PN ABT 099
91006

10.197

FINAL REPORT

August 30, 1990-April 30, 1994

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

Identification and Conservation of Indigenous Tilapiine
Genetic Resources

Principal Investigator: Dr. James H. Howard
Grantee Institution: Frostburg State University

Co-Collaborators:
Dr. Joseph Gopo-University of Zimbabwe
Ms. Fortune Shonhiwa-National Parks and Wild Life
Management, Zimbabwe

Project Number: 10.197
Grant Number: COM-5542-G-00-0016-00

A.I.D. Project Officer: Dr. Lamarr Trott
S & T/AGR/RNR
Agency for International Development
Room 406 E. SA-18
Washington, DC 20523

Project Duration: August 30, 1990 to October 30, 1993*

*Permission has been granted for a no cost extension of six months to complete additional analyses.

Cr. Dosh D

This project is designed to identify cryptic species of a commercially important group of fish in Zimbabwe and assess the threat to these fishes through hybridization with exotic species. This project has also provided equipment and training opportunities for personnel at the University of Zimbabwe (UZ) and National Parks and Wild Life Management (NPWLM) to conduct future studies in the genetic management of natural populations of organisms.

NPWLM research officer, Fortune Shonhiwa, has defended her Master's thesis and is currently enrolled in a Ph.D. program to further her training. Dr. Joseph Gopo and Dr. W.K. Nduku have received the equipment necessary to establish biochemical genetics laboratories at UZ and NPWLM, respectively.

We have completed the allozyme analysis of over 1000 fish samples from 40 populations collected throughout the rivers and reservoirs in Zimbabwe. In addition, we have completed morphological analyses and mtDNA analyses on smaller subsets of those collections. Our results indicate that we can unambiguously identify the species of interest using biochemical techniques. We have also found extensive evidence of hybridization among forms not naturally sympatric in several major reservoirs. These data indicate that introduction of exotic forms pose a significant threat to the genetic integrity of the most economically important group of Zimbabwean fishes. Recommendations are being made to NPWLM concerning management strategies to mitigate this threat and to utilize pure genetic stocks for aquaculture trials.

Table of Contents

	Page #
Executive Summary:	1
A). Research Objectives	3
B). Methods and Results	5
C). Impact, Relevance and Technology Transfer	17
D). Project Activities/Outputs	18
E). Project Productivity	19
F). Future Work	20
G). Literature Cited	21
H). Financial Reports	26
I). Tables	28
J). Figures	49

Appendix I.	Publication
Appendix II	MS Thesis
Appendix III	Abstracts
Appendix IV	Gene Frequencies

The use of cichlid fishes in Africa as a source of relatively inexpensive protein has grown remarkably over the past several decades and the demand is expected to accelerate. In Zimbabwe alone, it is estimated that total fish catches should increase from 20-30,000 tons per year (estimated in the 1980s) to as much as 160,000 tons per year by the end of the century (Balarin 1984, 1986, Gurure 1985).

Tilapiine fish species are particularly suitable for fish farming, having excellent growth rates on a wide range of both natural and artificial foods (Wohlfarth and Hulata 1983). They are also resistant to disease and tolerant of crowding. These fish represent the best potential for successful aquaculture development in Zimbabwe.

The genetic integrity of indigenous tilapiine species in Zimbabwe is being compromised, however, by aquacultural practices. The development of aquaculture projects to meet the increased demand for fish protein has lead to many accidental and intentional releases of exotic species (Beattie, 1986). Many Zimbabwean species not previously sympatric are now found in sympatry due to these manipulations and some naturally allopatric species of tilapias will freely hybridize when placed in sympatry (Bell-Cross and Minshull 1988). Extensive introductions and translocations of tilapias make it more difficult to identify either pure stocks or hybrids (McAndrew and Majumdar 1983).

In recent years the government of Zimbabwe, through the Department of National Parks and Wild Life Management, has expressed concern over the long term consequences of such manipulations on native fish fauna. To address their concerns two issues must be resolved: 1). pure stocks of native tilapiine fishes must be identified unambiguously; and 2). the threat hybridization poses to indigenous fishes must be assessed.

The identification of pure stocks is problematic due to substantial morphological variation and broad overlap in meristic characteristics considered important in species identification within most genera (Fryer and Illes 1972, McAndrew and Majumdar 1983, Wohlfarth and Hulata 1983, Van der Bank et al. 1989). The difficulty of obtaining pure stocks for basic biological research or aquaculture trials has already been noted by several investigators (e.g. McAndrew and Majumdar 1984, Thompson 1984, Beattie 1986, Van der Bank et al. 1989). Because of the difficulties in attempting to identify species using

traditional characteristics this study employed electrophoretic genetic markers as an alternative methodology.

The taxonomic utility of allozymes detected by electrophoresis is well known (Avise 1974, Thorpe 1979, Ferguson 1980). Cruz et al. (1982), McAndrew and Majumdar (1983, 1984) and Van der Bank et al. (1989) have demonstrated the use of detectable enzyme loci as diagnostic characters in tilapiine species. Once these enzyme differences between potential parent species were established, interspecific hybrids and introgressed backcrosses could be identified (Cruz et al. 1982, Macaranas et al. 1986).

This study focused on tilapiine species indigenous to Zimbabwe and characterized these species using enzyme loci as species-specific genetic markers. Eight species (five in the genus Oreochromis and three in the genus Tilapia) are currently recognized in Zimbabwe (Table 1). In addition, Oreochromis niloticus and Oreochromis aureus have been introduced into the country for aquacultural purposes.

Specific objectives of the study were to:

1. Electrophoretically examine samples from tilapiine fish populations collected throughout Zimbabwe.

2. Summarize the genetic differences among all tilapiine species and among populations within species, particularly with reference to fixed allelic differences.

3. Genetically characterize samples from areas where documented introductions have occurred and determine the incidence of hybridization.

4. Examine the fish carcasses, with a priori knowledge of species identification, for morphological features that will unambiguously distinguish the species of interest.

The results described below and in the appendices could not have been possible without significant funding by the U.S. Agency for International Development and the use of field personnel and equipment donated by Zimbabwe's Department of National Parks and Wildlife Management and the University of Zimbabwe. In addition, Frostburg State University (University of Maryland System) provided laboratory support, an international graduate student fellowship, sabbatical support for Dr. Gopo, as well as providing time for the Principal Investigator to complete the study.

Methods and Results

Allozyme analysis

Sample Collection

Samples of 4-40 fish per site were collected from the major river systems of Zimbabwe (Fig. 1, Table 2). Collections were made in native ranges of each species, and in areas where introductions of other species were unlikely. Between May 1989 and August 1991, 16 populations of *Tilapia rendalli*, 13 of *Oreochromis mossambicus*, nine of *O. mortimeri*, three of *O. macrochir*, three of *O. andersonii*, three of *T. sparrmanii* and two populations of *O. placidus* were sampled (not all collections were analyzed in this study). In addition, nine *O. niloticus* individuals and five *O. aureus* individuals were collected from the Edwards and Son Farm located just outside of Harare. These two species are not indigenous to Zimbabwe but are currently being farmed there. The strains at the Edwards farm were imported from Stirling, U.K. in 1985 and are believed to have come from cultures in Israel. Their purity could not be ascertained. *Tilapia ruweti*, one of the three *Tilapia* species listed as indigenous to Zimbabwe, was not collected due to its scarcity in the Upper Zambezi System.

Collecting Techniques

Specimens were collected using seines and gill nets. Muscle, liver, eye, heart, and brain tissues were dissected from freshly killed fish, labelled, placed in Eppendoff tubes and immediately frozen in liquid nitrogen at the collection site. The tissues were then taken to the University of Zimbabwe and were stored in an ultra-cold freezer at -80°C. Tissues were shipped on dry ice to Frostburg State University, Maryland, U.S.A. for analysis.

Carcasses were preserved in 10% formalin and stored in 50% ethanol. These were also shipped to Frostburg State University.

Sample Preparation

In the laboratory, tissues were prepared for electrophoresis by adding an equal volume of distilled water to the tissue and homogenizing with a tissue grinder for 1 min The homogenate was then centrifuged at 12,800 x g for three minutes and the supernatant was absorbed onto 1.5 x 0.3 cm strips of Whatman No. 3 filter paper for loading in gels.

Gel Preparation

Gel preparation procedures followed those outlined in Selander et al. (1971) and May (1975), employing 9.5 x 20 x 1 cm and 9.5 x 20 x 2 cm molds. Gels consisted of 13% Sigma hydrolyzed starch and one of the buffer solutions listed in Table 3. The mixture was then aspirated for approximately 1 min. with constant swirling to remove air bubbles, poured in the Plexiglass mold and allowed to cool at room temperature.

Electrophoresis

Horizontal starch gel electrophoresis was carried out following procedures outlined in Selander et al. (1971), May (1975), Allendorf et al. (1977), and Aebersold et al. (1987). Electrophoresis was continued for 3-5 hr. until the dye marker had moved to a distance of 6 cm. The resulting gel was sliced into 1.5 mm thick replicates and each was stained with specific histochemical solutions as outlined by May (1975) and Allendorf et al. (1977).

Assay

In the first five months of laboratory analysis, an assay for the Zimbabwean tilapias was developed. Initially all five tissues (eye, liver, muscle, heart and brain) were run on the same gel and on each of the four buffer systems. The enzyme systems which showed good to fair resolution and activity on the different buffer systems were selected for further use in the electrophoretic analysis of tilapias. As the analysis proceeded, only those enzymes which produced scorable banding patterns were used. Stains and appropriate buffers used in the study are listed in Table 4. Enzyme nomenclature largely follows that revised by the American Fisheries Society (Shaklee et al. 1990), and that used for tilapias by McAndrew and Majumdar (1983) and Van der Bank et al. (1989). Relative mobilities were calculated based on the most common allele that is designated 100 for an anodal locus or -100 for a cathodal locus.

Data Analysis

Electrophoretic data were analyzed primarily with the BIOSYS-I program (Swofford and Selander 1981). This program computes genetic variability, determines conformance to expected genotypic frequencies based on Hardy-Weinberg equilibrium, and calculates F-statistics. Significant allelic differences among populations were tested using Chi-square contingency tables.

Genetic variability within each population was estimated by calculating percentage of polymorphic loci and mean heterozygosity. Average heterozygosity was calculated based on Hardy-Weinberg expectations using Nei's (1978) unbiased estimate.

Allelic frequencies were also tested for conformance to Hardy-Weinberg expectations. The Chi-square goodness-of-fit test corrected for small sample sizes and G-tests for samples that violated Chi-square test assumptions were used. Genetic differentiation of populations was analyzed using F-statistics (Wright 1965, 1978, Nei 1977).

In addition, Nei's (1978) and Rogers's (1972) similarity and distance coefficients were calculated. These indices were used for comparisons among species and populations. They were then used to construct Distance Wagner trees (Farris 1970) and UPGMA phenograms (Sneath and Sokal 1973). FREQPARS (Swofford and Berlocher 1987) trees were also constructed.

Results of Collections

Extensive field collections produced some unanticipated results; specimens which could be classified as O. mossambicus (or O. mortimeri) were collected in the Nata system at three sites. Bell-Cross and Minshull (1988) do not list the presence of any tilapias in the Nata system. Collections of T. rendalli were also made in the Budzi system although there is no previous listing of this species in the system. A search for tilapias in the Pungwe system was unsuccessful. This finding agrees with previous research (Bell-Cross and Minshull 1988). Although T. ruweti (2 specimens) was collected previously in the Upper Zambezi system at Kazungula (Bell-Cross and Minshull 1988), no specimens were taken for this study.

Results of Allozyme Analysis

Out of the 64 enzyme systems tested, the assay resulted in the resolution of 38 allozyme loci from 28 enzyme systems. These enzyme systems were included in all subsequent electrophoretic analyses. Allelic frequencies for all loci studied are listed in Appendix IV.

A total of 603 fish from 34 indigenous populations was examined for the 38 loci described above and yielded a total of *106* presumptive alleles. *Twenty-seven* loci showed variation in at least one population, while 11 loci were monomorphic for all species. Of the 11 monomorphic loci, six were monomorphic for the same allele for all species examined, while the remaining five showed fixed allelic differences among species (Appendix IV).

No fixed allelic differences were found among O. mossambicus (ten populations) and O. mortimeri (nine populations). The only differences among these nineteen populations were in allelic frequencies at some loci.
The nine O. mortimeri populations were fixed for one allele at the sAAT-2 and GP-2 loci, while some O. mossambicus specimens carried another allele at these loci. On the other hand, O. mossambicus populations were fixed for one allele at GDA-1, G3PDH-1, and PGM-1 while some O. mortimeri were heterozygous at the same loci. Also, at GPI-1, the heterozygotes in O. mortimeri demonstrated a different allele than the heterozygotes in O. mossambicus. Results from the contigency Chi-square analysis showed significant differences

in allele frequencies ($p<0.05$) between O. mossambicus and O. mortimeri at sAAT-2, ADA-1, GDA-1, sMDH-1, PGM-1, sIDDH-1, SOD-1 and TPI-1.

sMEP-1 was the only locus which showed a fixed allelic difference between O. andersonii and the O. mossambicus\O. mortimeri complex.

Of the Tilapia spp., both T. rendalli and T. sparrmanii, have more than 13 diagnostic loci which distinguish them from any of the Oreochromis spp. The diagnostic loci are not similar in all cases as the Tilapia spp. have 16 loci that are diagnostic between them.

Chi-square tests for conformance to Hardy-Weinberg proportions were performed for each population at all polymorphic loci. Twenty-four out of the 178 (not including the exotic species) Chi-square tests deviated significantly at the 0.05 level. One of the thirty-four indigenous populations (not including exotics) included in this study was responsible for six of the deviations.

Genetic variation within populations was measured using percentage polymorphism and average heterozygosity (H) (Table 5). A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. The percentages of loci which were polymorphic ranged from 0% in a T. rendalli population to 21.1% in an O. mossambicus population collected from the Ruti Dam. The lowest mean heterozygosity was 0.0 in T. rendalli and the highest was 0.088 in O. mossambicus from Ruti Dam.

F-statistics were calculated for all populations where $n>10$. Means of 0.078 for F_{IS}, 0.679 for F_{IT}, and 0.783 for F_{ST} were found when all Oreochromis species were included. The O. mortimeri/O. mossambicus complex yielded an average F_{ST} of 0.337. The loci which are responsible for the major differences among Oreochromis populations were CK-2, EST-1, LDH-C, GP-2, GPI-1, LDH-B, MDH-3, ME-1 and ME-2, all of which showed a F_{ST} value greater than 0.80.

The calculated values for Nei's (1978) unbiased distances (D_n) and Rogers (1972) genetic distances for population comparisons are given in Table 6 and for species comparisons in Table 7. Phenograms and dendrograms were produced for the data from individual populations and from the collapsed species data (Figs. 2 and 3). To get species data, the population data for each species was collapsed to give a pooled value for each locus. Phenograms were based

on the unweighted pair group method using arithmetic averaging (UPGMA) algorithm, with Nei's (1978) unbiased genetic distance. Dendrograms were produced by Distance Wagner procedure (Farris 1972) using Rogers's (1972) genetic distance index.

The UPGMA phenograms show T. sparrmanii and T. rendalli as outliers to Oreochromis species. The collapsed data phenogram (Fig. 2) shows that within the genus Oreochromis there are two groupings; O. macrochir and O. placidus in one cluster and the rest of the Oreochromis species in another. Fish identified as O. mossambicus and O. mortimeri all show a close relationship.

The Distance Wagner tree (Fig. 3) shows distinct separation of Tilapia species from the Oreochromis species. The Tilapia spp. form their own clade. Unlike the UPGMA tree, which indicates a sister group relationship between O. macrochir and O. placidus, the Distance Wagner trees do not support such a relationship. In the Distance Wagner topology, O. macrochir branches off after the Tilapia ssp.. Oreochromis placidus is also a distinct clade from the O. mossambicus, O. mortimeri and O. andersonii cluster.

Oreochromis mossambicus, O. mortimeri, and O. andersonii cluster together in all Distance Wagner trees which is consistent with the UPGMA topologies. The UPGMA topology (Fig. 12, Appendix II) show O.mossambicus and O. mortimeri clustering together and O. andersonii as a sister taxa to that cluster. The Distance Wagner topology reverses this situation and has O. mortimeri clustering with O. andersonii and O. mossambicus as the next closest taxon. The Distance Wagner individual population topology (Fig. 13, Appendix II) reveals O. andersonii populations being clustered within the O. mortimeri population group.

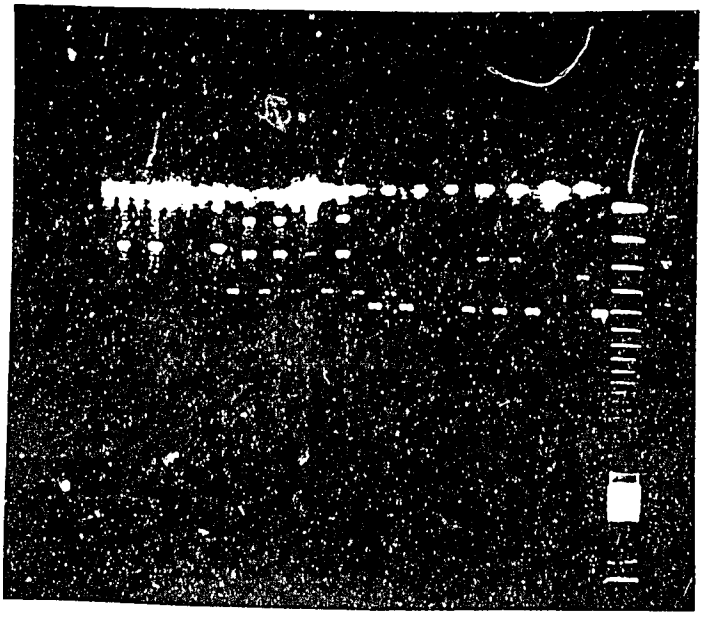
The uncertain status of the species described as O. mortimeri was evidenced by both the small distance coefficients separating this taxon from O. mossambicus and the dendrograms. Some pairs of O. mortimeri populations have greater genetic distances separating them than some O. mossambicus-O. mortimeri pairs. In an attempt to further evaluate the species status we decided to use an estimator of genetic differentiation which evolves at a faster rate than do nuclear genes. The section below summarizes our attempts to isolate and amplify mtDNA genes which could then be digested with restriction enzymes to reveal restriction fragment length polymorphisms (RFLPs).

Mitochondrial DNA Analysis

DNA was isolated from 256 individuals including samples from four O. mortimeri populations and six O. mossambicus populations (Gustincich et al. 1991). Polymerase chain reactions were used to amplify two mtDNA genes (16S-rRNA and d-loop) using the methods of Williams et al. (1990). The primer sequences for the amplification of these genes was provided by Kornfield (pers. comm.) and the sequences were synthesized at the University of Florida Core Facility and by Operon Technologies, Inc.. Although not all samples have been examined, several individuals from populations of O. mortimeri and O. mossambicus were included. The amplified mtDNA was subjected to restriction enzyme digests and the resulting fragments separated with electophoresis on 2% agarose gels. After electrophoresis, the fragments were stained with ethidium bromide and visualized with UV light. The assay used 16 enzymes, primarily four-base cutters, and individuals from the two species were run in adjacent lanes for comparison. A list of the restriction enzymes used is found below with a copy of a representative gel. Because we failed to find diagnostic differences between O. mortimeri and O. mossambicus in our allozyme assay we hoped that by examining genes which evolve at higher rates than nuclear genes we would find loci which enable us to distinguish unambigously between these taxa. We intentionally chose these two genes because one of them (16S-rRNA gene) is conservative and the other (d-loop region) is not conserved. This should have allowed us to "bracket" detectable differences with two different rates of evolutionary change.

Restriction Enzymes

Alu I	AGCT	Rsa I	GTAC
BstU I	CGCG	Dde I	CTNAG
Cfo I	GCGC	Hinf I	GANTC
Hae III	GGCC	EcoR II	CCA/TGG
Mbo I	GATC	Ava II	GGA/TCC
Msp I	CCGG	Tru 9I	PuGGNCCPy
Taq I	TCGA	EcoR I	GAATTC
Nci I	CCC/GGG	Sau96 I	GGNCC



Enzyme digest pattern for two samples of O. mortimeri and two samples of O. mossambicus. Each sample repeated with 16S and d-loop amplified genes. Total of eight samples repeated with three different enzymes.

Results of MtDNA analysis

Although differences in digest patterns were detected during this analysis, as with the allozyme data, no fixed genetic differences were found that could aways distinguish these two putative taxa. This finding coupled with the allozyme similarities leads us to the conclusion that O. mortimeri is not a valid species and should be synonomized with O. mossambicus.

Hybridization

We have examined samples of over 400 fish from three reservoirs where introductions are known to have occurred for evidence of hybridization. At each location hybridization was detected using principle components analysis.

Multivariate statistical methods are commonly used to examine hybridization. Multivariate analysis can be applied to morphological characters or to genetic data such as allozyme data (Rainboth et al. 1989). Detecting hybridization is especially problematic when character states overlap between parental species, as is the case for Oreochromis species.

Principle component analysis (PCA) reduces the dimensionality of the data while retaining much of the original variation (Joliffe 1986). Often, areas of multivariate space can be defined which correspond to the parental species and to the hybrids (Neff and Smith 1979). PCA also provides a way to visualize the degree of backcrossing of the hybrids to either parental species.

We scored each polymorphic locus as either 0,1 (for the homozygous states), or 0.5 (for the heterozygous condition). All variable loci contained two alleles except for mMDH-1 in O. mortimeri which contained three alleles. For this locus we pooled the rare alleles due to the low frequency of each of the rare alleles. Lake Kyle and Lake MacIllwaine both contain the species O. macrochir and O. mossambicus, which have six variable loci: LDH-B, EST-1, CK-2, GPI-1, PROT-1, mMDH-1. In Lake Kariba, which contained O. mortimeri and O. macrochir, we found sMEP-1 to be polymorphic in addition to the six loci listed above. The major sources of variation in these discrete data were summarized from a correlation matrix of the numerically coded allelic character states and principle component scores were calculated using normalized eigenvectors (Table 8). The first two principle component values were then plotted for each individual in order to show its relationship to other individuals. The first two principle components described between 69.4% and 98.1% of the total variation in the origninal data set (Table 9).

Lake MacIllwaine contains some individuals which are pure O. macrochir (n=148, PC1 < 2), two fish which are likely first generation hybrids between O. mossambicus and O. macrochir (PC1 > 6), and five fish which are hybrids backcrossed to O. macrochir (0 < PC1 < 2) (Fig. 4). Lake Kyle contains individuals of both parental species, O. macrochir (n=160, PC1 = -0.39 and PC2 = 0.03) and O. mossambicus (n=23, PC1 = 2.74 and PC2 = 0.48), two fish which are likely to be F1 hybrids (PC2 < -5.0), and two fish which are hybrids backcrossed to O. mossambicus (2.0 < PC1 < 2.5) (Fig. 5). Since backcrossing is rare in Lake Kyle, the data suggest that either the introduction has occurred recently so that hybridization has not had time to proceed beyond the first generation or the introduction was large enough that species can usually locate memeber of their own taxa thus making hybridization rare. Samples from subsequent years would help distinguish between these hypotheses.

The data from Lake Kariba are more difficult to interpret that the data from the other two lakes in part due to the variability within O. mortimeri. In Lake Kariba we detected pure O. mortimeri (n=98) (PC1 = 0.38 and PC2 = 0.15), F1 hybrids (n=5, PC2 < -3.0), and individuals produced by hybrids which have backcrossed to O. macrochir (n = 7, -3.0 < PC1 < 0 and -1.0 < PC2 < 3.0) (Fig. 6). Lake Kariba has more backcrossing than either of the other lakes. The abundance of

hybrids may be due to either an earlier introduction or may reflect difference in species involved in hybridization or a difference in breeding habitat availability..

Morphological Analysis

Populations of the five indigenous Zimbabwean cichlid species (Oreochromis andersoni, O. macrochir, O. mortimeri, O. mossambicus, and O. placidus) were analyzed morphologically. The individuals examined were collected from the localities indicated in Table 10. Twenty-nine mensural and nine meristic characters were recorded from 244 individuals that were assayed genetically (Table 11). Morphometric characters were recorded from a set of 18 truss measurements (Bookstein et al. 1985) plus 11 auxiliary measurements. Dial calipers were used to obtain all measurements, which were recorded to the nearest 0.1 mm. Meristic characters (serially repeated elements) were those shown by Trewavas (1983) to be taxonomically important for this group of fishes.

Sheared principal components analysis (Humphries et al. 1981, Bookstein et al. 1985) of the morphometric data was used in order to discriminate among individuals that vary in size. This technique allows for the determination of size-free shape differences among specimens by: 1) converting scores from a principal components analysis to zero mean within group, 2) regressing the second component on the group size component, 3) estimating the residuals from the regression, and 4) using the estimated residuals as a new linear combination of principal component scores (Humphries et al. 1981). Meristic data were analyzed using principal components analysis. Multivariate analysis of variance (MANOVA) was used to determine if the species clusters were statistically significant at P = 0.05. The SAS package of programs (SAS Institute, Inc. 1985) was used for all statistical analyses and the shearing technique was performed using a SAS program developed by D.L. Swofford.

Initially, two separate comparisons were made between pairs of similar species: O. andersonii (N = 14, 2 localities) versus O. macrochir (N = 27, 4 localities) and O. mortimeri (N = 69, 7 localities) versus O. mossambicus (N = 108, 11 localities). Following these analyses, all five species were analyzed simultaneously. The preliminary results are outlined below.

Morphological analyses of the O. andersonii/O. macrochir species pair indicated that these species overlap considerably in meristic characters, but exhibit shape differences that separate most individuals in these two species (Figure 7). MANOVA results indicated the clusters were signficantly different. Variables contributing to separation of these species were those associated with head shape. Oreochromis andersonii individuals have deeper heads and a greater snout-to-nape length than O. macrochir individuals.

Multivariate morphological analyses were less successful in resolving differences between the O. mortimeri/O. mossambicus pair. Considerable overlap of O. mortimeri and O. mossambicus along the major axes of shape and meristic variation was evident (Figure 8), and the two species clusters were not significantly different as determined by MANOVA. Distances from the most posterior dorsal- and anal-fin rays to the hypural plate and counts of median fin rays and gill rakers contribute most to the separation of these two species. Oreochromis mossambicus individuals tend to have longer caudal peduncles and lower gill raker and median fin ray counts than O. mortimeri. The large amount of morphological overlap may be due to phenotypic plasticity in one or both species. Since samples of both taxa were collected from a large geographic area, environmental differences among localities may contribute to increased morphological variation.

The simultaneous analysis of all five species resulted in the separation of all species clusters with the exception of O. mortimeri and O. mossambicus. Oreochromis placidus differed from other species in the number of anal fin spines, which contributed strongly to principal component I for the meristic data. As before, head shape and caudal peduncle length were highly correlated with sheared PC II, and median fin rays and the number of gill rakers on the lower first arch loaded high on principal component one for the meristic data.

Impact, Relevance and Technology Transfer:

The most significant impact of our research for National Parks and Wild Life Management of Zimbabwe will be in the identification of pure fish stocks. By starting with pure stocks, NPWLM will be able to produce aquaculture trials that can be reliably replicated and produce hybrids for experimentation of known ancestry. This should be a significant advantage in developing coordinated, long-range aquaculture trials. In addition, the data we have gathered on hybridization indicate that the traditional methods that fisheries officers use to produce the fry for distribution around the country may endanger the integrity of wild fish gene pools. In order to avoid problems of "polluting" gene pools of wild fish stocks and possibly endangering some species, NPWLM may need to change their procedures for producing fry. These findings have been communicated to NPWLM officers informally but our formal recommendations will be sent to NPWLM along with a copy of this report. We have analyzed enough populations that NPWLM can use the data from our study sites to collect pure gene stocks for their aquaculture trials. We will recommend to NPWLM that they initiate a culture program where pure stocks, collected from sites we have sampled, are maintained in separate culture facilities as a "gene bank" for future experimentation and reintroduction in the wild. Future introductions should only occur within the native ranges of the species being introduced and stocks should be verified genetically. In addition, significant safe-guards against the accidental escape of experimentally produced hybrids must be provided. NPWLM and UZ now have the appropriate equipment and trained personnel so stocks of questionable purity can be easily verified from small tissue samples. Finally, the evidence we have provided on genetic variation will help NPWLM choose stocks with the greatest potential response to selective breeding trials.

Zimbabwean institutions have been strengthened as a result of this project primarily because of increases in technical expertise and the acquisition of new equipment. National Parks and Wild Life Management have a fisheries officer (Fortune Shonhiwa) trained in population genetics and fisheries, skills they did not previously possess. In addition, Mrs. Shonhiwa has enrolled in a Ph.D. program at West Virginia University to continue her training. NPWLM also has obtained the necessary equipment to conduct future investigations on the genetics of wild populations. This is a capability that NPWLM has expressed

a genuine need for in their management program. The University of Zimbabwe also has received several critical pieces of equipment they require to conduct genetic studies. Because of this project, Dr. Joseph Gopo has already been able to initiate new kinds of investigations not previously possible in Zimbabwe. Partly because of the new equipment and partly because of the opportunity he had in the US to visit other laboratories and gather information about new technologies, Dr. Gopo has been able to share these technologies with his students and increase their research potential.

Project Activities/Outputs:

As a result of this project we delivered three papers summarizing portions of our findings, the first was delivered at the Network Meeting in Manila, Phillipines, (August, 1991) sponsored by BOSTID and the National Research Council. Subsequent papers were delivered at the annual meetings of the Association of Southeastern Biologists in April, 1992 (University of Alabama) and at the annual meetings of the Society for Conservation Biology in June, 1992 (Virginia Polytechnic University) (abstracts included in Appendix III). In addition, the PI traveled to a special symposia sponsored by Ohio State University and the Columbus Zoo from October 30-November 2, 1993. This symposia was entiled "Conservation Genetics and Evolutionary Ecology-A Case Study of Cichlid Fauna of Lake Victoria." At this meeting the PI made the contacts necessary to add the mtDNA analysis of cichlid fishes discussed above. The PI has also delivered two invited addresses based on our work; at Towson State University in Maryland (October 7, 1993-Allozyme analysis of tilapiine fish of Zimbabwe) and the University of Montana (October 29, 1993-Cryptic species and hybridization in African cichlid fishes).

Ms. Fortune Shonhiwa has finished all the coursework required for her MS in Applied Ecology and Conservation Biology and her thesis is ready for publication (Appendix II). She also has enrolled in a Ph.D. program at West Virginia University to continue her studies. She also has been the principal author on two paper presentations at the regional and national meetings outlined above and was the first author on the article found in Appendix I. The

completion of other papers has been delayed by the addition of the mtDNA analysis and Ms. Shonhiwa's Ph.D. program. We do anticipate two completed manuscripts, one of the electrophoretic characterization of Zimbabwean tilapiine species and the second on the species status of O. mortimeri, will be under review within a few months. Also, Mr. Ron Gregg, of the University of Montana, and I will be completing a manuscript this summer on the hybridization studies. Dr. Richard Raesly is preparing a manuscript on the morphological analysis and Dr. Brian Masters of Towson State University, Maryland and I will complete a mtDNA analysis of all species to compare with the allozyme studies already completed. As these papers are accepted for publication, copies will be mailed to the Office of the Science Advisor bearing the project number, 10.197.

Project Productivity:

We completed all the necessary collections and developed an excellent allozyme assay for tilapiine fishes as a result of this project. We have examined over 1000 individual fish from all populations of interest and can distinguish all the cryptic species (except O. mossambicus and O. mortimeri which should be synonomized) unambiguously using genetic techniques. We also have estimated the amount of genetic variation in each population and determined the incidence of hybridization in three large Zimbabwean reservoirs. We have been unable to develop a morphological key that will be useful to fisheries officers in the field. Although our detailed morphological analysis does permit us to separate most species, the O. mossambicus/O. mortimeri, O. andersonii complex is problematic due to significant phenotypic plasticity and a broad range of character state overlap.

Future Work:

We have a significant number of tissues remaining from this study with a great deal of valuable information remaining. In the months ahead we hope to continue to gather mtDNA data to augment our allozyme data base. There are few studies available that contain such a large data base on population structure from two independently derived genetic sources (nuclear and mtDNA). That comparision by itself is of significant scientific interest. In addition, we hope that NPWLM will be encouraged to incorporate this study in their aquaculture experimentation and we would be happy to assist in any way. This data, on

sources of pure genetic stocks and population differences in heterozygosity, can provide the foundation for well-documented, repeatable breeding trials.

Literature Cited

Aebersold, P.B., G.A. Winans, D.J. Teel, G.B. Milner, and F. Utter. 1987. Manual for starch gel electrophoresis: A method for the detection of genetic variation. NOAA Technical Report. NMFS 61:20 pp.

Allendorf, F.W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (Salmo trutta): detection and interpretation from population data. Hereditas 86: 179-190.

Balarin, J.D. 1984. National review for aquaculture development in Africa. FAO Fish Circular 770.1. 71pp.

Balarin, J.D. 1986. The status of fish farming in Zimbabwe and future prospects. Zimbabwe Agricultural Journal 83:3-9.

Beattie, I.H. 1986. Summary of a talk on "The Development of Pure Strains." Proceedings of the Fish Forum held at Henderson Research Station on 21st March 1986. pp. 35-38.

Bell-Cross, G. and J. Minshull. 1988. The fishes of Zimbabwe. National Museums & Monuments of Zimbabwe. Harare. 294 pp.

Bookstein, F., B. Chernoff, R. Elder, J. Humphries, G. Smith, and R. Strauss. 1985. Morphometrics in evolutionary biology. Spec. Publ. 15, Acad. Nat. Sci. Phila., Philadelphia.

Clayton, J.W. and D.N. Tretiak. 1972. Amino-citrate buffers for pH control in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29:1169-1172.

Cruz, T.A., J.P. Thorpe and R.S.V. Pullin. 1982. Enzyme electrophoresis in Tilapia zilli: a pattern for determining biochemical genetic markers for use in tilapia stock identification. Aquaculture 29:311-329.

Farris, J.S. 1970. Methods of computing Wagner tress. Systematic Zoology 19:83-92.

Farris, J.S. 1972. Estimating phylogenetic trees from distance matrices. American Naturalist 106:645-668.

Ferguson, A. 1980. Biochemical Systematics and Evolution. Blackie and Sons, Glasgow. 194 pp..

Fryer, G. & T.D. Illes. 1972. The Cichlid Fishes of the Great Lakes of Africa. Oliver Boyd, Edinburgh. 641 pp..

Gurure, R. 1985. Aquaculture in Rural Development Planning in Zimbabwe. Freetown Proceedings of the Commission of the Secretariat Consultant Workshop on Village Level Aquaculture in Africa: 18 pp..

Gustinicich, S., G. Mafioletti, G. Del Sal and C. Scheider. 1991. Rapid identification of genetic variation and pathotype of Leptoshaeria maculans by random amplified polymorphic DNA assay. Appl. Environ. Microbiol. 57:2482-2486.

Humphries, J.M., F.L. Bookstein, B. Chernoff, G.R. Smith, R.L. Elder, and S.G. Poss. 1981. Multivariate discrimination by shape in relation to size. Syst. Zool. 30:291-308.

Joliffe, I.T. 1986. Principle component analysis. Springer-Verlag. New York, New York.

Macaranas, J.M., N. Taniguchi, M. Pante, J.B. Capili and R. Pullin. 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial Oreochromis niloticus (L.) stocks in the Philippines. Aquaculture and Fish Management 17:249-261.

Markert, C.L. and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. Journal of Experimental Zoology 159:319-332.

May, B. 1975. Electrophoretic variation in the genus Oncorhynchus: The methodology, genetic basis, and practical applications to research and management. Master's Thesis, University of Washington.

McAndrew, B.J. & K.C. Majumdar. 1983. Tilapia stock identification using electrophoretic markers. Aquaculture 30:249-261.

McAndrew, B.J. & K.C. Majumdar. 1984. Evolutionary relationships within three Tilapiine genera (Pisces:Cichlidae). Zoological Journal of Linnean Society 80:421-435.

Neff, N.A. and G.R. Smith. 1979. Multivariate analysis of hybrid fishes. Syst. Zool. 28:176-196.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Rainboth, W.J., D.G. Buth and F.B. Turner. Allozyme variation in Mojave populations of the desert tortoise, Gopherus agassizi.

Ridgeway, G.J., S.W. Shelborne, and R.D. Lewis. 1970. Polymorphism in esterases of the Atlantic herring. Transactions of the American Fisheries Society 99:147-151.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas Publications 7213:145-153.

SAS Institute, Inc. 1985. SAS user's guide: statistics. Cary, North Carolina.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Studies in Genetics VI. University of Texas Publications 7103:49-90.

Shaklee, J.B., F.W. Allendorf, D.C. Morizot and G.S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 111:2-15.

Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis cf enzymes - a compilation of recipes. Biochemical Genetics 4:297-320.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy. W.H. Freeman and Co., San Francisco. 573 pp.

Sodsuk, P. and B.J. McAndrew. 1991. Molecular systematics of three tilapiine genera Tilapia, Sarotherodon and Oreochromis using allozyme data. Journal of Fish Biology 39(Supplement A):301-308.

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-I: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Swofford, D.L. and S.H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Systematic Zoology 36: 293-325.

Thompson, P.J. 1984. Hybridization of indigenous Oreochromis species in Zimbabwe and production of all-male offspring. Aquaculture in South Africa 1984 Joint Symposium by the CSIR and SAAU. pp. 1-11.

Thorpe, J.P. 1979. Enzyme variation and taxonomy: the estimation of sampling errors in measurements of interspecific genetic similarity. Journal of the Linnean Society. London (Biology) 11:369-386.

Trewavas, E. 1983. Tilapiine Fishes of the Genera Sarotherodon, Oreochromis, and Danakilia. British Museum (Natural History). London. 583 pp.

Van der Bank, F.H., W.S. Grant, J.T. Ferreira. 1989. Electrophoretically detectable genetic data for fifteen Southern African cichlids. Journal of Fish Biology 34(3):465-483.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. acids Res., 18:6531-6535.

Wohlfarth, G.W. and G. Hulata. 1983. Applied Genetics of Tilapias. Second Edition. ICLARM Studies and Reviews 6. 26 pp. International Center for Living Aquatic Resources Management, Manila, Philippines.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 9:395-420.

Wright, S. 1978. Evolution and the Genetics of Populations.IV. Variability Within and Among Natural Populations. Univ. of Chicago, IL. 580 pp

Table 1: The distribution of tilapiine fish species in Zimbabwean river systems. (Adapted from: Bell-Cross and Minshull 1988).

Species	UZ	LK	MZ	LZ	N	L	P	B	URS	LRS
O. andersonii	x									
O. macrochir	x	i	i	i					i	
O. mortimeri		x	x							
O. mossambicus				x	x*	x		x	x	x
O. placidus						x			i	x
T. sparrmanii	x	x	x			x			x	x
T. rendalli rendalli	x	x	x	x				x*	i	x
T. rendalli swierstrae						x				
T. ruweti	x									

key: Drainage Systems:

UZ: Upper Zambezi — LK: Lake Kariba
MZ: Middle Zambezi — LZ: Lower Zambezi
N: Nata — L: Limpopo
B: Budzi — P: Pungwe
URS: Upper Save/Runde — LRS: Lower Save/Runde

x: specimens collected there in the past.

i: fish introduced into the system

x*: new collection records from this study

TABLE 2. Locations and drainage systems where samples of tilapiine fish species were collected in Zimbabwe.

Pop. #	Population Name	Drainage System	
	T. rendalli		
1.	TR1 Tokwe River	Upper Save/Runde	USR
2.	TR2 Beitbridge Dam	Limpopo	
3.	TR3 Deka River	Middle Zambezi	L
4.	TR4 Mana Pools	Middle Zambezi	MZ
5.	TR5 Save/Runde Jct	Lower Save/Runde	LSR
6.	TR6 Budzi River	Budzi	B
7.	TR7 Hippo River	Upper Zambezi	Uz
	T. sparrmanii		
8.	TS1 U. Zambezi River	Upper Zambezi	UZ
9.	TS2 Deka River	Middle Zambezi	MZ
	O. placidus		
10.	OPI Save/Runde Jct	Lower Save/Runde	LSR
	O. mortimeri		
11.	OMO1 Sinamatella River	Middle Zambezi	MZ
12.	OMO2 Mandavu Dam	Middle Zambezi	MZ
13.	OMO3 Deka River	Middle Zambezi	MZ
14.	OMO4 Dolilo Saltpan	Middle Zambezi	MZ
15.	OMO5 Gwayi River	Middle Zambezi	MZ
16.	OMO6 Mana Pools	Middle Zambezi	MZ
17.	OMO7 Mlibizi	Lake Kariba	LK
18.	OMO8 Sinamwenda	Lake Kariba	LK
19.	OMO9 Kariba	Lake Kariba	LK
	O. mossambicus		
20.	OMS1 Gwavazabuya River	Nata	N
21.	OMS2 Amanzamnyama River	Nata	N
22.	OMS3 Beitbridge Dam	Limpopo	L
23.	OMS4 Mwenezi River	Limpopo	L
24.	OMS5 Tokwe River	Upper Save/Runde	USR
25.	OMS6 Runde River	Upper Save/Runde	USR
26.	OMS7 Ruti Dam	Upper Save/Runde	USR
27.	OMS8 Save/Runde Jct	Lower Save/Runde	LSR
28.	OMS9 Budzi River	Budzi	B
29.	OMS10 Mazowe River	Lower Zambezi	LZ

TABLE 2. (continued)

Pop. #	Population Name	Drainage System	
	O. macrochir		
30.	OMA1 Hippo River	Upper Zambezi	UZ
31.	OMA2 Katombora Rapids	Upper Zambezi	UZ
32.	OMA3 Udu Dam	Lower Zambezi	LZ
	O. andersonii		
33.	OAN1 Hippo River	Upper Zambezi	UZ
34.	OAN2 Katombora Rapids	Upper Zambezi	UZ
	O. niloticus		
35.	ON1 Edwards Farm	----------	
	O. aureus		
36.	OAU Edwards Farm	----------	

Note: The numbers allocated to the different populations apply to all other tables and figures.

Table 3: Buffer systems used in this study

System	Reference	Gel Buffer	Electrode Buffer	Gel composition
RW	Ridgeway, Sherborne and Lewis (1970)	0.03M Tris 0.005m Citric acid pH 8.5	0.06m Lithium hydroxide 0.3 M Boric Acid pH 8.1	99% gel buffer, 1% electrode buffer
AC	Clayton and Tretiak (1972)	1:20 dilution of Electrode: H_2O pH 6.1	0.04 M Citric acid pH 6.1	pH was adjusted with N- (3-aminopropyl)-morpholine.
MF	Markert & Faulhaber (1965)	1:20 dilution of stock: H_2O pH 6.1	1.5 dilution of stock: H_2O	Stock 0.9M Tris 0.5M Boric acid 0.02M Na EDTA pH 8.7
TC	Shaw and Prasad (1970)	1:14 dilution of Electrode: H_2O pH 7.5	56.4g Tris 27.0g Citric acid 3000 ml H_2O pH 7.5	Undiluted stock

TABLE 4. Summary table of enzymes screened in the tilapiine fish species of Zimbabwe. Locus abbreviations, Enzyme Commission numbers, subunit structures (from Shaklee et al. (1990)), buffers giving the best results, and tissues reflecting the greatest activities are given for each enzyme system.

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
Aspartate aminotransferase	2.6.1.1	dimer		
sAAT-1			RW, AC	E, M
sAAT-2			RW, AC	E, M
mAAT-1			RW	E
Adenosine deaminase	3.5.4.4	monomeric		
ADA-1			RW	E
Adenylate kinase	2.7.4.3	monomeric		
AK-1			AC, RW	E
Alcohol dehydrogenase	1.1.1.1	dimeric		
ADH-1			TC	L
Creatine kinase	2.7.3.2	dimeric		
CK-1			RW	E
CK-2			RW	E
Esterases	3.1.1.-	monomeric (dimeric)		
EST-1			AC	E
Fructose bisphosphate	3.1.3.11	dimeric or tetrameric		
FBP-1			TC	M
Galactosaminidase	-.-.-.-	dimeric		

TABLE 4. (continued).

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
GAM-1			RW	M
General protein	-.-.-.-	monomeric		
PROT-1			RW	M
PROT-2			RW	M
Glucose-6-phosphate isomerase	5.3.1.9	dimeric		
GPI-A			RW	M
GPI-B			RW	M
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	tetrameric		
GAPDH-1			AC	M
Glycerol-3-phosphate dehydrogenase	1.1.1.8	dimer	AC	M
G3PDH-1				
Guanine deaminase	3.5.4.3	dimeric		
GDA-1			MF	L
L-lditol dehydrogenase	1.1.1.14	tetrameric		
sIDDH-1			RW	L
Isocitrate dehydrogenase	1.1.1.42	dimeric		
mIDHP-1			TC	M
L-Lactate dehydrogenase	1.1.1.27	tetrameric		

TABLE 4. (continued).

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
LDH-A			RW, MF	M
LDH-B			RW, MF	M
			RW, MF	L
			RW	E
LDH-C			RW	E
Malate dehydrogenase	1.1.1.37	dimeric		
sMDH-A			MF, TC	M
sMDH-B			MF, TC	M
mMDH-1			MF	L
			TC	M
Malic Enzyme ($NADP^+$)	1.1.1.40	tetrameric		
sMEP-1			RW, TC	M
mMEP-1			RW, TC	L
			RW	E
Mannose-6-phosphate isomerase	5.3.1.8	monomeric		
MP1-1			TC	L
Peptidases	3.4.-.-	dimeric & monomeric		
PEPA-1			RW	M
PEPB-1			MF	M
PEPC-1			RW	M
PEPD-1			TC	M

TABLE 4. (continued).

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
6-Phosphogluconate dehydrogenase PGD-1	1.1.1.44	dimeric	AC	L
Phosphoglucomutase PGM-1	5.4.2.2	monomeric	TC	L
Superoxide dismutase SOD-1	1.15.1.1	dimeric	-	L
Triose-phosphate isomerase TPI-1	5.3.1.1	dimeric	RW	L

+ see Table 3. * E, Eye; L, Liver; M, Muscle

TABLE 5. Genetic variability at 38 loci in all populations.

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean Heterozygosity	
				Direct count	Hardy-Weinberg expected
1. TR1 TOKWE USR	18.0	1.1	5.3	0.024	0.018
2. TR2 BEITBRIDGE	14.3	1.0	0.0	0.000	0.000
3. TR3 DEKA	12.9	1.1	2.6	0.007	0.012
4. TR4 MANA	9.4	1.2	13.2	0.042	0.041
5. TR5 S/R JCT	10.2	1.1	7.9	0.013	0.016
6. TR6 BUDZI	9.7	1.0	0.0	0.000	0.000
7. TR7 HIPPO	11.0	1.1	5.3	0.018	0.021
8. TSI U ZAMBEZI UZ	7.1	1.2	10.5	0.041	0.042
9. TS2 DEKA	11.7	1.3	18.4	0.044	0.048
10. OP1 SRJCT LSR	18.9	1.3	13.2	0.039	0.041
11. OMO1 SINAMI MZ	11.1	1.1	5.3	0.016	0.024
12. OMO2 MANDAVU MZ	4.1	1.1	13.2	0.039	0.041
13. OMO3 DEKA MZ	17.4	1.1	5.3	0.029	0.028
14. OMO4 DOLILO MZ	9.9	1.1	13.2	0.037	0.032
15. OMO5 GWAYI MZ	24.6	1.3	21.1	0.043	0.064
16. OMO6 MANAPS MZ	16.1	1.2	10.5	0.033	0.033
17. OMO7 MLIBIZI LK	5.9	1.2	13.2	0.056	0.058
18. OMO8 SINAMWEN LK	5.1	1.2	13.2	0.047	0.057
19. OMO9 KARIBA LK	13.3	1.2	5.3	0.025	0.031
20. OMS1 GWAVAZAB N	12.4	1.2	13.2	0.045	0.051
21. OMS2 AMANZAMNY N	21.5	1.2	7.9	0.031	0.036
22. OMS3 BEITBRIDG L	15.9	1.2	18.4	0.031	0.033
23. OMS4 MWENEZI L	13.2	1.3	13.2	0.043	0.051
24. OMS5 TOKWE USR	16.8	1.2	13.2	0.024	0.038
25. OMS6 RUNDE USR	16.3	1.1	10.5	0.022	0.031
26. OMS7 RUTI USR	9.6	1.3	21.1	0.095	0.088

TABLE 5 (continued).

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean Heterozygosity	
				Direct count	Hardy-Weinberg expected
27. OMS8 SRJCT LSR	10.5	1.2	15.8	0.049	0.051
28. OMS9 BUDZI B	13.1	1.3	15.8	0.034	0.035
29. OMS10 MAZOE LZ	13.1	1.1	5.3	0.023	0.026
30. OMA1 HIPPO UZ	5.0	1.2	15.8	0.070	0.071
31. OMA2 KATOMBOR UZ	26.8	1.3	13.2	0.055	0.061
32. OMA3 UDU DAM LZ	23.0	1.2	13.2	0.048	0.058
33. OAN1 HIPPO UZ	7.5	1.2	15.8	0.052	0.055
34. OAN2 KATOMBOR UZ	14.7	1.2	13.2	0.024	0.030

TABLE 6. Matrix of genetic similarity and/or distance coefficients.
Below diagonal: Nei (1978) unbiased genetic distance.
Above diagonal: Rogers (1972) genetic distance.

Population	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI
1. TR1 TOKWE USR	*****	0.012	0.019	0.028	0.019	0.012	0.021	0.475
2. TR2 BEITBRIDGE	0.003	*****	0.007	0.024	0.009	0.000	0.012	0.477
3. TR3 DEKA	0.004	0.001	*****	0.029	0.014	0.007	0.020	0.482
4. TR4 MANA	0.004	0.003	0.005	*****	0.028	0.024	0.029	0.472
5. TR5 S/R JCT	0.004	0.001	0.003	0.004	*****	0.009	0.019	0.472
6. TR6 BUDZI	0.003	0.000	0.001	0.003	0.001	*****	0.012	0.477
7. TR7 HIPPO	0.005	0.003	0.004	0.004	0.004	0.003	*****	0.469
8. TSI U ZAMBEZI UZ	0.634	0.641	0.645	0.616	0.627	0.641	0.623	*****
9. TS2 DEKA	0.625	0.632	0.634	0.608	0.619	0.632	0.615	0.004
10. OP1 SRJCT LSR	0.581	0.592	0.595	0.571	0.569	0.592	0.581	0.537
11. OMO1 SINAMA MZ	0.705	0.715	0.694	0.688	0.691	0.715	0.704	0.765
12. OMO2 MANDAVU MZ	0.689	0.699	0.681	0.672	0.675	0.699	0.688	0.749
13. OMO3 DEKA MZ	0.683	0.693	0.672	0.667	0.669	0.693	0.682	0.742
14. OMO4 DOLILO MZ	0.688	0.698	0.678	0.672	0.674	0.698	0.688	0.745
15. OMO5 GWAYI MZ	0.681	0.690	0.670	0.666	0.666	0.690	0.680	0.744
16. OMO6 MANAPS MZ	0.673	0.682	0.661	0.657	0.658	0.682	0.671	0.730
17. OMO7 MLIBIZI LK	0.735	0.743	0.722	0.718	0.718	0.743	0.733	0.798
18. OMO8 SINAMWEN LK	0.698	0.707	0.686	0.683	0.682	0.707	0.696	0.758
19. OMO9 KARIBA LK	0.684	0.693	0.672	0.667	0.669	0.693	0.682	0.740
20. OMS1 GWAVAZAB N	0.662	0.672	0.651	0.646	0.648	0.672	0.661	0.698
21. OMS2 AMANZAMNY N	0.673	0.683	0.662	0.656	0.659	0.683	0.672	0.696
22. OMS3 BEITBRIDG L	0.670	0.681	0.660	0.654	0.657	0.681	0.670	0.701
23. OMS4 MWENEZI L	0.662	0.672	0.652	0.646	0.649	0.672	0.662	0.685
24. OMS5 TOKWE USR	0.666	0.676	0.656	0.650	0.653	0.676	0.666	0.678
25. OMS6 RUNDE USR	0.666	0.676	0.656	0.650	0.652	0.676	0.665	0.673
26. OMS7. RUTI USR	0.665	0.675	0.654	0.648	0.651	0.675	0.664	0.646
27. OMS8 SRJCT LSR	0.621	0.631	0.611	0.605	0.608	0.631	0.621	0.634
28. OMS9 BUDZI B	0.664	0.674	0.655	0.648	0.651	0.674	0.664	0.663

TABLE 6 (continued)

Population	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI
29. OMS10 MAZOE LZ	0.671	0.682	0.661	0.656	0.658	0.682	0.671	0.685
30. OMA1 HIPPO UZ	0.654	0.663	0.667	0.631	0.652	0.663	0.652	0.553
31. OMA2 KATOMBOR UZ	0.656	0.665	0.669	0.634	0.654	0.665	0.654	0.551
32. OMA3 UDU DAM LZ	0.654	0.663	0.667	0.635	0.652	0.663	0.653	0.536
33. OAN1 HIPPO UZ	0.776	0.784	0.762	0.759	0.761	0.784	0.774	0.842
34. OAN2 KATOMBOR UZ	0.782	0.789	0.767	0.763	0.764	0.789	0.779	0.850

TABLE 6 (continued)

Population	TS2	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
1. TR1 TOKWE USR	0.471	0.448	0.510	0.506	0.499	0.502	0.500	0.492
2. TR2 BEITBRIDGE	0.473	0.452	0.514	0.510	0.503	0.506	0.504	0.496
3. TR3 DEKA	0.475	0.457	0.507	0.503	0.496	0.499	0.497	0.488
4. TR4 MANA	0.467	0.445	0.505	0.502	0.495	0.498	0.495	0.487
5. TR5 S/R JCT	0.468	0.443	0.505	0.502	0.495	0.498	0.495	0.487
6. TR6 BUDZI	0.473	0.452	0.514	0.510	0.503	0.506	0.504	0.496
7. TR7 HIPPO	0.465	0.446	0.509	0.505	0.498	0.501	0.499	0.490
8. TSI U ZAMBEZI UZ	0.033	0.428	0.542	0.538	0.531	0.533	0.535	0.524
9. TS2 DEKA	*****	0.421	0.530	0.526	0.520	0.521	0.523	0.514
10. OP1 SRJCT LSR	0.528	*****	0.241	0.228	0.232	0.232	0.243	0.231
11. OMO1 SINAMA MZ	0.749	0.249	*****	0.018	0.011	0.011	0.033	0.033
12. OMO2 MANDAVU MZ	0.734	0.233	0.000	*****	0.022	0.018	0.039	0.042
13. OMO3 DEKA MZ	0.728	0.238	0.002	0.000	*****	0.008	0.029	0.026
14. OMO4 DOLILO MZ	0.731	0.239	0.001	0.000	0.000	*****	0.026	0.028
15. OMO5 GWAYI MZ	0.729	0.241	0.005	0.005	0.003	0.003	*****	0.034
16. OMO6 MANAPS MZ	0.718	0.240	0.012	0.012	0.005	0.006	0.005	*****
17. OMO7 MLIBIZI LK	0.780	0.272	0.019	0.016	0.022	0.021	0.020	0.025
18. OMO8 SINAMWEN LK	0.741	0.251	0.007	0.013	0.012	0.011	0.002	0.009
19. OMO9 KARIBA LK	0.727	0.247	0.006	0.006	0.002	0.002	0.003	0.000
20. OMS1 GWAVAZAB N	0.687	0.214	0.018	0.016	0.011	0.011	0.014	0.009
21. OMS2 AMANZAMNY N	0.684	0.211	0.017	0.014	0.011	0.011	0.015	0.013
22. OMS3 BEITBRIDG L	0.689	0.204	0.017	0.010	0.010	0.010	0.017	0.019
23. OMS4 MWENEZI L	0.674	0.190	0.032	0.023	0.024	0.024	0.032	0.031
24. OMS5 TOKWE USR	0.666	0.190	0.045	0.037	0.039	0.038	0.046	0.047

TABLE 6 (continued)

Population	TS2	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
25. OMS6 RUNDE USR	0.663	0.186	0.047	0.039	0.039	0.039	0.047	0.046
26. OMS7 RUTI USR	0.633	0.169	0.041	0.037	0.038	0.038	0.045	0.045
27. OMS8 SRJCT LSR	0.623	0.165	0.044	0.036	0.034	0.035	0.043	0.043
28. OMS9 BUDZI B	0.652	0.184	0.037	0.029	0.029	0.029	0.037	0.038
29. OMS10 MAZOE LZ	0.674	0.195	0.045	0.038	0.037	0.037	0.045	0.044
30. OMA1 HIPPO UZ	0.536	0.184	0.284	0.275	0.273	0.274	0.277	0.265
31. OMA2 KATOMBOR UZ	0.535	0.188	0.275	0.266	0.263	0.264	0.264	0.252
32. OMA3 UDU DAM LZ	0.520	0.195	0.305	0.292	0.293	0.292	0.285	0.280
33. OAN1 HIPPO UZ	0.820	0.315	0.047	0.056	0.057	0.055	0.050	0.055
34. OAN2 KATOMBOR UZ	0.828	0.327	0.058	0.068	0.067	0.066	0.063	0.063

TABLE 6 (continued)

Population	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5
1. TR1 TOKWE USR	0.526	0.509	0.499	0.486	0.492	0.492	0.489	0.490
2. TR2 BEITBRIDGE	0.530	0.512	0.503	0.490	0.496	0.496	0.493	0.494
3. TR3 DEKA	0.523	0.505	0.496	0.483	0.489	0.489	0.486	0.487
4. TR4 MANA	0.521	0.504	0.494	0.482	0.488	0.487	0.484	0.485
5. TR5 S/R JCT	0.521	0.504	0.494	0.482	0.488	0.488	0.484	0.485
6. TR6 BUDZI	0.530	0.512	0.503	0.490	0.496	0.496	0.493	0.494
7. TR7 HIPPO	0.524	0.507	0.498	0.484	0.491	0.491	0.487	0.488
8. TSI U ZAMBEZI UZ	0.558	0.540	0.529	0.512	0.511	0.514	0.505	0.499
9. TS2 DEKA	0.546	0.528	0.518	0.503	0.501	0.505	0.498	0.489
10. OP1 SRJCT LSR	0.264	0.249	0.238	0.218	0.214	0.203	0.196	0.188
11. OMO1 SINAMA MZ	0.040	0.033	0.022	0.044	0.034	0.043	0.064	0.070
12. OMO2 MANDAVU MZ	0.051	0.051	0.031	0.055	0.045	0.040	0.054	0.066
13. OMO3 DEKA MZ	0.051	0.043	0.016	0.037	0.027	0.037	0.057	0.064
14. OMO4 DOLILO MZ	0.051	0.044	0.015	0.041	0.030	0.037	0.058	0.064
15. OMO5 GWAYI MZ	0.055	0.036	0.029	0.054	0.049	0.059	0.080	0.087
16. OMO6 MANAPS MZ	0.049	0.032	0.013	0.034	0.033	0.042	0.062	0.070
17. OMO7 MLIBIZI LK	*****	0.040	0.046	0.064	0.068	0.077	0.084	0.103
18. OMO8 SINAMWEN LK	0.014	*****	0.035	0.058	0.060	0.072	0.088	0.095
19. OMO9 KARIBA LK	0.021	0.008	*****	0.034	0.030	0.042	0.062	0.069
20. OMS1 GWAVAZAB N	0.032	0.021	0.009	*****	0.019	0.031	0.039	0.049
21. OMS2 AMANZAMNY N	0.037	0.026	0.011	0.002	*****	0.024	0.035	0.042
22. OMS3 BEITBRIDG L	0.040	0.034	0.016	0.008	0.004	*****	0.027	0.038
23. OMS4 MWENEZI L	0.046	0.047	0.029	0.009	0.007	0.004	*****	0.029
24. OMS5 TOKWE USR	0.068	0.058	0.044	0.019	0.017	0.014	0.006	*****

TABLE 6 (continued)

Population	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5
25. OMS6 RUNDE USR	0.067	0.062	0.045	0.015	0.014	0.012	0.002	0.003
26. OMS7 RUTI USR	0.048	0.049	0.042	0.015	0.021	0.023	0.012	0.016
27. OMS8 SRJCT LSR	0.068	0.060	0.041	0.023	0.020	0.018	0.016	0.021
28. OMS9 BUDZI B	0.061	0.053	0.036	0.012	0.007	0.006	0.002	0.006
29. OMS10 MAZOE LZ	0.063	0.057	0.043	0.013	0.018	0.015	0.006	0.012
30. OMA1 HIPPO UZ	0.305	0.285	0.268	0.241	0.240	0.247	0.238	0.235
31. OMA2 KATOMBOR UZ	0.290	0.270	0.256	0.233	0.240	0.246	0.240	0.246
32. OMA3 UDU DAM LZ	0.319	0.289	0.287	0.267	0.272	0.271	0.265	0.265
33. OAN1 HIPPO UZ	0.049	0.030	0.051	0.065	0.074	0.087	0.096	0.101
34. OAN2 KATOMBOR UZ	0.061	0.041	0.059	0.071	0.083	0.099	0.104	0.105

TABLE 6 (continued)

Population	OMS6	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3
1. TR1 TOKWE USR	0.489	0.501	0.468	0.491	0.491	0.485	0.483	0.481
2. TR2 BEITBRIDGE	0.493	0.505	0.472	0.495	0.495	0.488	0.486	0.485
3. TR3 DEKA	0.486	0.498	0.465	0.487	0.487	0.493	0.491	0.490
4. TR4 MANA	0.484	0.496	0.463	0.484	0.486	0.475	0.473	0.473
5. TR5 S/R JCT	0.484	0.497	0.464	0.486	0.486	0.484	0.482	0.480
6. TR6 BUDZI	0.493	0.505	0.472	0.495	0.495	0.488	0.486	0.485
7. TR7 HIPPO	0.487	0.499	0.466	0.489	0.489	0.482	0.480	0.479
8. TSI U ZAMBEZI UZ	0.495	0.495	0.481	0.496	0.502	0.443	0.438	0.426
9. TS2 DEKA	0.488	0.484	0.475	0.488	0.496	0.429	0.472	0.416
10. OP1 SRJCT LSR	0.184	0.198	0.176	0.189	0.196	0.200	0.198	0.203
11. OMO1 SINAMA MZ	0.070	0.079	0.076	0.063	0.066	0.275	0.266	0.284
12. OMO2 MANDAVU MZ	0.063	0.079	0.071	0.060	0.067	0.274	0.266	0.284
13. OMO3 DEKA MZ	0.064	0.071	0.067	0.054	0.060	0.267	0.258	0.276
14. OMO4 DOLILO MZ	0.064	0.073	0.070	0.057	0.060	0.267	0.259	0.277
15. OMO5 GWAYI MZ	0.086	0.099	0.092	0.078	0.085	0.274	0.263	0.273
16. OMO6 MANAPS MZ	0.068	0.091	0.075	0.063	0.067	0.251	0.237	0.261
17. OMO7 MLIBIZI LK	0.097	0.098	0.107	0.094	0.095	0.288	0.274	0.293
18. OMO8 SINAMWEN LK	0.093	0.103	0.100	0.087	0.090	0.274	0.261	0.268
19. OMO9 KARIBA LK	0.068	0.081	0.074	0.062	0.064	0.255	0.247	0.268
20. OMS1 GWAVAZAB N	0.043	0.063	0.053	0.043	0.038	0.242	0.227	0.247
21. OMS2 AMANZAMNY N	0.042	0.062	0.048	0.036	0.038	.0244	0.234	0.252
22. OMS3 BEITBRIDG L	0.031	0.068	0.044	0.028	0.035	0.253	0.242	0.260
23. OMS4 MWENEZI L	0.019	0.053	0.038	0.031	0.026	0.245	0.233	0.250
24. OMS5 TOKWE USR	0.019	0.063	0.045	0.030	0.033	0.235	0.240	0.254

TABLE 6 (continued)

Population	OMS6	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3
25. OMS6 RUNDE USR	*****	0.057	0.043	0.023	0.022	0.235	0.234	0.251
26. OMS7 RUTI USR	0.011	*****	0.059	0.057	0.042	0.263	0.250	0.273
27. OMS8 SRJCT LSR	0.018	0.013	*****	0.036	0.045	0.261	0.248	0.265
28. OMS9 BUDZI B	0.002	0.013	0.012	*****	0.029	0.237	0.234	0.254
29. OMS10 MAZOE LZ	0.004	0.007	0.023	0.008	*****	0.252	0.234	0.256
30. OMA1 HIPPO UZ	0.236	0.244	0.259	0.229	0.252	*****	0.031	0.052
31. OMA2 KATOMBOR UZ	0.241	0.239	0.260	0.235	0.246	0.003	*****	0.038
32. OMA3 UDU DAM LZ	0.266	0.272	0.285	0.260	0.276	0.013	0.012	*****
33. OAN1 HIPPO UZ	0.110	0.088	0.111	0.105	0.105	0.330	0.316	0.343
34. OAN2 KATOMBOR UZ	0.117	0.091	0.120	0.115	0.110	0.336	0.323	0.357

TABLE 6 (continued)

Population	OAN1	OAN2
1. TR1 TOKWE USR	0.541	0.542
2. TR2 BEITBRIDGE	0.545	0.545
3. TR3 DEKA	0.537	0.538
4. TR4 MANA	0.536	0.536
5. TR5 S/R JCT	0.536	0.536
6. TR6 BUDZI	0.545	0.545
7. TR7 HIPPO	0.539	0.539
8. TSI U ZAMBEZI UZ	0.572	0.575
9. TS2 DEKA	0.560	0.562
10. OP1 SRJCT LSR	0.289	0.292
11. OMO1 SINAMA MZ	0.080	0.079
12. OMO2 MANDAVU MZ	0.091	0.094
13. OMO3 DEKA MZ	0.090	0.090
14. OMO4 DOLILO MZ	0.089	0.088
15. OMO5 GWAYI MZ	0.087	0.094
16. OMO6 MANAPS MZ	0.078	0.082
17. OMO7 MLIBIZI LK	0.080	0.091
18. OMO8 SINAMWEN LK	0.064	0.074
19. OMO9 KARIBA LK	0.080	0.079
20. OMS1 GWAVAZAB N	0.098	0.097
21. OMS2 AMANZAMNY N	0.107	0.107
22. OMS3 BEITBRIDG L	0.115	0.116
23. OMS4 MWENEZI L	0.119	0.122
24. OMS5 TOKWE USR	0.123	0.116

TABLE 6 (continued)

Population	OAN1	OAN2
25. OMS6 RUNDE USR	0.124	0.122
26. OMS7 RUTI USR	0.136	0.132
27. OMS8 SRJCT LSR	0.138	0.139
28. OMS9 BUDZI B	0.131	0.029
29. OMS10 MAZOE LZ	0.127	0.120
30. OMA1 HIPPO UZ	0.298	0.304
31. OMA2 KATOMBOR UZ	0.287	0.294
32. OMA3 UDU DAM LZ	0.304	0.313
33. OAN1 HIPPO UZ	*****	0.028
34. OAN2 KATOMBOR UZ	0.002	*****

TABLE 7. Matrix of similarity/distance coefficients averaged by species. Coefficient: Nei (1978) unbiased genetic distance.

Species	No. of pops.	1	2	3	4	5	6	7
1. *T. rendalli*	7	0.003						
2. *T. sparrmanii*	2	0.628	0.004					
3. *O. placidus*	1	0.583	0.533	****				
4. *O. mortimeri*	9	0.689	0.745	0.246	0.008			
5. *O. mossambicus*	10	0.659	0.670	0.191	0.035	0.012		
6. *O. macrochir*	3	0.655	0.539	0.189	0.280	0.252	0.009	
7. *O. andersonii*	2	0.774	0.835	0.321	0.055	0.098	0.334	0.002

Table 8. Factor matrix showing contribution of each polymorphic locus to each of the first two principle components.

	Kyle		MacIllwaine		Kariba	
	PC1	PC2	PC1	PC2	PC1	PC2
LDH-2	0.99	-0.10	0.77	-0.28	0.80	-0.11
EST	0.97	0.14	0.77	-0.19	0.59	-0.57
CK-2	0.98	-0.13	0.98	-0.04	0.82	0.11
GPI-1	0.97	0.14	0.98	-0.04	0.69	0.10
MDH-3	0.99	0.02	0.98	-0.04	0.88	-0.18
PROT	0.98	-0.06	0.70	0.69	0.40	0.77
ME-1					0.89	0.12

Table 9. Eigenvalues and percentage of total variation explained by the first two principle components in Lakes Kyle, MacIllwaine, and Kariba.

	PC1	PC2	Cumulative %
Kyle	5.81	0.0	
	(96.9%)	(1.2%)	98.1%
MacIllwaine	4.56	0.59	
	(76.1%)	(9.8%)	85.9%
Kariba	3.85	1.00	
	(55.0%)	(14.4%)	69.4%

Table 10. Collection locations of Oreochromis specimens used in morphological analyses.

Oreochromis andersonii

Katombora Rapids - 8 June 1991
Hippo River - 21 June 1989

Oreochromis macrochir

Katombora Rapids - 27 November 1989
Udu Dam - 17 November 1989
Hippo River - 21 June 1989
Katombora Rapids - 22 June 1989

Oreochromis mortimeri

Sinamatella River - 16 June 1989
Manduvu Dam - 16 June 1989
Deka River - 18 June 1989
Mana Pools - 8 December 1989
Mlibizi - 27 June 1989
Sinamwenda - 31 July 1989
Kariba Basin - 3 August 1989
Gwayi River - 10 June 1991

Oreochromis mossambicus

Runde River - 9 May 1989
Ruti Dam/Save River - May 1989
Gwavazambuya River - 14 June 1989
Dolilo Saltpan - 18 June 1989
Save/Runde Junction - 10 May 1989
Mazoe River - December 1989
Buzi River - 12 May 1989
Mwenezi River - 7 May 1989
Tokwe River - 4 May 1989
Beitbridge Dam - 11 June 1990
Amanzamnyama River - 13 June 1989

Oreochromis placidus

Mwenezi River, Malipati - 7 May 1989
Save/Runde Junction - 10 May 1989

Table 11. Measurements and counts recorded from Zimbabwean *Oreochromis* specimens.

Distance Measures

Standard length
Snout-to-nape length
Dorsal fin base
Base of last dorsal-fin ray to dorsal terminus of hypural plate
Depth at hypural plate
Ventral terminus of hypural plate to base of last anal-fin ray
Anal fin base
Base of first anal-fin spine to pelvic-fin insertion
Pelvic fin insertion to snout
Base of first dorsal-fin ray to pelvic-fin insertion
Base of first dorsal-fin ray to base of first anal-fin spine
Base of first dorsal-fin ray to base of last anal-fin ray
Base of last dorsal-fin ray to pelvic-fin insertion
Base of last dorsal-fin ray to base of first anal-fin spine
Base of last dorsal-fin ray to base of last anal-fin ray
Base of last dorsal-fin ray to ventral terminus of hypural plate
Dorsal terminus of hypural plate to base of last anal-fin ray
Pectoral-fin length
Pelvic-fin length
Head length
Postorbital head length
Head depth at nape
Length of maxilla
Horizontal eye diameter
Vertical eye diameter
Cheek depth
Preorbital bone length

Counts

Dorsal-fin spines
Dorsal-fin rays
Anal-fin spines
Anal-fin rays
Pectoral-fin rays
Lateral line scales
Scale rows on cheek
Gill rakers on first arch
Number of tooth rows in upper jaw
Number of tooth rows in lower jaw

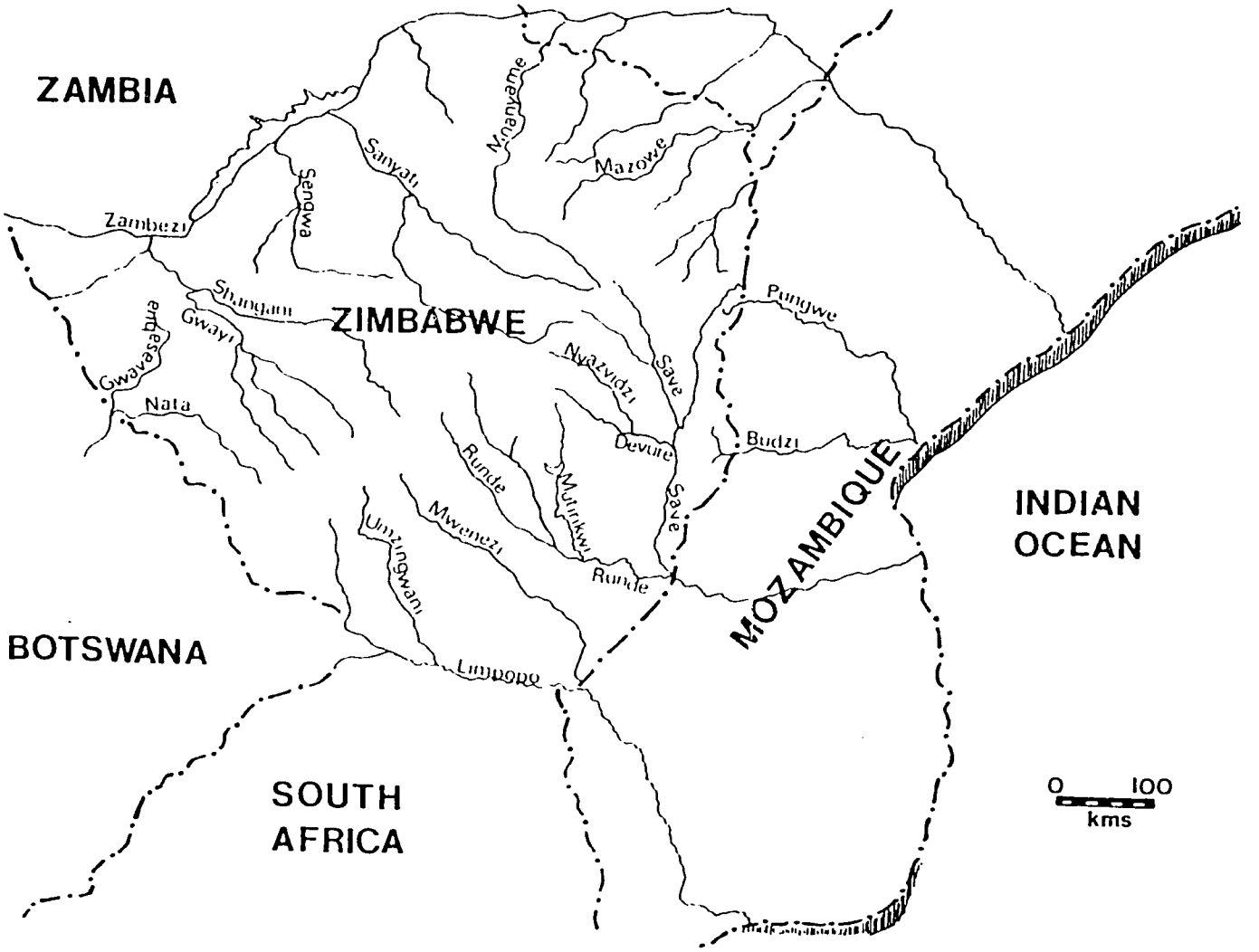

ZAMBIA
ZIMBABWE
BOTSWANA
SOUTH AFRICA
MOZAMBIQUE
INDIAN OCEAN
Zambezi
Sanyati
Mnanyame
Mazowe
Sanawe
Shangani
Gwayi
Gwavasabua
Nata
Nyazvidzi
Save
Devure
Budzi
Pungwe
Runde
Mutirikwi
Mwenezi
Umzingwani
Limpopo
0
100
kms

Figure 1

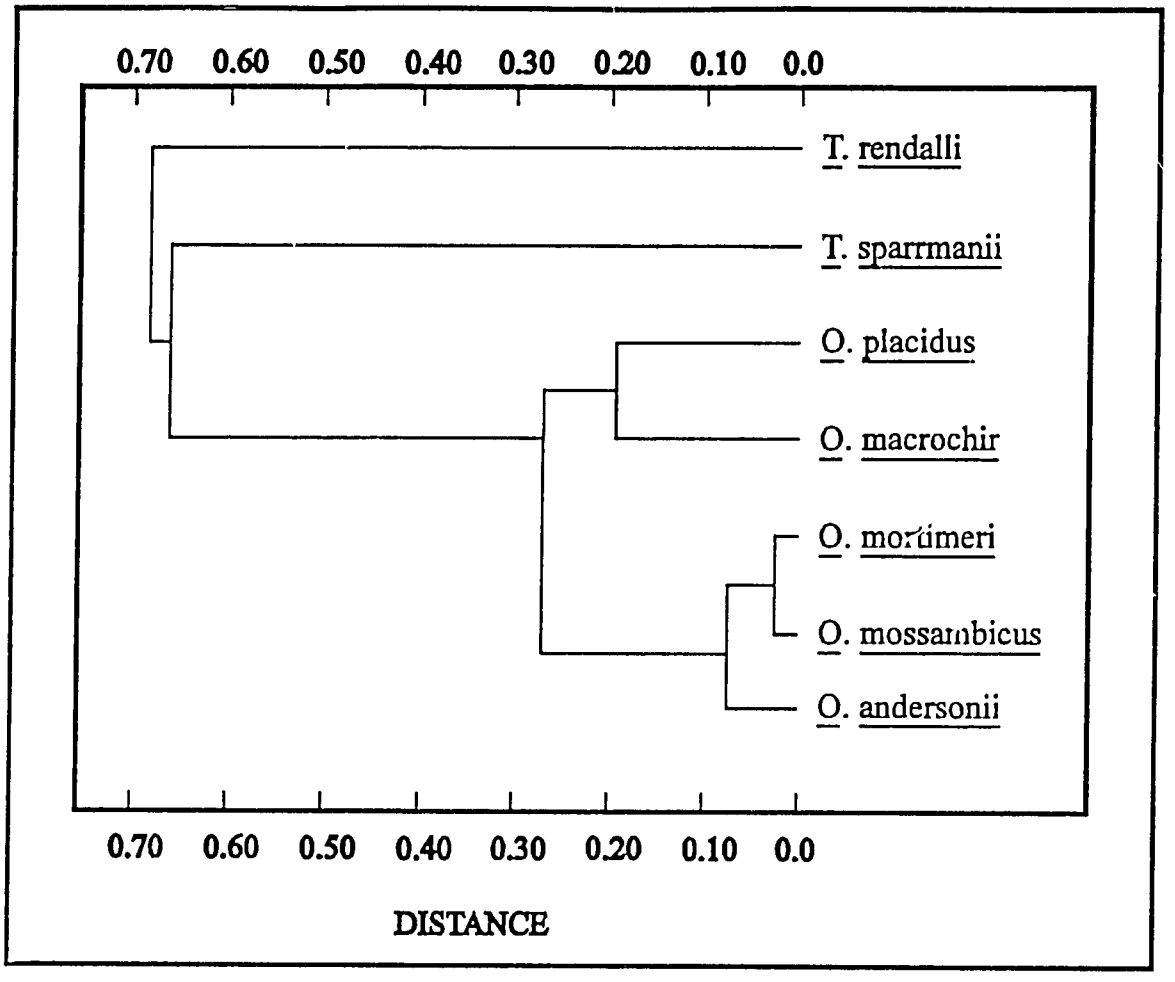


FIGURE 2. Cluster analysis using unweighted pair group method. Coefficient used: Nei (1978) unbiased genetic distance.

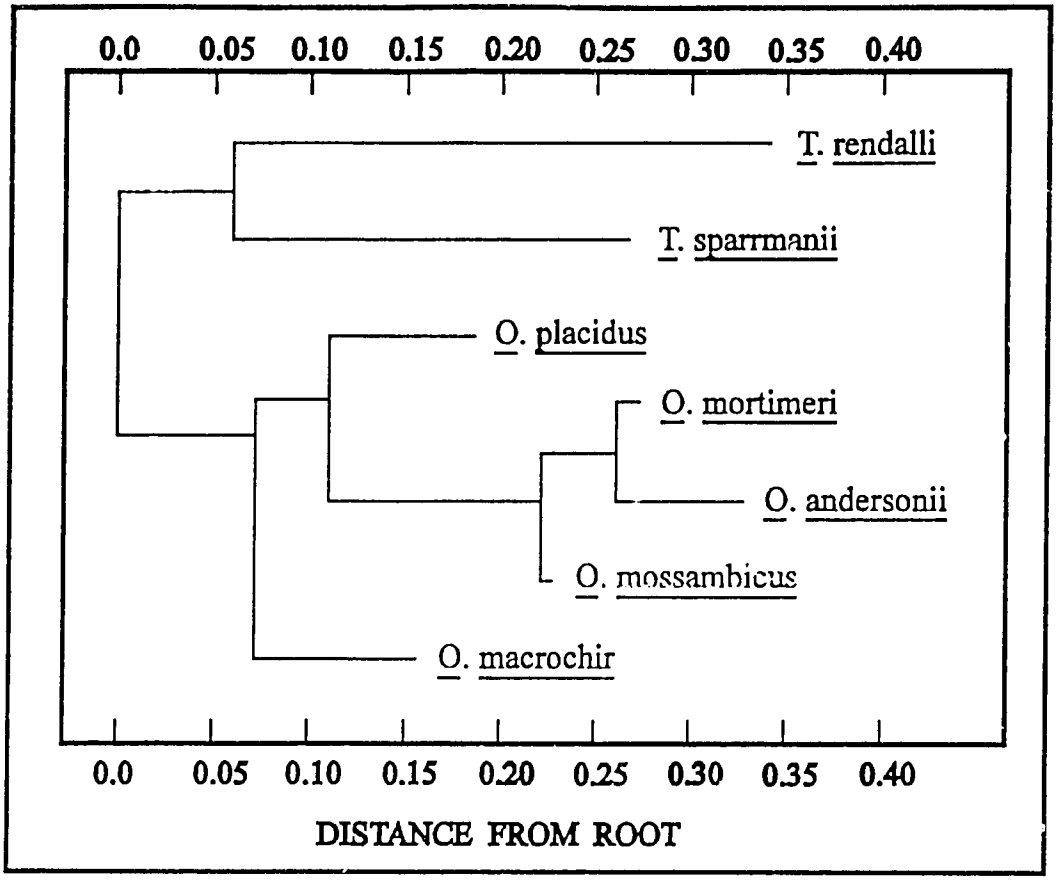


FIGURE 3. Wagner tree produced by rooting at midpoint of longest path.

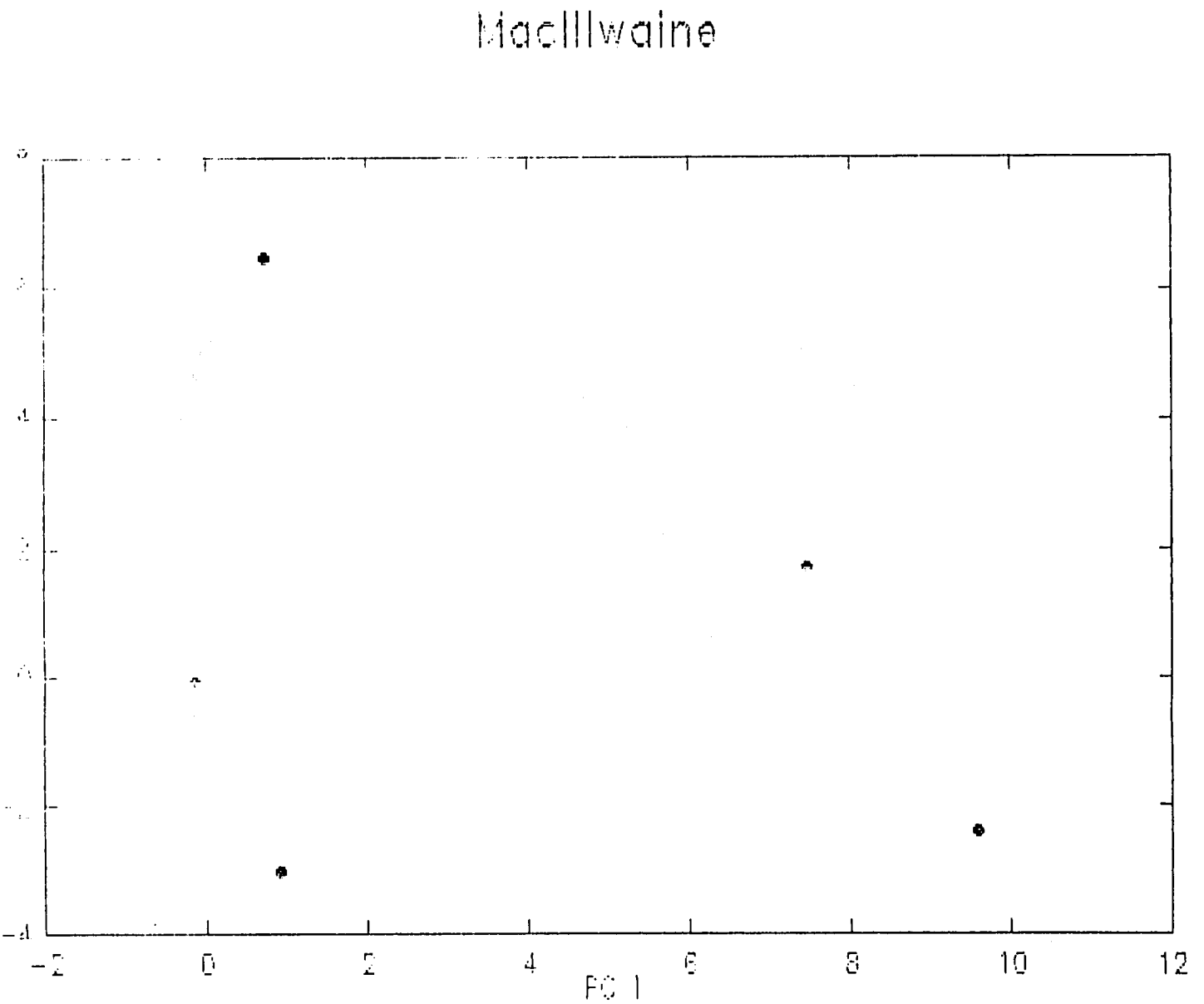


Figure 4. First two principle component scores for fish in Lake MacIlwaine.

BEST AVAILABLE DOCUMENT

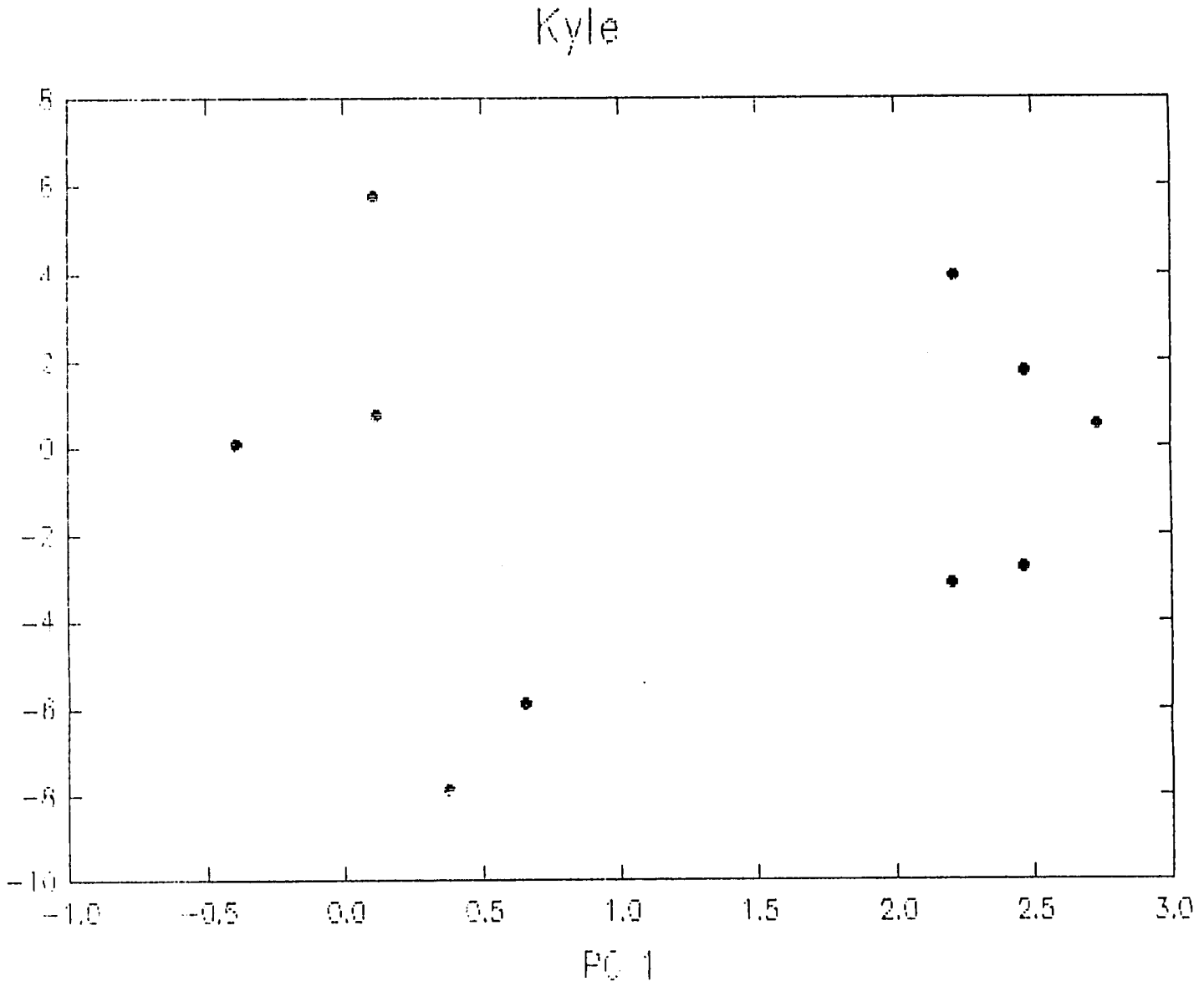


Figure 5. First two principle component scores for fish in Lake Kyle.

BEST AVAILABLE DOCUMENT

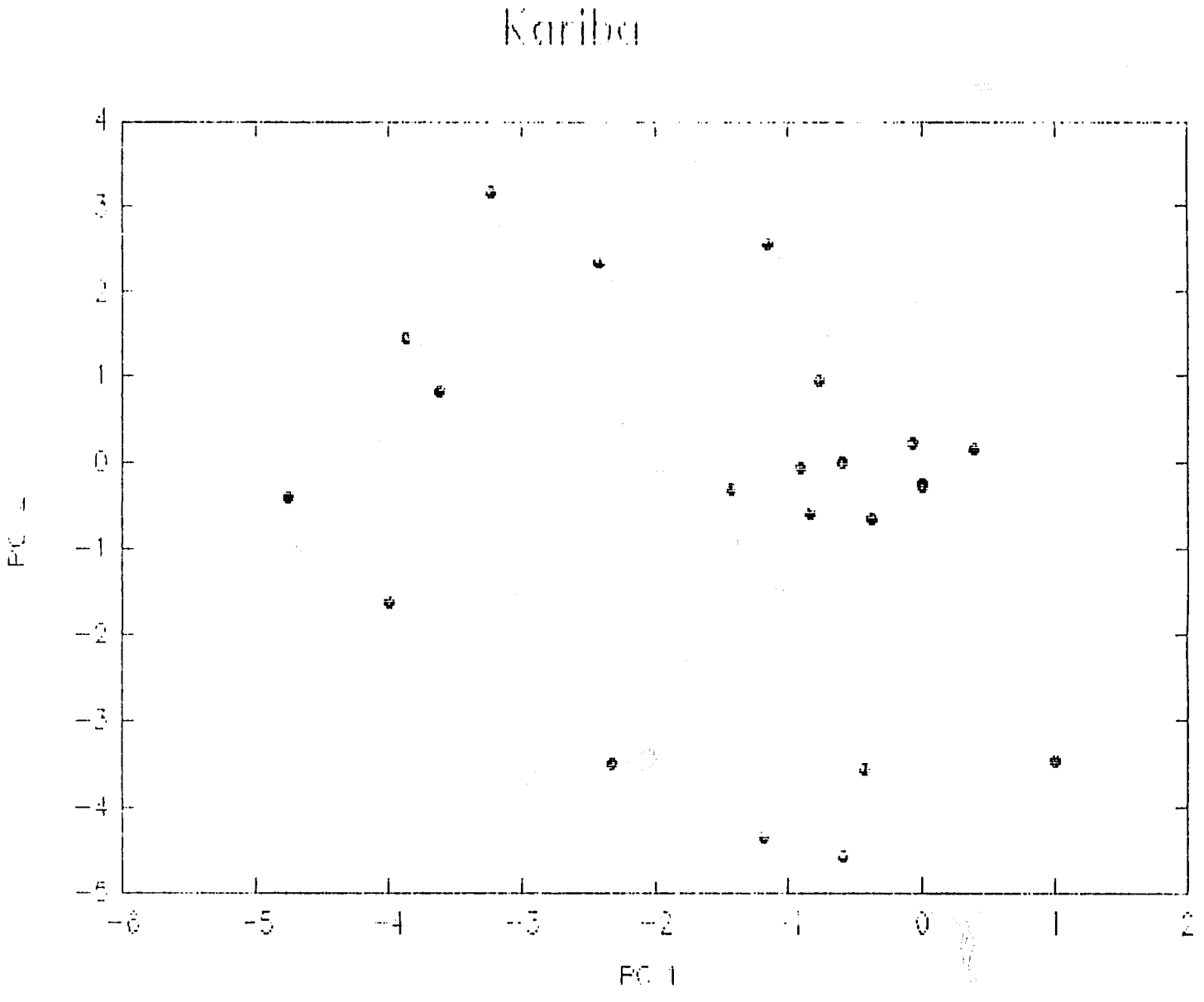


Figure 6 First two principle component scores for fish in Lake Kariba

BEST AVAILABLE DOCUMENT

Figure 7.

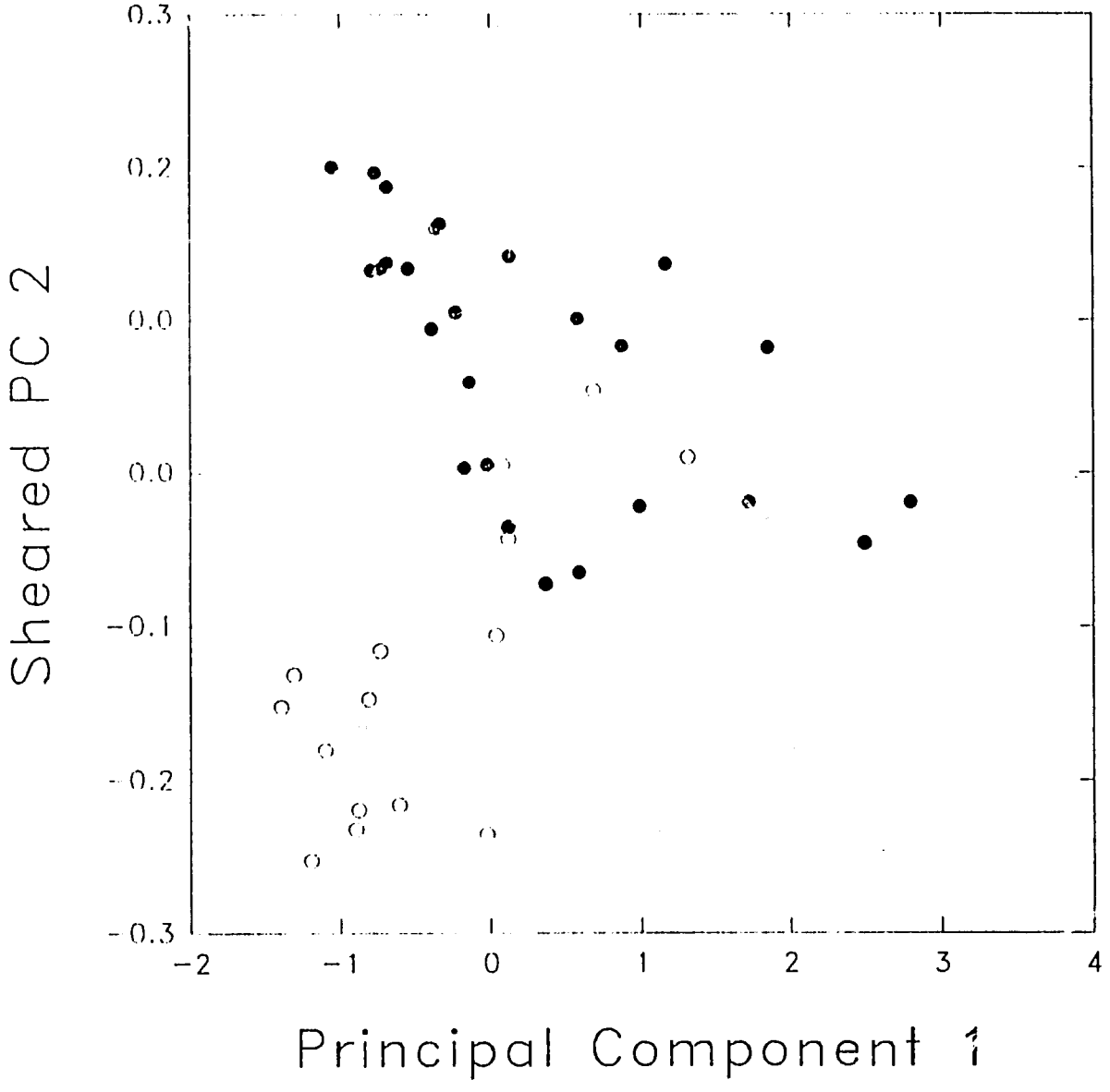
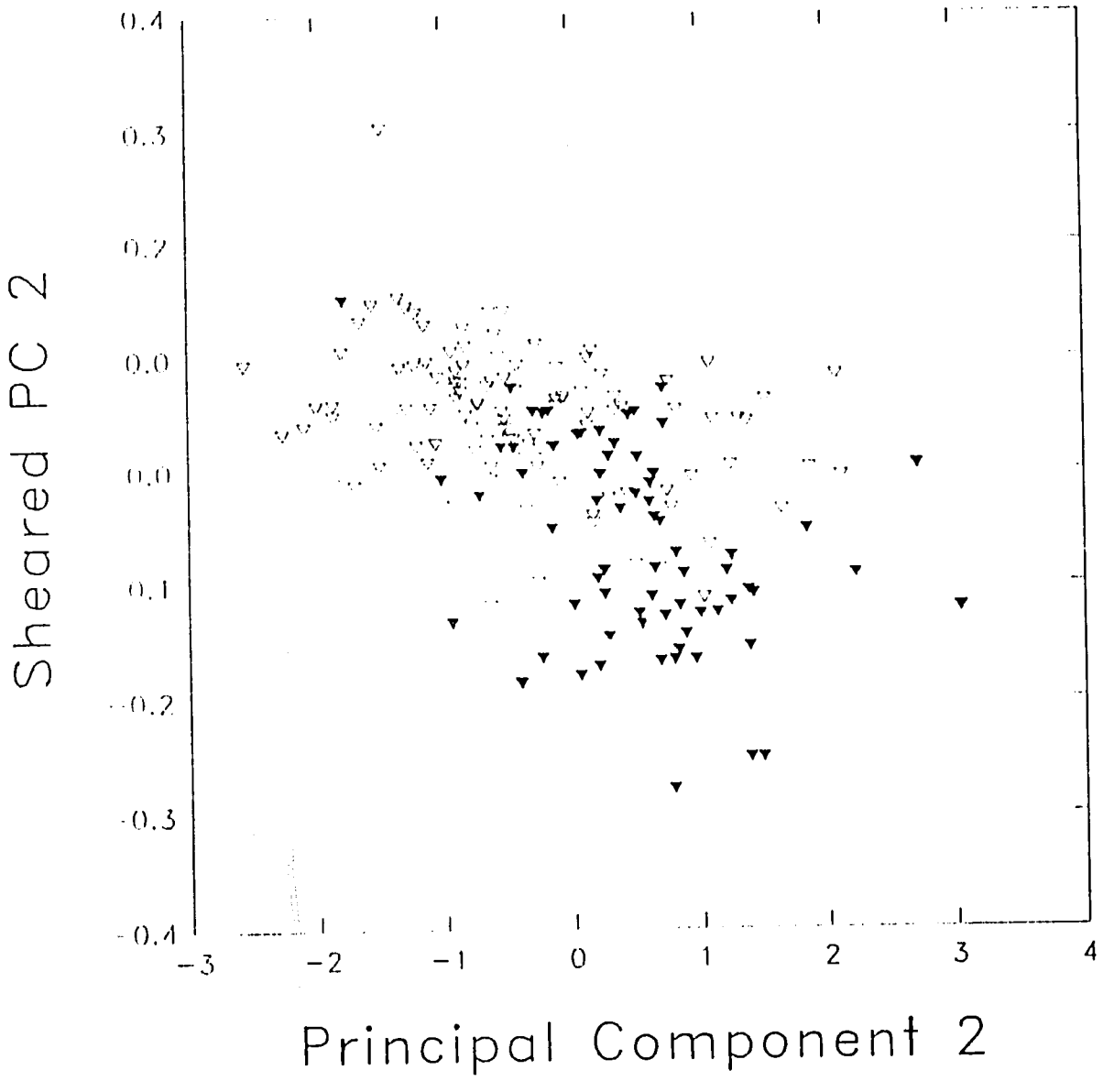

0.3
0.2
0.0
0.0
-0.1
-0.2
-0.3
-2
-1
0
1
2
3
4
Sheared PC 2
Principal Component 1

Figure 8.


0.4
0.3
0.2
0.0
0.0
0.1
-0.2
-0.3
-0.4
-3
-2
-1
0
1
2
3
4
Sheared PC 2
Principal Component 2

Appendix I.

Identification and Conservation of Indigenous Tilapiine Genetic Resources in Zimbabwe pp 130-138; In: National Research Council 1992: Proceedings of a Network Meeting Held in Manila, Phillipines, August 6-10, 1991. National Academy Press, Washington, DC. 288 pp.

IDENTIFICATION AND CONSERVATION OF INDIGENOUS TILAPIINE GENETIC RESOURCES OF ZIMBABWE

F. SHONHIWA and J.H. HOWARD

Department of Biology, Frostburg State University
Frostburg, Maryland

ABSTRACT

Use of tilapiine fishes for aquaculture in Zimbabwe has increased greatly in recent years. Unfortunately, many allopatric species of tilapias will freely hybridize when placed in sympatry. The common practice for fisheries officers has been to collect broodstock from a variety of locations, pool them, allow them to breed, and distribute the fry throughout the country for stock ponds and reservoirs. There is a concern, therefore, that some indigenous tilapias may disappear as a direct result of hybridization.

Previously, the only method of identifying tilapias was morphology. However, that method has proven unreliable, especially for tilapias, due to overlap and variation in the taxonomic characteristics of the fish. Now, the utilization of biochemical techniques provides a means of unambiguous species and hybrid identification. In this study, starch gel electrophoresis was used to detect enzyme loci that are useful as diagnostic characters.

INTRODUCTION

Although the use of tilapiine species for aquaculture in Zimbabwe, and Africa as a whole, has greatly increased in recent years, it must continue to grow to keep pace with the demand for fish protein. In Zimbabwe it is estimated that catches should increase from the current 20,000-30,000 t/yr to perhaps as much as 160,000 t/yr by the end of the century. This growth is necessary to offset increased demand for fish protein from a population that is expected to double by the year 2000. Much of the technology necessary for such aquacultural programs is both simple and inexpensive. Imports do not represent a reasonable possibility because of a severe foreign currency problem. While more than 30 exotic fish species have been introduced into Zimbabwe, none have the production potential of the indigenous tilapias.

Programs to culture and release fish into non-native habitats to supplement or replace the harvest of natural populations have no significant parallel in terrestrial systems. A common practice for fisheries officers is to collect broodstock from a variety of locations, pool these stocks, allow them to breed, and distribute the fry throughout the country for release in stock ponds and reservoirs. Unfortunately, many allopatric species of tilapias will freely hybridize when placed together.

Therefore, we are concerned that some indigenous tilapias may disappear as a direct result of hybridization.

Although there is some confusion about the actual number of tilapias indigenous to Zimbabwean waters, the best current estimate is eight (five in the genus *Oreochromis* and three in the genus *Tilapia)* (Table 1).

TABLE 1. The distribution of tilapias in Zimbabwean river systems (after Bell-Cross and Minshull 1987).

Species	River systems									
	UZ	LK	MZ	LZ	N	L	P	B	USR	LSR
O. andersonii	o									
O. macrochir	o	i	i	i					i	
O. mortimeri		o	o							
O. mossambicus				o	*	o		o	o	o
O. placidus						o			i	o
T. sparrmanii	o	o	o			o			o	o
T. rendalli rendalli	o	o	o	o				*	i	o
T. rendalli swierstrae						o				
T. ruweri	o									

River system: UZ - Upper Zambezi, LK - Lake Kariba, MZ - Middle Zambezi, LZ - Lower Zambezi, N - Nata, L - Limpopo, B - Budzi, P - Pungwe, USR - Upper Save/Runde, LSR - Lower Save/Runde

Other: o - collected in the past, i - fish introduced into the system, * - new records from this study.

In addition to occasional disagreements regarding the number of species, hybridization of existing species makes it even more difficult for fisheries officers to identify fish stocks with certainty. Over the past 30–40 years, extensive introductions and translocations of both exotic and indigenous tilapia species have occurred. According to Bell-Cross and Minshull (1988), *Oreochromis andersonii*, *O. mortimeri*, and *O. mossambicus* may hybridize freely when they occur together. Philippart and Ruwet (1982) report extensive transfers of *O. macrochir*, *O. placidus*, *O. mossambicus*, and *O. mortimeri* into new reservoirs within Zimbabwe. This complex situation is worsened further by the introductions of exotics from other parts of Africa.

Hybridization between indigenous and introduced fish species can result in local extinctions, and over a broader range, threaten species with extinction. Among introduced populations of *O. macrochir* and *O. niloticus* in Madagascar, introgression ultimately resulted in the disappearance of *O. macrochir* (Moreau 1981). Other studies demonstrating the potential for introgression and such

61

losses in African cichlids are Mascaranus et al. (1986), using *O. mossambicus* and *O. niloticus* in the Philippines, and Elder et al. (1971), working with *O. spilurus* and *O. leucosticus* in Kenya. The difficulty of obtaining pure stocks in Zimbabwe has already been commented on by several investigators (e.g., McAndrew and Majumdar 1984, Van der Bank et. al. 1989, Beattie 1986).

Some interspecific hybridizations result in nearly all-male populations. Exploitation of this event in aquacultural practices can mean that most of the biomass consumed by all-male fish will be converted to flesh rather than reproduction, resulting in much higher farm-pond production. Preliminary research has shown that indigenous male *O. macrochir* and female *O. mossambicus* produce 100% male hybrid progeny (Thompson 1984). This work has also shown that tilapia species from different river systems do not yield similar results. In addition, some hybrids grow faster than the parental species and are superior in converting food or exhibit greater cold tolerance. These potentials make it imperative that controlled experiments be conducted on indigenous Zimbabwean tilapias and hybrids of known ancestry. Without unambiguous stock identification of these resources at the outset, it is probable that such experiments could not be duplicated. The utilization of biochemical techniques provides a means for the unambiguous identification of hybrids and species.

With this background in mind, the following two objectives were established:

1. To unambiguously identify all tilapiine species native to Zimbabwe using enzyme loci as species-specific genetic markers and once so identified, develop a key based on morphological characteristics; and
2. To ascertain the degree of hybridization where introductions are known to have occurred.

MATERIALS AND METHODS

Sample Collection

Samples consisting of 25 fish per sample were collected from the major river systems of Zimbabwe (Figure 1). Collections were made in the native ranges of the various species and in areas where introduction of other species were unlikely. Over a period of one-and-one-half years, the following populations were sampled: 16 *Tilapia rendalli*, 13 *Oreochromis mossambicus*, 9 *O. mortimeri*, 3 *O. macrochir*, 2 *O. andersonii*, 3 *T. sparrmanii*, and 2 *O. placidus*. In addition, five fish each of *O. niloticus* and *O. aureus* were collected from the Edwards and Son Farms located just outside of Harare. These two species are not indigenous to Zimbabwe, but are currently being farmed there. The strains at the Edwards farm were imported from Stirling, U.K., in 1985 and are believed to have come from cultures in Israel. *Tilapia ruweri* was not collected, probably due to its scarcity in the Upper Zambezi system.

Collecting Techniques

Specimens were collected using seine nets and gill nets. Muscle, liver, eye, heart, and brain tissues were dissected from freshly killed fish, labelled, placed in Eppendoff tubes, and immediately frozen in liquid nitrogen at the collection site. The tissues were then taken to the University of Zimbabwe and were stored in an ultra freezer at 80°C. The tissues were shipped on dry ice to Frostburg State University, Maryland, for analysis. The carcasses were preserved in 10% formalin and stored in 50% alcohol. These were also shipped to the United States.

62

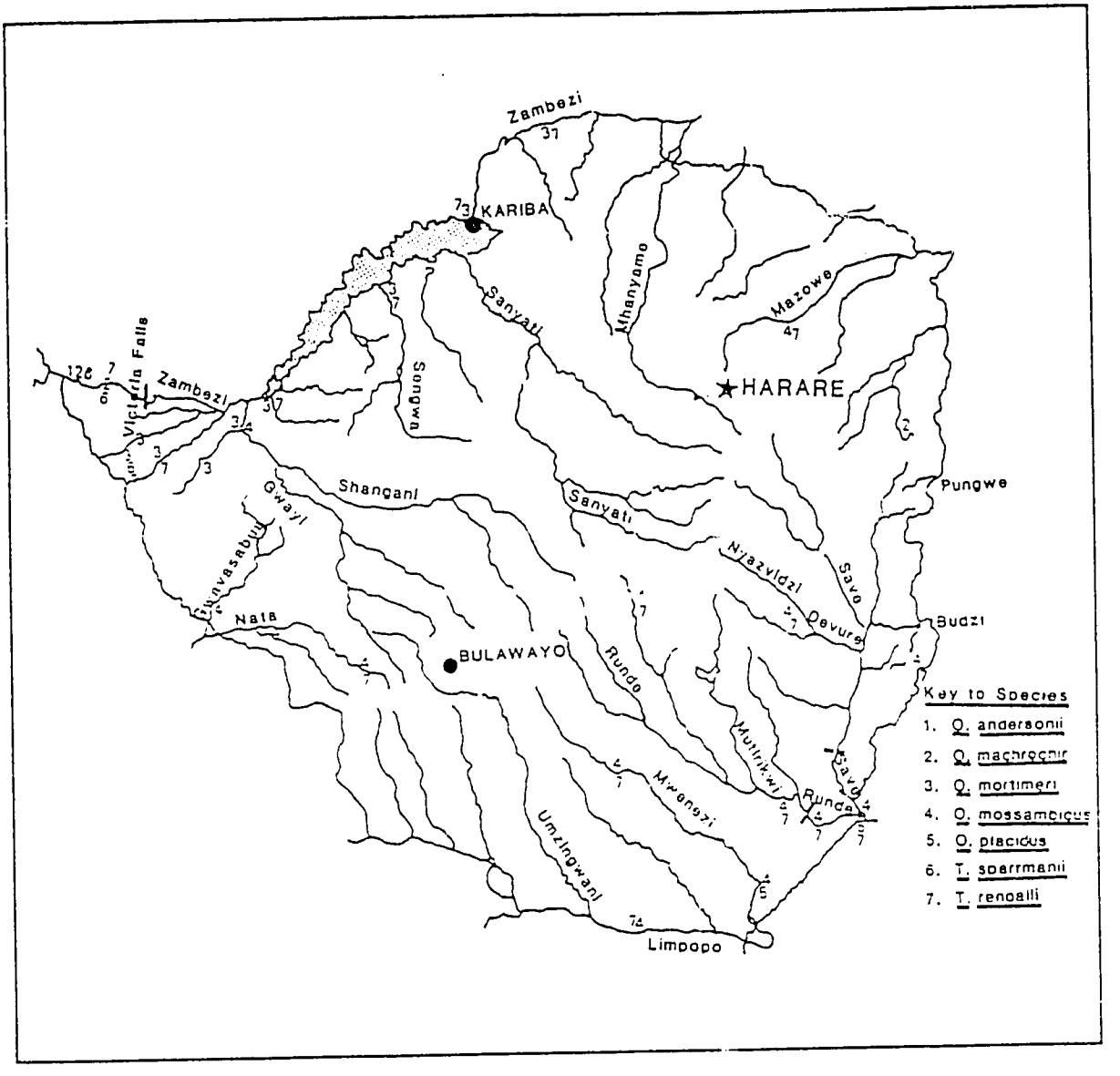


FIGURE 1. Map of drainage systems of Zimbabwe indicating collection sites of species.

Sample Preparation

In the laboratory, tissues were prepared for electrophoresis by adding equal amounts of water (1:1 v/v) to tissue and homogenized with a tissue grinder for one minute. The homogenized tissue was then centrifuged at 12 800 x g for 3 minutes and the supernant was absorbed onto 1.5 x 0.3 cm strips of Whatman No. 3 filter paper.

BEST AVAILABLE DOCUMENT

TABLE 2. Buffer systems used in the study.

System	Reference	Gel Buffer	Electrode Buffer	Gel composed
RW	Ridgway, Shelborne, and Lewis (1970)	0.03M Tris 0.005m Citric acid pH 8.5	0.06m Lithium hydroxide 0.3 M Boric acid pH 8.1	of 99% gel buffer, 1% electrode buffer
AC	Clayton and Tretiak (1972)	1:20 dilution of electrode: H_2O pH 6.1	0.04 M Citric acid pH 6.1	pH was adjusted with N- (3-aminopropyl) - morpholine.
MF	Markert and Faulhaber (1965)	1:20 dilution of stock. H_2O pH 6.1	1.5 dilution of stock: H_2O	Stock 0.9M Tris 0.5M Boric acid 0.02M Na EDTA pH 8.7
TC	Shaw and Prasad (1970)	1:14 dilution of electrode H_2O pH 7.5	56.4g Tris 27.0g Citric acid 3,000 ml H_2O pH 7.5	Undiluted stock

Gel Preparation

Gel preparation procedures followed those outlined in Selander et al. (1971) and May (1975), employing the use of 9.5 x 20 x 1 cm and 9.5 x 20 x 2 cm molds. Gels consisted of 13% Sigma hydrolyzed starch and one of the buffer solutions listed in Table 2. Two-thirds of the gel buffer was heated in a microwave oven for two minutes. The last third was mixed thoroughly with the starch in a 1,000 ml Erlenmeyer flask. The heated buffer was then added to the starch/buffer mixture and heated for three minutes in a microwave. This mixture was swirled every 15 seconds to ensure a smooth, lump-free gel mixture as the end product.

The mixture was then aspirated for approximately one minute with constant swirling to remove air bubbles. At the end of this period, the starch solution was poured in the plexiglass mold, formed, and allowed to cool at room temperature. The gels were kept covered with plastic wrap overnight.

Electrophoresis

Horizontal starch gel electrophoresis was carried out following procedures outlined in Selander et al. (1971), May (1975), Allendorf et al. (1977), and Abersold et al. (1987), employing the use of Model 1P-17 Heath Kit power supplies. Voltage was maintained at 300 mv for gels made with RW and MF buffers and those made with TC and AC buffers were maintained at 200 mv (Table 2). Gels were run over a duration of 3-5 hr until the dye marker had moved to a distance of 6 mm. The resulting gel was sliced into 1.5 mm replicates and each was stained with specific histochemical solutions as outlined by Allendorf et al. (1977) and May (1975).

Data Analysis

The electrophoretic data were analyzed using the BIOSYS-I program (Swofford and Selander 1981). This program allows for the computation of allele frequencies and associated measures of genetic variability, determination of conformance to expected genotypic frequencies based on the Hardy-Weinberg equilibrium, and calculation of F-statistics. The F statistics developed by Wright (1978) were useful in the analysis of population structure. In addition, BIOSYS-I was used to calculate similarity and distance coefficients as developed by Nei (1978). BIOSYS-I was also used in constructing distance Wagner trees (Farris 1970) and UPGMA phenograms.

Assay

A laboratory enzyme assay for the Zimbabwean tilapias was developed using two fish each from *O. mossambicus* and *T. rendalli* populations. Initially, all five tissues, eye, muscle, heart, and brain, were run on the same gel and on each of the four buffer systems.

PRELIMINARY RESULTS

Collections

Bell-Cross and Minshull (1988) did not list the presence of any tilapias in the Nata River system. However, specimens that could be classified as *O. mossambicus* or *O. mortimeri* were collected in the Nata system at three sites (Table 1). *Tilapia rendalli* was also collected in the Budzi system, although no prior tilapia records exist in this system. A search for tilapias in the Pungwe system was unsuccessful. No *T. ruweri* were collected, probably due to their scarcity.

Currently, data have been gathered on the activity and resolution of 64 enzymes on four buffer systems and five different tissues. Of these systems, 28 provide reasonable resolution and activity in three of the tissues: the muscle, liver and eye, and should provide data on over 38 loci (Table 3). Although the assay is essentially complete, we expect it will become refined as we proceed. We have now started the analysis of some experimental populations collected in Zimbabwe. No diagnostic loci were found between *O. mossambicus* and *O. mortimeri* from the loci used in the study.

TABLE 3. Enzyme loci among the tilapiine fish species of Zimbabwe that are diagnostic between at least one species pair[1].

Enzyme	*Tilapia*		*Oreochromis*						
			Indigenous					Exotics	
	rendalli	*sparrmanii*	*placidus*	*mortimeri*	*mossambicus*	*macrochir*	*andersonii*	*niloticus*	*aureus*
SAAT-2	c[2]	-[3]	a	b	-	-	b	-	a
AK-1	a	b	a	a	a	a	a	a	a
CK-2	c	d	b	a	-	b	a	a	c
EST-1	d	e	a	a	-	c	-	a	c
GDA-1	c	c	-	-	a	-	-	-	b
GP-2	a	b	-	a	-	a	a	b	b
GPI-1	b	d	b	-	-	b	a	-	c
GPI-2	b	a	a	-	a	a	-	a	a
G3P-1	cd	e	a	-	a	a	-	a	a
IDH-1	a	c	a	a	a	a	a	a	b
LDH-1	b	b	a	a	a	a	a	a	a
LDH-2	a	a	a	a	-	b	a	a	a
LDH-3	a	c	a	-	-	a	a	a	a
MDH-1	a	a	-	-	-	-	b	-	a
MDH-2	b	b	a	a	a	a	a	a	a
MDH-3	c	-	a	a	-	b	a	a	a
ME-1	a	a	b	a	a	b	a	a	a
ME-2	a	a	a	a	a	a	b	a	a
PEPLG	b	a	a	a	a	a	a	a	a
PEPPA	b	a	a	a	a	a	a	a	a
PGM-1	a	d	a	-	a	a	a	c	a
SDH-1	d	-	-	-	-	-	-	c	c
6PGDH	b	c	a	a	a	a	a	a	a
SOD-1	b	b	a	-	-	-	-	b	b
TPI-1	b	a	b	a	-	a	a	-	a

[1] SAAT-1, MAAT-1, ADA-1, ALD-1, ADH-1, CK-1, FBP-1, GAM-1, GAP-1, GP-1, MPI-1, PEPGL AND PEPLL did not exhibit diagnostic mobilities.

[2] mobilities are based on "a" as the fastest, and decreasing as you go down the alphabet.

[3] (-) indicates multiple alleles.

REFERENCES CITED

Abersold, P.B., G.A. Winans, D.J. Teel, G.B. Milner, and F. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation, National Oceanic and Atmospheric Administration (NOAA) Tech. Rep. National Marine Fisheries Service 61:20 pp.

Allendorf, F.W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (*Salmo trutta*): detection and interpretation from population data. Hereditas 86:179-190.

Beattie, I.H. 1986. The Development of Pure Strains. pp. 35-38. *in* Proc. of Fish Forum held at Henderson Research Station, 21 March, 1986. Department of National Parks and Wildlife, Zimbabwe.

Bell-Cross, G. and J. Minshull. 1988. The Fishes of Zimbabwe. National Museums and Monuments of Zimbabwe, Harare.

Clayton, J.W. and D.N. Tretiak. 1972. Amino-citrate buffers for pH control in starch gel electrophoresis. J. Fish Res. Board. Can. 29:1169-1172.

Elder, H.Y., D.J. Garrod, and P.J.P. Whitehead. 1971. Natural hybrids of the African cichlid fishes *Tialpia spirulus nigra* and *Tilapia leucostrica*: a case of hybrid introgression. Biol. J. Linn. Soc. 3:103-146.

Farris, J.S. 1970. Methods of computing Wagner trees. Syst. Zoo. 19:83-90.

Macaranus, J.M., N. Taniguchi, M. Pante, J.B. Capili, and R. Pullin. 1986. Electrophoretic evidence for *Oreochromis niloticus* (L.) stocks in the Philippines. Aquacult. Fish Manag. 17:249-261.

Markert, C.L. and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159:319-332.

May, B. 1975. Electrophoretic variation in the genus *Oncorhynchus*: the methodology, genetic basis, and practical applications to research and management. Master of Science Thesis, Washington State Univ., Dept. of Fisheries.

McAndrew, B.J. and K.C. Majumdar. 1984. Evolutionary relationships within three Tilapiine genera (Pisces: Cichlidae). Zoo. J. Linn. Soc. 80:421-435.

Moreau, J. 1981. Application trial of a model to study the fisheries in tropical floodplains: the Alaotra Lake (Madagascar). Hydrobiologia 13:83-92.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetica 89:583-590.

Philippart, J-Cl. and J-Cl. Ruwet. 1982. Ecology and distribution of tilapias. pp. 15-59. *in* Pullin, R.S.V. and R.H. Lowe-McConnell. (ed.). The biology and culture of tilapias. ICLARM Conference Proceedings. Manila, Philippines.

Ridgeway, G.J., S.W. Shelborne, and R.D. Lewis. 1970. Polymorphism in the esterases of the Atlantic herring. Trans. Am. Fish Soc. 99:147-151.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson, and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse *Peromyscus polionotus*). Studies in Genetics IV. Univ. Tex. Publ. 7103:49-90.

Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Gen. 4:297-320.

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-I: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Heredity 72:281-283.

Thompson, P.J. 1984. Hybridization of indigenous *Oreochromis* species in Zimbabwe and the production of all-male offspring. pp. 1-11. *in* Aquaculture in southern Africa. Joint Symposium of CSIRO and SAAU. Cathedral Peak. 3-4 May 1984.

67

Van der Bank, F.H., W.S. Grant, and J.T. Ferreira. 1989. Electrophoretically detectable genetic data for fifteen southern African cichlids. J. Fish Biol. 34:465-483.

Wright, S. 1978. *Evolution and the Genetics of Populations. IV. Variability Within and Among Natural Populations*. Univ. of Chicago, Illinois.

Appendix II

Electrophoretic Identification of Indigenous Tilapiine Fish Species of Zimbabwe and an Investigation of Their Phylogenetic Relationships-A Master of Science Thesis for a graduate degree in Applied Ecology and Conservation Biology

Frostburg State University

- Graduate Studies -

Electrophoretic Identification of Indigenous Tilapiine Fish Species of Zimbabwe and an Investigation of Their Phylogenetic Relationships

A Thesis in

Applied Ecology and Conservation Biology

by

Fortune Shonhiwa

Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science

August 1994

70

ABSTRACT

Tilapiine fish species (Cichlidae) are endemic to Africa. Eight native species are recognized in Zimbabwe; three in the genus Tilapia and five in Oreochromis. These fishes represent the best hope for developing strong aquaculture programs using indigenous species in tropical regions. However, identification using meristic and morphometric analyses are difficult because of the phenotypic plasticity characteristic of these species.

This study employs genetic markers as an alternative method for identification. Twenty nine populations of seven indigenous and two exotic species were collected in Zimbabwe rivers. Horizontal starch gel electrophoresis using aqueous extracts of eye, liver and muscle tissue resolved 38 loci. Twenty three loci were polymorphic while nine were monomorphic but had fixed allelic differences among the species; six were monomorphic for the same allele in all species. A maximum of 18 diagnostic loci was found between the Tilapia spp. versus Oreochromis spp. These loci were not similar in all cases as 17 diagnostic loci distinguished the two Tilapia spp. No fixed allelic differences were found between O. mossambicus and O. mortimeri, and only one locus distinguished O. andersonii from O. mossambicus and O. mortimeri. Nei's (1978) genetic identity values ranged from 0.940 to 1.000 for the comparisons of all 19 populations in

the O. mossambicus/O. mortimeri complex. However, pairwise comparisons of O. mossambicus or O. mortimeri populations frequently showed a greater degree of difference within the species than was evident in some O. mossambicus x O. mortimeri pairs. Results of this study failed to confirm O. mortimeri as a distinct species from O. mossambicus.

Average heterozygosity for all 29 populations was 0.042 and was higher than that found for cichlids in previous studies. F_{ST} values were considerably higher when the two genera were combined and lower when only Oreochromis spp. were analyzed. Hierarchical F-statistics showed that populations of the same species were genetically differentiated according to drainage system.

Both phenetic (UPGMA) and cladistic (Distance Wagner and FREQPARS) methodologies produced topologies with many similarities. The substrate spawning Tilapia were a distinct clade from the mouthbrooding Oreochromis in the Distance Wagner topology. Oreochromis mossambicus, O. mortimeri and O. andersonii consistently clustered together as did O. placidus and O. macrochir in all phenograms and trees. The principal relationships revealed in these topologies are consistent with other cichlid studies.

I dedicate this thesis to Upenyu.

Acknowledgements

I extend sincere gratitude to my major professor, Dr James H. Howard for professional guidance and his unwilting patience. I thank members of my committee, Dr R.L. Raesly and Dr R.P. Morgan, Jr. for their advice and critical comments on the thesis. This research would not have been possible without the tremendous support of the Director of Zimbabwe National Parks and Wild Life Management Department, Dr W. Nduku and the Chief Ecologist (Aquatic), Mr S. Chimbuya. I thank all National Parks personnel who were involved in the collection of fish samples. Special thanks go to the personnel who spent weeks with me living under very difficult conditions. I also thank Dr J.P. Gopo for facilitating the opportunity for me to do this project and for use of his laboratory at the University of Zimbabwe. I am grateful to the Biology Department, Frostburg State University for use of equipment and necessary supplies. Several graduate students worked with me in the laboratory, particularly R.E. Gregg and R.J. Kwaramba.

Special thanks go to my husband Temba Shonhiwa whose patience I marvel at every day. A special thank you go to the Feresu family for their unfailing support and encouragement. I thank my Mom and Dad for laying the special foundation that keep us going. The Howard family who became my second family.

This research was supported in part under grant No.

Com-5542-G-00-0016-00, Program in Science and Technology Cooperation, Office of the Science Advisor, U.S. Agency for International Development. Additional funding was provided by Frostburg State University, the University of Zimbabwe and the Zimbabwe Department of National Parks and Wild Life Management.

TABLE OF CONTENTS:

	Page
ABSTRACT	iii
DEDICATION	v
ACKNOWLEDGEMENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
INTRODUCTION	1
MATERIALS AND METHODS	12
Sample collection	12
Collecting techniques	12
Sample preparation	13
Gel preparation	13
Electrophoresis	14
Assay	14
Data analysis	15
RESULTS	19
DISCUSSION	37
Allelic variability	38
Chi-square tests and G-tests	40
Genetic variability	42
Genetic distances	45
Taxonomic Considerations	48
REFERENCES	52

76

LIST OF TABLES

Table	Page
1. The distribution of tilapiine fish species in Zimbabwean river systems.	62
2. Locations and drainage systems where samples of tilapiine fishes were collected in Zimbabwe.	63
3. Buffer systems used in this study.	64
4. Summary table of enzymes screened in the tilapiine fish species of Zimbabwe.	65
5. Allelic frequencies of all loci for the 29 populations of seven indigenous and two exotic tilapiine species resident in Zimbabwean waters.	69
6. Enzyme loci among the tilapiine fish species of Zimbabwe that are diagnostic between at least one pair.	101
7a. Loci revealing fixed allelic differences between O. mossambicus and other tilapiine fishes indigenous to Zimbabwe + two exotics.	102
7b. Loci revealing fixed allelic differences between O. mortimeri and other tilapiine fishes indigenous to Zimbabwe + two exotics.	103
7c. Loci revealing fixed allelic differences between O. andersonii and other tilapiine fishes indigenous to Zimbabwe + two exotics.	104
7d. Loci revealing fixed allelic differences between O. placidus and other tilapiine fishes indigenous to Zimbabwe + two exotics.	105
7e. Loci revealing fixed allelic differences between O. macrochir and other tilapiine fishes indigenous to Zimbabwe + two exotics.	106

7f. Loci revealing fixed allelic differences between T. rendalli and other tilapiine fishes indigenous to Zimbabwe + two exotics. 107

7g. Loci revealing fixed allelic differences between T. sparrmanii and other tilapiine fishes indigenous to Zimbabwe + two exotics. 108

7h. Loci revealing fixed allelic differences between O. niloticus and tilapiine fish species indigenous to Zimbabwe + O. aureus. 109

7i. Loci revealing fixed allelic differences between O. aureus and tilapiine fish species indigenous to Zimbabwe + O. niloticus. 110

8. Results from Chi-square/G-tests for conformance to Hardy-Weinberg proportions for all polymorphic loci from 27 tilapiine populations collected in Zimbabwe river systems and two exotic species. 111

9. Genetic variability at 38 loci in 29 populations. 113

10. Summary of F-statistics at all loci in the analysis of nine species of tilapiine fishes of Zimbabwe. 116

11. Genetic heterogeneity analysis among Zimbabwe tilapiine species using F-statistics over all polymorphic loci. 117

12. Summary of F-statistics at all loci in the analysis of five Oreochromis species of Zimbabwe. 118

13. Matrix of Nei's (1978) unbiased genetic similarity and distance coefficients of 29 populations of nine tilapiine fish species collected in Zimbabwean river systems. 119

14. Matrix of Nei's (1978) unbiased genetic similarity and distance coefficients (averaged by species) among tilapiine fishes of Zimbabwe. 124

15. Roger's (1972) genetic distance coefficients of 29 populations of nine tilapiine fish species collected in Zimbabwean river systems. 128

16. Roger's (1972) genetic distance coefficients (averaged by species) among tilapiine fishes of Zimbabwe. 131

LIST OF FIGURES

Figure	Page
1. Map of Zimbabwe drainage systems indicating the points at which they flow into the Indian Ocean	133
2. Map of Africa showing the natural distribution of *Oreochromis andersonii*.	134
3. Map of Africa showing the natural distribution of *Oreochromis macrochir*.	135
4. Map of Africa showing the natural distribution of *Oreochromis mortimeri*.	136
5. Map of Africa showing the natural distribution of *Oreochromis mossambicus*.	137
6. Map of Africa showing the natural distribution of *Oreochromis placidus*.	138
7. Map of Africa showing the natural distribution of *Tilapia rendalli*.	139
8. Map of Africa showing the natural distribution of *Tilapia sparrmanii*.	140
9. Map of Africa showing the natural distribution of *Oreochromis aureus*.	141
10. Map of Africa showing the natural distribution of *Oreochromis niloticus*.	142
11. Map of drainage systems of Zimbabwe indicating collection sites for this study of tilapiine species indigenous to Zimbabwe.	143
12. Phenogram of 29 populations of tilapiine fish species of Zimbabwe based on the UPGMA procedure using Nei's (1978) unbiased genetic distance coefficients.	144

13. Dendrogram of 29 populations of tilapiine fish species of Zimbabwe based on the Distance Wagner procedure using Rogers (1972) genetic distance coefficients. 145

14. Phenogram of nine tilapiine fish species of Zimbabwe based on the UPGMA procedure using Nei's (1978) unbiased genetic distance coefficients. 146

15. Dendrogram of nine tilapiine fish species of Zimbabwe based on the Distance Wagner procedure using Rogers (1972) genetic distance coefficients. 147

16. Dendrogram of 9 tilapiine fish species of Zimbabwe based on the FREQPARS procedure. 148

INTRODUCTION

The use of tilapiine species for aquaculture in Zimbabwe, and Africa as a whole, has greatly increased in recent years. In Zimbabwe it is estimated that total fish catches should increase from the current 20-30,000 tons per year to as much as 160,000 tons per year by the end of the century (Balarin 1984, 1986, Gurure 1985). This increase in fish harvest is necessary to offset an increasing demand for fish protein from a population which is expected to double by the year 2000. Imported fish do not represent a reasonable solution to this problem because of a severe foreign currency problem. Over 30 exotic fish species have been introduced, but none have the production potential of indigenous tilapiine species (Balarin 1986, Gurure 1985).

Several attributes make tilapiine fish species suitable for fish farming. These fish have excellent growth rates and grow on a wide range of both natural and artificial foods (Wohlfarth and Hulata 1983). They are generally hardy, resistant to disease, and have a high yield potential due to their tolerance to crowding and their ability to survive at low oxygen tensions, as well as in a wide range of salinities (Wohlfarth and Hulata 1983). High market acceptability make them a suitable domesticated species in the tropics and subtropics. In addition, much of the technology necessary for tilapiine fish farming is both

simple and inexpensive.

Tilapiine fish species (Cichlidae: Tilapiini), commonly known as tilapias, were formerly placed in one genus, Tilapia. Since the 1920's, several attempts have been made by taxonomists to divide the tilapias into smaller groups (summarized in McAndrew and Majumdar 1984). Initially, morphological characters were utilized but as more information on tilapiine biology has become available, systematists have included behavioral characteristics such as breeding and brooding. Recently, Trewavas (1983) suggested the substrate-spawners retain the generic name Tilapia while the mouth-brooders become Oreochromis (maternal mouth-brooders), Sarotherodon (paternal or biparental mouth-brooders) or Danakilia (containing only D. franchetti). These divisions clash with those of Peters and Berns (1978, 1982) who believe there is no strong justification for any divisions and prefer to leave all species in one genus, Tilapia.

Both groups of workers agree that mouthbrooders evolved from a substrate-spawning ancestor. However, Peters and Berns (1978, 1982) suggest that mouthbrooding was independently evolved from substrate spawning in several lineages, while Trewavas (1973, 1983) suggests that the substrate-spawners gave rise to the mouthbrooding branch which then quickly divided into Sarotherodon, Oreochromis and Danakilia. In this study, I follow the taxonomy of

Trewavas (1983).

Although baseline research on many aspects of the biology and culture of tilapias is available (summarized in Pullin and Lowe-McConnell 1982, Pullin 1985), the annual world production of tilapias is still low (FAO 1978, Wohlfarth and Hulata 1983). One of the major reasons is because the literature, from both field biology and experimental culture work, has been confusing. This is because there have been several cases of species misidentification and nomenclatural changes. Among tilapiine species, much of this confusion has been due to broad overlap in meristic characteristics considered important in species identification within most genera (Fryer and Illes 1972, McAndrew and Majumdar 1983, Wohlfarth and Hulata 1983, Van der Bank et al. 1989). Consequently, the 70 or more species in the tribe Tilapiini are not morphologically very distinctive (McAndrew and Majumdar 1984).

Furthermore, allopatric species of tilapias, for example Oreochromis andersonii, O. mortimeri and O. mossambicus, will freely hybridize when placed in sympatry (Bell-Cross and Minshull 1988). Extensive introductions and translocations of tilapias, both worldwide and within their native ranges, make it more difficult to identify either pure stocks or hybrids, which are usually intermediate in appearance to parental species (McAndrew and Majumdar 1983).

These factors have contributed to the paucity of phylogenetic studies conducted on tilapiine cichlids.

This study focuses on tilapiine species indigenous to Zimbabwe. Eight species (five in the genus Oreochromis and three in the genus Tilapia) are currently recognized in Zimbabwe (Table 1). In addition, Oreochromis niloticus and Oreochromis aureus have been introduced into the country for aquacultural purposes.

About 20% of Zimbabwe exceeds 1,200 m (4,000 ft) above sea level. This high ground forms a large plateau that transverses the country from northeast to southwest, and creates a central watershed drained by tributaries of six independent river systems: the Zambezi, Pungwe, Budzi, Save/Runde and Limpopo rivers which flow eastward to the Indian Ocean, and the Nata River which loses itself in the landlocked Makarikari Basin in Botswana (Fig. 1).

The Zambezi River is the largest African River that flows into the Indian Ocean. Natural and manmade barriers have further divided the Zambezi River into the Upper Zambezi above Victoria Falls, the Middle Zambezi down to the Kabrabassa Rapids, Lake Kariba which was built in 1953 on the Middle Zambezi, and the Lower Zambezi from the Kabrabassa Rapids to the Indian Ocean. The Upper Zambezi includes the Zambezi and its tributaries, the Chobe and Okavango rivers, which originate in Angola. The Okavango River ends in Okavango swamps but is connected to the

Zambezi through the Chobe swamps during periods of heavy floods. The Upper Zambezi has a rich fish fauna compared with the Middle and Lower Zambezi (Jubb 1967, Bell-Cross and Minshull 1988). Eighty-four fish species are found in the Zambezi River above Victoria Falls as compared to 70 species below, with only 46 species common to both systems (Bell-Cross and Minshull 1988).

The Middle Zambezi River system comprises the Batoka Gorge which starts just below the Victoria Falls to the Kabrabassa Rapids where the Kabora Bassa Dam is now situated. In between, several tributaries such as the Matetsi, Gwayi, Sanyati, and Manyame join the Zambezi.

The Lower Zambezi covers the section from Kabora Bassa Dam to the Indian Ocean. From Zimbabwe, the Lower Zambezi River is joined by the Mazowe, Inyangombe and Gairezi rivers which drain the north-eastern area of the country. The Lower and Middle Zambezi systems have basically similar fish fauna (Jubb 1967, Bell-Cross and Minshull 1988).

The Pungwe River rises in the north-eastern part of the country and loses altitude rapidly within Zimbabwe. The dense forest canopy along its banks and low water temperatures limits the existence of warm-water species such as tilapias (Jubb 1967). Most of the Pungwe River is located in Mozambique and flows independently to the Indian Ocean.

The Budzi River in Zimbabwe also covers a small catchment area in the eastern highlands, with the rest of

the river in Mozambique. As the Budzi itself and its major tributaries are relatively small, and the water temperatures are low within Zimbabwe, previous authors (Jubb 1967, Bell-Cross and Minshull 1988) expected larger fish species such as tilapias to be absent in the system.

The Save and Runde Rivers, together with their tributaries, drain most of the south-eastern region of Zimbabwe. Above their confluence, practically at the Zimbabwe-Mozambique border, they pass through an extensive granite area, followed by gorges and rapids which have been impassable barriers to upstream migration of fishes (Jubb 1967, Bell-Cross and Minshull 1988). The Chivirira Falls on the Save River, and the Selawandoma Falls on the Runde River, effectively demarcate the boundary between the Upper (39 spp) and Lower (41 spp) Save/Runde systems, with only 21 species common to both (Bell-Cross and Minshull 1988). The Lower Save/Runde system has many Middle and Lower Zambezi River species absent in the Upper Save/Runde.

The Limpopo River system straddles the Tropic of Capricorn and drains southern Zimbabwe. No major barrier seems to affect fish movement on the Limpopo River included in Zimbabwean territory. Bell-Cross and Minshull (1988) report that the fauna of this river system is not well known. In its fish fauna, remnants of the Zambezi fauna as well as entirely new species are found (Jubb 1967). Forty-six species have been recorded from the Zimbabwean

Limpopo system (Bell-Cross and Minshull 1988).

The Nata River and its tributaries, the Gwavazabuya and Amanzamnyama rivers, flow through much of the semi-desert area of the western part of Zimbabwe. Most of the year the rivers are made up of a series of isolated pools. Only four species have been collected from this system (Bell-Cross and Minshull 1988).

Even though the mouths of these independent river systems are widely separated, their headwaters come into close proximity on the Zimbabwean plateau either as swamps or streams. Little is known about the zoogeography of the Zimbabwean fish fauna. Jubb (1967) and Bell-Cross and Minshull (1988) put forward some speculations on origins of Zimbabwean fishes. They suggest that Zimbabwe's fish fauna originated in the Zaire and Nile river systems and migrated southwards in successive invasions. Bell-Cross and Minshull (1988) advocate the theory that deteriorating physico-chemical conditions and associated habitats caused the depauperate fauna found in several river systems such as the Middle and Lower Zambezi and Pungwe and Lower Save/Runde Rivers. One reason accounting for the differences in the fish fauna of the Upper Zambezi, Middle Zambezi, Pungwe and Upper and Lower Save/Runde system is that their faunas were probably derived from different sources. The Upper Zambezi was colonized by forms from the south-western Zaire system while the other eastward flowing rivers derived their faunas

from the south-eastern Zaire system and the Nile system. Stream piracy on the plateau has been responsible for some limited faunal exchanges among drainage systems (Jubb 1967).

Continental distributions of the nine tilapiine fish species examined in this study are shown in Figures 2-10. The local distributions in Zimbabwe are listed in Table 1.

The native ranges of T. rendalli and T. sparrmanii are quite similar and include most of the rivers in Zimbabwe except the Nata, Budzi, and Pungwe. Tilapia sparrmanii is also not present in the Lower Zambezi system (Bell-Cross 1976, Bell-Cross and Minshull 1988, Philippart and Ruwet 1982). Only two specimens of Tilapia ruweti have been found in Zimbabwe at Kazungula in the Upper Zambezi River. Oreochromis andersonii and O. macrochir are both present in the Upper Zambezi, Kafue and Okavango basins (Bell-Cross and Minshull 1988, Trewavas 1982b). Oreochromis placidus and O. mossambicus are sympatric in the Lower Save/Runde system. Oreochromis mossambicus is widely distributed over the eastern and southern part of the country, which includes the Limpopo, the Save/Runde systems, and Lower Zambezi. Oreochromis mortimeri occupies the Middle Zambezi and its tributaries between Victoria Falls and Kabrabassa Rapids.

Because tilapia identification is difficult, especially at the fingerling stage, ichthyologists and fisheries research officers in Zimbabwe have tended to rely heavily on identifying fish according to where they were

collected. Coloration patterns have also been used as taxonomic characters. The fact that tilapias alter color during breeding seasons, and for camouflage in different habitats, has further complicated the situation. Extensive introductions and translocations of both exotic and indigenous tilapia species has also occurred over much of Zimbabwe in the past 30 to 40 years (Beattie, 1986). The common practice for fisheries officers has been to collect broodstock from a variety of locations, allow them to breed, and then distribute fry throughout the country for pond and reservoir rearing. Philippart and Ruwet (1982) report transfers of O. macrochir, O. mossambicus, O. mortimeri and O. placidus into new reservoirs within Zimbabwe. Hubbs (1955), Behnke (1972), Busack and Gall (1981), Campton (1987), and Allendorf and Leary (1988) have documented hybridization between indigenous and introduced fish species in a number of taxonomically diverse fish families; a possibility in Zimbabwean tilapiine species. The difficulty of obtaining pure native stocks has already been noted by several investigators (e.g. McAndrew and Majumdar 1984, Thompson 1984, Beattie 1986, Van der Bank et al. 1989).

Because of the difficulties in attempting to identify species using traditional characteristics (Jubb 1961, 1967, Bell-Cross and Minshull 1988), this study employs electrophoretic genetic markers as an alternative methodology. The taxonomic utility of allozymes detected by

electrophoresis is well known (Avise 1974, Thorpe 1979, Ferguson 1980). Cruz et al. (1982), McAndrew and Majumdar (1983, 1984) and Van der Bank et al. (1989) have demonstrated the use of detectable enzyme loci as diagnostic characters in tilapiine species. Once enzyme differences between the parent species have been established, interspecific hybrids and introgressed backcrosses can be identified (Cruz et al. 1982, Macaranas et al. 1986).

Another advantage of enzyme electrophoresis for species identification is that age, sex, physiological state of the fish or environmental factors do not affect electrophoretic characters to the same degree as morphological characters (McAndrew and Majumdar 1983). F-1 hybrids and introgressed backcrosses are relatively easy to distinguish as diagnostic allozyme characters act in an additive fashion.

This study seeks to characterize indigenous tilapiine species of Zimbabwe using enzyme loci as species-specific genetic markers. Molecular-genetic variation has also proven useful in solving systematic problems and creating phylogenetic trees (Mickevich and Johnson 1976). Only Kornfield et al. (1979), McAndrew and Majumdar (1984) and Sodsuk and McAndrew (1991) have extended allozymic analyses to examine phylogenetic relationships in tilapias. Enzymatic variation discovered in this study will also be used to resolve phylogenetic relationships among tilapia fish species of Zimbabwe.

Specific objectives of the study are:

1) to develop an assay of enzyme systems which may be used to genetically characterize tilapiine fish species native to Zimbabwe,
2) to use enzyme loci as diagnostic markers for the identification of each species,
3) to estimate genetic variation within and among species, and
4) to reconstruct the phylogenetic relationships of these species.

METHODS AND MATERIALS

Sample Collection

Samples of 4-31 fish per site were collected from the major river systems of Zimbabwe (Fig. 11, Table 2). Collections were made in native ranges of each species, and in areas where introductions of other species were unlikely. Between May 1989 and August 1991, 16 populations of *Tilapia rendalli*, 13 of *Oreochromis mossambicus*, nine of *O. mortimeri*, three of *O. macrochir*, three of *O. andersonii*, three of *T. sparrmanii* and two populations of *O. placidus* were sampled (not all collections were used in this study). In addition, nine *O. niloticus* individuals and five *O. aureus* individuals were collected from the Edwards and Son Farm located just outside of Harare. These two species are not indigenous to Zimbabwe but are currently being farmed there. The strains at the Edwards farm were imported from Stirling, U.K. in 1985 and are believed to have come from cultures in Israel. Their purity could not be ascertained. *Tilapia luweti*, one of the three *Tilapia* species listed as indigenous to Zimbabwe, was not collected due to its scarcity in the Upper Zambezi System.

Collecting Techniques

Specimens were collected using seines and gill nets. Muscle, liver, eye, heart, and brain tissues were dissected from freshly killed fish, labelled, placed in Eppendoff

tubes and immediately frozen in liquid nitrogen at the collection site. The tissues were then taken to the University of Zimbabwe and were stored in an ultra-cold freezer at -80°C. Tissues were shipped on dry ice to Frostburg State University, Maryland, U.S.A. for analysis.

Carcasses were preserved in 10% formalin and stored in 50% ethanol. These were also shipped to Frostburg State University.

Sample Preparation

In the laboratory, tissues were prepared for electrophoresis by adding an equal volume of distilled water to the tissue and homogenizing with a tissue grinder for 1 min. The homogenate was then centrifuged at 12,800 x g for three minutes and the supernatant was absorbed onto 1.5 x 0.3 cm strips of Whatman No. 3 filter paper.

Gel Preparation

Gel preparation procedures followed those outlined in Selander et al. (1971) and May (1975), employing 9.5 x 20 x 1 cm and 9.5 x 20 x 2 cm molds. Gels consisted of 13% Sigma hydrolyzed starch and one of the buffer solutions listed in Table 3. Two-thirds of the gel buffer was heated in a microwave oven for two minutes. The last third was mixed thoroughly with the starch in a 1000 ml Erlenmeyer flask. The heated buffer was then added to the starch/buffer

mixture and heated for three minutes in a microwave. This mixture was swirled every 15 sec. to ensure a smooth, lump-free gel mixture as the end product.

The mixture was then aspirated for approximately 1 min. with constant swirling to remove air bubbles. At the end of this period, the starch solution was poured in the Plexiglass mold and allowed to cool at room temperature. Gels were kept covered with plastic wrap overnight.

Electrophoresis

Horizontal starch gel electrophoresis was carried out following procedures outlined in Selander et al. (1971), May (1975), Allendorf et al. (1977), and Aebersold et al. (1987), and using Model 1P-17 Heath Kit power supplies. Voltage was maintained at 300 mv for gels made with RW and MF buffers while those made with TC and AC buffers were maintained at 200 mv (for buffer systems abbreviations see Table 3). Electrophoresis was continued for 3-5 hr. until the dye marker had moved to a distance of 6 cm. The resulting gel was sliced into 1.5 mm thick replicates and each was stained with specific histochemical solutions as outlined by May (1975) and Allendorf et al. (1977).

Assay

In the first five months of laboratory analysis, an assay for the Zimbabwean tilapias was developed. Initially

all five tissues (eye, liver, muscle, heart and brain) were run on the same gel and on each of the four buffer systems. Two specimens each of O. mossambicus and T. rendalli were used in the initial screens. The enzyme systems which showed good to fair resolution and activity on the different buffer systems were selected for further use in the electrophoretic analysis of tilapias. As the analysis proceeded, only those enzymes which produced scorable banding patterns were used. Stains and appropriate buffers used in the study are listed in Table 4. Enzyme nomenclature largely follows that revised by the American Fisheries Society (Shaklee et al. 1990), and that used for tilapias by McAndrew and Majumdar (1983) and Van der Bank et al. (1989). Relative mobilities were calculated based on the most common allele that is designated 100 for an anodal locus or -100 for a cathodal locus.

Data Analysis

Electrophoretic data were analyzed primarily with the BIOSYS-I program (Swofford and Selander 1981). This program computes genetic variability, determines conformance to expected genotypic frequencies based on Hardy-Weinberg equilibrium, and calculates F-statistics. Significant allelic differences among populations were tested using Chi-square contingency tables.

Genetic variability within each population was

estimated by calculating percentage of polymorphic loci and mean heterozygosity. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Average heterozygosity was calculated in two ways: (1) the proportion of individuals sampled that were actually heterozygous ("direct count") and (2) an estimate based on Hardy-Weinberg expectations using Nei's (1978) unbiased estimate.

Allelic frequencies were also tested for conformance to Hardy-Weinberg expectations. The Chi-square goodness-of-fit test corrected for small sample sizes and G-tests for samples that violated Chi-square test assumptions were used.

Genetic differentiation of populations was analyzed using F-statistics (Wright 1965, 1978, Nei 1972). The basic formula used is

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST}),$$

where F_{IT} and F_{IS} are fixation indices for individuals relative to the total population and its subpopulations, respectively. F_{ST} measures the amount of differentiation among subpopulations relative to the limiting amount under complete fixation. A hierarchical analysis of population differentiation was done using the formulation of Wright (1978). Populations had been hierarchically arranged by: genus, species, and drainage system.

In addition, BIOSYS-I was used to calculate Nei's (1978) and Rogers's (1972) similarity and distance

coefficients. The choice of distance/similarity coefficients was based on the fact that both Nei's (1978) and Rogers's (1972) have been widely used in systematics literature. This would enable comparison of results from this study with others (e.g. McAndrew and Majumdar 1983, Van der Bank et al 1989). Nei's genetic distance has been reported to measure the mean number of electrophoretically detectable substitutions per locus that are accumulated since two populations diverged from their common ancestor. This is an advantage as it is a measure of a biological property. This coefficient can also be corrected for error due to small sample sizes (Nei 1978). The disadvantage is that it is nonmetric and does not satisfy the triangle inequality, causing negative branch lengths to be produced. This is undesirable and biologically uninterpretable for use in reconstructing phylogenies (Farris 1981). Rogers' distance has been found to be superior to several other distances (Rogers 1986). This is because this coefficient is metric and is a simple arithmetic average of single-locus distances more suitable for constructing minimum-length trees from allele frequencies. Its disadvantage is that it requires extensive computer time (Rogers 1986) and has not been corrected for small sample sizes (Buth 1984).

These indices were used for comparisons among species and populations. They were then used to construct Distance Wagner trees (Farris 1970) and UPGMA phenograms (Sneath and

Sokal 1973). FREQPARS (Swofford and Berlocher 1987) trees were also constructed. The algorithm used in this program finds the tree which requires the least amount of change, while at the same time ensuring that allele frequencies in hypothetical ancestors add up to one (Swofford and Berlocher 1987).

RESULTS

Extensive field collections produced some unanticipated results; specimens which could be classified as O. mossambicus (or O. mortimeri) were collected in the Nata system at three sites. Bell-Cross and Minshull (1988) do not list the presence of any tilapias in the Nata system. Collections of T. rendalli were also made in the Budzi system although there is no previous listing of this species in the system. A search for tilapias in the Pungwe system was unsuccessful. This finding agrees with previous research (Bell-Cross and Minshull 1988). Although T. ruweti (2 specimens) was collected previously in the Upper Zambezi system at Kazungula (Bell-Cross and Minshull 1988), no specimens were taken for this study.

Out of the 64 enzyme systems tested, the assay resulted in the resolution of 38 allozyme loci from 25 enzyme systems. These were included in all subsequent electrophoretic analyses. The subunit structures for all enzyme systems are listed in Table 4. Allelic frequencies for all loci studied are listed in Table 5. Further details are given below under separate headings for each enzyme.

Aspartate aminotranferase (AAT)

Three loci were detected from eye: two cytosolic (sAAT-1 and sAAT-2) and one mitochondrial (mAAT-1). sAAT-1

and sAAT-2 were also expressed in muscle. sAAT-1 was monomorphic in all species except Oreochromis placidus which contained two alleles. sAAT-2 was variable in some species and proved to be a species-specific marker for Tilapia rendalli, distinguishing this species from the rest of the species tested. mAAT-1 was monomorphic and migrated cathodally in the RW buffer system. Macaranas et al. (1986) resolved two loci (one anodal and one cathodal) from liver tissue. The anodal AAT was polymorphic in the tilapias they studied.

Adenosine deaminase (ADA)

A single locus with a large number of alleles was revealed. This highly polymorphic system, which showed homozygotes as single bands and heterozygotes as double bands, is interpreted as a monomer. Several mobilities are unique to certain species (Table 6). McAndrew and Majumdar (1983) reported the same high levels of ADA polymorphism in cichlids they studied.

Adenylate kinase (AK)

One locus from eye tissue was used in this study while a second slower eye locus was discarded due to inconsistencies in scoring. A different single AK locus was observed in both liver and muscle but was not used because of inconsistencies in scoring. Cruz et al. (1982) observed

four different AK loci in both liver and eye in Tilapia zilli. Van der Bank et al. (1989) observed three loci, two in liver (AK-1 and AK-3) and two in heart (AK-2 and AK-3). They also reported one locus in muscle tissue which corresponded with AK-3 found in other tissues examined by the same authors. McAndrew and Majumdar (1983) reported only one locus in muscle tissue.

The AK locus in our study proved to be a species-specific marker for T. sparrmanii differentiating it from the rest of the species studied.

Alcohol dehydrogenase (ADH)

A single locus was detected in liver tissue. Mobility in the buffer system used was cathodal. Except for Tilapia sparrmanii this locus was monomorphic. Cruz et al. (1982), McAndrew and Majumdar (1983) and Van der Bank et al. (1989) also observed a single locus in the cichlids they studied.

Creatine kinase (CK)

Two loci were observed in both eye and liver and one in muscle tissue. The eye CK loci were scored in this study. CK-1 was invariant while CK-2 was variable in O. mossambicus only. CK-2 proved to be a species-specific marker for T. rendalli and for T. sparrmanii and also differentiated O. placidus and O. macrochir from O. mortimeri, O. andersonii, O. niloticus and O. aureus.

Esterases (EST)

The esterases in both muscle and liver were not easily scored. One consistently scorable locus in eye was used in this study. McAndrew and Majumdar (1983) also reported difficulty in scoring liver esterases. The eye EST locus used in this study was species-specific for T. rendalli and T. sparrmanii while O. macrochir and O. aureus shared the same allele and O. mossambicus, O. mortimeri, O. placidus, O. andersonii and O. niloticus shared a common alleles (Table 6).

Fructose-bisphosphate aldolase (FBALD)

A monomorphic single locus was expressed in all species in liver tissue.

Fructose-bisphosphate dehydrogenase (FBP)

Two loci were observed, one in muscle and one in liver. The liver locus was dropped from the study due to inconsistencies in scoring. The FBP locus scored was polymorphic in some O. mossambicus and O. andersonii populations only.

Galactosaminidase (GAM)

One monomorphic locus was detected in eye and muscle tissue from all species.

General Protein (PROT)

The products of two PROT loci were observed in the muscle tissue. PROT-1 was monomorphic in all species except T. sparrmanii which contained some heterozygous individuals. PROT-2 was polymorphic in O. placidus and O. mossambicus populations. Van der Bank et al. (1989) also observed two loci in muscle samples.

Glucose-6-phosphate isomerase (GPI)

Two GPI loci were detected and this result agrees with several other studies done on cichlids (Kornfield et al. 1979, Cruz et al. 1982, McAndrew and Majumdar 1983, Macaranas et al. 1986, Van der Bank et al. 1989). The nomenclature follows that in Van der Bank et al. (1989) in designating the least anodal locus GPI-A. GPI was stained for in muscle tissue and heterozygous individuals showed the expected dimeric pattern of three bands of activity at a single locus.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

A single monomorphic locus was scored from both eye and liver. Van der Bank et al. (1989) detected an invariant locus in muscle and liver.

Glycerol-3-phosphate dehydrogenase (G3PDH)

One dimeric locus was scored from muscle tissue. This result is consistent with that of Van der Bank et al. (1989) although they did not observe any heterozygotes in the populations they examined. I observed heterozygotes in O. andersonii, O. mortimeri and T. rendalli. Cruz et al. (1982) and McAndrew and Majumdar (1983) observed two or more loci in muscle tissue with the buffer systems they used. Van der Bank et al. (1989) labelled the muscle locus G3PDH-2 as they had observed G3PDH-1 and G3PDH-2 in liver tissue. The muscle locus scored in this study distinguished T. rendalli and T. sparrmanii from the Oreochromis spp.

Guanine deaminase (GDA)

A single locus was scored from liver tissue in this study. Van der Bank et al. (1989) also reported one locus in liver but two in muscle. The GDA locus was polymorphic in most of the Oreochromis and monomorphic in the Tilapia populations. This locus, like G3PDH, was diagnostic at the generic level.

L-lditol dehydrogenase ($_{S}$IDDH-1)

For this enzyme, previously known as sorbitol dehydrogenase, one polymorphic locus was detected in liver tissue. Tilapia rendalli had a fixed allelic difference at this locus compared to the rest of the species examined except for O. macrochir.

Isocitrate dehydrogenase ($_m$IDHP)

Cruz et al. (1982), McAndrew and Majumdar (1983), Macaranas et al. (1986) and Van der Bank et al. (1989) all recorded a muscle-specific IDHP and a liver-specific IDHP. The results of this study are consistent with these previous studies. Because of scoring difficulties, only the muscle-specific locus was used. This locus was a species-specific marker for T. sparrmanii.

Lactate dehydrogenase (LDH)

Three different regions of activity were detected. The locus which showed highest activity in muscle and was the least anodal was designated LDH-A. LDH-B is the fastest band in muscle and corresponds with the single band expressed in liver tissue. Eye tissue yielded both LDH-A and LDH-B plus a rapidly migrating band which was designated LDH-C. This nomenclature follows that designated by Van der Bank et al. (1989). The intermediate bands were presumed to be the heterotetrameric expression of the three different loci. The low level of polymorphism observed corresponds with the results reported by Chen and Tsuyuki (1970), Kornfield et al. (1979), Cruz et al. (1982), McAndrew and Majumdar (1983), and Van der Bank et al. (1989) in previous cichlid studies. LDH-A proved to be species-specific marker for T. sparrmanii. LDH-B revealed a diagnostic difference

between O. macrochir versus O. andersonii, O. mortimeri, O. placidus, O. niloticus, O. aureus, T. rendalli and T. sparrmanii but not O. mossambicus. LDH-C was diagnostic at the generic level.

Malate dehydrogenase (MDH)

MDH loci were resolved in all three tissues used in this study. Three anodal loci were detected and all three were expressed in muscle. sMDH-A and sMDH-B are results of isozyme mobility duplication in Oreochromis spp. (McAndrew and Majumdar 1983, Macaranas et al. 1986). Three-banded phenotypes were observed that showed either 1:6:9 or 1:2:1 banding intensities. sMDH-B, which was expressed in liver as a single locus, was monomorphic and exhibited the same mobility as the sMDH-A(b). sMDH-B proved to be a species-specific marker for T. rendalli and T. sparrmanii. mMDH-1 proved to be a species-specific marker for T. rendalli.

Malic Enzyme (MEP)

Good resolution was recorded for muscle and eye tissues on a Ridgeway buffer. Two loci were expressed. Both sMEP and mMEP were present in muscle. mMEP was more active in muscle and sMEP in eye and liver (resolution in liver was poor). Results of this study agree with those of Van der Bank et al. (1989). McAndrew and Majumdar (1983) also

recorded two MEP loci, but reported polymorphism in sMEP in one of the species they studied. mMEP proved to be a species-specific marker for both O. placidus and O. macrochir. A fixed difference at the sMEP locus proved to be the only diagnostic genetic character separating O. andersonii from O. mossambicus/O. mortimeri.

Mannose-6-phosphate isomerase (MPI)

Liver tissue provided the best activity and revealed a single monomorphic band for all species examined in this study. This is consistent with the results of Cruz et al. (1982), who observed one MPI locus in both liver and eye; however, they reported two loci from muscle tissue.

Peptidases (PEP)

PEP staining depends upon the release of an L-amino acid from a dipeptide or tripeptide that is sensitive to the action of L-amino acid oxidase contained in the staining solution. Four different substrates; L-glycyl-leucine (PEPA), L-leucycl-glycyl-glycine (PEPB), L-leucyl-leucyl-leucine (PEPC) and L-phenyl-alanine-proline (PEPD) were utilized. PEPA was stained for in muscle tissue and had a single locus which was monomorphic and the same except for two specimens from the Gwayi River O. mortimeri population, and two specimens from the O. niloticus sample. The variant O. mortimeri specimens were homozygous for a

different allele from the rest. These could either be rare alleles or could represent the presence of null alleles. The rare/null alleles were also observed in some individuals at PEPC. PEPB and PEPD proved to be species-specific markers for T. rendalli.

Phosphoglucomutase (PGM)

PGM was resolved in liver samples, where one locus was detected. This locus contained a few heterozygotes only in two O. mortimeri populations. Homozygotes had a single band while heterozyotes were double banded indicating a monomeric subunit structure. McAndrew and Majumdar (1983) reported one locus in muscle and Macaranas et al. (1986) observed one locus in eye. These probably represent the same locus I observed.

6-Phosphogluconate dehydrogenase (PGD)

A single locus was detected in liver for PGD. This result is similar to those of Cruz et al. (1982), McAndrew and Majumdar (1983), Macaranas et al. (1986) and Van der Bank et al. (1989). This locus proved to be a species-specific marker for T. rendalli and for T. sparrmanii. McAndrew and Majumdar (1983) recorded a fixed allelic difference between O. macrochir versus O. andersonii, O. aureus, O. mossambicus and O. niloticus. My study did not find such a difference.

Superoxide dismutase (SOD)

A polymorphic banding pattern consistent with a single locus was detected in liver tissue. Three-banded heterozygotes indicated that SOD is a dimer. McAndrew and Majumdar (1983) and Van der Bank et al. (1989) obtained similar mobilities. Almost all the tilapiine species carried both alleles in some of their populations except O. niloticus, O. aureus and T. sparrmanii which were monomorphic for the alternate allele.

Triose-phosphate Isomerase (TPI)

One locus was expressed in liver tissue in all species. Tilapia rendalli and O. placidus carried the same allele distinguishing them from T. sparrmanii, O. mortimeri, O. macrochir, O. andersonii and O. aureus. Oreochromis mossambicus and O. niloticus carried both alleles in some of their populations.

A total of 362 fish from 29 populations were examined for the 38 loci described above and yielded a total of 102 presumptive alleles. Twenty-three loci showed variation in at least one population, while 15 loci were monomorphic for all species. Of the 15 monomorphic loci, six were monomorphic for the same allele for all species examined, while the remaining nine showed fixed allelic differences among species (Tables 5 and 6).

No fixed allelic differences were found among O. mossambicus (ten populations) and O. mortimeri (nine populations) (Table 6). The only differences among these nineteen populations were in allelic frequencies at some loci.

The nine O. mortimeri populations were fixed for one allele at the sAAT-2, CK-2, EST-1, FBP-1, PROT-2, LDH-B, mMDH-1 and TPI-1 loci, while some O. mossambicus specimens carried another allele at these loci. On the other hand, O. mossambicus populations were fixed for one allele at GDA-1, GPI-B, G3PDH-1, PEPA-1 and PGM-1 while some O. mortimeri were heterozygous at the same loci. Also, at GPI-A, the heterozygotes in O. mortimeri demonstrated a different allele than the heterozygotes in O. mossambicus. Results from the contigency Chi-square analysis showed significant differences in allele frequencies ($p<0.05$) between O. mossambicus and O. mortimeri at sAAT-2, ADA-1, FBP-1, GDA-1, G3PDH-1, LDH-A, sMDH-1, PEPA-1, PEPC-1, PGM-1, sIDDH-1, SOD-1 and TPI-1.

sMEP-1 was the only locus which showed a fixed allelic difference between O. andersonii and the O. mossambicus\O. mortimeri complex.

The alleles demonstrating fixed differences among the species are given in Table 6.Tables 7a through 7i show diagnostic loci between the tilapiine fish species analyzed in this study.

///

Of the Tilapia spp., both T. rendalli and T. sparrmanii, have more than 13 diagnostic loci which distinguish them from any of the Oreochromis spp. (Tables 7f and 7g). The diagnostic loci are not similar in all cases as the Tilapia spp. have 17 loci that show diagnostic differences between them.

Chi-square tests for conformance to Hardy-Weinberg proportions were performed for each population at all polymorphic loci. Nine out of the 144 Chi-square tests deviated significantly at the 0.05 level (Table 8). G-tests were carried out for those populations which violated the Chi-square test assumption that sample sizes be larger than five individuals. Of these, all but three still showed a significant deviation from Hardy-Weinberg expectations.

Genetic variation within populations was measured using percentage polymorphism and average heterozygosity (H) (Table 9). The percentages of loci which were polymorphic ranged from 2.6% in the O. aureus population to 23.7% in an O. mossambicus population collected from the Ruti Dam (Table 9). The lowest mean heterozygosity was 0.007 in T. rendalli and the highest was 0.096 in O. mossambicus from Ruti Dam. The average for all 29 populations was 0.042.

F-statistics were calculated for all populations and resulted in a mean of 0.035 for F_{IS}; 0.784 for F_{IT}, and the mean F_{ST} was 0.776 when all species were included (Table 10). F-statistics of the Oreochromis species alone yielded

lower values (F_{IS}=0.040, F_{IT}=0.718 and F_{ST}=0.679; Table 11). A comparison of only the indigenous Oreochromis spp (O. mossambicus, O. mortimeri, O. macrochir, O. placidus and O. andersonii) yielded an even lower value of F_{ST}=0.664 (Table 12). The O. mortimeri/O. mossambicus complex yielded F_{ST} of 0.393 (Table 11). The loci which are mostly responsible for the major differences among all populations were AK-1, mIDHP-1, LDH-C, sMDH-B, sMEP-1, mMEP-1, PEPB-1, PEPD-1 and PGD-1, which all showed a F_{ST} value equal to 1.000 followed by CK-2, EST-1, PROT-2, GPI-A, LDH-B and mMDH-1 which had F_{ST} values higher than 0.900 (Table 10). PEPA-1 contributed least with an F_{ST} = 0.133. Among the indigenous Oreochromis only sMEP-1 and mMEP-1 had an F_{ST} = 1.000 but several loci had F_{ST} values above 0.900 (Table 12).

Hierarchical F-statistics showed that the populations were genetically differentiated by drainage as there was less variation (F_{XY} = 0.082) within a species in the same drainage and more across populations of the same species in different drainages (F_{XY} = 0.233). Genetic variation among species within a genus was high (F_{XY}=0.695). Unfortunately, due to the nature of how hierarchical F-statistics are calculated low variation is reported between the two genera (F_{XY}=0.040). This might not be the case.

The calculated values for Nei's (1978) unbiased genetic identities (I_n) and distances (D_n) for population comparisons are given in Table 13 and for species

comparisons in Table 14. Rogers' (1972) genetic distances were also calculated and are presented in Tables 15 and 16 for population and species comparisons, respectively.

Phenograms and dendrograms were produced for the data from individual populations (Figs. 12 and 13) and from the collapsed species data (Figs. 14 and 15). To get species data, the population data for each species was collapsed to give a pooled value for each locus. Phenograms (Figs. 12 & 14) were based on the unweighted pair group method using arithmetic averaging (UPGMA) algorithm, using Nei's (1978) unbiased genetic distance. Dendrograms were produced by Distance Wagner procedure (Farris 1972) using Rogers's (1972) genetic distance index (Figs. 13 and 15). Tables 13, 14, 15 and 16 show the genetic distance coefficients used in the construction of these dendrograms.

Lastly, Figure 16 shows a species dendrogram constructed using the FREQPARS procedure developed by Swofford and Berlocher (1987). When tree topologies were specified using trees constructed from the UPGMA and Distance Wagner algorithms the total tree lengths were longer, (87.2540 and 89.8290 respectively), than when no tree topology was specified (86.0370). Rearranging species order in the data set did not alter the length of the tree produced when tree topologies were not specified. The UPGMA species phenogram had a cophenetic correlation of 0.963 while that for the Distance Wagner tree

was 0.980.

The UPGMA phenograms (Figs. 12 and 14) show T. sparrmanii and T. rendalli as outliers to Oreochromis species. The collapsed data phenogram (Fig. 14) shows that within the genus Oreochromis there are two main groupings; O. placidus, O. macrochir, O. niloticus and O. aureus forming one group while O. mossambicus, O. mortimeri and O. andersonii form the other. Within the first group O. macrochir and O. placidus cluster together while O. niloticus and O. aureus cluster together. In the second group O. andersonii is interpreted as sister to the O. mossambicus/O. mortimeri cluster.

Fish identified as O. mossambicus and O. mortimeri all show a close relationship (Fig. 12). Among O. mossambicus populations, the Gwavazabuya and Amanzamnyama populations (Nata system) clustered together with the Beitbridge Dam (Limpopo system) population. The Mwenezi and Runde cluster together while the Ruti, Budzi and Mazoe populations (Fig. 12) clustered together. The Tokwe and Save/Runde junction populations were each separated from other clusters.

In the O. mortimeri complex, the Sinamatella, Mandavu, Deka and Dolilo populations clustered together. The Gwayi, Mlibizi, Sinamwenda, Manapools, and Kariba populations were clumped together.

The Distance Wagner trees (Figs. 13 and 15) show distinct separation of Tilapia species from the Oreochromis

species. The Tilapia spp. form their own clade. The two Oreochromis groupings have O. macrochir and O. placidus clustered together in one group and the rest of the Oreochromis species in the second group. In the second group O. niloticus and O. aureus cluster together and O. andersonii is interpreted as a sister taxon to the O. mossambicus/O. mortimeri cluster.

Oreochromis mossambicus, O. mortimeri, and O. andersonii cluster together in both Distance Wagner trees which is consistent with the UPGMA topologies. The UPGMA topology (Fig. 14) and the FREQPARS tree (Fig. 16) show O. mossambicus and O. mortimeri clustering together and O. andersonii as a sister taxa to that cluster. The Distance Wagner topology (Fig. 15) reverses this situation and has O. mortimeri clustering with O. andersonii and O. mossambicus as the next closest taxon. The Distance Wagner individual population topology (Fig. 13) reveals O. andersonii populations being clustered within the O. mortimeri population group.

The O. mossambicus populations maintain the same relative positions in the dendrograms produced by Distance Wagner (Fig. 13) and UPGMA (Fig. 12) algorithms. The sample collected in Ruti Dam on the Upper Save\Runde system branches off first, followed by the Mazoe River population and then the Save/Runde junction popualtion. The branching off continues with the Budzi River, the Runde River, the

Tokwe and then the Mwenezi River population in one cluster. The Beitbridge Dam (Limpopo System) branches off on its own while the Nata system populations, the Amanzamnyama and Gwavazabuya river collections are closely associated. Some minor variations are found when comparing the Distance Wagner population trees to the UPGMA trees. Most branch lengths separating populations within species are short and restrict the amount of information we can interpret from such topologies.

The FREQPARS tree (Fig. 16), which used the collapsed species data of the Oreochromis spp. and T. rendalli, was only slightly different from the UPGMA tree and the Distance Wagner trees in branching patterns. Both O. niloticus and O. aureus lie outside the clade of Zimbabwean Oreochromis. Within the Zimbabwean Oreochromis clade, O. placidus and O. macrochir are sister taxa.

DISCUSSION

The difficulty in relying on morphological identification of the different species in the genus Oreochromis has been noted by several investigators working with tilapias in Zimbabwe (Balon 1974, Beattie 1986, Bell-Cross and Minshull 1988). As aquaculture development became a priority of the Aquatic Branch of Zimbabwe's Department of National Parks and Wild Life Management, it was imperative to accurately identify the ichthyofaunal resources of the country. In order to establish a foundation for basic biological studies, a reliable method for identifying species was a priority.

The first objective of this study, therefore, was to develop a diagnostic assay using a large number of enzyme systems. After examining 38 loci systems on 29 populations of tilapiine fish species present in Zimbabwean waters, no two species showed the same mobility at all loci except O. mossambicus and O. mortimeri. The large number of systems allowed the investigator to analyze individual fish at a series of markers at one time. This was important because when delineating the boundary between species, one fixed difference occurring between two sympatric species, for example O. mossambicus and O. placidus or O. macrochir and O. andersonii, is sufficient to show that they are two distinct species (Sarich 1977, Richardson et al. 1986).

This is because the fixed difference proves that there is no gene flow between the species. Richardson et al. (1986) recommend that as few as five fish per species are sufficient for the electrophoretic detection of sympatric cryptic species. Therefore the use of as many loci as possible maximizes the probability of detecting fixed differences and is more important than screening a large number of individuals (Richardson et al. 1986, Van der Bank et al. 1989). Also when assessing the validity of allopatric species, e.g. O. placidus and O. macrochir or O. mortimeri/O. mossambicus and O. andersonii, Nei (1978), McAndrew and Majumdar (1983) and Richardson et al. (1986) all recommend the use of many loci rather than many individuals. Because a diploid individual possesses a pair of homologous chromosomes, a single locus carries two independent measurements for a character. Vertebrate populations have been shown to be monomorphic at approximately 85% of isozyme loci (Nevo 1978), therefore a single individual is representative of the whole population for at least 85% of electrophoretic characters.

Allelic variability

Allelic variation shown through electrophoretic mobility of proteins provides information about DNA variability (Avise 1974), as this type of molecular variation is largely genetically controlled and co-dominant.

Specific patterns of enzyme differentiation in related taxa provide valuable insight into the evolution of genomes and evolutionary divergence (Fisher and Whitt 1978).

In this study, nearly 20 putative populations of O. mossambicus and O. mortimeri were studied and no diagnostic difference was found between the two species. Sodsuk and McAndrew (1991), the only other study which has electrophoretically analyzed these two species, did not report the allelic variations they observed, therefore, I cannot compare results. My results indicate that allozyme electrophoresis either failed to detect a difference between these two species (which are morphologically very similar) or the differentiation into two species may not be valid. O. mortimeri was not described until 1966 (Trewavas 1966). The separation from O. mossambicus has been based primarily on the colors of the breeding males and the relatively shorter caudal peduncle in O. mortimeri (Trewavas 1983).

While this study found only one diagnostic difference sMEP-1 between O. andersonii and the O. mossambicus/O. mortimeri complex, McAndrew and Majumdar (1983) found four diagnostic loci; ICD-1, CK-1, EST-2 and ADA-1, between O. andersonii and O. mossambicus. The present study did not include ICD-1 and EST-2, and CK-1 was monomorphic at the same locus for all the nine species. Adenosine deaminase (ADA-1) was not diagnostic. Van der Bank et al. (1989) reported five diagnostic allelic differences; AK-2, ES-s,

GPI-B, sMDH-B and mMEP. Again some of the loci were not used in this study. The enzyme systems were not resolved from the same tissues and neither were the buffer systems similar in all three studies. The fact that I collected more populations over a greater part of the species' natural range also increased the number of variant alleles at some loci. There is a need for standardization of methods in different tilapia genetics laboratories so that consistent results can be obtained and compared.

The large number of diagnostic allelic differences between Tilapia and Oreochromis spp. support grouping them into two higher taxa as suggested by Trewavas (1983). The electrophoretic results of Sodsuk and McAndrew (1991) also show a distinct separation of these two groups.

Chi-square tests and G-tests

The deviations from Hardy-Weinberg expectations could be the result of several factors which include small sample sizes, non-equilibrium status in the populations sampled and/or scoring ambiguity. Only four specimens of T. sparrmanii were used in the analysis and this small number could account for some of the deviations in that population. A small sample size could also account for the deviation in EST in the O. andersonii population from the Hippo River (8 fish). The fact that some of the G-tests also showed significant deviation from Hardy-Weinberg expectations can

be attributed to sampling error.

Scoring ambiguity is suspected in PEPA-1 and PEPC-1 as these two enzyme systems consistently showed a few putative homozygotes for an alternate allele and no heterozygotes. In this situation, null alleles are suspected as the A/null-heterozygote can be difficult to detect (Richardson et al. 1986). Post-translational modification which may include: a) combination with other chains to form a protein, b) attachment of small molecules and c) `aging' of tissue in vivo (treatment of the protein molecule during collection and storage can lead to changes in shape and charge from one stable form to another) can all affect how the enzyme systems will be interpreted on the electrophoretic gel (Richardson et al. 1986).

Many of the deviations from the Hardy-Weinberg expectations may be explained by the Wahlund effect. The Wahlund effect occurs when a deficiency in the number of heterozygotes is observed in a sample. This may be because the sample was collected from a population which has become divided into subunits in which allele frequencies at a locus differ. This is highly likely in my samples as Zimbabwean rivers are seasonal. As the rivers dry up, fish become congregated in pools. Most of our samples were collected in these pools where it is likely that mixing of different subpopulations might have occurred.

Secondly, since Hardy-Weinberg equilibrium assumes a

large population size, the annual drying up of many Zimbabwean rivers may lead to bottlenecks which might disrupt the expected gene frequencies. Founder effects resulting from the fish which manage to survive the dry season and genetic drift can lead to shifts in allele frequencies from one generation to the next (Nei et al. 1975).

Genetic variability

The genic variation of a population is usually measured by the proportion of polymorphic loci or the average heterozygosity per locus. Nei (1975) considered the definition of polymorphism as arbitrary, and concluded that heterozygosity was a better measure of genetic variability.

The average heterozygosities calculated for the tilapiine species used in this study appear to be higher than that of other cichlids as summarized by Kornfield et al. (1979), Cruz et al. (1982), McAndrew and Majumdar (1983) and Van der Bank et al. (1989). The overall average heterozygosity estimates in this study are still lower than the typical mean heterozygosity value reported for fishes. Nevo (1978) reports a value of 0.050 and Ayala (1982) gives the average heterozygosity value for fishes as 0.078. However, a comparison of heterozygosity estimates is not particularly reliable as these values depend on the types and number of loci analyzed. The observed differences in

heterozygosity in this study compared to the other studies on tilapias can therefore be accounted for by the analysis of different populations, sample sizes, and the number of different loci studied.

The effective population size at present or in the past can also affect heterozygosity estimates (Avise and Selander 1972, Nei 1975). This factor could account for the low values (0.016 -0.033) of some of the populations collected in seasonal rivers such as the Sinamatella, Deka, Amanzamnyama, Tokwe, Budzi and Mazowe rivers where populations go through bottlenecks every year. A high value (0.100) was observed in a population of O. mossambicus collected from Ruti Dam which is a remote high water table dam where fishing is prohibited and lake levels are always high. Values of samples collected on the Zambezi River which is not a seasonal river, were also higher than average (Table 9).

The F_{ST} value, which measures the amount of genetic variance between subpopulations was considerably higher (0.776) when the two Tilapia spp. (T. rendalli and T. sparrmanii) were included in the analysis. This is not surprising since this value represents the correlation of gametes among subpopulations relative to gametes drawn at random from the entire population (Hartl and Clark 1989) and in this case the entire population included two genera. When only the Oreochromis spp. indigenous to Zimbabwe were

analyzed, the F_{ST} value dropped to 0.664. Although lower, this value is still high since the analysis includes several species. Since the O. mossambicus and O. mortimeri populations had no diagnostic loci among them, a high F_{ST} value (Table 11) was unexpected. This value can be explained partly by the small sample sizes used which might have meant the exclusion of some alleles present in the population. The wide geographical area from which samples were collected also meant allelic variation in populations caused by genetic drift could lead to the high F_{ST} values as genetic drift may lead eventually to fixation of different alleles at a locus in different populations.

Unbalanced hierarchies in hierarchical F-statistics often result when more populations for some species than other species are sampled (Chakraborty and Leimar 1987). Because only two populations in the genus Tilapia, each representing the two species, were compared to 27 Oreochromis populations the hierarchical F-statistics in this study are probably unbalanced. Additionally, the two Tilapia species had 17 fixed allelic differences between them. This lowers the relative difference between the two genera as these two species are as different from each other, when compared within a genus, as when they are compared with species from another genus. The low F_{XY} value found for the comparison of the two genera is not surprising in such circumstances.

Genetic Distances

Nine populations of O. mortimeri were collected throughout the species' range and genetic similarities (Nei, 1978) vary from 0.984 to 1.000. The samples collected in the rivers in the northwest corner of Zimbabwe (all draining into the Zambezi River between the Victoria Falls and Gwayi River) were virtually identical (In = 1.000). The identity value between the Mlibizi (beginning of Lake Kariba) and the Sinamwenda (mid-way down Lake Kariba) populations was 1.000. Comparing these two populations to the Kariba (close to dam wall) population gave identity values of 0.993 and 0.997 respectively. The small sample analyzed for the Mlibizi and Sinamwenda populations could account for the slight differences when compared to the Kariba population.

The O. mossambicus population comparisons revealed genetic similarities that varied between 0.978 and 0.999. These populations were collected from virtually all the eastern and southern Zimbabwean river systems where O. mossambicus is endemic and all populations were remarkably closely related.

Identity values from 0.933 to 1.000 were obtained for comparisons of all 19 populations in the O. mossambicus/O. mortimeri complex. Morphologically O. mossambicus and O. mortimeri only differ in the length of the caudal peduncle and breeding coloration (Trewavas 1983). The proposed recognition of the two species resulted from a two week

field experience by Dr. Trewavas (Trewavas, 1983). Trewavas suggested that the recognition of O. mortimeri be regarded as an hypothesis, since breeding coloration differences often mediate a degree of reproductive isolation. Balon (1974), on the other hand suggests that O. mortimeri be considered as a subspecies of O. mossambicus. His reasoning is that the two taxa are morphologically similar and also occupy adjacent geographical areas with no real barrier to gene flow. Geographically, they share the Zambezi river; O. mossambicus occupying the lower Zambezi from Kabrabassa Rapids to the Indian Ocean and O. mortimeri occupying the middle Zambezi from Kabrabassa Rapids up to Victoria Falls. I found high identity values between the two species, and some pair wise comparisons of O. mossambicus populations indicated a greater degree of differences than some O. mossambicus x O. mortimeri pairs (Tables 13 and 15).

Oreochromis andersonii is another Oreochromis species which is morphologically very similar to both O. mortimeri and O. mossambicus and syntopic with them in the Zambezi River. It is endemic to the region above Victoria Falls. This species showed a close genetic relationship with populations of the other two species; with a lowest value of In=0.935 when compared with O. mortimeri populations and In=0.888 when compared with O. mossambicus populations.

Once all populations were collapsed into their respective species, the average similarity value (In) for O.

mortimeri versus O. mossambicus was 0.966. Oreochromis andersonii showed a close relationship with O. mortimeri (In=0.946) and was somewhat more distant from O. mossambicus (In=0.904). The only other study which reported genetic relationships in these same three species is by Sodsuk and McAndrew (1991). They obtained the following identity values: In=0.852 for O. mortimeri versus O. mossambicus; In=0.852 for O. mortimeri versus O. andersonii; and In=0.700 for O. mossambicus versus O. andersonii. The differences between our studies probably arise as a result of the use of different buffer and enzyme systems, and highlights the variation inherent in using electrophoresis as a systematics tool.

Tilapia sparrmanii and T. rendalli were genetically quite different from other taxa examined in this study (eg. In=0.534, In=561 respectively in their comparison with O. placidus; In=0.505 and In=533, respectively in the comparison with O. andersonii). Tilapia sparrmanii was also not very similar to T. rendalli (In=0.530), the only other species from the same genus included in the analysis.

Sodsuk and McAndrew (1991) obtained D values which consistently showed greater distances between the same species when compared to my study. Choice of loci and possible differences in the electrophoretic techniques in the two laboratories could be responsible for these differences.

Taxonomic Considerations

Much controversy still surrounds the choice of algorithms for dendrogram construction using allozyme data in taxonomic or evolutionary studies (reviewed in Swofford and Olsen 1990). In this study both phenetic (UPGMA) and cladistic (Distance Wagner and FREQPARS) methodologies were adopted for comparative purposes and these produced topologies with many similarities.

The present study reveals two distinct clades; the substrate spawning Tilapia and the mouthbrooding Oreochromis in the Distance Wagner topology. This division is consistent with Sodsuk and McAndrew (1991) and supports the division of genera as suggested by Trewavas (1980, 1982a, 1982b, 1983).

The major objective of this project was to establish a method to distinguish the Oreochromis species. The Tilapia species can be easily separated visually from the Oreochromis species. The grouping of species within the Oreochromis clade is generally consistent with the visual identification of these fish. Of the species which are indigenous to Zimbabwe, O. macrochir is relatively easy to distinguish from the others. With some experience O. placidus can also be separated visually. The presence of four anal spines (the others have three) in O. placidus is a distinguishing characteristic. The remaining three species, O. mossambicus, O. mortimeri and O. andersonii cannot be

easily separated and the collection location has historically been the only distinguishing feature (Minshull, Gurure, Beattie, Chimbuya per. comm.).

The only other study which has analyzed the phylogenetic relationships of these Oreochromis species (excluding O. placidus) using electrophoretic data is Sodsuk and McAndrew (1991). The three species, O. mossambicus, O. mortimeri and O. andersonii consistently group together in all phenograms and trees in both this study and that of Sodsuk and McAndrew (1991). Geographically O. mortimeri and O. mossambicus have no major physical barrier separating them. On the other hand, O. andersonii is separated from these two taxa by Victoria Falls. Through time, a derived character could have developed to separate O. andersonii from the O. mossambicus/O. mortimeri complex. Oreochromis andersonii did exhibit a fixed difference at the sMEP locus (and possibly others not analyzed in this study) that could be used to argue this case. The UPGMA trees (Figs. 12 and 14) support this separation. However, in the Distance Wagner population topology (Fig. 13) O. andersonii comes out in the middle of the O. mortimeri population cluster. This situation is inconsistent with the monophyletic origin of each species. In this case, the validity of O. andersonii is questionable. The species topologies also show O. mossambicus and O. mortimeri clustering most closely in the UPGMA tree (Fig. 14) but O. andersonii and O. mortimeri

clustering together in the Distance Wagner tree (Fig. 15). The dendrograms in Sodsuk and McAndrew (1991) show O. mossambicus and O. mortimeri consistently clustering together and O. andersonii always as the close relative. This analysis is questionable, however, since the O. mortimeri sample used in their study was collected in the Upper Zambezi system (Zimbabwe) which is the region where O. andersonii is endemic and O. mortimeri should not occur (Jubb 1967, Trewavas 1982b, 1983, Bell-Cross and Minshull 1988). Clearly, there is a need for further investigations and analyses and the use of more sensitive methodology such as mtDNA analysis in order to justify the recognition of all three species in this group.

Oreochromis placidus is a sister taxon to O. macrochir in the FREQPARS topology in contrast to the suggestion by Trewavas (1983). Trewavas placed O. macrochir in a separate subgenus Nyasalapia and the other four species O. mossambicus, O. mortimeri, O. andersonii and O. placidus in the subgenus Oreochromis.

In summary, a total of 38 loci was examined in Zimbabwean tilapiine fishes, many of which were useful as diagnostic markers. Electrophoresis can be successfully applied to stock identification of tilapias for aquaculture experiments and in the conservation of wild stocks. The allozymes described in this study provide a basis for investigating genetic variation within and among species of

the tilapiine fish species resident in Zimbabwe waters. The principal phylogenetic relationships revealed by this study are consistent with phylogenies suggested by morphological data.

REFERENCES

Aebersold, P.B., G.A. Winans, D.J. Teel, G.B. Milner, and F. Utter. 1987. Manual for starch gel electrophoresis: A method for the detection of genetic variation. NOAA Technical Report. NMFS 61:20 pp.

Allendorf, F.W. and R.F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology 2:170-184.

Allendorf, F.W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (Salmo trutta): detection and interpretation from population data. Hereditas 86: 179-190.

Avise, J.C. 1974. Systematic value of electrophoretic data. Systematic Zoology 23:465-481.

Avise, J.C. and R.K. Selander. 1972. Evolutionary genetics of cave dwelling fishes of genus Astyanax. Evolution 26:1-19.

Ayala, F.J. 1982. Population and Evolutionary Genetics: a primer. The Benjamin/Cummings Publishing Company Inc. 54 pp.

Balarin, J.D. 1984. National review for aquaculture development in Africa. FAO Fish Circular 770.1. 71pp.

Balarin, J.D. 1986. The status of fish farming in Zimbabwe and future prospects. Zimbabwe Agricultural Journal

83:3-9.

Balon, E.K. 1974. Fish production of a tropical ecosystem. pp. 249-573. In: E.K. Balon and Coche, A.G. (eds). Lake Kariba: A man-made tropical ecosystem in Central Africa The Hague: Dr W. Junk.

Beattie, I.H. 1986. Summary of a talk on "The Development of Pure Strains." Proceedings of the Fish Forum held at Henderson Research Station on 21st March 1986. pp. 35-38.

Behnke, R.J. 1972. The systematics of salmonid fishes of recently glaciated lakes. Journal of the Fisheries Research Board of Canada 29:639-671.

Bell-Cross, G. 1976. The fishes of Rhodesia. National Museum of Rhodesia Publ. Salisbury. 286 pp.

Bell-Cross, G. and J. Minshull. 1988. The fishes of Zimbabwe. National Museums & Monuments of Zimbabwe. Harare. 294 pp.

Busack, C.A. & G.A.E. Gall. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (Salmo clarki seleniris). Canadian Journal of Fisheries and Aquatic Sciences 38:939-951.

Buth, D.G. 1984. The application of electrophoretic data in systematic studies. Annual Reviews Ecology and Systematics 15:501-522.

Campton, D.E. 1987. Natural hybridization and introgression in fishes. pp. 161-192. In: N. Ryman and Utter F.

(eds.). Population Genetics and Fishery Management. University of Washington Press. Seattle, Washington. 420 pp.

Chakraborty R. and O. Leimar. 1987. Genetic variation within a subdivided population. pp. 89-120. In: N. Ryman, N. and Utter F. (eds.). Population Genetics and Fishery Management. University of Washington Press. Seattle, Washington. 420 pp.

Chen, F.Y. and M. Tsuyuki. 1970. Zone electrophoretic studies on the proteins of Tilapia mossambicus and T.hornorum and their F1 hybrids, T. zilli and T. melanopleura. Journal of the Fisheries Research Board of Canada 27:2167-2177.

Clayton, J.W. and D.N. Tretiak. 1972. Amino-citrate buffers for pH control in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29:1169-1172.

Cruz, T.A., J.P. Thorpe and R.S.V. Pullin. 1982. Enzyme electrophoresis in Tilapia zilli: a pattern for determining biochemical genetic markers for use in tilapia stock identification. Aquaculture 29:311-329.

FAO. 1978. Yearbook of fisheries statistics 44. 1977. Food and Agriculture Organization of the United Nations, Rome, Italy.

Farris, J.S. 1970. Methods of computing Wagner tress. Systematic Zoology 19:83-92.

Farris, J.S. 1972. Estimating phylogenetic trees from distance matrices. American Naturalist 106:645-668.

Farris, J.S. 1981. Distance data in phylogenetic analysis. pp. 3-23. In: V.A. Funk and Brooks, D.R. (eds.). Advances in Cladistics, Vol. 1. Proceedings of the First Meeting of the Willi Hennig Society. New York Botanical Gardens, New York. 250 pp..

Ferguson, A. 1980. Biochemical Systematics and Evolution. Blackie and Sons, Glasgow. 194 pp..

Fisher, S.E. and G.S. Whitt. 1978. Evolution of isozyme loci and their differential tissue expressions: creatine kinase as a model system. Journal of Molecular Evolution 12:25-55.

Fryer, G. & T.D. Illes. 1972. The Cichlid Fishes of the Great Lakes of Africa. Oliver Boyd, Edinburgh. 641 pp..

Gurure, R. 1985. Aquaculture in Rural Development Planning in Zimbabwe. Freetown Proceedings of the Commission of the Secretariat Consultant Workshop on Village Level Aquaculture in Africa: 18 pp..

Hartl, D.L. and A.G. Clark. 1989. Principles of Population Genetics. 2nd edition. Sinauer Associates Inc., Publ. Sunderland, Massachusetts. 682 pp.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Systematic Zoology 4:1-20.

Jubb, R.A. 1961. Freshwater Fishes of the Zambezi River,

Lake Kariba, Pungwe, Sabi, Lundi, and Limpopo Rivers. Stewart Manning, Bulawayo. 294 pp.

Jubb, R.A. 1967. Freshwater Fishes of Southern Africa. A.A. Balkema. Cape Town/Amsterdam. 310 pp..

Kornfield, I.L., U. Ritte, C. Richler and J. Wahrman. 1979. Biochemical and cytological differentiation among cichlid fishes of sea of Galilee. Evolution 33:1-14.

Macaranas, J.M., N. Taniguchi, M. Pante, J.B. Capili and R. Pullin. 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial Oreochromis niloticus (L.) stocks in the Philippines. Aquaculture and Fish Management 17:249-261.

Markert, C.L. and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. Journal of Experimental Zoology 159:319-332.

May, B. 1975. Electrophoretic variation in the genus Oncorhynchus: The methodology, genetic basis, and practical applications to research and management. Master's Thesis, University of Washington.

McAndrew, B.J. & K.C. Majumdar. 1983. Tilapia stock identification using electrophoretic markers. Aquaculture 30:249-261.

McAndrew, B.J. & K.C. Majumdar. 1984. Evolutionary relationships within three Tilapiine genera (Pisces: Cichlidae). Zoological Journal of Linnean Society 80:421-435.

Mickevich, M.F. and M.S. Johnson. 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Systematic Zoology 25:260-270.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283-292.

Nei. M. 1975. Molecular Population Genetics and Evolution. American Elsevier Publishing Co., Inc., New York. 288 pp.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei, M., T. Maruyama and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. Theoretical Population Biology 13:121-177.

Peters, H.M. and S. Berns. 1978. Uber die vorgeschicht der maulbrutender Cichliden. Aquarien Magazin, 1978(5): 211-217 and (7): 324-331. (cited by McAndrew and Majumdar 1984).

Peters, H.M. and S. Berns. 1982. Die Maulbrutpflege der Chichliden Untersuchugen zur Evolution eines Verhaltensmusters. Zeitschrift fur Zoologische Systematik und Evolutionsforschung 20: 18-52. (cited

by McAndrew and Majumdar 1984).

Philippart, J-Cl. and J-Cl. Ruwet. 1982. Ecology and distribution of tilapias. p. 15-59. In: R.S.V. Pullin & R.H. Lowe-McConnell (eds.). The Biology and Culture of Tilapias. ICLARM Conference Proceedings. Manila, Philippines. 432 pp.

Pullin, R.S.V. 1985. Tilapias: "everyman's fish". Biologist 35(2):84-88.

Pullin, R.S.V. and R.H. Lowe-McConnell (eds.). 1982. The Biology and Culture of Tilapias. ICLARM Conference Proc. 7. 432 pp.

Richardson, B.J., P.R. Baverstock and M. Adams. 1986. Allozyme Electrophoresis. Academic Press Inc., Orlando, Florida. 410 pp.

Ridgeway, G.J., S.W. Shelborne, and R.D. Lewis. 1970. Polymorphism in esterases of the Atlantic herring. Transactions of the American Fisheries Society 99:147-151.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas Publications 7213:145-153.

Rogers, J.S. 1986. Deriving phylogenetic trees from allelic frequencies: a comparison of nine genetic distances. Systematic Zoology 35:297-310.

Sarich, V.M. 1977. Rates, sample sizes and the neutrality hypothesis for electrophoresis in evolutionary studies.

Nature 265:24-28.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Studies in Genetics VI. University of Texas Publications 7103:49-90.

Shaklee, J.B., F.W. Allendorf, D.C. Morizot and G.S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 111:2-15.

Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochemical Genetics 4:297-320.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy. W.H. Freeman and Co., San Francisco. 573 pp.

Sodsuk, P. and B.J. McAndrew. 1991. Molecular systematics of three tilapiine genera Tilapia, Sarotherodon and Oreochromis using allozyme data. Journal of Fish Biology 39(Supplement A):301-308.

Swofford, D.L. and S.H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Systematic Zoology 36: 293-325.

Swofford, D.L. and G.J. Olsen. 1990. Phylogeny reconstruction. pp. 411-501 In: D.M. Hillis and Moritz, C. (eds.). Molecular Systematics. Sinauer Associates,

Inc., Publ. Sunderland Massachusetts. 588 pp..

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-I: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Thompson, P.J. 1984. Hybridization of indigenous Oreochromis species in Zimbabwe and production of all-male offspring. Aquaculture in South Africa 1984 Joint Symposium by the CSIR and SAAU. pp. 1-11.

Thorpe, J.P. 1979. Enzyme variation and taxonomy: the estimation of sampling errors in measurements of interspecific genetic similarity. Journal of the Linnean Society. London (Biology) 11:369-386.

Trewavas, E. 1966. A preliminary review of fishes of the genus Tilapia in the eastward-flowing rivers of Africa, with proposals of two new specific names. Revue de Zoologie et de Botanique Africaines 74:50-62.

Trewavas, E. 1973. On the cichlid fishes of the genus Pelmatochromis with proposal of a new genus for P. congicus on the relationship between Pelmatochromis and Tilapia and the recognition of Sarotherodon as a distinct genus. Bulletin of the British Museum (Natural History) Zoology 25:1-26.

Trewavas, E. 1980. Tilapia and Sarotherodon. Buntbarshe Bulletin 81:1-6.

Trewavas, E. 1982a. Generic grouping of Tilapiini used in

aquaculture. Aquaculture 27:79-81.

Trewavas, E. 1982b. Tilapias: Taxonomy & Speciation. p. 3-13. In: R.S.V. Pullin and R.H. Lowe-McConnell (eds.). The Biology and Culture of Tilapias. ICLARM Conference Proceedings. Manila, Philippines.432 pp.

Trewavas, E. 1983. Tilapiine Fishes of the Genera Sarotherodon, Oreochromis, and Danakilia. British Museum (Natural History). London. 583 pp.

Van der Bank, F.H., W.S. Grant, J.T. Ferreira. 1989. Electrophoretically detectable genetic data for fifteen Southern African cichlids. Journal of Fish Biology 34(3):465-483.

Wohlfarth, G.W. and G. Hulata. 1983. Applied Genetics of Tilapias. Second Edition. ICLARM Studies and Reviews 6. 26 pp. International Center for Living Aquatic Resources Management, Manila, Philippines.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 9:395-420.

Wright, S. 1978. Evolution and the Genetics of Populations. IV. Variability Within and Among Natural Populations. Univ. of Chicago, IL. 580 pp.

TABLE 1. The distribution of tilapiine fish species in Zimbabwean river systems. (Adapted from: Bell-Cross and Minshull 1988).

Species	UZ	LK	MZ	LZ	N	L	P	B	URS	LRS
O. andersonii	x									
O. macrochir	x	i	i	i					i	
O. mortimeri		x	x							
O. mossambicus				x	x*	x		x	x	x
O. placidus						x			i	x
T. sparrmanii	x	x	x			x			x	x
T. rendallii rendalli	x	x	x	x				x*	i	x
T. rendalli swierstrae						x				
T. ruweti	x									

key: Drainage Systems:

UZ: Upper Zambezi
LK: Lake Kariba
MZ: Middle Zambezi
LZ: Lower Zambezi
N: Nata
L: Limpopo
B: Budzi
P: Pungwe
URS: Upper Save/Runde
LRS: Lower Save/Runde

x: specimens collected there in the past.

i: fish introduced into the system

x*: new collection records from this study

TABLE 2. Locations and drainage systems where samples of tilapiine fish species were collected in Zimbabwe.

Pop. #	Population Name	Drainage System	
	T. rendalli		
1.	TR1 Tokwe River	Upper Save/Runde	USR
	T. sparrmanii		
2.	TS1 U. Zambezi River	Upper Zambezi	UZ
	O. placidus		
3.	OPI Save/Runde Jct	Lower Save/Runde	LSR
	O. mortimeri		
4.	OMO1 Sinamatella River	Middle Zambezi	MZ
5	OMO2 Mandavu Dam	Middle Zambezi	MZ
6.	OMO3 Deka River	Middle Zambezi	MZ
7.	OMO4 Dolilo Saltpan	Middle Zambezi	MZ
8.	OMO5 Gwayi River	Middle Zambezi	MZ
9.	OMO6 Mana Pools	Middle Zambezi	MZ
10.	OMO7 Mlibizi	Lake Kariba	LK
11.	OMO8 Sinamwenda	Lake Kariba	LK
12.	OMO9 Kariba	Lake Kariba	LK
	O. mossambicus		
13.	OMS1 Gwavazabuya River	Nata	N
14.	OMS2 Amanzamnyama River	Nata	N
15.	OMS3 Beitbridge Dam	Limpopo	L
16.	OMS4 Mwenezi River	Limpopo	L
17.	OMS5 Tokwe River	Upper Save/Runde	USR
18.	OMS6 Runde River	Upper Save/Runde	USR
19.	OMS7 Ruti Dam	Upper Save/Runde	USR
20.	OMS8 Save/Runde Jct	Lower Save/Runde	LSR
21.	OMS9 Budzi River	Budzi	B
22.	OMS10 Mazowe River	Lower Zambezi	LZ
	O. macrochir		
23.	OMA1 Hippo River	Upper Zambezi	UZ
24.	OMA2 Katombora Rapids	Upper Zambezi	UZ
25.	OMA3 Udu Dam	Lower zambezi	LZ
	O. andersonii		
26.	OAN1 Hippo River	Upper Zambezi	UZ
27.	OAN2 Katombora Rapids	Upper Zambezi	UZ
	O. niloticus		
28.	ON1 Edwards Farm	----------	
	O. aureus		
29.	OAU Edwards Farm	----------	

Note: The numbers allocated to the different populations apply to all other tables and figures.

TABLE 3. Buffer systems used in this study.

System	Reference	Gel Buffer	Electrode Buffer	Gel composition
RW	Ridgeway, Sherborne and Lewis (1970)	0.03M Tris 0.005m Citric acid pH 8.5	0.06m Lithium hydroxide 0.3 M Boric Acid pH 8.1	99% gel buffer, 1% electrode buffer
AC	Clayton and Tretiak (1972)	1:20 dilution of Electrode: H_2O pH 6.1	0.04 M Citric acid pH 6.1	pH was adjusted with N- (3-aminopropyl)-morpholine.
MF	Markert & Faulhaber (1965)	1:20 dilution of stock: H_2O pH 6.1	1.5 dilution of stock: H_2O	Stock 0.9M Tris 0.5M Boric acid 0.02M Na EDTA pH 8.7
TC	Shaw and Prasad (1970)	1:14 dilution of Electrode: H_2O pH 7.5	56.4g Tris 27.0g Citric acid 3000 ml H_2O pH 7.5	Undiluted stock

TABLE 4. Summary table of enzymes screened in the tilapiine fish species of Zimbabwe. Locus abbreviations, Enzyme Commission numbers, subunit structures (from Shaklee et al. (1990)), buffers giving the best results, and tissues reflecting the greatest activities are given for each enzyme system.

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
Aspartate aminotransferase	2.6.1.1	dimer		
sAAT-1			RW, AC	E, M
sAAT-2			RW, AC	E, M
mAAT-1			RW	E
Adenosine deaminase	3.5.4.4	monomeric		
ADA-1			RW	E
Adenylate kinase	2.7.4.3	monomeric		
AK-1			AC, RW	E
Alcohol dehydrogenase	1.1.1.1	dimeric		
ADH-1			TC	L
Creatine kinase	2.7.3.2	dimeric		
CK-1			RW	E
CK-2			RW	E
Esterases	3.1.1.-	monomeric		
EST-1		(dimeric)	AC	E
Fructose bisphosphate	3.1.3.11	dimeric or		
FBP-1		tetrameric	TC	M
Galactosaminidase	-.-.-.-	dimeric		

TABLE 4. (continued).

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
GAM-1			RW	M
General protein	-.-.-.-	monomeric		
PROT-1			RW	M
PROT-2			RW	M
Glucose-6-phosphate isomerase	5.3.1.9	dimeric		
GPI-A			RW	M
GPI-B			RW	M
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	tetrameric		
GAPDH-1			AC	M
Glycerol-3-phosphate dehydrogenase	1.1.1.8	dimer	AC	M
G3PDH-1				
Guanine deaminase	3.5.4.3	dimeric		
GDA-1			MF	L
L-lditol dehydrogenase	1.1.1.14	tetrameric		
sIDDH-1			RW	L
Isocitrate dehydrogenase	1.1.1.42	dimeric		
mIDHP-1			TC	M
L-Lactate dehydrogenase	1.1.1.27	tetrameric		

TABLE 4. (continued).

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
LDH-A			RW, MF	M
LDH-B			RW, MF	M
			RW, MF	L
			RW	E
LDH-C			RW	E
Malate dehydrogenase	1.1.1.37	dimeric		
sMDH-A			MF, TC	M
sMDH-B			MF, TC	M
mMDH-1			MF	L
			TC	M
Malic Enzyme ($NADP^+$)	1.1.1.40	tetrameric		
sMEP-1			RW, TC	M
mMEP-1			RW, TC	L
			RW	E
Mannose-6-phosphate isomerase	5.3.1.8	monomeric		
MP1-1			TC	L
Peptidases	3.4.-.-	dimeric & monomeric		
PEPA-1			RW	M
PEPB-1			MF	M
PEPC-1			RW	M
PEPD-1			TC	M

148

TABLE 4. (continued).

Enzyme system	E.C. No.	Subunit Structure	Buffer+	Tissue*
6-Phosphogluconate dehydrogenase PGD-1	1.1.1.44	dimeric	AC	L
Phosphoglucomutase PGM-1	5.4.2.2	monomeric	TC	L
Superoxide dismutase SOD-1	1.15.1.1	dimeric	-	L
Triose-phosphate isomerase TPI-1	5.3.1.1	dimeric	RW	L

+ see Table 3. * E, Eye; L, Liver; M, Muscle

TABLE 5. Allelic frequencies of all proteins studied for the 29 populations of seven indigenous and two exotic tilapiine fish species resident in Zimbabwean waters. Species are designated by abbreviations; for names see Table 2.

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
sAAT1									
(N)	20	4	20	10	4	20	10	24	10
a	1.000	1.000	.725	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.275	.000	.000	.000	.000	.000	.000
sAAT2									
(N)	20	4	15	10	4	20	10	24	10
a	.000	1.000	1.000	.000	.000	.000	.000	.000	.000
b	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000
c	1.000	.000	.000	.000	.000	.000	.000	.000	.000
mAAT1									
(N)	10	4	15	10	4	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1									
(N)	10	4	20	10	4	9	10	24	10
a	.000	.000	.075	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.025	.000	.000	.000	.000	.000	.000
f	1.000	.000	.000	.000	.000	.000	.000	.000	.000
g	.000	.000	.050	.000	.000	.000	.000	.000	.000
h	.000	.000	.850	.000	.000	.000	.000	.000	.000
i	.000	.000	.000	.000	.000	.000	.000	.000	.000
j	.000	.625	.000	.000	.000	.000	.000	.000	.000
k	.000	.375	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
ADH-1									
(N)	10	4	20	10	4	10	10	11	10
a	1.000	.125	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.875	.000	.000	.000	.000	.000	.000	.000
AK-1									
(N)	20	4	20	10	4	10	10	14	10
a	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	1.000	.000	.000	.000	.000	.000	.000	.000
FBALD-1									
(N)	20	4	15	10	4	10	10	14	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	20	4	15	10	4	20	10	24	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2									
(N)	20	4	15	10	4	20	10	24	10
a	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	1.000	.000	.000	.000	.000	.000	.000
c	1.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	1.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
EST-1									
(N)	10	4	20	10	4	9	10	24	10
a	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	1.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	1.000	.000	.000	.000	.000	.000	.000	.000
FBP-1									
(N)	20	4	20	10	4	18	10	24	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAM-1									
(N)	20	4	20	10	4	20	10	24	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-1									
(N)	20	4	20	10	4	20	10	24	10
a	1.000	1.000	1 .000	1.000	1.000	1.000	1.000	1.000	1.000
GDA-1									
(N)	20	4	18	10	4	19	9	21	10
a	.000	.000	.250	1.000	1.000	1.000	1.000	.690	.700
b	.000	.000	.750	.000	.000	.000	.000	.310	.300
c	1.000	1.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
PROT-1									
(N)	10	4	20	10	4	10	10	16	10
a	1.000	.625	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.375	.000	.000	.000	.000	.000	.000	.000
PROT-2									
(N)	10	4	20	10	4	10	10	16	10
a	1.000	.000	.033	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	1.000	.967	.000	.000	.000	.000	.000	.000
GPI-A									
(N)	20	4	20	10	4	18	10	26	10
a	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	.950
b	1.000	.000	1.000	.000	.000	.000	.000	.000	.000
c	.000	1.000	.000	.000	.000	.000	.000	.000	.050
GPI-B									
(N)	20	4	20	10	4	18	10	26	10
a	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	.000	.000	.000	.000	.000	.000	.000
G3PDH-1									
(N)	20	4	20	10	4	18	10	26	10
a	.000	.000	1.000	1.000	1.000	1.000	1.000	.904	1.000
b	.000	.000	.000	.000	.000	.000	.000	.096	.000
c	.900	.000	.000	.000	.000	.000	.000	.000	.000
d	.100	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	1.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
mIDHP-1									
(N)	10	4	10	10	4	8	10	27	10
a	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	1.000	.000	.000	.000	.000	.000	.000	.000
LDH-A									
(N)	20	4	20	10	4	18	10	16	10
a	1.000	.000	1.000	1.000	1.000	1.000	.950	.938	1.000
b	.000	.000	.000	.000	.000	.000	.050	.063	.000
c	.000	1.000	.000	.000	.000	.000	.000	.000	.000
LDH-B									
(N)	20	4	20	10	4	18	10	27	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-C									
(N)	15	4	20	10	4	20	10	24	10
a	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	1.000	.000	.000	.000	.000	.000	.000	.000
sMDH-A									
(N)	20	4	20	10	4	18	10	27	10
a	1.000	1.000	.950	.300	.500	.639	.600	.463	.800
b	.000	.000	.050	.700	.500	.361	.400	.500	.200
c	.000	.000	.000	.000	.000	.000	.000	.037	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
sMDH-B									
(N)	20	4	20	10	4	18	10	27	10
a	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	1.000	.000	.000	.000	.000	.000	.000	.000
mMDH-1									
(N)	20	4	20	10	4	18	10	26	10
a	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	1.000	.000	.000	.000	.000	.000	.000	.000
c	1.000	.000	.000	.000	.000	.000	.000	.000	.000
mMEP-1									
(N)	20	4	20	10	4	18	10	26	10
a	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	1.000	.000	.000	.000	.000	.000	.000
sMEP-1									
(N)	20	4	20	10	4	18	10	26	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
MPI-1									
(N)	10	2	10	6	2	9	10	17	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPA-1									
(N)	20	4	20	10	4	18	10	27	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.926	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.074	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
PEPB-1									
(N)	10	4	20	10	4	18	10	26	10
a	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	.000	.000	.000	.000	.000	.000	.000
PEPC-1									
(N)	20	3	10	10	4	18	10	27	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.852	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.111	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.037	.000
PEPD-1									
(N)	20	4	20	10	4	18	10	26	10
a	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1									
(N)	10	4	15	10	4	10	10	18	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.833	1.000
b	.000	.000	.000	.000	.000	.000	.000	.167	.000

TABLE 5. (continued).

POPULATION

LOCUS	TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6
sIDDH-1									
(N)	10	4	20	10	4	9	10	24	10
a	.000	.000	.900	1.000	.875	1.000	.950	.979	1.000
b	.000	.000	.050	.000	.125	.000	.050	.021	.000
c	.000	1.000	.050	.000	.000	.000	.000	.000	.000
d	1.000	.000	.000	.000	.000	.000	.000	.000	.000
PGD-1									
(N)	20	4	20	10	4	19	10	24	10
a	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	1.000	.000	.000	.000	.000	.000	.000	.000
SOD-1									
(N)	20	4	20	10	4	16	10	24	10
a	.050	.000	1.000	.700	.875	.688	.700	.333	.300
b	.950	1.000	.000	.300	.125	.313	.300	.667	.700
TPI-1									
(N)	10	4	20	10	4	10	10	17	10
a	.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	1.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
sAAT1									
(N)	5	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
sAAT2									
(N)	5	5	14	10	20	10	10	7	10
a	.000	.000	.000	.600	.725	.600	.850	1.000	1.000
b	1.000	1.000	1.000	.400	.275	.400	.150	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
mAAT1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1									
(N)	5	5	14	10	10	10	10	7	10
a	.800	.700	1.000	.650	1.000	.850	.400	.857	.350
b	.200	.300	.000	.250	.000	.050	.150	.143	.050
c	.000	.000	.000	.050	.000	.100	.150	.000	.450
d	.000	.000	.000	.000	.000	.000	.050	.000	.050
e	.000	.000	.000	.000	.000	.000	.250	.000	.100
f	.000	.000	.000	.000	.000	.000	.000	.000	.000
g	.000	.000	.000	.000	.000	.000	.000	.000	.000
h	.000	.000	.000	.000	.000	.000	.000	.000	.000
i	.000	.000	.000	.050	.000	.000	.000	.000	.000
j	.000	.000	.000	.000	.000	.000	.000	.000	.000
k	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
ADH-1									
(N)	5	5	10	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
AK-1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
FBALD-1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	5	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2									
(N)	5	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
EST-1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	1.000	.950	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.050	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.000	.000
FBP-1									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	.950	.800	1.000	.950
b	.000	.000	.000	.000	.000	.050	.200	.000	.050
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAM-1									
(N)	5	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-1									
(N)	5	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
GDA-1									
(N)	5	5	14	5	20	10	9	7	10
a	.800	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
b	.200	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
PROT-1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
PROT-2									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	.950	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.050	.000	.000	.000
GPI-A									
(N)	9	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	.900	1.000	.857	.950
b	.000	.000	.000	.000	.000	.100	.000	.143	.050
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
GPI-B									
(N)	9	5	14	10	21	10	10	7	10
a	.944	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.056	.000	.000	.000	.000	.000	.000	.000	.000
G3PDH-1									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
mIDHP-1									
(N)	5	5	7	10	11	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-A									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	.964	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.036	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-B									
(N)	9	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-C									
(N)	9	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
sMDH-A									
(N)	9	5	14	10	20	10	10	7	10
a	.333	.500	.750	.700	.825	.750	.900	.786	1.000
b	.667	.500	.250	.300	.175	.250	.100	.214	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued)

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
sMDH-B									
(N)	9	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
mMDH-1									
(N)	9	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	.950	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.050	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
mMEP-1									
(N)	9	5	14	10	11	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
sMEP-1									
(N)	9	5	14	10	11	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
MPI-1									
(N)	5	5	11	10	10	10	2	7	3
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPA-1									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
PEPB-1									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEPC-1									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	.950	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.050	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEPD-1									
(N)	5	5	14	10	21	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.100	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6
sIDDH-1									
(N)	5	5	14	10	10	10	10	7	10
a	1.000	1.000	.964	1.000	1.000	.950	.950	.643	.850
b	.000	.000	.036	.000	.000	.050	.050	.357	.150
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGD-1									
(N)	5	5	14	10	20	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
SOD-1									
(N)	5	5	13	10	16	10	10	7	10
a	.100	.200	.385	.650	.594	1.000	1.000	1.000	1.000
b	.900	.800	.615	.350	.406	.000	.000	.000	.000
TPI-1									
(N)	5	5	5	10	10	10	10	7	10
a	1.000	1.000	1.000	1.000	1.000	1.000	.950	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.050	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
sAAT-1									
(N)	5	10	14	14	4	24	10	6	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
sAAT-2									
(N)	5	10	13	14	4	23	10	6	15
a	.900	.650	.923	.786	1.000	.761	.300	.000	.000
b	.100	.350	.077	.214	.000	.239	.700	1.000	1.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
mAAT-1									
(N)	5	10	7	14	3	14	10	7	15
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1									
(N)	5	10	10	6	4	14	28	6	16
a	.500	.650	.800	.833	.375	.464	.482	.250	.250
b	.200	.200	.150	.167	.125	.536	.107	.167	.125
c	.100	.150	.050	.000	.250	.000	.054	.333	.594
d	.200	.000	.000	.000	.250	.000	.250	.250	.000
e	.000	.000	.000	.000	.000	.000	.000	.000	.000
f	.000	.000	.000	.000	.000	.000	.036	.000	.031
g	.000	.000	.000	.000	.000	.000	.000	.000	.000
h	.000	.000	.000	.000	.000	.000	.000	.000	.000
i	.000	.000	.000	.000	.000	.000	.071	.000	.000
j	.000	.000	.000	.000	.000	.000	.000	.000	.000
k	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
ADH-1									
(N)	5	10	7	7	3	24	10	7	2
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
AK-1									
(N)	5	10	14	14	4	24	10	6	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
FBALD-1									
(N)	5	10	13	14	4	24	10	6	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	5	10	14	14	6	24	31	8	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2									
(N)	5	10	14	14	6	24	31	8	16
a	1.000	.900	1.000	1.000	.000	.000	.000	1.000	1.000
b	.000	.100	.000	.000	1.000	1.000	1.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
EST-1									
(N)	5	10	14	14	6	9	31	8	15
a	1.000	1.000	1.000	1.000	.000	.000	.000	.813	1.000
b	.000	.000	.000	.000	.000	.000	.000	.188	.000
c	.000	.000	.000	.000	1.000	1.000	1.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.000	.000
FBP-1									
(N)	5	10	14	14	3	20	10	7	16
a	.700	1.000	1.000	1.000	1.000	1.000	1.000	.857	1.000
b	.300	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.143	.000
GAM-1									
(N)	5	10	14	14	4	24	10	6	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-1									
(N)	5	10	14	14	4	24	10	6	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GDA-1									
(N)	5	9	11	13	6	25	30	9	12
a	1.000	1.000	1.000	1.000	.833	.800	.367	.889	.917
b	.000	.000	.000	.000	.167	.200	.633	.111	.083
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
PROT-1									
(N)	5	10	14	14	8	20	31	9	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
PROT-2									
(N)	5	10	14	14	8	20	31	9	16
a	.800	1.000	1.000	1.000	.000	.000	.000	1.000	1.000
b	.200	.000	.000	.000	1.000	1.000	1.000	.000	.000
GPI-A									
(N)	5	10	14	14	8	20	31	9	21
a	1.000	1.000	1.000	1.000	.000	.000	.000	1.000	1.000
b	.000	.000	.000	.000	1.000	1.000	1.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
GPI-B									
(N)	5	10	14	14	8	20	31	9	21
a	1.000	1.000	1.000	1.000	1.000	1.000	.935	1.000	.952
b	.000	.000	.000	.000	.000	.000	.065	.000	.048
G3PDH-1									
(N)	5	10	14	14	3	20	10	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.938
b	.000	.000	.000	.000	.000	.000	.000	.000	.063
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION / LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
mIDHP-1									
(N)	5	10	14	14	3	14	10	6	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-A									
(N)	5	10	14	14	5	20	31	7	16
a	.800	1.000	1.000	.964	1.000	1.000	1.000	1.000	1.000
b	.200	.000	.000	.036	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-B									
(N)	5	10	14	14	8	20	31	9	16
a	.900	1.000	.929	1.000	.000	.000	.000	1.000	1.000
b	.100	.000	.071	.000	1.000	1.000	1.000	.000	.000
LDH-C									
(N)	5	10	14	14	6	24	31	8	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
sMDH-A									
(N)	5	10	14	14	8	19	31	9	21
a	.700	1.000	1.000	.964	.813	.842	.968	.000	.000
b	.300	.000	.000	.036	.000	.158	.032	1.000	1.000
c	.000	.000	.000	.000	.188	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION									
LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
sMDH-B									
(N)	5	10	14	14	8	19	31	9	21
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
mMDH-1									
(N)	5	10	14	14	8	19	31	9	21
a	1.000	1.000	1.000	1.000	.000	.000	.000	1.000	1.000
b	.000	.000	.000	.000	1.000	1.000	1.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
mMEP-1									
(N)	5	10	14	14	8	19	31	9	21
a	1.000	1.000	1.000	1.000	.000	.000	.000	1.000	1.000
b	.000	.000	.000	.000	1.000	1.000	1.000	.000	.000
sMEP-1									
(N)	5	10	14	14	8	19	31	9	21
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000
b	.000	.000	.000	.000	.000	.000	.000	1.000	1.000
MPI-1									
(N)	5	10	7	7	1	15	10	3	8
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPA-1									
(N)	5	10	14	14	3	20	10	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
PEPB-1									
(N)	5	10	14	14	3	20	10	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEPC-1									
(N)	5	10	14	14	3	20	5	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.643	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.286	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000
e	.000	.000	.000	.000	.000	.000	.000	.071	.000
PEPD-1									
(N)	5	10	14	14	3	20	10	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1									
(N)	5	10	14	14	3	24	10	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION

LOCUS	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
sIDDH-1									
(N)	5	10	14	14	5	24	31	7	16
a	1.000	.800	.929	1.000	.400	.458	.000	.929	.938
b	.000	.200	.071	.000	.000	.000	.000	.071	.063
c	.000	.000	.000	.000	.600	.521	1.000	.000	.000
d	.000	.000	.000	.000	.000	.021	.000	.000	.000
PGD-1									
(N)	5	10	14	14	3	24	10	7	16
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
SOD-1									
(N)	5	10	14	14	3	22	10	7	16
a	.800	.950	1.000	1.000	.667	.136	.450	.143	.000
b	.200	.050	.000	.000	.333	.864	.550	.857	1.000
TPI-1									
(N)	5	10	5	5	3	23	10	7	2
A	.600	.250	.900	.600	1.000	1.000	1.000	1.000	1.000
B	.400	.750	.100	.400	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
sAAT-1		
(N)	10	5
a	1.000	1.000
b	.000	.000
sAAT-2		
(N)	10	5
a	.950	1.000
b	.050	.000
c	.000	.000
mAAT-1		
(N)	10	5
a	1.000	1.000
ADA-1		
(N)	10	5
a	.100	.000
b	.200	.000
c	.000	.000
d	.000	.000
e	.000	.000
f	.700	1.000
g	.000	.000
h	.000	.000
i	.000	.000
j	.000	.000
k	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
ADH-1		
(N)	5	5
a	.900	.300
b	.100	.700
AK-1		
(N)	10	5
a	1.000	1.000
b	.000	.000
FBALD-1		
(N)	10	5
a	1.000	1.000
CK-1		
(N)	10	5
a	1.000	1.000
CK-2		
(N)	10	5
a	1.000	1.000
b	.000	.000
c	.000	.000
d	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
EST-1		
(N)	10	5
a	1.000	.000
b	.000	.000
c	.000	1.000
e	.000	.000
FBP-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
c	.000	.000
GAM-1		
(N)	10	5
a	1.000	1.000
GAPDH-1		
(N)	10	5
a	1.000	1.000
GDA-1		
(N)	9	5
a	.833	.000
b	.167	1.000
c	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
PROT-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
PROT-2		
(N)	9	5
a	.000	.000
b	1.000	1.000
GPI-A		
(N)	9	5
a	.056	.000
b	.944	.000
c	.000	1.000
GPI-B		
(N)	9	5
a	1.000	1.000
b	.000	.000
G3PDH		
(N)	9	5
a	1.000	1.000
b	.000	.000
c	.000	.000
d	.000	.000
e	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
mIDHP-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
LDH-A		
(N)	9	5
a	1.000	1.000
b	.000	.000
c	.000	.000
LDH-B		
(N)	9	5
a	1.000	1.000
b	.000	.000
LDH-C		
(N)	10	5
a	1.000	1.000
b	.000	.000
sMDH-A		
(N)	9	5
a	.667	1.000
b	.333	.000
c	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
sMDH-B		
(N)	9	5
a	1.000	1.000
b	.000	.000
mMDH-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
c	.000	.000
mMEP-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
sMEP-2		
(N)	9	5
a	1.000	1.000
b	.000	.000
MPI-1		
(N)	5	5
a	1.000	1.000
PEPA-1		
(N)	9	5
a	.833	1.000
b	.167	.000
c	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
PEPB-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
PEPC-1		
(N)	9	5
a	.944	1.000
b	.000	.000
c	.000	.000
d	.056	.000
e	.000	.000
PEPD-1		
(N)	9	5
a	1.000	1.000
b	.000	.000
PGM-1		
(N)	10	5
a	1.000	1.000
b	.000	.000

TABLE 5. (continued).

POPULATION		
LOCUS	ONI	OAU
sIDDH-1		
(N)	10	5
a	.000	.000
b	.000	.000
c	1.000	1.000
d	.000	.000
PGD-1		
(N)	10	5
a	1.000	1.000
b	.000	.000
c	.000	.000
SOD-1		
(N)	10	5
a	.000	.000
b	1.000	1.000
TPI-1		
(N)	5	5
a	.300	1.000
b	.600	.000
c	.100	.000

TABLE 6. Enzyme loci among the tilapiine fish species of Zimbabwe that are diagnostic between at least one species pair[1].

Enzyme	Tilapia		Oreochromis						
			Indigenous					exotics	
	rendalli	sparrmanii	placidus	mortimeri	mossambicus	macrochir	andersonii	niloticus	aureus
sAAT-2	c[2]	a	a	b	ab	ab	b	ab	a
ADA-1	f	jk	aegh	ab	abcdei	abcdfi	abcdf	abf	f
AK-1	a	b	a	a	a	a	a	a	a
CK-2	d	e	b	a	ab	c	a	a	a
EST-1	d	e	a	a	ab	c	ab	a	c
GDA-1	c	c	ab	ab	a	ab	ab	ab	b
PROT-2	a	b	ab	a	ab	b	a	b	b
GPI-A	b	c	b	ac	ab	b	a	ab	c
GPI-B	b	a	a	ab	a	ab	ab	a	a
G3PDH-1	cd	e	a	ab	a	a	ab	a	a
sIDDH-1	d	c	abc	ab	ab	acd	ab	c	c
mIDPH-1	a	b	a	a	a	a	a	a	a
LDH-A	a	c	a	ab	ab	a	a	a	a
LDH-B	a	a	a	a	ab	b	a	a	a
LDH-C	b	b	a	a	a	a	a	a	a
sMDH-A	a	a	ab	ab	ab	abc	b	ab	a
sMDH-B	b	b	a	a	a	a	a	a	a
mMDH-1	c	b	a	a	ab	b	a	a	a
mMEP-1	a	a	b	a	a	b	a	a	a
sMEP-1	a	a	a	a	a	a	b	a	a
PEPB-1	b	a	a	a	a	a	a	a	a
PEPD-1	b	a	a	a	a	a	a	a	a
PGD-1	b	c	a	a	a	a	a	a	a
TPI-1	b	a	b	a	ab	a	a	abc	a

1* sAAT-1, mAAT-1, FBALD-1, ADH-1, CK-1, FBP-1, GAM-1, GAPDH-1, PROT-1, MPI-1, PEPA-1, PEPC-1, PGM-1 and SOD-1 did not exhibit diagnostic mobilities.

2** mobilities are based on "a" as the fastest, "b" as the second fastest, etc.

TABLE 7a. Loci revealing fixed allelic differences between O. mossambicus and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Tilapia		Oreochromis					
		Indigenous				Exotics	
rendalli	sparrmanii	macrochir	placidus	andersonii	mortimeri	niloticus	aureus
sAAT-2	ADA-1	EST-1	mMEP-1	sMEP-1		sIDDH-1	ADA-1
ADA-1	AK-1	mMEP-1					EST-1
CK-2	CK-2						GDA-1
EST-1	EST-1						GPI-A
GDA-1	GDA-1						sIDDH-1
GPI-B	GPI-A						
G3PDH-1	G3PDH-1						
sIDDH-1	sIDDH-1						
LDH-C	mIDPH-1						
sMDH-B	LDH-A						
mMDH-1	LDH-C						
PEPB-1	sMDH-B						
PEPD-1	PGD-1						
PED-1							

TABLE 7b. Loci revealing fixed allelic differences between O. mortimeri and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Tilapia		Oreochromis					
		Indigenous				Exotics	
rendalli	sparrmanii	macrochir	placidus	andersonii	mossambicus	niloticus	aureus
sAAT-2	sAAT-2	CK-2	sAAT-2	sMEP-1		PROT-2	sAAT-2
ADA-1	ADA-1	EST-1	CK-2			sIDDH-1	ADA-1
CK-2	AK-1	PROT-2					EST-1
EST-1	CK-2	GPI-A					PROT-2
GDA-1	EST-1	LDH-B					sIDDH-1
GPI-A	GDA-1	mMDH-1					
G3PDH-1	PROT-2	mMEP-1					
sIDDH-1	G3PDH-1						
LDH-C	sIDDH-1						
sMDH-B	mIDPH-1						
mMDH-1	LDH-A						
PEPB-1	LDH-C						
PEPD-1	sMDH-B						
PGD-1	mMDH-1						
TPI-1	PGD-1						

TABLE 7c. Loci revealing fixed allelic differences between O. andersonii and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Tilapia		Oreochromis					
		Indigenous				Exotics	
rendalli	sparrmanii	macrochir	placidus	mossambicus	mortimeri	niloticus	aureus
sAAT-2	sAAT-2	CK-2	sAAT-2	sMEP-1	sMEP-1	PROT-2	sAAT-2
CK-2	ADA-1	EST-1	CK-2			sIDDH-1	EST-1
EST-1	AK-1	PROT-2	GPI-A			sMEP-1	GPI-A
GDA-1	CK-2	GPI-A	mMEP-1				PROT-2
GPI-A	EST-1	LDH-B	sMEP-1				sIDDH-1
G3PDH-1	GDA-1	mMDH-1	TPI-1				sMDH-A
sIDDH-1	GPI-A	mMEP-1					sMEP-1
LDH-C	PROT-2	sMEP-1					
sMDH-A	G3PDH-1						
sMDH-B	mIDDH-1						
mMDH-1	mIDPH-1						
sMEP-1	LDH-A						
PEPB-1	LDH-C						
PEPD-1	sMDH-B						
PGD-1	mMDH-1						
TPI-1	sMEP-1						
	PGD-1						

TABLE 7d. Loci revealing fixed allelic differences between O. placidus and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Talapia		Oreochromis					
		Indigenous				Exotics	
rendalli	sparrmanii	macrochir	mossambicus	mortimeri	andersonii	niloticus	aureus
sAAT-2	ADA-1	EST-1	mMEP-1	sAAT-2	sAAT-2	CK-2	ADA-1
ADA-1	AK-1	LDH-B		CK-2	CK-2	mMEP-1	CK-2
CK-2	CK-2	mMDH-1		GDA-1	GDA-1		EST-1
EST-1	EST-1	TPI-1		mMEP-1	mMEP-1		GPI-A
GDA-1	GDA-1			TPI-1	sMEP-1		mMEP-1
GPI-B	GPI-A				TPI-1		TPI-1
G3PDH-1	G3PDH-1						
sIDDH-1	mIDPH-1						
LDH-C	LDH-A						
sMDH-B	LDH-C						
mMDH-1	sMDH-B						
mMEP-1	mMDH-1						
PEPB-1	mMEP-1						
PEPD-1	PGD-1						
PGD-1	TPI-1						

TABLE 7e. Loci revealing fixed allelic differences between *O. macrochir* and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Tilapia		Oreochromis					
		Indigenous				Exotics	
rendalli	*sparrmanii*	*placidus*	*mossambicus*	*mortimeri*	*andersonii*	*niloticus*	*aureus*
sAAT-2	ADA-1	EST-1	EST-1	CK-2	CK-2	CK-2	CK-2
CK-2	AK-1	LDH-B	mMEP-1	EST-1	EST-1	EST-1	GPI-A
EST-1	CK-2	mMDH-1		PROT-2	PROT-2	LDH-B	LDH-B
GDA-1	EST-1	TPI-1		GPI-A	GPI-A	mMDH-1	mMDH-1
PROT-2	GDA-1			LDH-B	LDH-B	mMEP-1	mMEP-1
G3PDH-1	GPI-A			mMDH-1	mMDH-1		
LDH-B	G3PDH-1			mMEP-1	mMEP-1		
LDH-C	mIDPH-1				sMEP-1		
sMDH-B	LDH-A						
mMDH-1	LDH-B						
mMEP-1	LDH-C						
PEPB-1	sMDH-B						
PEPD-1	mMEP-1						
PGD-1	PGD-1						
TPI-1							

TABLE 7f. Loci revealing fixed allelic differences between *T. rendalli* and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Tilapia	*Oreochromis*						
	Indigenous					Exotics	
sparrmanii	*macrochir*	*placidus*	*mossambicus*	*mortimeri*	*andersonii*	*niloticus*	*aureus*
sAAT-2	sAAT-2	sAAT-2	sAAT-2	sAAT-2	sAAT-2	sAAT-2	sAAT-2
ADA-1	CK-2	ADA-1	ADA-1	ADA-1	ADA-1	CK-2	CK-2
AK-1	EST-1	CK-2	CK-2	CK-2	CK-2	EST-1	EST-1
CK-2	GDA-1	EST-1	EST-1	EST-1	EST-1	GDA-1	GDA-1
EST-1	PROT-2	GDA-1	GDA-1	GDA-1	GDA-1	PROT-2	PROT-2
PROT-2	G3PDH-1	GPI-B	GPI-B	GPI-B	GPI-B	GPI-B	GPI-A
GPI-A	LDH-B	G3PDH-1	G3PDH-1	G3PDH-1	G3PDH-1	G3PDH-1	GPI-B
GPI-B	LDH-C	sIDDH-1	sIDDH-1	sIDDH-1	sIDDH-1	sIDDH-1	G3PDH-1
G3PDH-1	sMDH-B	LDH-C	LDH-C	LDH-C	LDH-C	LDH-C	sIDDH-1
sIDDH-1	mMDH-1	sMDH-B	sMDH-B	sMDH-B	sMDH-A	sMDH-B	LDH-C
mIDPH-1	mMEP-1	mMDH-1	mMDH-1	mMDH-1	sMDH-B	mMDH-1	sMDH-B
LDH-A	PEPB-1	mMEP-1	PEPB-1	PEPB-1	mMDH-1	PEPB-1	mMDH-1
mMDH-1	PEPD-1	PEPB-1	PEPD-1	PEPD-1	sMEP-1	PEPD-1	PEPB-1
PEPB-1	PGD-1	PEPD-1	PGD-1	PGD-1	PEPB-1	PGD-1	PEPD-1
PEPD-1	TPI-1	PGD-1		TPI-1	PEPD-1		PGD-1
PGD-1					PGD-1		TPI-1
TPI-1					TPI-1		

TABLE 7g. Loci revealing fixed allelic differences between *T. sparrmanii* and other tilapiine fishes indigenous to Zimbabwe + two exotics.

Tilapia	*Oreochromis*						
	Indigenous					Exotics	
rendalli	*macrochir*	*placidus*	*mossambicus*	*mortimeri*	*andersonii*	*niloticus*	*aureus*
sAAT-2	ADA-1	ADA-1	ADA-1	sAAT-2	sAAT-2	ADA-1	ADA-1
ADA-1	AK-1	AK-1	AK-1	ADA-1	ADA-1	AK-1	AK-1
AK-1	CK-2	CK-2	CK-2	CK-2	AK-1	CK-2	CK-2
CK-2	EST-1	EST-1	EST-1	EST-1	CK-2	EST-1	EST-1
EST-1	GDA-1	GDA-1	GDA-1	GDA-1	EST-1	GDA-1	GDA-1
PROT-2	GPI-A	GPI-A	GPI-A	PROT-2	GDA-1	GPI-A	G3PDH-1
GPI-A	G3PDH-1	G3PDH-1	G3PDH-1	G3PDH-1	GPI-A	G3PDH-1	mIDPH-1
GPI-B	mIDPH-1	mIDPH-1	sIDDH-1	sIDDH-1	PROT-2	mIDPH-1	LDH-A
G3PDH-1	LDH-A	LDH-A	mIDPH-1	mIDPH-1	G3PDH-1	LDH-A	LDH-C
sIDDH-1	LDH-B	LDH-C	LDH-A	LDH-A	sIDDH-1	LDH-C	sMDH-B
mIDPH-1	LDH-C	mMDH-1	LDH-C	LDH-C	mIDPH-1	sMDH-B	mMDH-1
LDH-A	sMDH-B	sMDH-B	sMDH-B	sMDH-B	LDH-A	mMDH-1	PGD-1
mMDH-1	mMEP-1	mMEP-1	PGD-1	mMDH-1	LDH-C	PGD-1	
PEPB-1	PGD-1	PGD-1		PGD-1	sMDH-A		
PEPD-1		TPI-1			sMDH-B		
PGD-1					mMDH-1		
TPI-1					sMEP-1		
					PGD-1		

TABLE 7h. Loci revealing fixed allelic differences between O. niloticus and tilapiine fishes indigenous to Zimbabwe + O. aureus.

Tilapia		Oreochromis					
		Indigenous					Exotic
rendalli	sparrmanii	macrochir	placidus	mossambicus	mortimeri	andersonii	aureus
sAAT-2	ADA-1	CK-2	CK-2	sIDDH-1	PROT-2	PROT-2	EST-1
CK-2	AK-1	EST-1	mMEP-1		sIDDH-1	sIDDH-1	GPI-A
EST-1	CK-2	LDH-B					
GDA-1	EST-1	mMDH-1					
PROT-2	GDA-1	mMEP-1					
GPI-B	GPI-A						
G3PDH-1	G3PDH-1						
sIDDH-1	mIDPH-1						
LDH-C	LDH-A						
sMDH-B	LDH-C						
mMDH-1	sMDH-B						
PEPB-1	mMDH-1						
PEPD-1	PGD-1						
PED-1							

190

TABLE 7i. Loci revealing fixed allelic differences between *O. aureus* and tilapiine fishes indigenous to Zimbabwe + *O. niloticus*.

Tilapia		Oreochromis					
		Indigenous					Exotic
rendalli	*sparrmanii*	*macrochir*	*placidus*	*mossambicus*	*mortimeri*	*andersonii*	*niloticus*
sAAT-2	ADA-1	CK-2	ADA-1	ADA-1	ADA-1	sAAT-2	EST-1
CK-2	AK-1	GPI-A	CK-2	EST-1	sAAT-2	EST-1	GPI-A
EST-1	CK-2	LDH-B	EST-1	GDA-1	EST-1	PROT-2	
GDA-1	EST-1	mMDH-1	GPI-A	GPI-A	PROT-2	GPI-A	
PROT-2	GDA-1	mMEP-1	mMEP-1	sIDDH-1	sIDDH-1	sIDDH-1	
GPI-B	G3PDH-1		TPI-1			sMDH-A	
G3PDH-1	mIDPH-1					sMEP-1	
sIDDH-1	LDH-A						
LDH-C	LDH-C						
sMDH-B	sMDH-B						
mMDH-1	mMDH-1						
PEPB-1	PGD-1						
PEPD-1							
PED-1							
TPI-1							

TABLE 8. Results from Chi-square/G-tests for conformance to Hardy-Weinberg proportions for all polymormophic loci from 27 tilapiine populations collected in Zimbabwe river systems and two exotic species.

Population Name	# Polymorphic loci	# deviant	# deviant G-test	Loci deviant
TR1 Tokwe River	2			
TS1 U. Zambezi River	3			
OP1 Save/Runde Jct	7	1	1	sIDDH-1
OMO1 Sinamatella River	2			
OMO2 Mandavu Dam	3			
OMO3 Deka River	2			
OMO4 Dolilo Saltpan	4			
OMO5 Gwayi River	9	2	2	PEPA-1, PEPC-1
OMO6 Mana Pools	4			
OMO7 Mlibizi	5			
OMO8 Sinamwenda	4			
OMO9 Kariba	4			
OMS1 Gwavazabuya River	6			
OMS2 Amanzamnyama River	3			
OMS3 Beitbridge Dam	8			
OMS4 Mwenezi River	6			
OMS5 Tokwe River	4			
OMS6 Runde River	4			
OMS7 Ruti Dam	9			
OMS8 Save/Runde Jct	6			
OMS9 Budzi River	5			
OMS10 Mazoe River	5	1	1	TPI-1
OMA1 Hippo River	5			
OMA2 Katombora Rapids	6	1		sMDH-A
OMA3 Udu Dam	6	1	1	ADA-1

TABLE 8. (continued).

Population Name	# Polymorphic loci	# deviant	# deviant G-test	Loci deviant
OAN1 Hippo River	7	1		EST-1
OAN2 Katombora Rapids	5	1	1	GDA-1
ON1 Edwards Farm	9	1		GDA-1
OAU Edwards Farm	1			

TABLE 9. Genetic variability at 38 loci in all 29 populations (standard errors in parentheses).

Population	Mean sample size/locus	Mean no. of alleles/locus	Percentage of loci polymorphic	Mean heterozygosity Direct count	Mean heterozygosity Hdy Wbg expected
1 TR1 TOKWE	16.7	1.1	5.3	.008	.007
	(.8)	(.0)		(.006)	(.005)
2 TS1 U ZAMBEZI	3.9	1.1	7.9	.033	.035
	(.1)	(.0)		(.021)	(.020)
3 OP1 SRJCT	18.1	1.3	15.8	.035	.041
	(.5)	(.1)		(.014)	(.017)
4 OMO1 SINAMATELLA	9.9	1.1	5.3	.016	.023
	(.1)	(.0)		(.012)	(.016)
5 OMO2 MANDAVU DAM	3.9	1.1	7.9	.026	.028
	(.1)	(.0)		(.016)	(.017)
6 OMO3 DEKA	15.5	1.1	5.3	.026	.024
	(.7)	(.0)		(.018)	(.017)
7 OMO4 DOLILO	10.0	1.1	10.5	.032	.030
	(.0)	(.1)		(.019)	(.018)
8 OMO5 GWAYI	22.3	1.3	21.1	.044	.065
	(.8)	(.1)		(.018)	(.023)
9 OMO6 MANAPOOLS	10.0	1.1	10.5	.029	.035
	(.0)	(.1)		(.016)	(.018)
10 OMO7 MLIBIZI	5.9	1.1	13.2	.041	.039
	(.3)	(.1)		(.019)	(.018)
11 OMO8 SINAMWENDA	5.0	1.1	10.5	.026	.042
	(.0)	(.1)		(.013)	(.021)
12 OMO9 KARIBA	13.4	1.1	5.3	.021	.027
	(.3)	(.1)		(.012)	(.016)

TABLE 9. (continued).

Population	Mean sample size/locus	Mean no. of alleles/locus	Percentage of loci polymorphic	Mean heterozygosity Direct count	Mean heterozygosity Hdy Wbg expected
13 OMS1 GWAVAZABUYA	9.9	1.2	15.8	.045	.057
	(.1)	(.1)		(.022)	(.025)
14 OMS2 AMANZAMNYAMA	16.3	1.1	7.9	.033	.032
	(.8)	(.0)		(.020)	(.018)
15 OMS3 BEITBRIDGE	10.0	1.2	21.1	.042	.047
	(.0)	(.1)		(.016)	(.019)
16 OMS4 MWENEZI	9.8	1.2	15.8	.047	.046
	(.2)	(.1)		(.023)	(.023)
17 OMS5 TOKWE	7.0	1.1	10.5	.030	.036
	(.0)	(.1)		(.015)	(.018)
18 OMS6 RUNDE	9.8	1.2	10.5	.032	.031
	(.2)	(.1)		(.020)	(.020)
19 OMS7 RUTI DAM	5.0	1.3	23.7	.100	.096
	(.0)	(.1)		(.034)	(.031)
20 OMS8 SRJCT	10.0	1.2	15.8	.053	.054
	(.0)	(.1)		(.022)	(.023)
21 OMS9 BUDZI	13.0	1.2	13.2	.027	.026
	(.4)	(.1)		(.013)	(.012)
22 OMS10 MAZOE	13.2	1.1	7.9	.024	.035
	(.4)	(.1)		(.014)	(.013)
23 OMA1 HIPPO	4.9	1.2	13.2	.077	.066
	(.3)	(.1)		(.035)	(.030)
24 OMA2 KATOMBORA	20.6	1.2	15.8	.047	.059
	(.6)	(.1)		(.020)	(.024)

TABLE 9. (continued).

Population	Mean sample size/locus	Mean no. of alleles/locus	Percentage of loci polymorphic	Mean heterozygosity Direct count	Mean heterozygosity Hdy Wbg expected
25 OMA3 UDU DAM	19.7 (1.7)	1.3 (.1)	13.2	.053 (.025)	.061 (.027)
26 OAN1 HIPPO	7.3 (.2)	1.3 (.1)	18.4	.073 (.033)	.067 (.028)
27 OAN2 KATOMBORA	15.8 (.7)	1.2 (.1)	10.5	.024 (.015)	.028 (.016)
28 ONI EDWARDS FARM	9.1 (.2)	1.3 (.1)	23.7	.044 (.015)	.070 (.025)
29 OAU EDWARDS FARM	5.0 (.0)	1.0 (.0)	2.6	.016 (.016)	.012 (.012)

TABLE 13. Matrix of Nei's (1978) unbiased genetic similarity/distance coefficients of 29 populations of 9 tilapiine fish species collected in Zimbabwe river systems.
Below diagonal: genetic distance
Above diagonal: genetic identity

Population			TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5
1	TR1	TOKWE	****	.530	.561	.551	.554	.561	.560	.564
2	TS1	U ZAMBEZI	.636	****	.534	.524	.526	.534	.534	.540
3	OP1	SRJCT	.577	.628	****	.788	.796	.796	.795	.789
4	OMO1	SINAMA	.596	.645	.238	****	1.000	.998	.999	.993
5	OMO2	MANDAVU	.591	.642	.228	.000	****	1.000	1.000	.990
6	OMO3	DEKA	.579	.627	.228	.002	.000	****	1.000	.993
7	OMO4	DOLILO	.580	.626	.229	.001	.000	.000	****	.993
8	OMO5	GWAYI	.572	.616	.237	.008	.010	.007	.007	****
9	OMO6	MANAPS	.549	.592	.224	.012	.012	.006	.006	.003
10	OMO7	MLIBIZI	.558	.608	.246	.010	.016	.012	.012	.003
11	OMO8	SINAMWENDA	.560	.605	.242	.008	.012	.007	.007	.004
12	OMO9	KARIBA	.561	.605	.234	.007	.006	.002	.002	.005
13	OMS1	GWAVAZABUYA	.559	.578	.194	.015	.012	.011	.011	.019
14	OMS2	AMANZAMNYAM	.562	.573	.197	.021	.018	.015	.015	.023
15	OMS3	BEITBRIDGE	.575	.593	.184	.017	.010	.012	.012	.028
16	OMS4	MWENEZI	.575	.588	.179	.038	.030	.031	.031	.049
17	OMS5	TOKWE	.572	.579	.180	.039	.030	.034	.033	.050
18	OMS6	RUNDE	.571	.574	.177	.052	.041	.042	.042	.061
19	OMS7	RUTI	.561	.576	.156	.036	.032	.032	.031	.044
20	OMS8	SRJCT	.522	.612	.151	.045	.034	.035	.035	.053
21	OMS9	BUDZI	.573	.586	.178	.040	.029	.030	.030	.048
22	OMS10	MAZOE	.554	.601	.166	.035	.026	.026	.026	.044
23	OMA1	HIPPO	.647	.518	.153	.280	.272	.270	.270	.286
24	OMA2	KATOMBORA	.623	.510	.176	.274	.274	.265	.266	.266
25	OMA3	UDU	.626	.520	.185	.288	.280	.276	.276	.277

TABLE 13. (continued).

Population			TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5
26	OAN1	HIPPO	.638	.689	.306	.052	.062	.061	.060	.049
27	OAN2	KATOMBORA	.622	.677	.309	.056	.068	.065	.064	.053
28	ONI	EDWARDS FARM	.482	.469	.147	.164	.165	.159	.158	.152
29	OAU	EDWARDS FARM	.525	.424	.216	.256	.252	.243	.244	.224

Population			OMO6	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4
1	TR1	TOKWE.	.577	.573	.571	.571	.572	.570	.563	.563
2	TS1	U ZAMBEZI	.553	.545	.546	.546	.561	.564	.553	.555
3	OP1	SRJCT	.799	.782	.785	.791	.824	.821	.832	.836
4	OMO1	SINAMA	.988	.990	.992	.993	.985	.979	.983	.962
5	OMO2	MANDAVU	.988	.984	.988	.994	.988	.983	.990	.971
6	OMO3	DEKA	.994	.988	.993	.998	.989	.985	.988	.970
7	OMO4	DOLILO	.994	.988	.993	.998	.989	.985	.988	.970
8	OMO5	GWAYI	.997	.997	.996	.995	.981	.977	.972	.952
9	OMO6	MANAPS	****	.994	.995	.999	.984	.982	.975	.958
10	OMO7	MLIBIZI	.006	****	1.000	.993	.979	.972	.964	.946
11	OMO8	SINAMWENDA	.005	.000	****	.997	.986	.978	.972	.957
12	OMO9	KARIBA	.001	.007	.003	****	.987	.985	.981	.963
13	OMS1	GWAVAZABUYA	.017	.021	.014	.013	****	.998	.997	.993
14	OMS2	AMANZAMNYAM	.018	.028	.022	.015	.002	****	.996	.989
15	OMS3	BEITBRIDGE	.025	.037	.028	.020	.003	.004	****	.995
16	OMS4	MWENEZI	.043	.055	.044	.037	.007	.011	.005	****
17	OMS5	TOKWE	.046	.059	.050	.040	.010	.009	.005	.007
18	OMS6	RUNDE	.053	.069	.057	.048	.013	.016	.010	.002
19	OMS7	RUTI	.041	.047	.038	.036	.007	.011	.008	.003

TABLE 13. (continued).

Population			OMO6	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4
20	OMS8	SRJCT	.045	.061	.049	.040	.020	.022	.017	.016
21	OMS9	BUDZI	.041	.058	.047	.036	.008	.007	.004	.003
22	OMS10	MAZOE	.037	.054	.043	.032	.009	.009	.005	.006
23	OMA1	HIPPO	.272	.287	.277	.271	.235	.236	.226	.232
24	OMA2	KATOMBORA	.252	.256	.250	.255	.237	.241	.245	.257
25	OMA3	UDU	.261	.279	.278	.271	.272	.276	.267	.288
26	OAN1	HIPPO	.061	.039	.042	.059	.066	.082	.086	.100
27	OAN2	KATOMBORA	.062	.039	.042	.060	.070	.085	.091	.106
28	ONI	EDWARDS FARM	.146	.141	.138	.148	.125	.129	.138	.137
29	OAU	EDWARDS FARM	.209	.221	.226	.229	.204	.206	.216	.213

Population			OMS5	OMS6	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2
1	TR1	TOKWE	.565	.565	.571	.593	.564	.575	.524	.536
2	TS1	U ZAMBEZI	.560	.563	.562	.542	.556	.548	.596	.600
3	OP1	SRJCT	.835	.838	.856	.860	.837	.847	.858	.839
4	OMO1	SINAMA	.961	.949	.965	.956	.961	.966	.756	.760
5	OMO2	MANDAVU	.971	.959	.969	.966	.971	.975	.762	.761
6	OMO3	DEKA	.967	.959	.969	.966	.971	.974	.763	.767
7	OMC4	DOLILO	.968	.958	.969	.966	.970	.974	.763	.766
8	OMO5	GWAYI	.951	.940	.957	.948	.953	.957	.751	.766
9	OMO6	MANAPS	.955	.948	.960	.956	.960	.964	.762	.777
10	OMO7	MLIBIZI	.943	.933	.954	.941	.943	.947	.750	.775
11	OMO8	SINAMWENDA	.951	.945	.962	.952	.954	.958	.758	.779

TABLE 13. (continued).

Population			OMS5	OMS6	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2
12	OMO9	KARIBA	.960	.953	.965	.961	.965	.968	.763	.775
13	OMS1	GWAVAZABUYA	.990	.987	.993	.980	.992	.991	.791	.789
14	OMS2	AMANZAMNYAM	.991	.984	.989	.978	.993	.991	.790	.786
15	OMS3	BEITBRIDGE	.995	.990	.992	.983	.996	.995	.797	.783
16	OMS4	MWENEZI	.993	.998	.997	.984	.997	.994	.793	.774
17	OMS5	TOKWE	****	.993	.989	.980	.997	.992	.801	.782
18	OMS6	RUNDE	.007	****	.990	.980	.995	.989	.800	.775
19	OMS7	RUTI	.011	.010	****	.991	.994	.997	.802	.789
20	OMS8	SRJCT	.020	.020	.009	****	.987	.997	.782	.768
21	OMS9	BUDZI	.003	.005	.006	.013	****	.999	.798	.779
22	OMS10	MAZOE	.008	.011	.003	.003	.001	****	.786	.771
23	OMA1	HIPPO	.222	.223	.221	.246	.226	.240	****	.990
24	OMA2	KATOMBORA	.245	.255	.238	.264	.249	.260	.010	****
25	OMA3	UDU	.277	.286	.278	.288	.282	.290	.023	.023
26	OAN1	HIPPO	.110	.112	.090	.113	.113	.109	.339	.313
27	OAN2	KATOMBORA	.116	.115	.096	.118	.119	.115	.345	.315
28	ONI	EDWARDS FARM	.128	.135	.107	.125	.139	.134	.195	.186
29	OAU	EDWARDS FARM	.204	.205	.203	.231	.215	.225	.189	.183

Population			OMA3	OAN1	OAN2	ONI	OAU
1	TR1	TOKWE	.535	.528	.537	.617	.591
2	TS1	U ZAMBEZI	.594	.502	.508	.626	.654
3	OP1	SRJCT	.831	.736	.734	.863	.806
4	OMO1	SINAMA	.750	.949	.945	.849	.774
5	OMO2	MANDAVU	.756	.940	.935	.848	.777
6	OMO3	DEKA	.759	.941	.937	.853	.784

TABLE 13. (continued).

Population			OMA3	OAN1	OAN2	ONI	OAU
7	OMO4	DOLILO	.759	.942	.938	.854	.784
8	OMO5	GWAYI	.758	.952	.948	.859	.799
9	OMO6	MANAPS	.770	.941	.940	.864	.811
10	OMO7	MLIBIZI	.756	.962	.962	.868	.802
11	OMO8	SINAMWENDA	.757	.959	.959	.871	.798
12	OMO9	KARIBA	.762	.943	.942	.863	.795
13	OMS1	GWAVAZABUYA	.762	.936	.932	.882	.815
14	OMS2	AMANZAMNYAM	.759	.921	.918	.879	.814
15	OMS3	BEITBRIDGE	.765	.918	.913	.871	.805
16	OMS4	MWENEZI	.750	.905	.900	.872	.808
17	OMS5	TOKWE	.758	.896	.890	.880	.815
18	OMS6	RUNDE	.751	.894	.891	.874	.815
19	OMS7	RUTI	.758	.914	.909	.899	.816
20	OMS8	SRJCT	.750	.893	.889	.882	.793
21	OMS9	BUDZI	.755	.893	.888	.870	.806
22	OMS10	MAZOE	.748	.896	.891	.875	.798
23	OMA1	HIPPO	.978	.731	.708	.823	.828
24	OMA2	KATOMBORA	.978	.731	.730	.830	.832
25	OMA3	UDU	****	.712	.707	.814	.840
26	OAN1	HIPPO	.340	****	.996	.831	.961
27	OAN2	KATOMBORA	.346	.004	****	.835	.760
28	ONI	EDWARDS FARM	.206	.185	.180	****	.929
29	OAU	EDWARDS FARM	.175	.273	.274	.073	****

TABLE 14. Matrix of genetic similarity/distance coefficient averaged by species of Tilapiine fishes of Zimbabwe.

Below diagonal: Nei (1978) unbiased genetic identity
Above diagonal: Nei (1978) unbiased genetic distance

Species	No. of pops.	1	2	3	4	5
1. T. rendalli	1	******	.636 (.636-.636)	.577 (.577-.577)	.572 (.549-.596)	.562 (.522-.575)
2. T. sparmanii	1	.530 (.530-.530)	******	.628 (.628-.628)	.618 (.592-.645)	.586 (.573-.612)
3. O. placidus	1	.561 (.561-.561)	.534 (.434-.534)	******	.234 (.224-.246)	.176 (.151-.197)
4. O. mortimeri	9	.565 (.551-.577)	.539 (.524-.553)	.791 (.782-.799)	******	.035 (.010-.069)
5. O. mossambicus	10	.570 (.563-.593)	.557 (.542-.564)	.839 (.821-.860)	.966 (.933-.990)	******

TABLE 14. (continued).

Species	No. of pops.	6	7	8	9
1. *T. rendalli*	1	.632 (.623-.647)	.630. (.622-.638)	482. (.482-.482)	525 (.525-.525)
2. *T. sparmanii*	1	.516 (.510-.520)	.683 (.677-.689)	.469 (.469-.469)	.424 (.424-.424)
3. *O. placidus*	1	.171 (.153-.185)	.308 (.308-.309)	.147 (.147-.147)	.216 (.216-.216)
4. *O. mortimeri*	9	.272 (.250-.288)	.055 (.039-.068)	.152 (.138-.165)	.234 (.209-.256)
5. *O. mossambicus*	10	.254 (.221-.290)	.101 (.066-.119)	.130 (.107-.139)	.212 (.203-.231)

TABLE 14. (continued).

Species	No. of pops.	1	2	3	4	5
6. O. macrochir	3	.532 (.524-.536)	.597 (.594-.600)	.842 (.831-.858)	.762 (.750-.779)	.776 (.748-.802)
7. O. andersonii	2	.533 (.528-.537)	.505 (.542-.548)	.735 (.734-.736)	.946 (.935-.962)	.904 (.888-.936)
8. O. niloticus	1	.617 (.617-.617)	.626 (.526-.626)	.863 (.863-.863)	.859 (.848-.871)	.878 (.870-.899)
9. O. aureus	1	.591 (.591-.591)	.654 (.654-.654)	.806 (.806-.806)	.792 (.774-.811)	.809 (.793-.816)

TABLE 14. (continued).

Species	No. of pops.	6	7	8	9
6. O. macrochir	3	******	.333 (.313-.346)	.196 (.186-.206)	.182 (.175-.189)
7. O. andersonii	2	.717 (.707-.731)	******	.183 (.108-.185)	.273 (.273-.274)
8. O. niloticus	1	.822 (.814-.830)	.833 (.831-.835)	******	.075 (.075-.075)
9. O. aureus	1	.833 (.828-.840)	.761 (.760-.761)	.929 (.929-.929)	******

TABLE 15. Rogers (1972) genetic distance coefficients of 29 populations of 9 tilapiine fish species collected in Zimbabwe river systems.

Population			TR1	TS1	OP1	OMO1	OMO2	OMO3	OMO4	OMO5
1	TR1	TOKWE	****							
2	TS1	U ZAMBEZI	.477	****						
3	OP1	SRJCT	.445	.478	****					
4	OMO1	SINAMA	.455	.488	.233	****				
5	OMO2	MANDAVU	.453	.485	.223	.013	****			
6	OMO3	DEKA	.446	.478	.224	.009	.012	****		
7	OMO4	DOLILO	.448	.478	.225	.011	.011	.004	****	
8	OMO5	GWAYI	.448	.478	.243	.037	.040	.036	.035	****
9	OMO6	MANAPS	.428	.460	.222	.033	.036	.024	.028	.025
10	OMO7	MLIBIZI	.433	.468	.242	.029	.040	.035	.037	.034
11	OMO8	SINAMWENDA	.437	.468	.240	.029	.032	.027	.029	.032
12	OMO9	KARIBA	.436	.466	.230	.022	.023	.013	.013	.030
13	OMS1	GWAVAZABUYA	.437	.456	.207	.038	.041	.029	.033	.062
14	OMS2	AMANZAMNYAM	.436	.452	.203	.036	.038	.026	.030	.058
15	OMS3	BEITBRIDGE	.445	.463	.197	.047	.038	.038	.039	.073
16	OMS4	MWENEZI	.444	.457	.188	.067	.057	.058	.059	.093
17	OMS5	TOKWE	.442	.449	.189	.064	.051	.055	.056	.090
18	OMS6	RUNDE	.438	.444	.182	.074	.061	.066	.066	.101
19	OMS7	RUTI	.452	.464	.191	.079	.077	.071	.071	.104
20	OMS8	SRJCT	.414	.471	.169	.078	.065	.069	.070	.104
21	OMS9	BUDZI	.440	.454	.184	.062	.051	.053	.054	.088
22	OMS10	MAZOE	.432	.465	.180	.062	.055	.053	.055	.088
23	OMA1	HIPPO	.483	.423	.172	.262	.260	.253	.255	.278
24	OMA2	KATOMBORA	.467	.418	.190	.267	.265	.257	.259	.264
25	OMA3	UDU	.470	.422	.192	.272	.270	.263	.265	.269
26	OAN1	HIPPO	.478	.509	.290	.087	.097	.096	.095	.091
27	OAN2	KATOMBORA	.465	.497	.281	.077	.087	.086	.085	.086
28	ONI	EDWARDS FARM	.401	.396	.174	.182	.180	.173	.175	.176
29	OAU	EDWARDS FARM	.413	.353	.213	.239	.237	.230	.232	.231

TABLE 15. (continued).

Population			OMO6	OMO7	OMO8	OMO9	OMS1	OMS2	OMS3	OMS4
9	OMO6	MANAPS	****							
10	OMO7	MLIBIZI	.028	****						
11	OMO8	SINAMWENDA	.030	.019	****					
12	OMO9	KARIBA	.015	.032	.024	****				
13	OMS1	GWAVAZABUYA	.048	.052	.040	.037	****			
14	OMS2	AMANZAMNYAM	.037	.057	.049	.028	.019	****		
15	OMS3	BEITBRIDGE	.054	.069	.060	.043	.026	.027	****	
16	OMS4	MWENEZI	.073	.085	.073	.063	.039	.037	.031	****
17	OMS5	TOKWE	.069	.083	.075	.060	.042	.036	.027	.036
18	OMS6	RUNDE	.079	.093	.082	.071	.047	.044	.035	.022
19	OMS7	RUTI	.091	.097	.085	.078	.051	.056	.057	.044
20	OMS8	SRJCT	.084	.095	.084	.074	.050	.052	.047	.046
21	OMS9	RUDZI	.068	.080	.071	.058	.038	.032	.029	.023
22	OMS10	MAZOE	.068	.080	.072	.058	.039	.032	.033	.030
23	OMA1	HIPPO	.258	.270	.267	.258	.231	.232	.236	.234
24	OMA2	KATOMBORA	.240	.243	.243	.247	.232	.231	.240	.245
25	OMA3	UDU	.248	.264	.266	.256	.256	.259	.261	.272
26	OAN1	HIPPO	.093	.072	.077	.091	.104	.117	.123	.125
27	OAN2	KATOMBORA	.085	.059	.068	.081	.100	.107	.115	.121
28	ONI	EDWARDS FARM	.164	.160	.167	.167	.153	.155	.163	.161
29	OAU	EDWARDS FARM	.207	.217	.221	.220	.209	.204	.216	.210

TABLE 15. (continued).

Population	OMS5	OMS6	OMS7	OMS8	OMS9	OMS10	OMA1	OMA2
17 OMS5 TOKWE	****							
18 OMS6 RUNDE	.028	****						
19 OMS7 RUTI	.063	.060	****					
20 OMS8 SRJCT	.051	.045	.061	****				
21 OMS9 BUDZI	.025	.023	.050	.037	****			
22 OMS10 MAZOE	.036	.037	.043	.028	.018	****		
23 OMA1 HIPPO	.224	.222	.237	.249	.230	.246	****	
24 OMA2 KATOMBORA	.239	.248	.252	.255	.244	.251	.038	****
25 OMA3 UDU	.268	.270	.279	.273	.268	.276	.058	.059
26 OAN1 HIPPO	.141	.130	.141	.146	.137	.142	.304	.290
27 OAN2 KATOMBORA	.132	.119	.139	.137	.127	.132	.307	.290
28 ONI EDWARDS FARM	.151	.159	.147	.153	.160	.156	.211	.201
29 OAU EDWARDS FARM	.200	.196	.224	.227	.206	.219	.200	.194

Population	OMA3	OAN1	OAN2	ONI	OAU
25 OMA3 UDU	****				
26 OAN1 HIPPO	.310	****			
27 OAN2 KATOMBORA	.310	.032	****		
28 ONI EDWARDS FARM	.225	.207	.193	****	
29 OAU EDWARDS FARM	.187	.260	.251	.106	****

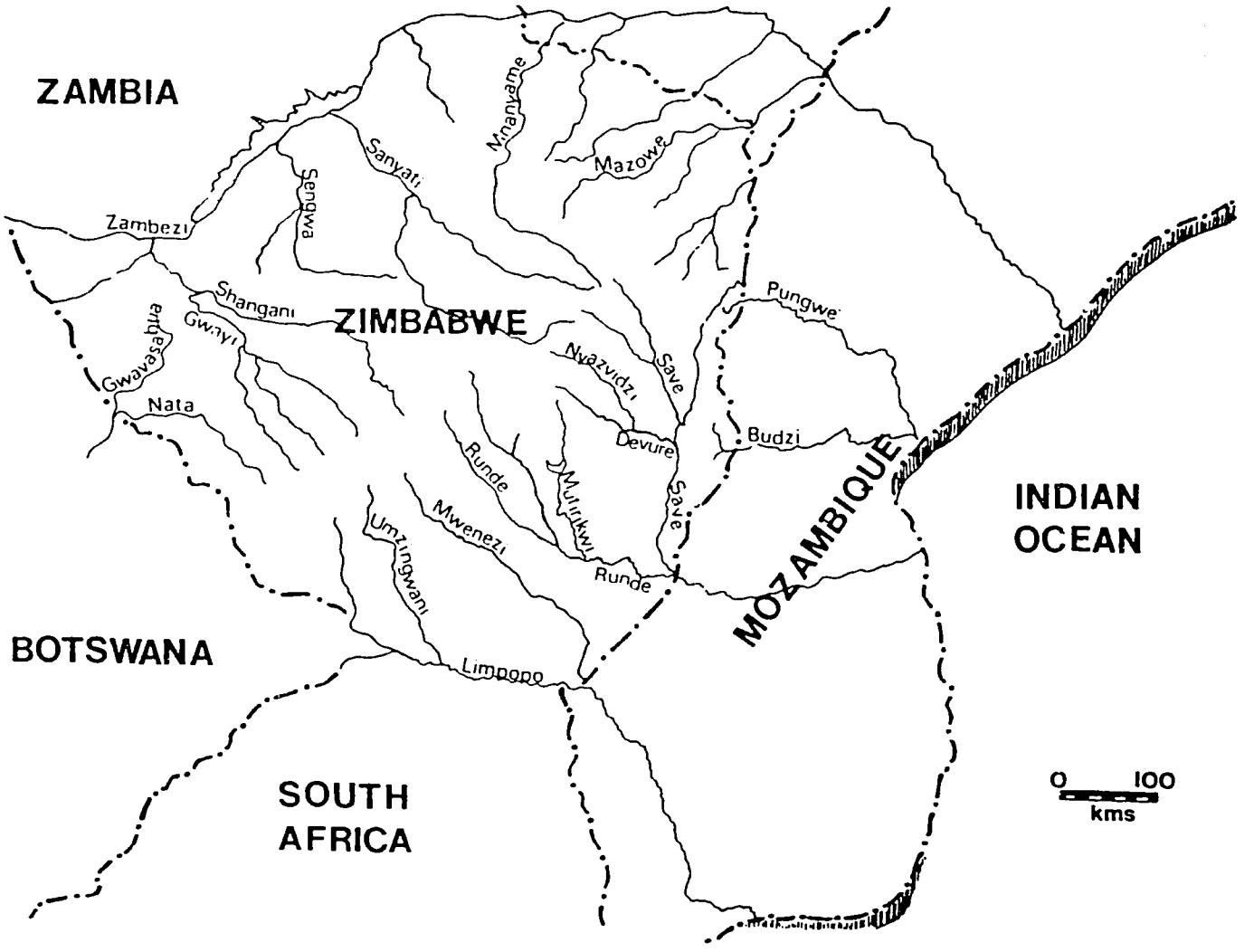
TABLE 16. Roger's (1972) genetic distance coefficients (averaged by species) among tilapiine fishes of Zimbabwe.

Species	No. of pops.	1	2	3	4	5
1. *T. rendalli*	1	******				
2. *T. sparmanii*	1	.477 (.477-.477)	******			
3. *O. placidus*	1	.445 (.445-.445)	.478 (.478-.478)	******		
4. *O. mortimeri*	9	.443 (.428-.455)	.474 (.460-.488)	.231 (.222-.243)	******	
5. *O. mossambicus*	10	.438 (.414-.452)	.457 (.444-.471)	.189 (.169-.207)	.064 (.026-.104)	******
6. *O. macrochir*	3	.473 (.467-.483)	.421 (.418-.423)	.185 (.172-.192)	.260 (.240-.278)	.249 (.222-.279)
7. *O. andersonii*	2	.471 (.465-.478)	.503 (.497-.509)	.286 (.281-.290)	.084 (.059-.097)	.127 (.100-.146)
8. *O. niloticus*	1	.401 (.401-.401)	.396 (.396-.396)	.174 (.174-.174)	.171 (.160-.182)	.156 (.147-.163)
9. *O. aureus*	1	.413 (.413-.413)	.353 (.353-.353)	.213 (.213-.213)	.226 (.207-.239)	.211 (.196-.227)

Table 16. (continued).

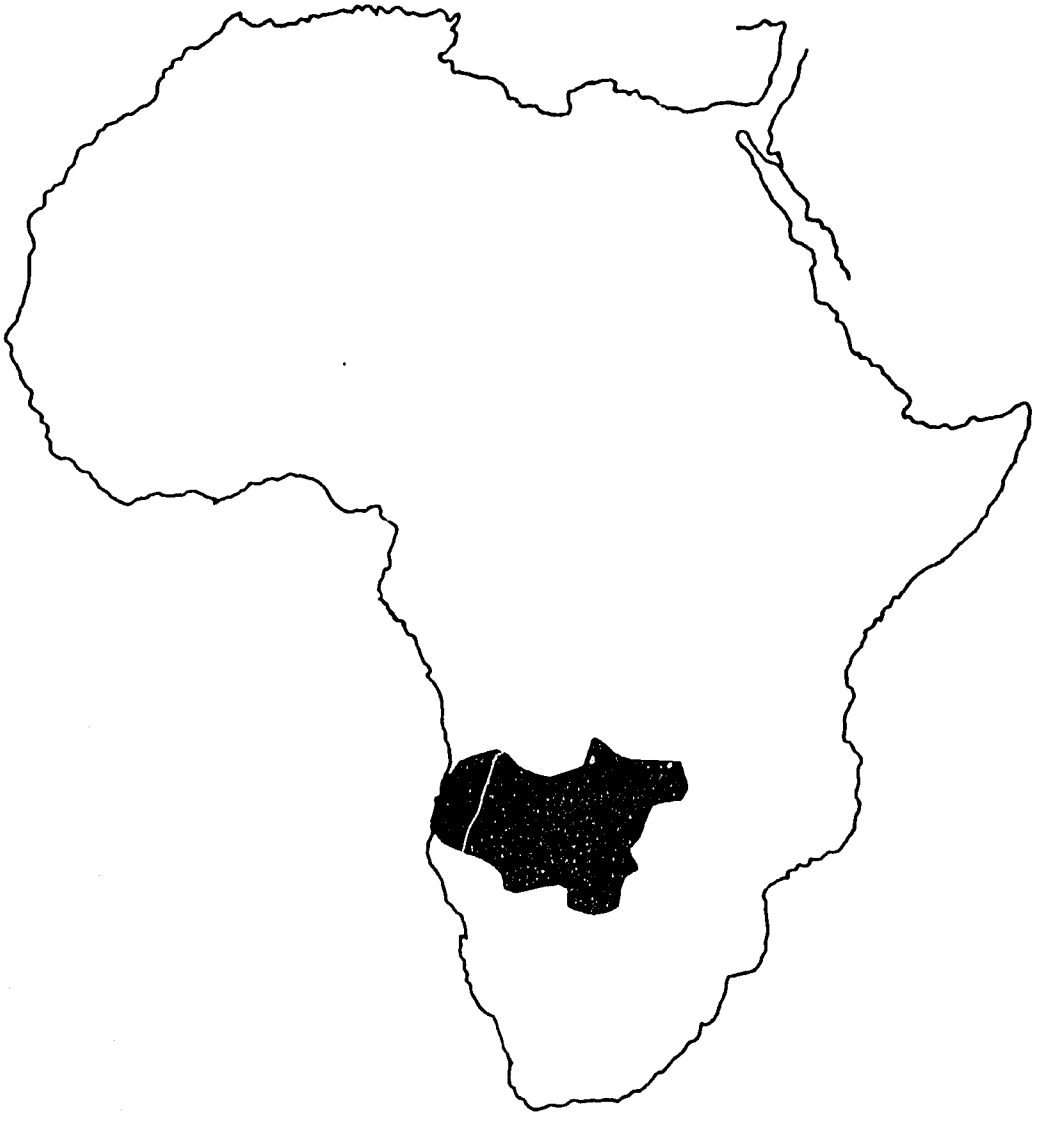
Species	No. of pops.	6	7	8	9
6. O. macrochir	3	******			
7. O. andersonii	2	.302 (.290-.310)	******		
8. O. niloticus	1	.212 (.201-.225)	.200 (.193-.207)	******	
9. O. aureus	1	.194 (.187-.200)	.255 (.251-.260)	.106 (.106-.106)	******

Figure 1. Map of Zimbabwe drainage systems indicating the at points at which they flow into the Indian Ocean.


ZAMBIA
ZIMBABWE
BOTSWANA
SOUTH AFRICA
MOZAMBIQUE
INDIAN OCEAN
Zambezi
Sengwa
Sanyati
Mnanyame
Mazowe
Shangani
Gwayi
Gwayasabia
Nata
Nyazvidzi
Save
Pungwe
Devure
Budzi
Runde
Mutirikwi
Mwenezi
Umzingwani
Limpopo
0 100
kms

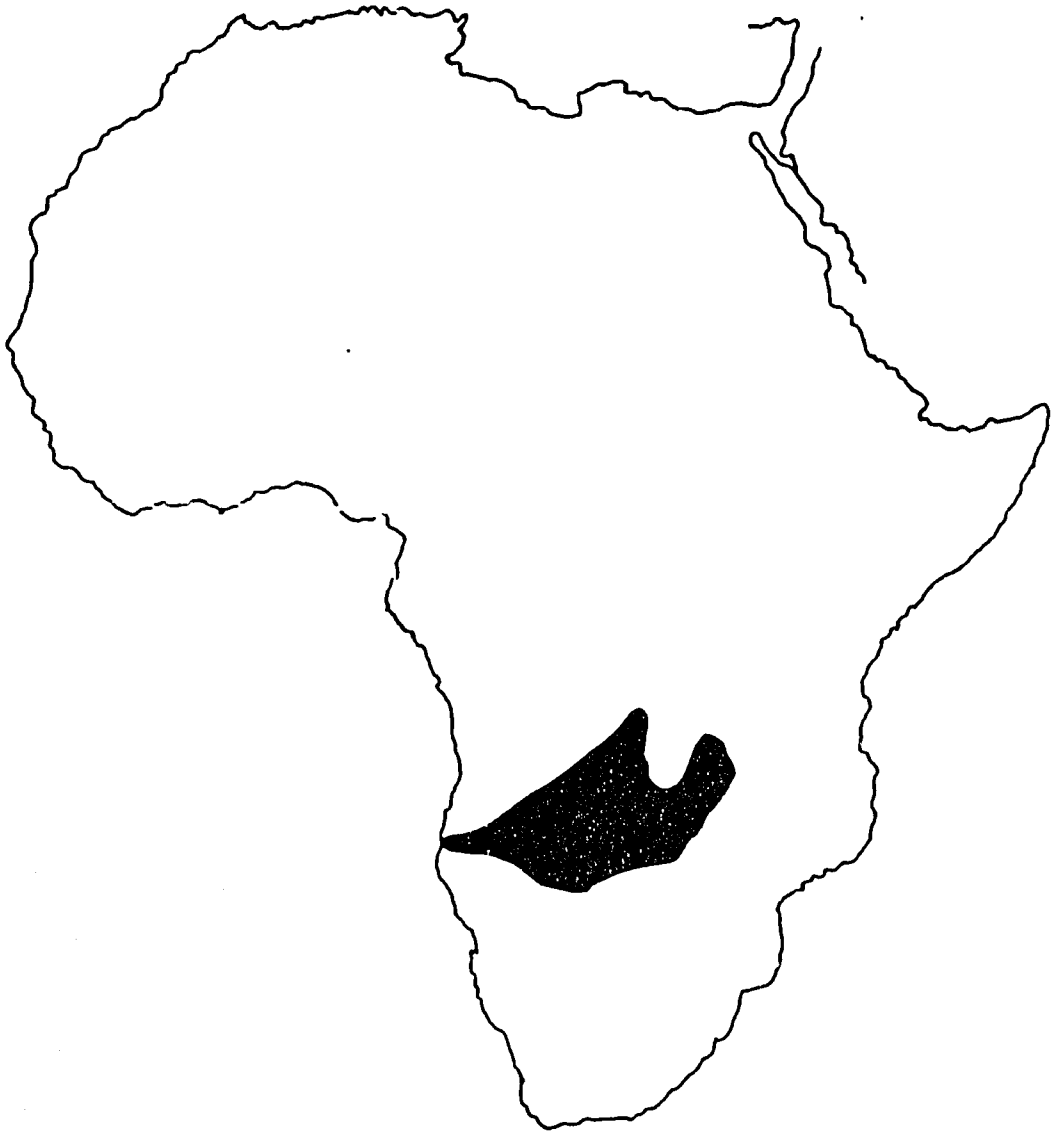
212

Figure 2. Map of Africa showing the natural distribution of *Oreochromis andersonii* (Trewavas 1982).



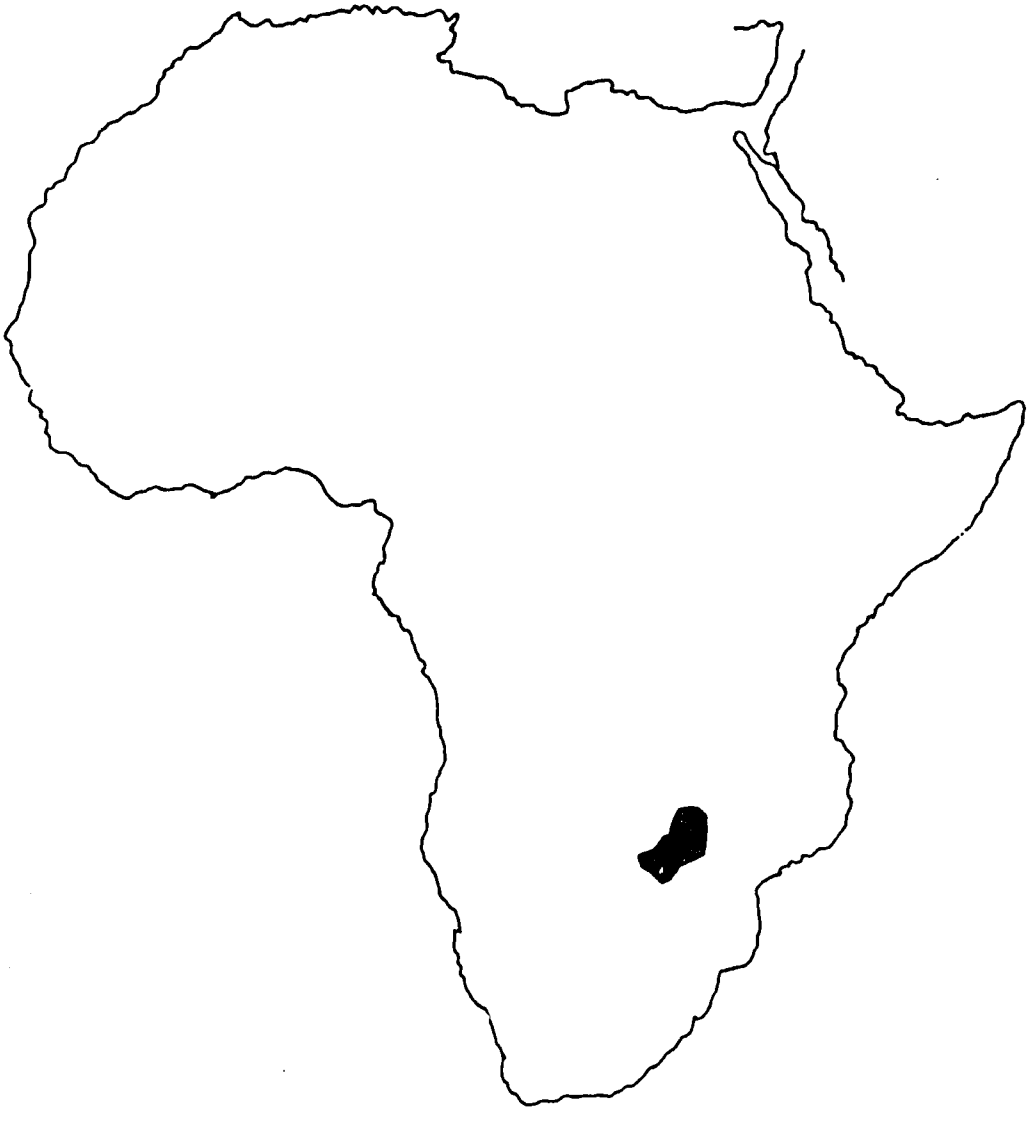
Natural Distribution of O. andersonii

Figure 3. Map of Africa showing the natural distribution of *Oreochromis macrochir* (Trewavas 1982).



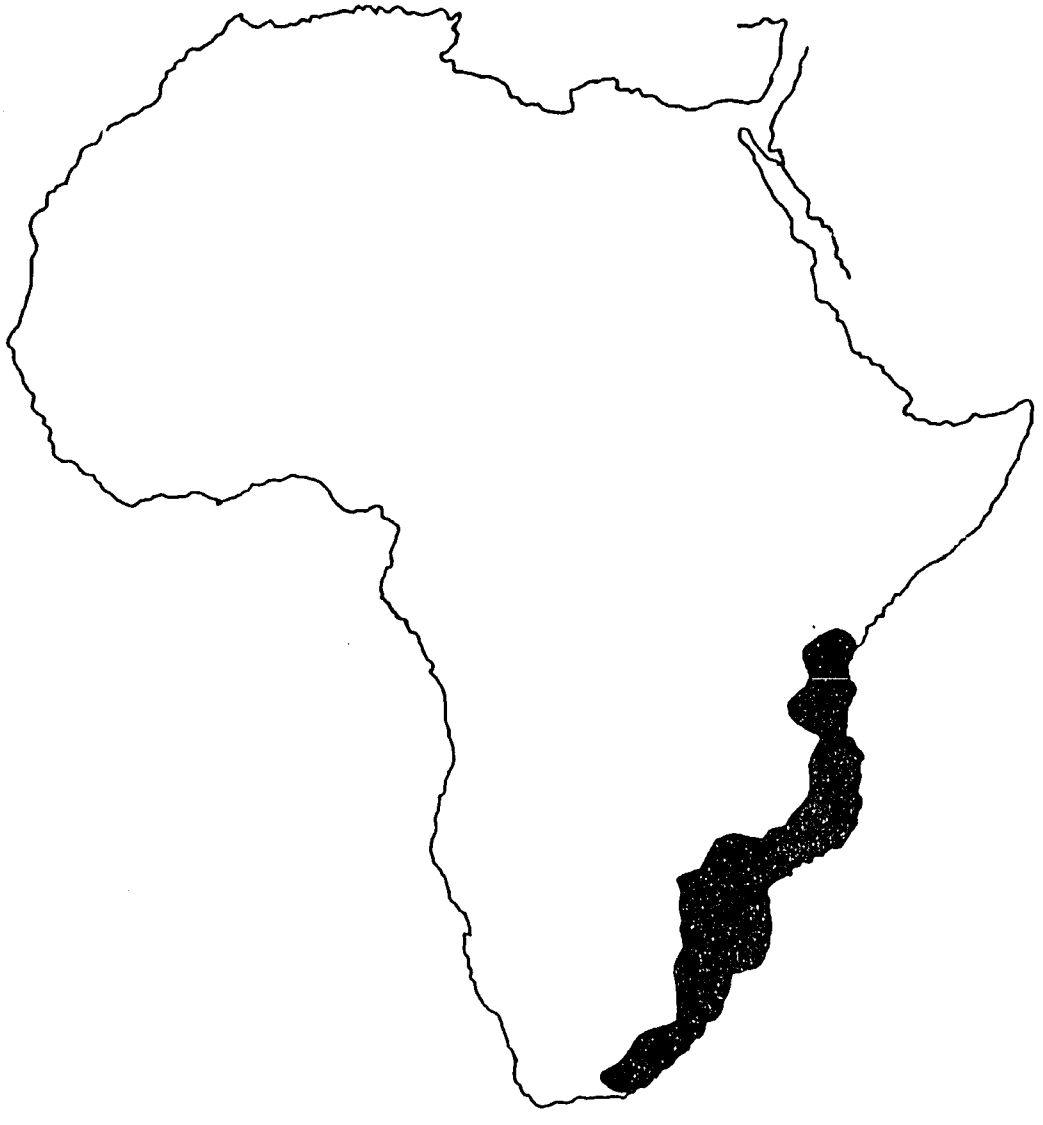
Natural Distribution of O. macrochir

Figure 4. Map of Africa showing the natural distribution of *Oreochromis mortimeri* (Trewavas 1982).



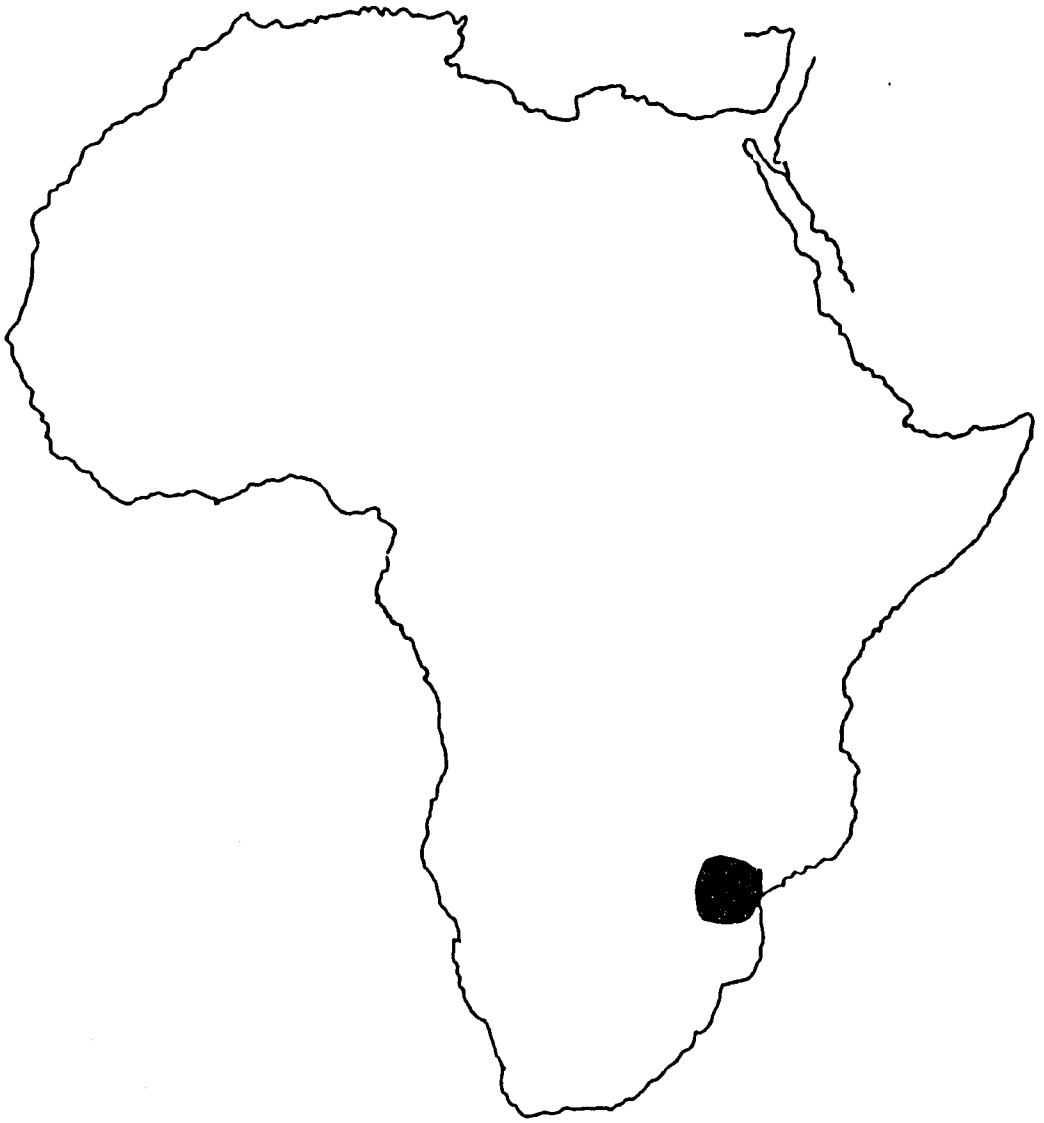
Natural Distribution of O. mortimeri

Figure 5. Map of Africa showing the natural distribution of *Oreochromis mossambicus* (Trewavas 1982).



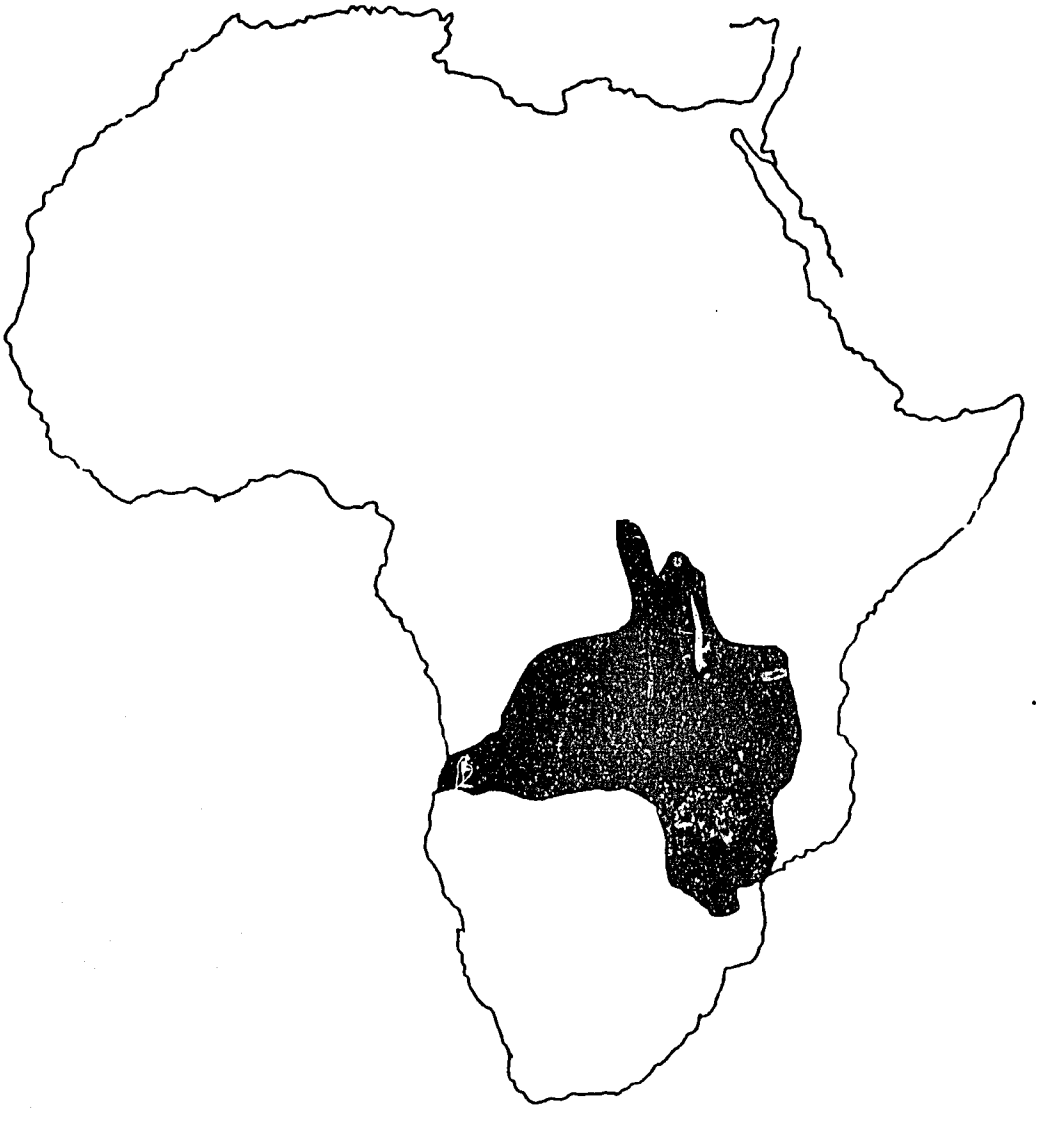
Natural Distribution of O. mossambicus

Figure 6. Map of Africa showing the natural distribution of *Oreochromis placidus* (Trewavas 1982).



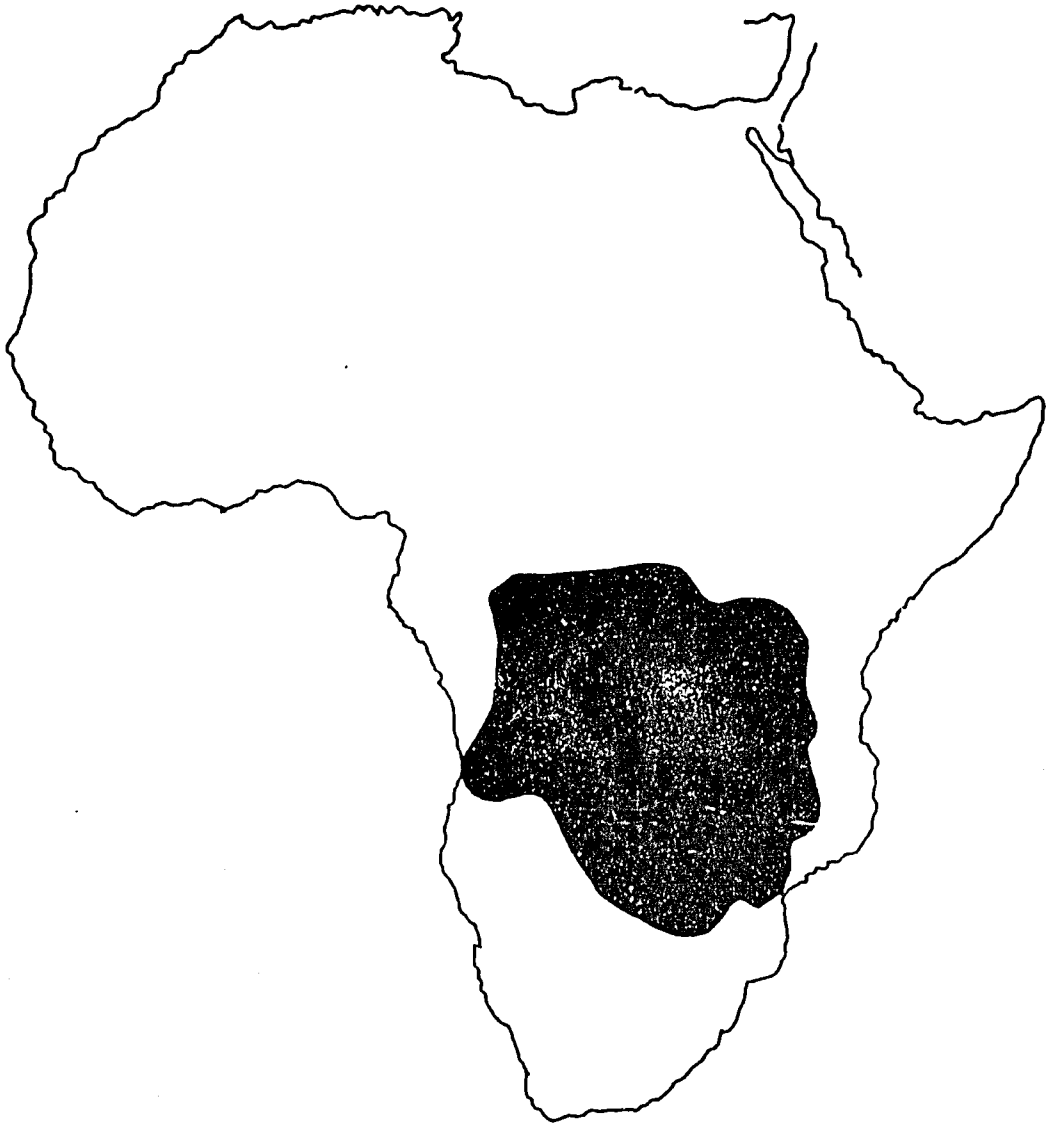
Natural Distribution of O. placidus

Figure 7. Map of Africa showing the natural distribution of *Tilapia rendalli* (Trewavas 1982).



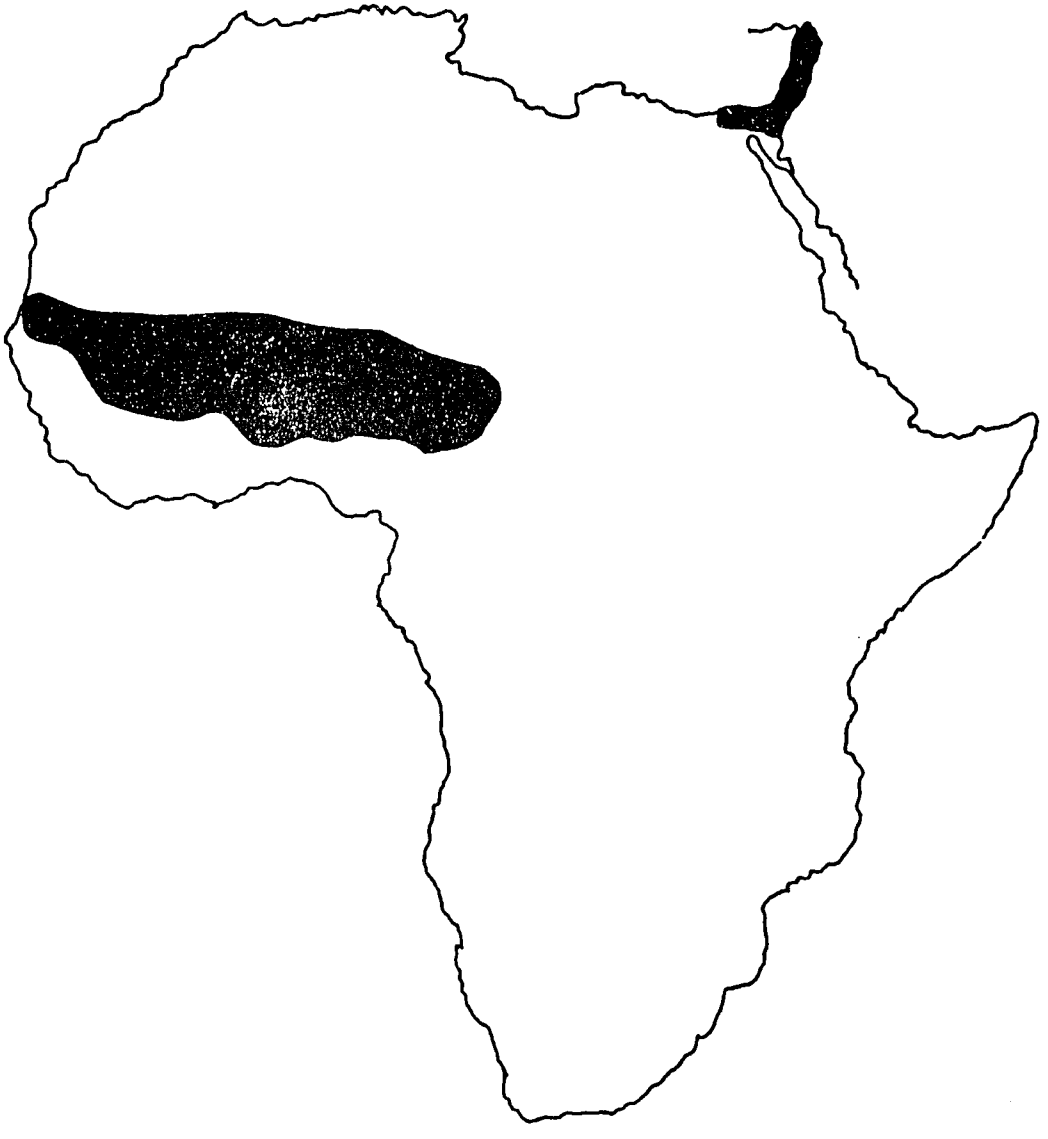
Natural Distribution of T. rendalli

Figure 8. Map of Africa showing the natural distribution of *Tilapia sparrmanii* (Trewavas 1982).



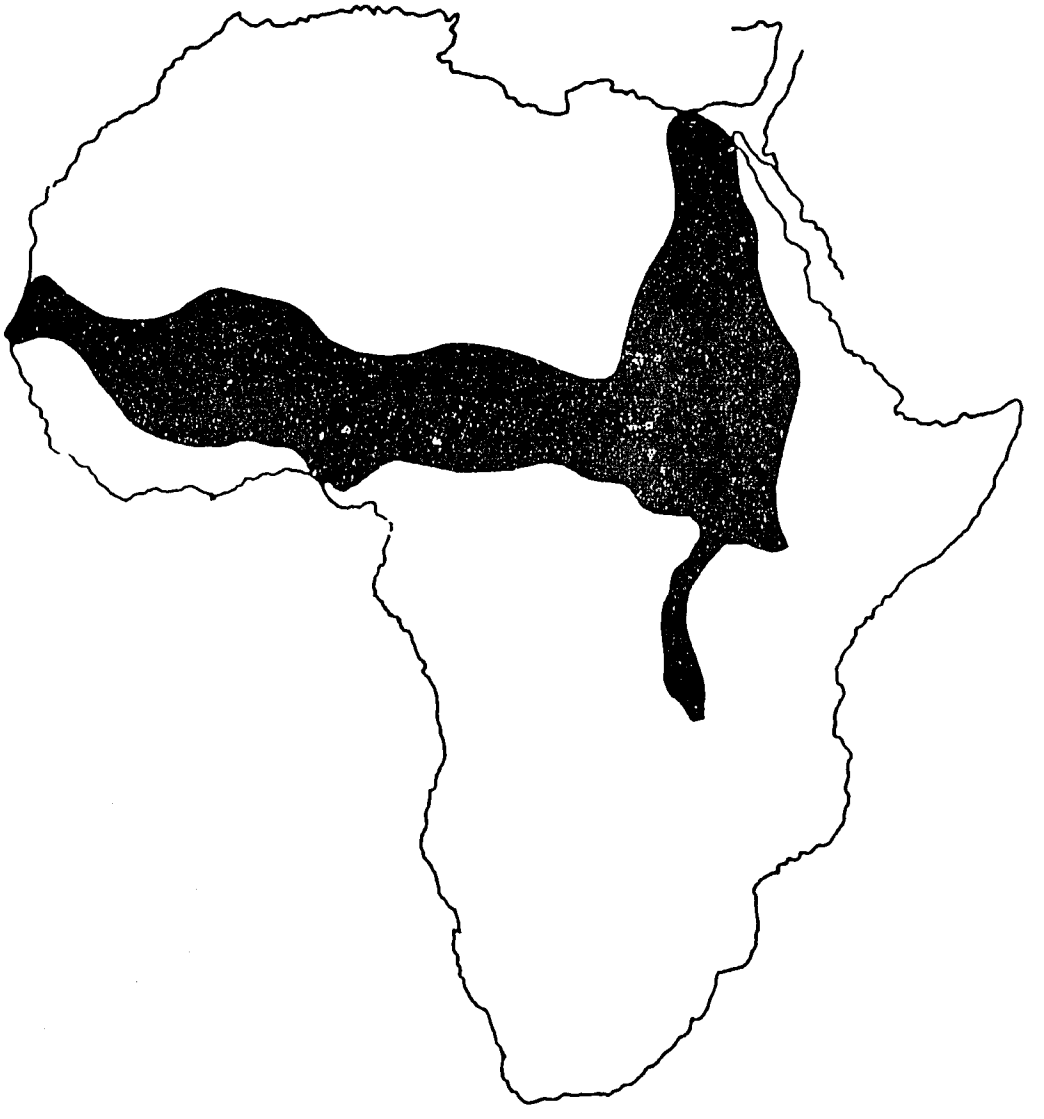
Natural Distribution of T. sparrmarii

Figure 9. Map of Africa showing the natural distribution of *Oreochromis aureus* (Trewavas 1982).



Natural Distribution of Q. aureus

Figure 10. Map of Africa showing the natural distribution of *Oreochromis niloticus* (Trewavas 1982).



Natural Distribution of O. niloticus

Figure 11. Map of drainage systems of Zimbabwe indicating collection sites of fish samples of tilapias indigenous to Zimbabwe.

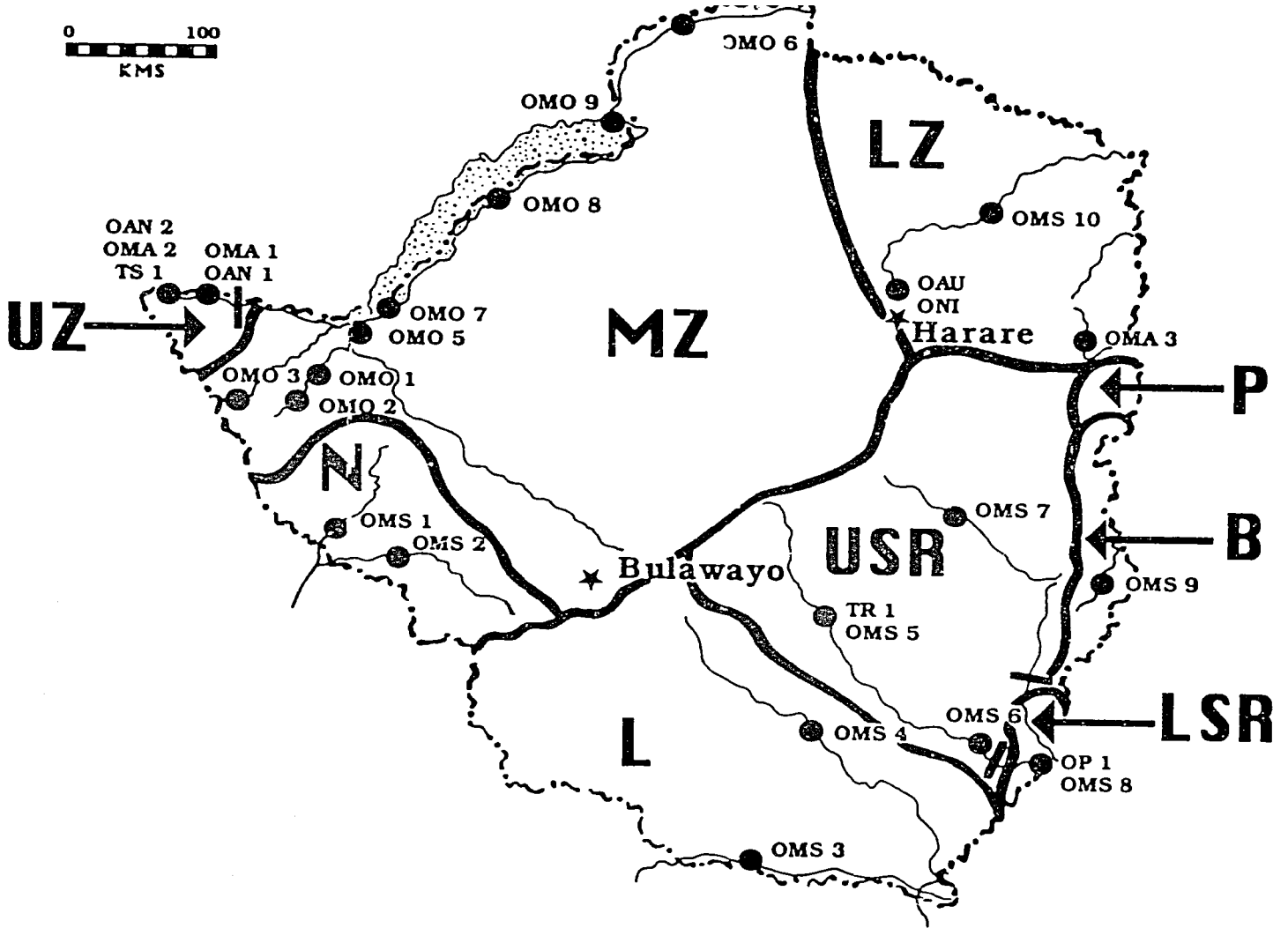

0
100
KMS
OMO 6
OMO 9
OMO 8
LZ
OMS 10
OAN 2
OMA 2
TS 1
OMA 1
OAN 1
OAU
ONI
Harare
OMA 3
UZ
OMO 7
OMO 5
MZ
OMO 3
OMO 1
OMO 2
P
N
OMS 7
OMS 1
OMS 2
USR
B
Bulawayo
OMS 9
TR 1
OMS 5
OMS 6
LSR
OMS 4
L
OP 1
OMS 8
OMS 3

Figure 12. Phenogram of 29 populations of tilapiine fish species of Zimbabwe based on the UPGMA procedure using Nei's (1978) unbiased genetic distance coefficients.

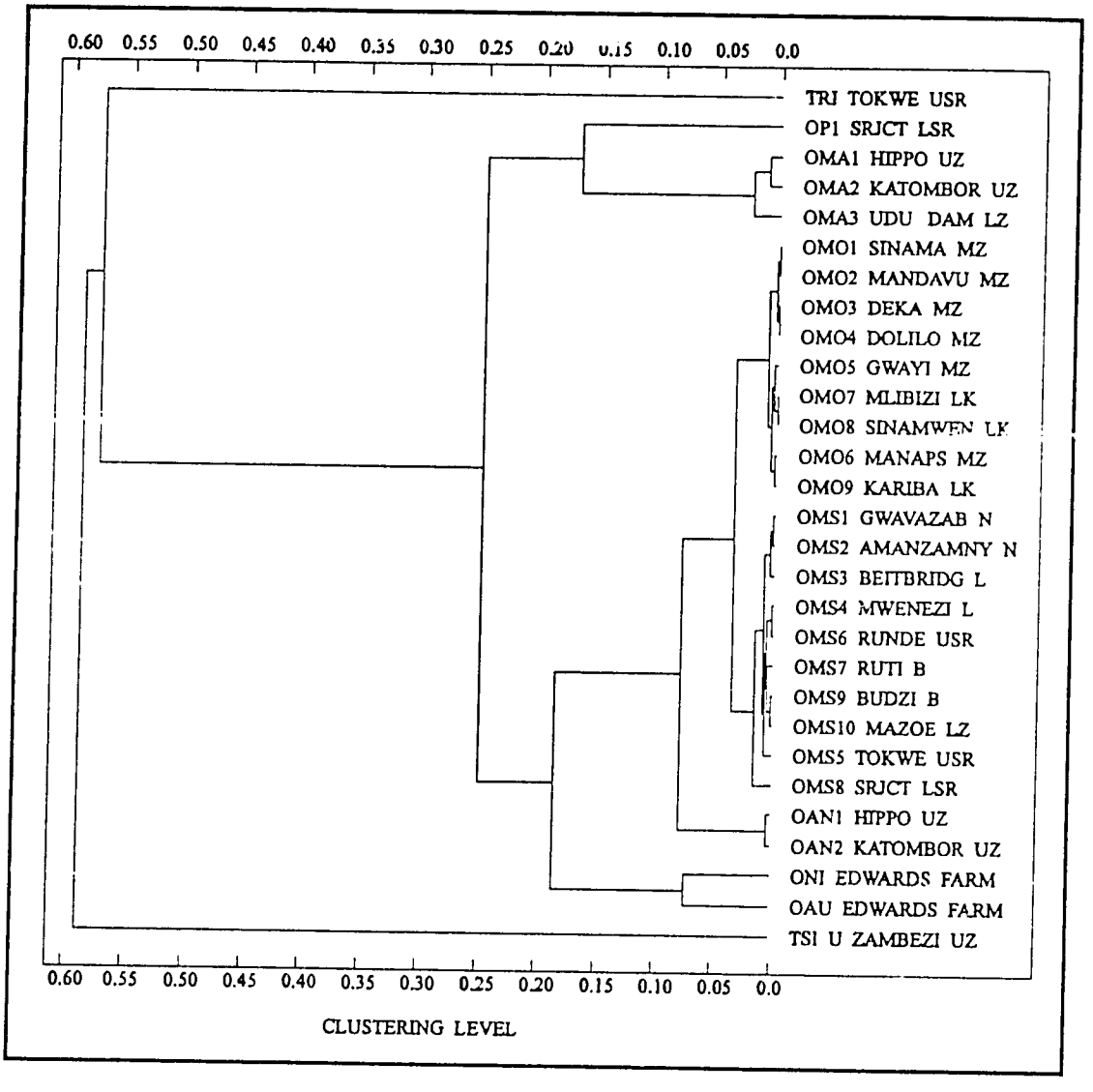

0.60 0.55 0.50 0.45 0.40 0.35 0.30 0.25 0.20 0.15 0.10 0.05 0.0
TRI TOKWE USR
OP1 SRJCT LSR
OMA1 HIPPO UZ
OMA2 KATOMBOR UZ
OMA3 UDU DAM LZ
OMO1 SINAMA MZ
OMO2 MANDAVU MZ
OMO3 DEKA MZ
OMO4 DOLILO MZ
OMO5 GWAYI MZ
OMO7 MLIBIZI LK
OMO8 SINAMWEN LK
OMO6 MANAPS MZ
OMO9 KARIBA LK
OMS1 GWAVAZAB N
OMS2 AMANZAMNY N
OMS3 BEITBRIDG L
OMS4 MWENEZI L
OMS6 RUNDE USR
OMS7 RUTI B
OMS9 BUDZI B
OMS10 MAZOE LZ
OMS5 TOKWE USR
OMS8 SRJCT LSR
OAN1 HIPPO UZ
OAN2 KATOMBOR UZ
ONI EDWARDS FARM
OAU EDWARDS FARM
TSI U ZAMBEZI UZ
0.60 0.55 0.50 0.45 0.40 0.35 0.30 0.25 0.20 0.15 0.10 0.05 0.0
CLUSTERING LEVEL

Figure 13. Dendrogram of 29 populations of tilapiine fish species of Zimbabwe based on the Distance Wagner procedure using Rogers (1972) genetic distance coefficients.

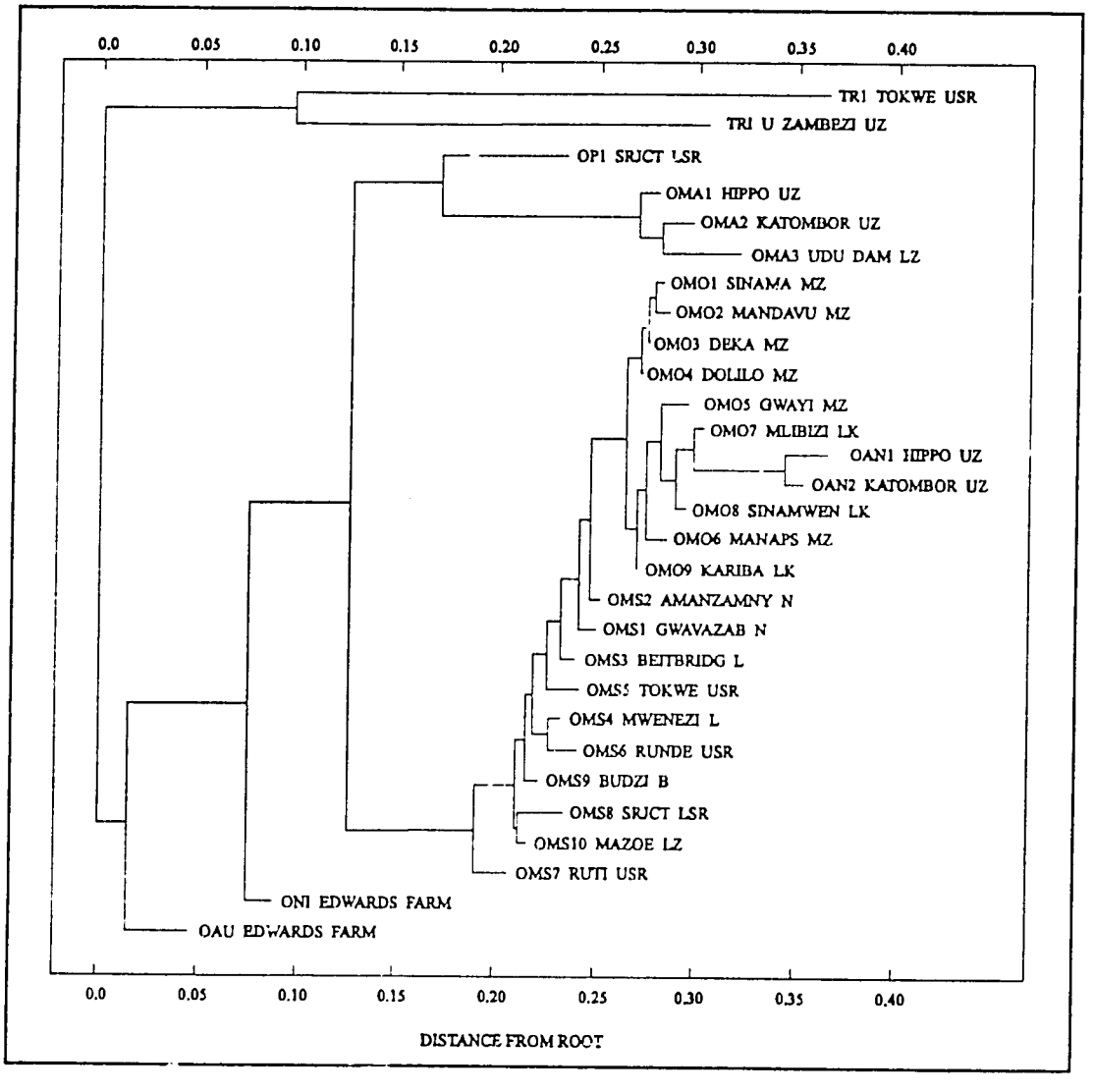

0.0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
TR1 TOKWE USR
TRI U ZAMBEZI UZ
OP1 SRJCT LSR
OMA1 HIPPO UZ
OMA2 KATOMBOR UZ
OMA3 UDU DAM LZ
OMO1 SINAMA MZ
OMO2 MANDAVU MZ
OMO3 DEKA MZ
OMO4 DOLILO MZ
OMO5 GWAYI MZ
OMO7 MLIBIZI LK
OAN1 HIPPO UZ
OAN2 KATOMBOR UZ
OMO8 SINAMWEN LK
OMO6 MANAPS MZ
OMO9 KARIBA LK
OMS2 AMANZAMNY N
OMS1 GWAVAZAB N
OMS3 BEITBRIDG L
OMS5 TOKWE USR
OMS4 MWENEZI L
OMS6 RUNDE USR
OMS9 BUDZI B
OMS8 SRJCT LSR
OMS10 MAZOE LZ
OMS7 RUTI USR
ONI EDWARDS FARM
OAU EDWARDS FARM
DISTANCE FROM ROOT

Figure 14. Phenogram of nine tilapiine fish species of Zimbabwe based on the UPGMA procedure using Nei's (1978) unbiased genetic distance coefficients.

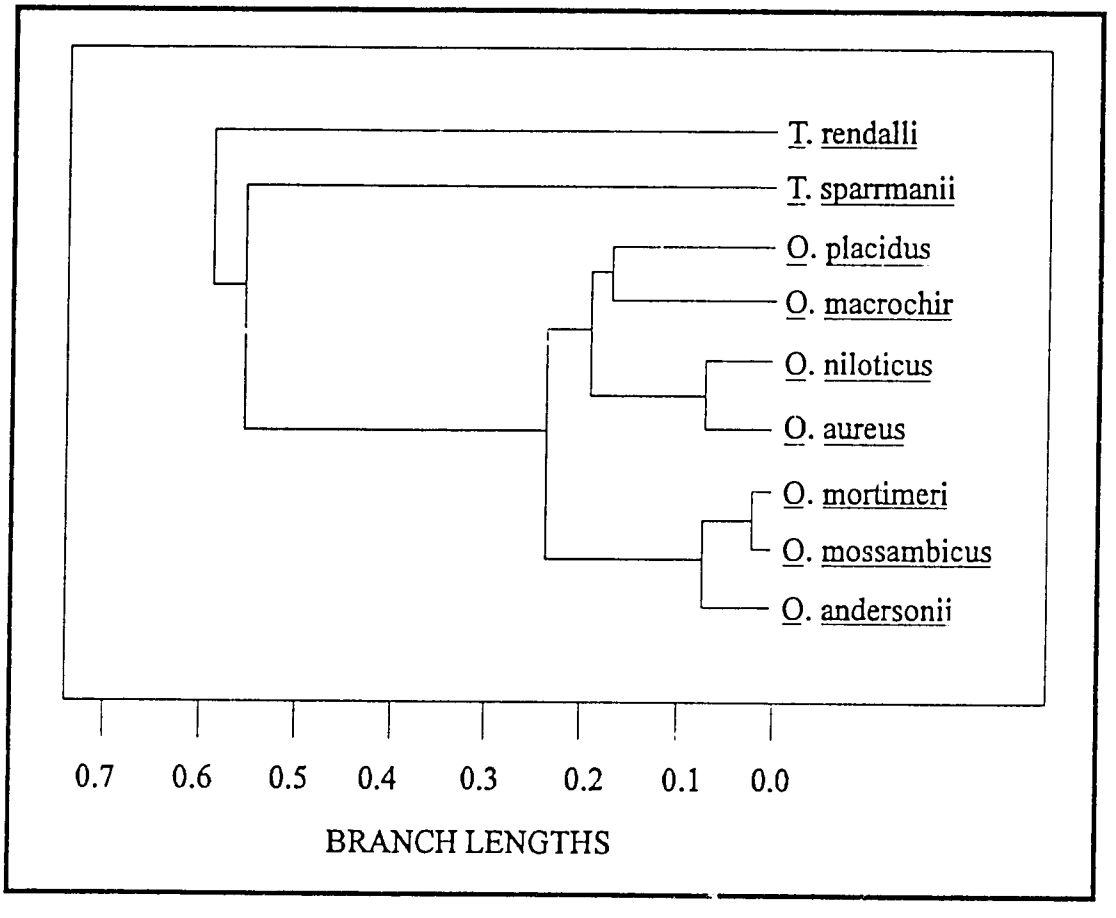

T. rendalli
T. sparrmanii
O. placidus
O. macrochir
O. niloticus
O. aureus
O. mortimeri
O. mossambicus
O. andersonii
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BRANCH LENGTHS

Figure 15. Dendrogram of nine tilapiine fish species of Zimbabwe based on the Distance Wagner procedure using Rogers (1972) genetic distance coefficients.

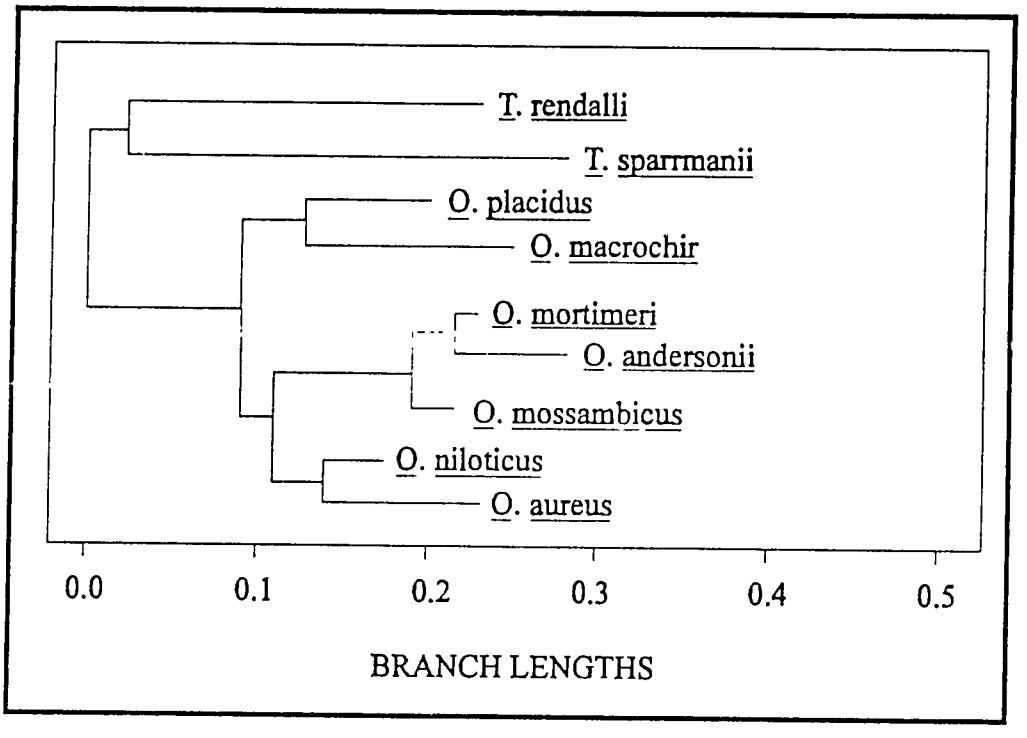
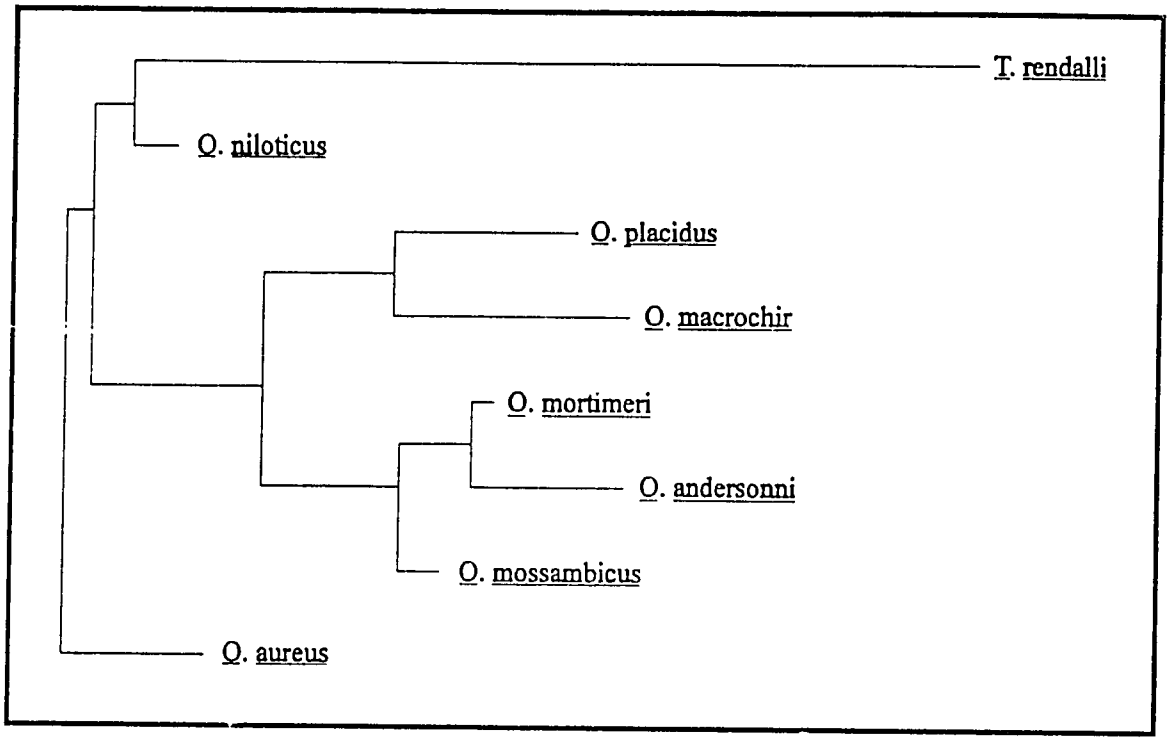

T. rendalli
T. sparrmanii
O. placidus
O. macrochir
O. mortimeri
O. andersonii
O. mossambicus
O. niloticus
O. aureus
0.0
0.1
0.2
0.3
0.4
0.5
BRANCH LENGTHS

Figure 16. Dendrogram of nine tilapiine fish species of Zimbabwe based on the FREQPARS procedure.


T. rendalli
O. niloticus
O. placidus
O. macrochir
O. mortimeri
O. andersonni
O. mossambicus
O. aureus

Appendix III

Published Abstracts for Regional and National Meetings
-Association of Southeastern Biologists-1992
-Society for Conservation Biology/The Wildlife Society-1992

6th ANNUAL MEETING • SOCIETY FOR CONSERVATION BIOLOGY in cooperation with THE WILDLIFE SOCIETY

ABSTRACT SUBMISSION FORM

SUBMIT ABSTRACT BY 23 MARCH 1992

Presenting Author Fortune Shonhiwa

Address Department of Biology, Frostburg State University, Frostburg, MD 21532

Telephone Number (301) 689-4166

Is this paper intended to be an oral presentation [x] or a poster* presentation [ ]

Eligible for student paper award [x] (Remember to submit an additional, expanded abstract — see directions)

Audiovisual Equipment Needed Slide Projector

Please check all boxes that apply (to be used only to project meeting attendance):

Presenting author is a member of the:

[x] Society for Conservation Biology [ ] The Wildlife Society

Presenting author: [ ] has [x] has not previously attended a Society for Conservation Biology Conference.

*Posters may be set up on Saturday. They must be taken down by 2:00 PM on Tuesday.

TYPE ABSTRACT IN THE BOX BELOW CONFORMING EXACTLY TO THE *DIRECTIONS FOR PREPARATION OF ABSTRACTS FOR PAPERS AND POSTERS*.

Please do not fold abstract within the boxed area.

MAIL TO: Jim Fraser, SCB Abstracts, Department of Fisheries and Wildlife, Virginia Tech, Blacksburg, VA 24061-0321

ELECTROPHORETIC IDENTIFICATION OF INDIGENOUS TILAPIINE GENETIC RESOURCES OF ZIMBABWE

Fortune Shonhiwa and James H. Howard. Frostburg State University, Department of Biology, Frostburg, Maryland, 21532

345 fish from seven Tilapiine species (two in the genus Tilapia and five in Oreochromis) from the major river drainages of Zimbabwe were examined using starch gel electrophoresis. Two additional samples from Oreochromis species introduced into Zimbabwe were also assayed. Of the 44 loci used in the assay, 29 were polymorphic in at least one of the 29 populations sampled. 12 loci were diagnostic between at least one pair of species. Two described species (O. mortimeri and O. mossambicus) were not distinguishable at any locus. The marker loci identified will be useful in assessing hybridization potential among species native to Zimbabwe and introduced tilapiine species.

SHONHIWA, FORTUNE AND JAMES HOWARD.
Frostburg State University--The validity of the Kariba Tilapia (Oreochromis mortimeri).

Samples of the Kariba Tilapia from eight locations on the Zambezi River were frozen in liquid nitrogen and transported to Maryland for genetic analysis. 43 presumptive enzyme loci were resolved using starch-gel electrophoresis. 25 loci were monomorphic in all populations and 18 were polymorphic in at least one population. The allozyme variation among these samples was compared to variation from ten samples of O. mossambicus collected from streams throughout its range in Zimbabwe. Although frequency diferences between O. mortimeri and O. mossambicus are significant at three loci (AAT-2, ADA, SOD), there are no fixed allelic differences between the two purported species. In addition, the Nei's genetic distance (D) between population pairs of O. mortimeri frequently exceeds the average genetic distance (D=0.07) between the two species. These data raise serious questions about the validity of the form designated O. mortimeri.

[illegible]

Rule a [illegible] on plain paper [illegible] Compose abstract to fit within the lines, or any portion of its length. The abstract should not exceed 200 words. This practice is important!

Type in the following order: AUTHOR'S NAME(S) (all capitalized; last name first for single or first author if more than one; start just to right of dashed line; end with period). Institution(s) (follow name directly and do not abbreviate); stay to right of dashed line; end with--). Title (start after 2 dashes without space; capitalize first letter of first word then only proper and scientific names as customary; underline all of title; maintain same indentation as name and institution; end with period). Text (start new line without indentation, maintain entire within box, but within all lines). See format examples on reverse side.

In case of more than two authors with different institutions, place all author names together first, followed by all institutions in same order; if necessary, key author to institution by a superscript number. See example.

Your abstract should be informative and contain a concise statement of the (1) problem; (2) experimental methods, if pertinent; (3) results, either citing quantitative data from representative experiments or summary data; (4) conclusions. It is not satisfactory to state: "The results will be discussed."

If grant number has to be acknowledged, place it at the [illegible] still within the box, but without new paragraph.

[illegible] Use [illegible]

BEST AVAILABLE DOCUMENT

Appendix IV

Gene Frequency Table for All Indigenous Populations Analyzed

APPENDIX 4. Allele frequencies.

POPULATION									
LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
sAAT1									
(N)	21	14	14	9	10	9	10	4	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT2									
(N)	20	14	13	9	10	9	10	4	15
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
mAAT1									
(N)	10	14	13	9	10	9	11	4	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1									
(N)	10	9	13	9	10	9	10	9	15
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	1.000	1.000	1.000	1.000	1.000	1.000	0.650	0.000	0.100
G	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.133
I	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.722	0.767
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
ADH-1									
(N)	15	10	11	9	10	9	12	5	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.800	0.625
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.375
AK-1									
(N)	20	14	13	9	10	9	12	4	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
ALD-1									
(N)	20	17	16	13	13	12	13	5	19
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	20	14	14	9	10	9	10	9	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2									
(N)	20	14	13	9	10	9	10	9	15
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
EST-1									
(N)	20	14	14	9	10	9	12	9	15
A	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
F	1.000	1.000	1.000	1.000	0.750	1.000	1.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
FBP-1									
(N)	21	15	13	10	10	10	10	4	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GAM-1									
(N)	21	15	13	9	10	9	10	11	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-1									
(N)	20	15	13	9	10	9	10	11	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GDA-1									
(N)	20	9	13	9	9	9	12	5	7
A	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	1.000	1.000	1.000	.899	1.000	1.000	1.000	1.000	1.000
GP-1									
(N)	15	15	13	10	10	10	11	10	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.0600	0.950
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.050
GP-2									
(N)	15	15	13	10	10	10	11	10	10
A	1.000	1.000	1.000	.899	1.000	1.000	1.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000

APPENDIX 4. (continued)
POPULATION

LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
GPI-1									
(N)	21	15	13	10	10	10	11	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	.899	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.950
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
GPI-2									
(N)	20	15	13	10	10	10	11	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	1.000	1.000	.899	1.000	1.000	1.000	0.000	0.000
G3P-1									
(N)	4	15	13	8	9	10	10	4	8
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
C	0.625	1.000	1.000	0.750	0.944	1.000	0.900	0.000	0.000
D	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.250	0.056	0.000	0.100	1.000	0.875
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
IDH-1									
(N)	22	15	13	10	8	11	11	8	10
A	0.977	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
B	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-1									
(N)	21	15	14	10	10	9	10	8	15
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

APPENDIX 4. (continued)
POPULATION

LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
LDH-2									
(N)	20	15	13	9	10	9	12	10	10
A	1.000	1.000	1.000	0.899	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000
LDH-3									
(N)	20	14	13	9	10	9	10	10	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
MDH-1									
(N)	19	19	17	12	13	13	15	10	17
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.853
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.147
MDH-2									
(N)	20	19	17	12	13	13	15	10	18
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	0.958	1.000	1.000	1.000	1.000	0.972
C	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028
MDH-3									
(N)	20	10	13	5	10	5	11	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.875	1.000
C	1.000	1.000	1.000	0.700	1.000	1.000	0.955	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.300	0.000	0.000	0.045	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
ME-1									
(N)	20	18	15	13	10	12	13	5	16
A	0.000	0.000	0.233	0.000	0.000	0.000	0.000	0.000	0.063
B	1.000	1.000	0.076	1.000	1.000	1.000	1.000	1.000	0.938
C	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000
ME-2									
(N)	20	19	17	12	13	13	14	4	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI-1									
(N)	15	15	13	9	10	8	10	8	8
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPGL									
(N)	20	15	13	9	10	10	11	8	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPLG									
(N)	20	15	13	10	10	10	8	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPLL									
(N)	20	10	12	10	10	10	9	4	8
A	0.000	0.000	0.042	0.100	0.050	0.000	0.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	1.000	1.000	0.958	0.900	0.950	1.000	1.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TSI	TS2
PEPPA									
(N)	20	15	13	10	10	10	12	8	9
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1									
(N)	20	10	5	9	10	9	12	1	11
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SDH-1									
(N)	3	15	5	4	10	10	5	9	8
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
6PDGH									
(N)	20	15	11	9	10	10	10	10	12
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
SOD-1									
(N)	20	15	11	9	10	10	10	5	8
A	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-1									
(N)	10	10	12	9	10	9	12	6	8
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
sAAT1									
(N)	20	11	5	21	10	27	13	5	6
A	0.725	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.225	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT2									
(N)	15	15	4	19	10	24	13	5	6
A	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mAA1									
(N)	22	11	4	18	10	18	10	5	6
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1									
(N)	22	11	4	8	10	24	17	5	5
A	0.068	1.000	1.000	1.000	1.000	1.000	0.912	0.800	0.800
B	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.200	0.100
C	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.100
D	0.068	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.864	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
ADH-1									
(N)	15	10	4	10	10	11	10	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AK-1									
(N)	20	10	5	16	10	17	13	5	6
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ALD-1									
(N)	15	10	4	14	10	14	13	5	6
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	21	11	4	22	10	28	20	7	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2									
(N)	13	11	4	21	10	24	20	7	5
A	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST-1									
(N)	22	15	5	23	10	29	20	5	6
A	1.000	1.000	1.000	0.978	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
FBP-1									
(N)	17	10	4	19	10	28	10	5	5
A	0.971	1.000	0.875	1.000	1.000	1.000	1.000	0.200	1.000
B	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.800	0.000
C	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GAM-1									
(N)	22	14	5	22	10	30	20	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-1									
(N)	22	14	4	21	10	25	20	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GDA-1									
(N)	17	10	4	19	9	21	10	5	2
A	0.029	1.000	1.000	1.000	1.000	0.690	0.800	0.800	0.500
B	0.059	0.000	0.000	0.000	0.000	0.310	0.100	0.200	0.500
C	0.912	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GP-1									
(N)	20	10	4	14	10	16	19	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GP-2									
(N)	20	10	4	8	10	26	20	8	5
A	0.025	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.975	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
GPI-1									
(N)	20	10	4	8	10	26	20	8	5
A	0.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000
B	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.950
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
GPI-2									
(N)	19	10	4	8	10	26	20	8	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G3P-1									
(N)	14	11	24	18	10	19	17	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1									
(N)	7	10	23	10	10	7	17	5	10
A	1.000	1.000	0.978	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000
LDH-1									
(N)	23	15	4	22	10	30	20	10	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
LDH-2									
(N)	14	20	27	24	19	20	21	25	12
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.980	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-3									
(N)	14	11	21	18	10	19	23	5	10
A	0.964	1.000	1.000	1.000	0.950	1.000	1.000	0.800	1.000
B	0.036	0.000	0.000	0.000	0.050	0.000	0.000	0.200	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-1									
(N)	14	12	25	18	10	20	23	5	10
A	0.750	0.917	0.880	0.944	1.000	0.875	1.000	0.700	1.000
B	0.250	0.083	0.120	0.056	0.000	0.125	0.000	0.030	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2									
(N)	14	12	26	18	21	26	24	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3									
(N)	14	12	22	18	10	20	16	5	10
A	1.000	1.000	1.000	0.944	1.000	0.979	1.000	1.000	1.000
B	0.000	0.000	0.000	0.056	0.000	0.021	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
ME-1									
(N)	14	12	25	18	10	7	16	5	10
A	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ME-2									
(N)	14	6	12	10	10	19	10	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI-1									
(N)	11	10	17	10	2	7	9	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPGL									
(N)	14	12	24	18	10	19	16	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPLG									
(N)	14	12	26	18	10	19	12	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPLL									
(N)	14	12	25	18	10	19	16	5	10
A	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OP1	OMO1	OMO2	OMO3	OMO4	OMO5	OMO6	OMO7	OMO8
PEPPA									
(N)	14	12	26	18	10	19	15	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1									
(N)	13	10	26	10	21	25	19	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SDH-1									
(N)	13	10	24	10	20	25	18	5	10
A	0.962	1.000	1.000	0.950	0.900	0.800	0.899	1.000	0.800
B	0.038	0.000	0.000	0.050	0.100	0.200	0.111	0.000	0.200
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PDGH									
(N)	13	10	21	10	10	7	15	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SOD-1									
(N)	13	9	21	10	11	25	14	5	10
A	0.385	0.500	0.619	1.000	0.955	1.000	1.000	0.800	0.950
B	0.615	0.500	0.381	0.000	0.045	0.000	0.000	0.200	0.050
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-1									
(N)	5	10	12	10	10	7	10	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.600	0.250
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.750

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
sAAT1									
(N)	14	20	27	24	19	20	13	25	12
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT2									
(N)	14	20	27	24	19	20	13	25	12
A	0.000	0.475	0.611	0.458	0.737	0.925	1.000	0.820	0.708
B	1.000	0.525	0.389	0.542	0.263	0.075	0.000	0.180	0.292
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mAAT1									
(N)	14	10	12	10	17	13	10	25	12
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1									
(N)	14	19	23	24	17	20	17	19	12
A	0.964	0.632	0.957	0.917	0.529	0.350	0.412	0.158	0.708
B	0.000	0.316	0.022	0.021	0.118	0.025	0.000	0.605	0.167
C	0.036	0.053	0.022	0.063	0.118	0.600	0.206	0.079	0.125
D	0.000	0.000	0.000	0.000	0.088	0.025	0.000	0.158	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.147	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
ADH-1									
(N)	11	10	10	10	10	7	10	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AK-1									
(N)	14	10	8	10	10	7	13	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ALD-1									
(N)	14	10	8	10	10	7	13	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	14	20	27	24	19	20	19	25	12
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2									
(N)	14	15	27	23	19	20	19	25	12
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST-1									
(N)	14	20	25	24	23	26	22	25	12
A	1.000	0.975	0.980	1.000	0.978	0.962	1.000	1.000	0.958
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.025	0.020	0.000	0.022	0.038	0.000	0.000	0.042
D	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
FBP-1									
(N)	14	10	23	10	10	7	10	5	10
A	1.000	1.000	1.000	0.950	0.800	1.000	0.950	0.800	1.000
B	0.000	0.000	0.000	0.050	0.200	0.000	0.050	0.200	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GAM-1									
(N)	14	10	23	10	10	7	19	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-1									
(N)	14	10	21	10	10	7	21	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GDA-1									
(N)	14	5	18	10	21	25	14	5	10
A	0.964	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GP-1									
(N)	14	12	22	18	10	19	19	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GP-2									
(N)	14	12	20	18	10	18	19	5	10
A	1.000	1.000	1.000	0.972	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
GPI-1									
(N)	14	12	16	18	10	19	20	5	10
A	1.000	1.000	1.000	0.944	0.900	0.921	0.925	1.000	1.000
B	0.000	0.000	0.000	0.056	0.100	0.079	0.075	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI-2									
(N)	14	12	16	18	10	19	20	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G3P-1									
(N)	14	12	16	18	10	19	17	5	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1									
(N)	21	14	4	22	10	30	20	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-1									
(N)	23	15	4	22	10	30	20	10	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
LDH-2									
(N)	22	15	4	22	10	27	20	10	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-3									
(N)	19	10	4	9	10	23	10	9	5
A	1.000	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-1									
(N)	22	15	4	21	10	30	20	9	5
A	0.932	0.367	0.500	0.690	0.650	0.633	0.900	0.333	0.300
B	0.068	0.633	0.500	0.310	0.350	0.367	0.100	0.667	0.700
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2									
(N)	22	14	5	20	10	30	20	8	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3									
(N)	22	10	4	19	10	24	17	8	5
A	1.000	1.000	1.000	0.974	1.000	0.979	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
ME-1									
(N)	21	10	4	20	10	29	10	8	5
A	0.000	1.000	0.875	1.000	0.950	0.931	1.000	1.000	1.000
B	1.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.050	0.069	0.000	0.000	0.000
ME-2									
(N)	15	10	4	12	10	30	10	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI-1									
(N)	16	6	2	16	10	17	17	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPGL									
(N)	21	10	4	18	10	27	17	5	5
A	1.000	1.000	1.000	1.000	1.000	0.926	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.074	0.000	0.000	0.000
PEPLG									
(N)	21	10	4	23	10	29	17	5	5
A	1.000	1.000	1.000	1.000	1.000	0.966	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000
PEPLL									
(N)	10	10	4	20	10	28	17	5	5
A	1.000	1.000	1.000	1.000	1.000	0.964	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMO9	OMS1	OMS2	OMS3	OMS4	OMS5	OMS6	OMS7	OMS8
PEPPA									
(N)	21	10	4	23	10	29	20	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1									
(N)	15	10	5	19	10	29	13	5	5
A	1.000	1.000	1.000	1.000	1.000	0.879	1.000	1.000	0.900
B	0.000	0.000	0.000	0.000	0.000	0.121	0.000	0.000	0.100
SDH-1									
(N)	18	10	4	19	10	24	17	5	5
A	0.833	1.000	0.875	1.000	0.950	0.938	0.971	1.000	1.000
B	0.139	0.000	0.125	0.000	0.050	0.063	0.029	0.000	0.000
C	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PDGH									
(N)	16	10	4	15	10	24	20	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SOD-1									
(N)	15	10	4	16	10	24	20	4	5
A	1.000	0.700	0.875	0.688	0.700	0.542	0.300	0.375	0.300
B	0.000	0.300	0.125	0.312	0.300	0.458	0.700	0.500	0.700
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-1									
(N)	15	10	4	11	10	17	10	5	5
A	0.000	1.000	1.000	0.955	1.000	1.000	1.000	1.000	1.000
B	1.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
sAAT1							
(N)	15	14	4	24	10	8	15
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT2							
(N)	13	14	4	25	10	6	15
A	0.923	0.786	1.000	0.740	0.700	0.000	0.000
B	0.077	0.214	0.000	0.260	0.300	1.000	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mAAT1							
(N)	7	14	4	10	10	6	14
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1							
(N)	14	14	4	27	34	6	13
A	0.750	0.179	0.500	0.407	0.368	0.500	0.231
B	0.143	0.607	0.125	0.481	0.279	0.167	0.231
C	0.107	0.000	0.375	0.111	0.353	0.333	0.538
D	0.000	0.214	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
ADH-1							
(N)	7	7	3	19	10	8	2
A	1.000	1.000	1.000	0.974	1.000	1.000	1.000
B	0.000	0.000	0.000	0.026	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AK-1							
(N)	15	14	4	26	10	6	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ALD-1							
(N)	14	14	4	24	10	6	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1							
(N)	15	14	6	34	37	8	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-2							
(N)	15	14	7	34	37	8	16
A	1.000	1.000	0.000	0.000	0.000	1.000	1.000
B	0.000	0.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST-1							
(N)	15	14	6	33	40	8	16
A	1.000	1.000	0.000	0.000	0.000	0.813	1.000
B	0.000	0.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.187	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
FBP-1							
(N)	15	14	4	26	10	7	16
A	1.000	1.000	1.000	1.000	1.000	.0857	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.143	0.000
GAM-1							
(N)	15	14	4	23	18	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-1							
(N)	14	14	4	23	18	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GDA-1							
(N)	11	13	5	23	27	7	14
A	1.000	1.000	0.900	0.826	0.389	0.714	0.929
B	0.000	0.000	0.100	0.130	0.611	0.286	0.071
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.043	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GP-1							
(N)	14	14	8	30	31	9	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GP-2							
(N)	14	14	8	24	31	9	16
A	0.964	1.000	0.000	0.000	0.000	1.000	1.000
B	0.036	0.000	1.000	1.000	1.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
GPI-1							
(N)	14	14	8	30	38	9	16
A	1.000	1.000	0.000	0.000	0.000	1.000	1.000
B	0.000	0.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI-2							
(N)	14	14	8	30	38	9	17
A	1.000	1.000	1.000	1.000	0.947	1.000	0.941
B	0.000	0.000	0.000	0.000	0.053	0.000	0.059
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G3P-1							
(N)	14	7	4	26	10	6	16
A	1.000	1.000	1.000	1.000	1.000	1.000	0.938
B	0.000	0.000	0.000	0.000	0.000	0.000	0.063
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1							
(N)	14	14	4	32	18	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-1							
(N)	14	14	9	34	38	10	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
LDH-2							
(N)	15	14	9	34	38	10	16
A	0.933	1.000	0.000	0.000	0.000	1.000	1.000
B	0.067	0.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-3							
(N)	12	14	4	30	38	7	13
A	1.000	0.964	1.000	1.000	1.000	1.000	1.000
B	0.000	0.036	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-1							
(N)	15	14	8	32	38	9	16
A	0.967	1.000	0.875	0.969	1.000	0.000	0.000
B	0.033	0.000	0.000	0.000	0.000	1.000	1.000
C	0.000	0.000	0.125	0.031	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2							
(N)	15	14	8	31	40	10	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3							
(N)	14	14	7	25	38	9	16
A	0.964	1.000	0.000	0.000	0.000	1.000	1.000
B	0.036	0.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.026	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
ME-1							
(N)	10	14	3	23	10	7	15
A	0.950	1.000	0.000	0.000	0.000	1.000	1.000
B	0.050	0.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ME-2							
(N)	10	14	8	25	10	7	15
A	1.000	1.000	1.000	1.000	1.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	1.000	1.000
MPI-1							
(N)	7	7	1	23	16	4	7
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEPGL							
(N)	14	14	3	27	17	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPLG							
(N)	14	7	3	33	17	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPLL							
(N)	14	14	3	24	12	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX 4. (continued)
POPULATION

LOCUS	OMS9	OMS10	OMA1	OMA2	OMA3	OAN1	OAN2
PEPPA							
(N)	14	14	3	31	17	7	16
A	1.000	1.000	0.667	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.333	0.000	0.000	0.000	0.000
PGM-1							
(N)	14	14	3	27	10	7	16
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SDH-1							
(N)	14	14	5	30	38	7	16
A	0.929	1.000	0.400	0.400	0.000	0.929	0.938
B	0.000	0.000	0.000	0.000	0.000	0.071	0.063
C	0.071	0.000	0.600	0.583	1.000	0.000	0.000
D	0.000	0.000	0.000	0.017	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PDGH							
(N)	14	14	3	24	17	8	14
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SOD-1							
(N)	14	14	3	17	10	7	16
A	1.000	1.000	0.333	0.265	0.450	0.143	0.000
B	0.000	0.000	0.667	0.735	0.550	0.857	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-1							
(N)	5	7	3	25	10	7	3
A	0.900	1.000	1.000	1.000	1.000	1.000	1.000
B	0.100	0.000	0.000	0.000	0.000	0.000	0.000