95 ±

0.04 tambala[1] per fingerling with a range of 1.20 - 2.00 tambala per fingerling while in the private farms the average price of fingerlings was 2.96±0.04 tambala with a range of 2.00 - 3.06.

During second stocking, only 14.6% of the 123 ponds were restocked. About 60% of those got fingerlings, for their first growout, from government stations while the other 40% produced their own fingerlings or bought from other farmers. The distribution of fingerlings from government stations to farmers declined from 65.0% of the ponds during first stocking to 46.6% during second stocking.

The initial batch of fingerlings for stocking fish ponds is normally provided to farmers by Fisheries Department and farmers are expected to produce their own fingerlings for subsequent stocking. It was observed in this study that most of the ponds (65.0%) were stocked with fingerlings provided by the government and the frequency of such ponds declined during second stocking. The general decline in the number of ponds that were restocked was due to lack of fingerlings and shortage of water. Most of the growout cycles in 1992 were not completed due to drought. The stocking rate recommended by Fisheries Department for tilapia is 2 fish/m^2 hence the average stocking rate was 1.90. It was generally observed that most of the ponds that were understocked were those that belonged to farmers who bought fingerlings from fellow farmers or produced their own.

[1] MK1.00 = 100 tambala

Table 6.3: Summary of pond stocking; figures in parentheses are percentages

Stocking density (number/M^2)	
Mean ± SE	1.90 ± 0.06
Range	0.20 - 5.11
Fingerling size at stocking (g)	
Mean ± SE	5.00 ± 0.24
Range	1.1 - 16.2
Source of fingerlings	
Farmer's own	8 (6.50)
Other farmers	35 (28.45)
Government stations	80 (65.05)
Price of fingerlings (t/piece)	
Government stations	
Mean ± SE	1.95 ± 0.04
Range	1.20 - 2.00
Private farms	
Mean + SE	2.96 ± 0.04
Range	2.00 - 3.06

6.4.4. *Cultural practices: case studies of MFF and NDF*

6.4.4.1 Mapendo Fish Farm

Mapendo Fish Farm (MFF) belonged to Mr. Binali, a village headman in Zomba district. Construction of fish ponds at the farm started in 1988 and was completed in 1989. There were seven ponds of varying sizes ranging from 50 m^2 to 1,000 m^2. All the ponds were constructed by the farmer himself. Water was from a perennial stream and drained through a battery of ponds starting from the ponds at highest elevation. In 1990, the ponds were stocked with fingerlings provided by NAC and had since been restocked with fingerlings produced as by-product of culture on the farm. In situations where the farm was in short supply of fingerlings, it supplemented by buying from MKB and NAC. Pond inputs for fish farming comprised on-farm waste from vegetable gardens, livestock and household waste like firewood ash, bran and food left-overs. The farmer also obtained chicken and goat manure, in kind, from relatives and friends who were given free fish at harvest.

6.4.4.2 Naming'azi Demonstration Farm:

The farm belonged to Blantyre Synod of the Church of Central African Presbyterian (CCAP) and was run by a Farm Director and a Farm Manager. It was a fish/crop/chicken integrated farm; all the subcomponents were operated at small scale level. It was a recent establishment, despite the fact that it had an old land ownership legend. The farm had a

204 m^2 pond constructed in 1992 and a reliable supply of water. All pond inputs were on-farm. Regular wage labor was used and the fish harvested was sold to farm workers and people from surrounding villages.

6.4.4.3 **Economic assessment:**

Economic assessment of growout operations at MFF and NDF is presented in Tables 6.4 and 6.5. The most common feedstuff were maize and rice bran and leafy vegetables; pond fertility was maintained by applying organic manure and firewood ash. Feeding and manure application rates were decided empirically by the farmers. It was common sense based on the color of water in the pond and availability of inputs. The rates were to a considerable extent constant at NDF while they varied at MFF. The economic indicators showed that growout operations were viable at both farms, the operational profits were MK666.40 and MK205.32 on MFF and NDF, respectively. Although operational profits were higher at MFF than NDF, the efficiency of land utilization, expressed in terms of return to land showed no considerable difference, MK0.98/m^2 on MFF and MK1.01/m^2 on NDF. Return to labor was higher on MFF (MK63.86/man-day) than on NDF (MK25.44/man-day).

The analysis of case studies shows that the major cultural practices carried out during growout were feeding, manure application and pond maintenance. Most of the inputs were obtained on the farm from other agricultural enterprises and from households. On-farm

recycling of resources has been recommended to the farmers from fish nutrition and pond fertilization trials carried out in aquaculture research stations and in small scale farms. Maize bran is the most common feed for fish on small scale farms. Use of maize bran and other resources as feed or manure has been reported to increase yield. A combination of 100 kg dry matterha^{-1}day^{-1} napier grass and 3% fish body weight maize bran produces 3,013 kgha^{-1}yr^{-1} net yield of *T. rendalli* and *O. shiranus* sp (Jamu and Costa-Pierce, 1994). Wood ash has been reported to have a neutralizing value of 79% and increases alkalinity, soil and water pH and total phosphorus in ponds when applied in combination with maize stover (Jamu and Costa-Pierce, 1994). Application of pumpkin leaves improves fertility and net fish yield of 1,444 kgha^{-1}yr^{-1} have been obtained, and waste cabbage leaf gives production of 678-1,024 t/ha (Jamu and Costa-Pierce, 1994).The economic assessment shows that growout operations were economically viable mostly because the inputs were solely obtained from on-farm. Return to labor was higher at MFF mainly because of labor inefficiency at NDF. It took 15 hours to maintain a 204 m^2 at NDF which was more than twice the time it took to maintain a 682 m^2 pond at MFF. The growout period was also shorter at MFF than at NDF, 191 and 221 days, respectively. The operator at MFF was more experienced than the wage labor at NDF which kept changing.

Table 6.4: Economic assessment of a growout cycle at Mapendo Fish Farm (MFF)

Input/Output	Cost/Revenue (MK)
Capital Inputs	
Pond construction	-
Operational Costs	
Fingerlings: 1400 @ MK0.05/piece	70.00
Maize and rice bran: 662.0 kg @ MK0.20/Kg	132.40
Vegetable matter: 33 Kg @ MK1.00/Kg	33.00
Manure (all forms)	-
Labor: 12.85 man-days @ MK12.00/man-day	154.20
Total operational costs	***389.60***
Revenues and Benefits	
Fish sold for cash: 65 Kg @ MK11.00/Kg	715.00
Fish for home consumption and gifts: 31 Kg	341.00
Total revenue and benefits	***1056.00***
Operational profits	***666.40***
Return to land	***0.98/m^2***
Return to labor	***63.86/man-day***

Table 6.5: Economic assessment of a growout cycle at Naming'azi Farm (NDF)

Input/Output	Cost/Revenue (MK)
Capital Inputs	
Pond construction	-
Operational Costs	
Fingerlings: 600 @ MK0.05/piece	30.00
Maize bran: 246.6 Kg @ MK0.20/Kg	49.32
Vegetable matter: 27 Kg @ MK1.00/Kg	27.00
Manure (all forms)	-
Labor: 15.28 man-days @ MK12.00/man-day	183.36
Total operational costs	***289.68***
Revenues and Benefits	
Fish sold for cash: 45 Kg @ MK11.00/Kg	495.00
Total revenue and benefits	***495.00***
Operational profits	***205.32***
Return to land	***1.01/m^2***
Return to labor	***25.44/man-day***

6.5 CONCLUSION:

Aquaculture has readily been adopted by small scale farmers in Malawi especially those who are in the remote areas from the lakes where fish is scarce. The majority of the farmers are in their economically active age group and married. Most of them have only attained primary education-hence the provision of extension services should be designed in such a way that it can be easily understood by them. Aquaculture is integrated with agriculture; the observed average pond size of 204 m^2 has been recommended to cater to an average household fish consumption. The government provides an initial batch of fingerlings to new farmers who are expected to produce their own for subsequent stocking. The drought which occurred in the country for three successive years led to most farmers being unable to restock their ponds. Preliminary economic assessment of fish farming operations shows that the enterprise is economically viable mainly because most of the inputs are obtained on-farm. These comprise crop, livestock and household wastes applied to fish ponds as feed and manure.

CHAPTER 7

GENERAL CONCLUSIONS

The variability of microsatellite markers has stimulated interest amongst scientists in their possible use in phylogenetic studies of tilapia and other cichlids. The microsatellite markers developed in this study show polymorphism across several genera of tilapia. The variability profile produced at the five loci varies both within individuals and within species. The general trend observed was that loci developed from sequences flanking long tandem repeats show higher variability than those made from sequences flanking shorter repeats.

Lack of reliable procedures for identifying cultured species in Malawi has deterred proper broodstock handling procedures and development of improved strains. The microsatellite DNA markers used in this study have been able to distinguish the accepted species of tilapia, and geographical subspecies of *O. shiranus*, previously postulated on morphological grounds (Trewavas 1983) have been confirmed. Results of the molecular systematics studies confirmed previous speculation that *O. shiranus* is genetically close to *O. mossambicus*, a species which is distributed to the south of Malawi. The assignment of the *O. shiranus* in Lakes Chilwa and Chiuta to a single subspecies is consistent with their geological union of these lakes until recently.

Microsatellite DNA probes have also been used to investigate the genetic diversity of lake, reservoir and farm populations of *O. shiranus*. The average number of alleles per locus and other measures of genetic diversity exhibited trends of decline in the domesticated populations compared to wild populations. The decline in genetic diversity within farm

stock shows a strong correlation with the time elapsed since their founding. The population genetic structure has been influenced strongly by exchange of germplasm among farmers. The original biogeographic relationship between genetic distance and geographical distance is no longer evident; in the southern region, farms growing different geographic subspecies of *O. shiranus* are closely intermingled. There is instead a strong correlation between genetic distance and a matrix of recorded or remembered stock transfers among farms, fisheries stations and reservoirs.

The Malawian situation may be typical of developing countries where little thought is given to genetic considerations during aquacultural development, despite a strong awareness of other aspects of biodiversity conservation. The result has been impoverishment, mixing and contamination of a unique genetic resource at the subspecies and species levels. Whether domestication selection has been improving the yield of *O. shiranus* (a fitness-related, quantitative genetic trait) in local farm environments while neutral microsatellite diversity is being lost is unknown, but not unlikely (Doyle *et al.* 1991). The combined genetic-socio-economic study reported here points to a novel strategy for conserving aquatic genetic resources in Malawi. Farmers who share common social characteristics tend to exchange genetic materials primarily among themselves and indirectly preserve the distinctness of pre-aquacultural zoogeographic groupings. This fact implies that governmental conservation and breed-improvement programs may be more successful if they are organised with due consideration to community boundaries rather than geographic boundaries.

Current domestication practices seem to promote the production of unplanned hybrids and yet management protocols for handling such hybrids have been established. The result be genetically detrimental if such hybrids were to escape and upset the reproductive barriers that exist in the wild. Experiences of interspecific hybridization mixing gene pools have been observed in this study. For example, introgressive hybridization has occurred due to uncontrolled spread of *O. shiranus* and *O. mossambicus* and the hybrids have wrongly been identified as *O. shiranus*.

The decline of effective allele number with time in the farm stocks indicates that microsatellite polymorphism is an effective tool for monitoring the accumulation of inbreeding and other small-population effects at the farm level. It may thus be a useful adjunct to selection programs.

The increasing pressure from human population growth and activities associated with the lakes on aquatic biodiversity in Malawi is of particular concern. *In situ* conservation offers achievable promise as a complementary alternative means of preserving the otherwise threatened biodiversity. Several reservoirs do exist in the country but most of them are underutilized for fishery production. The microsatellite DNA analysis suggests that with proper management, the reservoirs could be used for preserving genetic diversity within subspecies in the country.

The observed socio-economic characteristics of small scale farmers indicates that increase of profit can be achieved simultaneously with minimization of loss of genetic diversity in the farms. Most of the ponds are understocked leading to reduction of effective population sizes. Increased stocking density would result in higher profits through its direct effect on yield and would minimize the rate of erosion of genetic diversity through maintenance of high N_e. Although the demographic patterns observed here may not directly affect conservation of genetic diversity, they indirectly affect the rate of adoption of extension advice on minimizing the loss of genetic diversity at farm level and within community boundaries.

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