MOLECULAR GENETICS AND SYSTEMATICS OF TILAPIINE CICHLIDS USING ALLOZYMES AND MORPHOLOGICAL CHARACTERS

A thesis presented for the degree of Doctor of Philosophy to the University of Stirling

by

Panom Krachangpoj Sodsuk
B.Sc. (Aquaculture), M.Sc. (Fishery Science)

Institute of Aquaculture, University of Stirling
Stirling, Scotland

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TO MY PARENTS AND PI-TEM

DECLARATION

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged, the work described in this thesis has been conducted independently and has not been submitted for any other degree.

Signature of candidate: Panom K. Sodsak

Signature of supervisor: [illegible]

Date: 28/1/94

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ABSTRACT

Twenty-two tilapiine cichlid species in the three major genera, *Tilapia*, *Sarotherodon* and *Oreochromis*, were electrophoretically examined at 43 different enzyme loci, providing a large allozyme data base for these species. A number of comparative data based on behavioural, biogeographical and morphological characters at the generic, subgeneric and specific levels of the same species were collected from the literature and were numerically coded. A number of molecular phylogenies were produced by a variety of different analytical methods utilizing various treatments of the allozyme data set. The theoretically most robust and least restrictive analytical techniques were then used to assess the morphological data set. The phylogenies generated from the different data sets, allozymes and morphological characters, were compared and a conclusive consensus phylogeny generated.

The electrophoretic data were interpreted in a number of ways. The banding pattern of each enzyme locus was described. Allozyme differences between genus, subgenus and species were recorded as inter- generic, subgeneric and specific discriminating loci, providing a large number of genetic markers for species/stock management in this group. The estimated heterozygosities (*He*) ranged between 0.008 - 0.122 (± 0.008 - 0.034). The highest *He* were found in the lacustrine species, especially the three chambo species, *O. (Ny.) karongae*, *O. (Ny.) lidole* and *O. (Ny.) squamipinnis*, from Lake Malawi (≥ 0.110 ± 0.032). The fixation index (F-statistics, F_{ST}) observed within different levels of the taxa studied ranged between 0.734 - 0.907 within genera, and 0.378 - 0.749 within subgenera. The chambo showed the lowest F_{ST} (0.051) within any single species grouping, suggesting that very little genetic differentiation has occurred between these species. The observed genotypic frequencies in the various chambo species

did not significantly deviate from Hardy-Weinberg expectations ($\chi^2_{P \geq 0.05}$) but the allele frequencies observed between species were significantly different ($\chi^2_{P \leq 0.05}$) at most loci, suggesting large random mating populations with reproductive isolation of the chambo species. The results support the hypothesis that speciation in the Lake Malawi chambo was sympatric rather than allopatric.

Interspecific genetic distances ranged between 0.054 - 0.735 (arc distance of Cavalli-Sforza & Edwards, 1967) and 0.002 - 0.786 (unbiased distance of Nei, 1978) from the most similar pair of *T. (C.) tholloni* and *T. (C.) zillii* to the least similar pair of *T. (C.) rendalli* and *S. melanotheron* (aquarium stocks). However the averaged genetic distances within a group showed that the chambo share the closest relationship to each other (0.128, arc distance; 0.010, unbiased distance). The small genetic distances found in the chambo also suggest their recent speciation (estimated time since divergence about 100,000 - 250,000 y). The average inter-generic distances show that the two mouthbrooding genera were closer to each other than either were to the substrate spawning *Tilapia*.

Molecular and non-molecular phylogenies consistently supported the monophyly of the mouthbrooding taxa studied in relation to the substrate spawning *Tilapia*, supporting the classification proposed by Trewavas (1983). The relationships between two species pairs, *O. (O.) mossambicus* & *O. (O.) mortimeri* and *O. (O.) placidus* & *O. (O.) shiranus*, were consistently shown by the molecular phylogenies as closely related sister-species or subspecies. All intra- and inter- generic, subgeneric and specific evolutionary relationships shown in the phylogenies were discussed and placed in the context of the biogeography and distribution of the species in this group.

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CHAPTER 1

GENERAL INTRODUCTION (A LITERATURE OVERVIEW)

I. TILAPIINES IN AQUACULTURE & FISHERIES

The African group of cichlid fishes belonging to the tribe Tilapiini is composed of a number of separate genera (Trewavas, 1983). Four of these genera, *Tilapia*, *Sarotherodon*, *Oreochromis* and *Danakilia*, were formerly included in the broad genus *Tilapia* and are still commonly known as 'tilapia(s)' (Trewavas, 1982a, 1983).

Tilapias have become to be one of the most important groups of freshwater species in tropical aquaculture in recent years. Many species have been introduced into nearly every tropical and subtropical country of the world. The number of tilapia species cultured in ponds and aquaria, both experimentally and on a commercial scale, is quite large. Huet (1970) mentioned 16 species whilst Balarin & Hatton (1979) gave a list of 23 species which had been cultured at some stage. These fishes offer great advantages in aquaculture because of their general hardiness, resistance to disease, high yield potential, ability to grow on a wide range of natural and cheap artificial foods, ability to withstand low oxygen tensions, overcrowding and a wide range of salinities, and still produce a highly acceptable flesh (Pullin & Lowe-McConnell, 1982; Wohlfarth & Hulata, 1983). The species more commonly used in aquaculture are those belonging to the three major genera *Tilapia*, *Sarotherodon* and *Oreochromis*.

The information available on the most commonly cultured species in the three major genera was reviewed by Pullin (1988, 1991). The majority of the species cultured belong to the maternal mouthbrooders, the *Oreochromis* Günther group, and these tend to be restricted to two of the five subgenera, *O. (Oreochromis)*, in which the mature male lacks a genital tassel, and *O. (Nyasalapia)*, in which the mature male has a genital tassel. According to Trewavas's (1982b) suggestion, there is no need for zoologists or fish culturists who are not systematists to use these subgeneric names, and, therefore, these mouthbrooding tilapias may be referred to by the generic name *Oreochromis* followed by the specific name.

Nine species of the subgenus *O. (Oreochromis)* are used in aquaculture:

- *Oreochromis (O.) niloticus* (Linnaeus)
- *Oreochromis (O.) aureus* (Steindacher)
- *Oreochromis (O.) spilurus* (Günther)
- *Oreochromis (O.) urolepis hornorum* (Trewavas)
- *Oreochromis (O.) mossambicus* (Peters)
- *Oreochromis (O.) mortimeri* (Trewavas)
- *Oreochromis (O.) shiranus* (Boulenger)
- *Oreochromis (O.) andersonii* (Castelnau)
- *Oreochromis (O.) jipe* (Lowe-McConnell) (rather limited use)

The species in the subgenus *O. (Nyasalapia)* are also important in aquaculture and capture fisheries. The best known species of the subgenus are *O. (Ny.) macrochir*, which has been widely used in fish culture mainly in Central Africa, and a group of species in the Lake Malawi flock known as the 'chambo', which have been only recently started to be used in aquaculture since one of the species *O. (Ny.) karongae* has been found to spawn in ponds.

Among the biparental or paternal mouthbrooder species of the genus *Sarotherodon* Rüppell, only two species have been cultured, *S. galilaeus* (Linnaeus) and *S. melanotheron* Rüppell. *S. galilaeus* is an important commercial species in many lakes (including Lakes Kinneret, Turkana, Albert and Chad). *S. melanotheron* occurs in brackish lagoons and estuaries and rarely in neighboring freshwater or saltwater, from Senegal to lower Zaire.

Three substrate spawner species in the genus *Tilapia* A. Smith, have also been used in aquaculture. These are *T. rendalli* (Boulenger), *T. zillii* (Gervais) and *T. guineensis* (Bleeker). Of the two *Tilapia* species cultured in freshwater, *T. rendalli* appears to be superior to *T. zillii* as a food fish. The West African *T. guineensis* lives in brackishwater. *T. rendalli* and *T. zillii* have no overlapping areas of distribution.

Tilapia culture has made great advances in the last ten years in some Asian countries, such as the Philippines, Taiwan, Thailand and particularly the integrated crop-livestock-fish farming in China (Chen, 1988), but remains poorly developed in Africa and other regions. African countries hold the global wealth of the tilapia genetic resources, but many wild tilapia populations in Africa are under threat of irreversible change or loss from factors such as fish and water transfers and habitat disturbance (Pullin, 1988). Tilapias are playing an important role in aquaculture, but aquaculturists are undoing the work of natural evolution by transfers and bringing species together; therefore efforts should be made to protect some important resources (Thys van den Audenaerde, 1988). Accordingly, further work on the population genetics of wild and cultured tilapia stocks using various techniques of both biochemical and morphological characters for the documentation of tilapia genetic resources is recommended (Pullin, 1988).

II. GENERAL TAXONOMY

2.1 Generic Taxa & the Present Classification

There are over 70 different species in the Cichlidae Tribe Tilapiini. Trewavas (1983) defined the Tribe Tilapiini as an African and Levantine assemblage which included the following genera, *Tilapia, Sarotherodon, Oreochromis, Danakilia, Iranocichla, Tristramella, Pelmatochromis, Pterochromis*, the endemic genera of Barombi Mbo and probably some specialized genera of rapids (*Steatocranus, Gobiochromis*). The Tribe Tilapiini is basically distinguished from the Haplochromini, a related African tribe belonging to the Cichlidae, by the structure of the apophysis on the base of the skull for the articulation of the upper pharyngeal bones (Trewavas, 1983). In Tilapiini its facets are formed from the parasphenoid alone, whereas in the Haplochromini the basioccipital forms up to one half of each facet (see Figs. 1 & 2 of Trewavas, 1983). Of the other tilapiine genera Trewavas (1983) suggests that the least specialized is *Pelmatochromis* Steindachner (as restricted by Thys van den Audenaerde, 1968a), and this genus, and especially, *Pelmatochromis nigrofasciatus* (Pelligrin) may be representative of the ancestral group from which *Tilapia* diverged by an ancient dichotomy based on diet. Thys van den Audenaerde (1968b) proposed *Pelmatochromis* as a subgenus of the *Tilapia* and included the herbivorous *T. busumana* as *T. (P.) busamana*, a decision that Trewavas (1973) disputed. She classified *Pelmatochromis* as a separate genus and ancestral to the genus *Tilapia* because of the retention of certain morphological characters that are primitive in Cichlidae (see details in Trewavas, 1973, 1983). Trewavas (1973, 1983) commented on important differences between *Pelmatochromis* and *Tilapia* particularly in their diet, *Tilapia* being

mainly vegetarian in adults whereas *Pelmatochromis* usually eats small invertebrates, and in the structural features associated with their diets, especially dentition and intestinal morphology.

Chronologically the mouthbrooding species have been regrouped a number of times. Originally *Sarotherodon* and *Oreochromis* were described as genera by Rüppell (1852) and Günther (1889) respectively, with *S. melanotheron* and *O. hunteri* as the type species for each genus. Then the two genera were classified together as a subgenus (*Sarotherodon*) of the genus *Tilapia* by Regan (1920) and Trewavas (1966). In 1968 seven mouthbrooding subgenera (*Sarotherodon, Oreochromis, Alcolapia, Neotilapia, Nyasalapia, Loruwiala* and *Danakilia*) were recognised by Thys van den Audenaerde (1968b), and in 1971 he added one more subgenus *Nilotilapia*. Later Trewavas (1973, 1980, 1982b) raised *Sarotherodon* to a genus including all of Thys van den Audenaerde's subgenera. Subsequently she (Trewavas, 1981, 1982a) believed this was unsound and proposed the present classification (Trewavas, 1983), with *Sarotherodon* and *Oreochromis* as separate genera with the proviso that *Sarotherodon* and *Oreochromis* probably arose from substrate-brooding *Tilapia*, possibly from different species.

Trewavas (1983) also reclassified the subgenus *Danakilia* of Thys van den Audenaerde (1968b) to a genus because of some of its morphological characters she believes make it generically distinct. She suggests that *Danakilia* is clearly related to *Iranocichla* (Coad, 1982), which, although a mouthbrooder, has a lineage independent of that of either *Sarotherodon* or *Oreochromis*. *Iranocichla* is the southwestern Iranian tilapiine which Coad (1982) suggested was related to the Jordanian and Syrian genus *Tristramella* (Trewavas, 1942).

The present classification of the Tribe Tilapiini is summarized in Table 1.

Table 1. The present classification (Trewavas, 1983) of some genera in the Tribe Tilapiini. Compiled from Steinitz & Ben-Tuvia (1960), Thys van den Audenaerde (1968b), Trewavas (1973, 1982 a & b, 1983), and Coad (1982). [Scientific names are given with authorities. An authority given in brackets means the generic status of that species has been changed from the original use.]

Genus	Subgenus	Type species	Other examples	Breeding habit
Pelmatochromis Steindachner	-	*P. buettikoferi* Steindachner	*P. ocellifer* Boulenger *P. nigrofasciatus* (Pellegrin)	Substrate spawners (details unknown)
Tilapia A. Smith	*Tilapia* A. Smith	*T. (T.) sparrmanii* A. Smith	*T. (T.) ruweti* Poll & Thys *T. (T.) busamana* (Günther) etc.	Substrate spawners and guarders
	Trewavas Thys	*T. (Tr.) guinasana* Trewavas	-	As *Tilapia*
	Pelmatolapia Thys	*T. (P.) mariae* Boulenger	*T. (P.) cabrae* Boulenger *T. (P.) brevimanus* Boulenger etc.	As *Tilapia*
	Heterotilapia Regan	*T. (H.) buttikoferi* (Hubrecht)	*T. (H.) cessiana* Thys	As *Tilapia*
	Dagetia Thys	*T. (D.) rheophila* Daget	-	Probably as *Tilapia*
	Coptodon Gervais	*T. (C.) zillii* (Gervais)	*T. (C.) rendalli* (Boulenger) *T. (C.) tholloni* (Sauvage) etc.	As *Tilapia*
Tristramella Trewavas	-	*Tr. sacra* Günther	*Tr. magdalenae* (Lortet) *Tr. simonis* (Günther)	Biparental mouth-brooders
Danakilia Thys	-	*D. franchetti* (Vinciguerra)	-	Mouth-brooders (details unknown)
Iranocichla Coad	-	*I. hormuzensis* Coad	-	Mouth-brooders (details unknown)

Genus	Subgenus	Type species	Other examples	Breeding habit
Sarotherodon Rüppell	-	*S. melanotheron* Rüppell	*S. galilaeus* (Linnaeus) *S. linnellii* (Lönnberg)	Paternal, biparental or maternal mouth-brooders
Oreochromis Günther	*Oreochromis* Günther	*O. (O.) hunteri* Günther	*O. (O.) niloticus* (Linnaeus) *O. (O.) aureus* (Steindachner) *O. (O.) spilurus* (Günther) *O. (O.) urolepis hornorum* (Trewavas) *O. (O.) mossambicus* (Peters) *O. (O.) mortimeri* (Trewavas) *O. (O.) andersonii* (Castelnau) *O. (O.) placidus* (Trewavas) *O. (O.) shiranus* (Boulenger) *O. (O.) jipe* (Lowe) etc.	Arena spawners and maternal mouth-brooders
	Alcolapia Thys	*O. (A.) alcalicus grahami* (Boulenger)	*O. (A.) alcalicus alcalicus* (Hilgendorf)	Arena spawners and maternal mouth-brooders
	Vallicola Trewavas	*O. (V.) amphimelas* (Hilgendorf)	-	Probably as *Oreochromis*
	Nyasalapia Thys	*O. (Ny.) squamipinnis* (Günther)	*O. (Ny.) karongae* (Trewavas) *O. (Ny.) lidole* (Trewavas) *O. (Ny.) macrochir* (Boulenger) etc.	Arena spawners and maternal mouth-brooders
	Neotilapia Regan	*O. (Ne.) tanganicae* (Günther)	-	Maternal mouth-brooders (details unknown)

2.2 Different Evolutionary Theories Proposed & Disagreements on the Present Classification

The generic reclassification of the broad genus *Tilapia* (Thys van den Audenaerde, 1968b; 1971), in which some subgenera (i.e. *Sarotherodon, Oreochromis* and *Danakilia*) have been raised to the generic levels, by Trewavas (1973, 1982a, 1983) has not been accepted by all taxonomists and other scientists working with these species. Arguments on subdividing the broad genus *Tilapia* into more than one genus have been fully discussed and published elsewhere (Peters & Berns, 1978, 1982; Thys van den Audenaerde, 1978, 1980; Trewavas, 1983).

There are two main theories for the possible evolution of tilapiines (Trewavas, 1980; Peters & Berns, 1978, 1982). Both generally agree that substrate spawners (*Tilapia*) have given rise to mouthbrooding branches from time to time. Trewavas (1980) believes that both the mouthbrooding genera could have arisen from one or possibly two such splits from the ancestral line; one branch, *Sarotherodon*, remaining conservative and the other becoming the more progressive *Oreochromis*. Peters & Berns (1978, 1982) believe that a number of splits from the ancestral substrate spawners may have occured; the most ancient of these now represent the 'older' mouthbrooders, or maternal mouthbrooders compared with the more recently branched, 'younger' mouthbrooders or paternal and biparental mouthbrooders. Peters & Berns do not agree with Trewavas in her generic classification. They believe that the various forms should be called *Tilapia* and at best given subgeneric status based on their ideas of the evolution of the group. Thys van den Audenaerde (1980) also suggested that the broad generic name *Tilapia* should be retained for general use

and reserve the other names as subgeneric names for use by specialists only. The different ideas on the classification and evolution of tilapia are based on morphology and behaviour, which may not give enough systematic information to reveal the taxonomy and evolution of the species in this group clearly. Further to this, more systematic information at the molecular level of these species should be gained so that these problems can be resolved.

2.3 Difficulty & Confusion in Species Identification

It has become apparent that with the many introductions and fish movements both worldwide and within the tilapia's endemic range, there is great difficulty in identifying the actual nature of the fish species available. Many incidences of hybridization have been noted in natural water bodies, usually after introductions of non-indigenous species (McAndrew & Majumdar, 1983; and see Trewavas, 1983). Poor management of cultured stocks has allowed unwanted hybridization of previously pure species to occur by escapes into the wild and vice versa.

Despite the diversity of habitat and food requirements, the commercially important tilapia are remarkably similar in overall morphology with much emphasis placed on the breeding colouration of the adults in species identification. The morphometric and meristic characters used in identification are of limited value in that these characters often have overlapping distributions (see Fryer & Iles, 1972; Trewavas, 1983). Such characters are also affected by environmental factors, and with the widespread distribution of many species this

means that the fish are reared under quite different conditions to those in which they were originally described. These problems of identification are further complicated by the presence of hybrids which are usually intermediate in appearance to the parental species. Such problems have led to misidentification of species, particularly between *Oreochromis (O.) aureus* and *O. (O.) niloticus* (McAndrew & Majumdar, 1983).

Identification keys based on morphological characters of tilapias have been provided (Trewavas, 1983; Lowe-McConnell, 1988). However, in situations in which species have become mixed through introductions and transfers, such keys can prove very difficult to use. Lowe-McConnell (1988) accepted that the field keys she provided sometimes can be found difficult to use because some of the characters used in the keys, like male breeding colours and nest form, cannot be observed. Another difficulty is that small or juvenile specimens can be very difficult to identity. For example, juveniles of the *Oreochromis (Nyasalapia)* species in Lake Malawi (the chambo) which live in mixed shoals are very difficult to assign to the separate species. These species are best distinguished with breeding colouration in mature individuals only.

As introductions and transfers of tilapias are still essential for research and the future development of the culture industry, especially as new strains, hybrids and polyploids are being developed, a new system of strain identification is needed for cultured tilapias. This will have to involve techniques other than the purely morphological descriptions used for natural populations (Thys van den Audenaerde, 1988).

III. GENETIC STUDIES IN TILAPIINES

As the popularity of tilapias in aquaculture increases, it is important to know more about the genetic characteristics of the different species and their stocks and strains. In common with other species much of the earlier genetic work was based on the use of electrophoretic technique to study the population genetics of wild species or as means of managing cultured stocks. However all genetic work on this group of fish will be reviewed.

3.1 Chromosome Studies

Cytogenetic studies have been used to add to the information available to help clarify the evolutionary relationships, sex determination mechanisms, and to discriminate between species at the generic or subgeneric level of the tilapiine cichlids (Kornfield *et al.*, 1979; Thompson, 1979, 1981; Arai & Koike, 1980; Vervoort, 1980; Nijjhar *et al.*, 1983; Majumdar & McAndrew, 1986; Crosetti *et al.*, 1988). Although a karyotype consisting of 48 acrocentric chromosomes is quite common among fish species, usually closely related species in a group have distinct karyotypes (Sola *et al.*, 1981). About 20 of the 70 tilapiine species, in the genera of *Tilapia*, *Sarotherodon* and *Oreochromis*, have been analysed karyologically. The group show a high level homogeneity : all species having 2n = 44, with 2 pairs of marker chromosomes much larger than the others, but some minor differences in the number of biarmed chromosomes have been noted (Arai & Koike, 1980; Thompson, 1981; Majumdar & McAndrew, 1986; Crosetti *et al.*, 1988). Majumdar & McAndrew (1986) found in their study that the chromosome number of seven tilapiine species with examples from each of the

three genera was the same (2n = 44) but the arm number (NF) varied. They explained this was most likely caused by centrometric shifting and possibly pericentric inversions. They suggests that the arm number differences indicate the possible role of pericentric inversions in the karyotype evolution of these species but the occurrence of the karyotype evolution does not appear to be associated with speciation in this group. Crosetti *et al.* (1988) have reported that identification of the tilapia stocks of different parental species and their hybrid can be cytogenetically characterized using silver (Ag-NOR) staining and C- & G-banding techniques. They found that distribution of the biarmed chromosomes differentiated the *Oreochromis niloticus* stock, with the mode of 14 biarmed, from the *O. mossambicus* stock, with the mode of 6, and the hybrid stock was intermediate with a mode of 10. Using silver staining the nucleolus organiser regions (NORs) could be counted. This enabled the parental species (maximum 5-6 silver-stained NORs) to be discriminated from the hybrid (maximum 4 silver-stained NORs). C-banding identified several homologous pairs and constituative heterochromatin associated with ribosomal genes in some NOR-bearing chromosomes of both species. In tilapia only one report claims the presence of heteromorphic sex chromosomes (Nijjhar *et al.*, 1983). They said that they observed sexual dimorphism for size in the long marker chromosome in males and females of *Tilapia busamana, Sarotherodon multifasciatus* and *O. niloticus*. Other more detailed studies did not observe morphologically differentiated sex chromosomes in at least seven different tilapias (Majumdar & McAndrew, 1986; Crosetti *et al.*, 1988). This is possibly consistent with the postulation that a strong chromosomal sex determination is unlikely in fishes such as tilapias, where malleability of sexuality exists (Kornfield, 1984).

Cytogenetic studies in fish have been of considerable basic interest but of limited value to management programmes. They have however been useful in the development of gynogenetic, androgenetic and ploidy manipulation research. But chromosome analysis is still much more time consuming than alternative identification techniques such as nuclear and cell diameter techniques and protein electrophoresis. Consequently, chromosome markers will only be practical for stock identification where protein electrophoresis does not provide a clear distinction between groups, as in some comparisons within species (Thorgaard & Allen, 1987).

3.2 Electrophoretic Studies

3.2.1 Genetic Markers & Tilapiine Stock Identification

Electrophoresis has been widely used by population geneticists and taxonomists to clarify the status of species and other taxa; providing a wide set of markers to delineate stocks, indicating polymorphisms, estimating genetic distances and heterozygosity levels (Allendorf & Utter, 1979; Shaklee *et al.*, 1982, 1990b; Richardson *et al.*, 1986; Utter *et al.*, 1987; May & Krueger, 1990; Seeb *et al.*, 1990; Seeb & Miller, 1990). This type of molecular variation is largely genetically controlled and co-dominant, and on the whole, little affected by environmental disturbances. The variation observed over a number of protein loci allows not only pure species but also hybrid individuals to be identified. This technique has the advantage that it may be used at any stage of the fish life-cycle (McAndrew & Majumdar, 1983). A number of papers on tilapiine studies have been published, using protein electrophoresis for species identification of

both wild populations (Kornfield *et al.*, 1979; McAndrew & Majumdar, 1983; Van der Bank *et al.*, 1989) and cultured stocks (Herzberg, 1978; Avtalion, 1982; Cruz *et al.*, 1982; Wu & Wu, 1983; Basiao & Taniguchi, 1984; Galman & Avtalion, 1984; Wu *et al.*, 1984; Taniguchi *et al.*, 1985; Macaranas *et al.*, 1986; Brummett *et al.*, 1988; Galman *et al.*, 1988; Romana, 1988). These papers have used electrophoresis to provide genetic markers for the investigation of wild and cultured stocks in their studies, not only in natural population but also in hybridization and genetic manipulation studies.

SPECIES-STOCK IDENTIFICATION

Several workers have presented electrophoretic evidences that allozyme markers can be used for identification of tilapiine fishes (Kornfield *et al.*, 1979; Cruz *et al.*, 1982; McAndrew & Majumdar, 1983; Van der Bank *et al.*, 1989). Whereas Cruz *et al.* (1982)'s paper has reported 30 enzymes encoded by a total of about 60 gene loci in a single species (*Tilapia zillii*), the papers of Kornfield *et al.* (1979), McAndrew & Majumdar (1983) and Van der Bank *et al.* (1989) have provided, respectively, 21 variant loci in five tilapiine species, 22 variant loci in nine species and 32 variant loci in five species. The use of electrophoretic esterase patterns as markers for the identification and control of cultured tilapia stocks has also been reported : Herzberg (1978) using surface mucus; Avtalion (1982), Galman & Avtalion (1984) and Galman *et al.* (1988), blood serum; Wu *et al.* (1984), muscle tissue. Brummett *et al.* (1988) has developed dichotomous keys based on relative electrophoretic mobilities of four isozymes for the identification of four tilapia species, *Oreochromis niloticus, O. aureus, O. mossambicus* and *O. urolepis hornorum*.

This has been important because of the widespread hybridization that has occurred as a result of fish transplantations and disturbances within natural waterbodies. The escape of species and hybrids used in culture has also exacerbated the problem in many areas. It is important to know the specific status of any given strain which may be used in aquaculture and research.

HYBRIDIZATION

It is known that tilapia species hybridize easily, especially closely related species, when environmental conditions change -- the species are brought together in the wild or transferred to culture systems. In Madagascar, Daget & Moreau (1981) reported introgressive hybridization between two tilapia species, *O. niloticus* and *O. macrochir*, introduced into the country. *O. macrochir* entirely disappeared after extensive hybridization was observed. Subsequently two '*O. niloticus*' subpopulations with different growth rates emerged. A similar phenomenon was observed in Lake Naivasha, Kenya as a result of the introduction of *O. spilurus niger* and *O. leucosticus* (Elder *et al.*, 1971). The occurrence of unwanted hybridization in cultured tilapia stocks can be detected and evidenced by enzyme electrophoresis, as reported by Taniguchi *et al.* (1985) and Macaranas *et al.* (1986). The two papers reported introgressive hybridization of feral *O. mossambicus* into cultured strains of *O. niloticus* in the Philippines.

Avtalion (1982) used blood serum to look at variation in transferins, esterases and male sex-protein (MSP) as a way to identify the species status of *O. niloticus* and *O. aureus* in Israeli commercial strains so that all hybrids could be identified and removed from the breeding stocks. This technique was also used by a number of studies to identify the specific status of various stocks of red tilapia. This highly popular cultured strain was proposed by Kuo & Neal (1982)

as being a hybrid between *O. niloticus* and *O. mossambicus*. Later the blood serum technique developed by Galman & Avtalion (1984) revealed that the esterase patterns of red tilapias (from Taiwan and Philippines) were the result of different hybridization between two or more tilapia species including *O. aureus*, *O. niloticus*, and *O. mossambicus*. Wu & Wu (1983) and Wu *et al.* (1984) suggested that the red tilapia in Taiwan was a variant strain of *O. niloticus* as it shared more alleles of esterase loci with *O. niloticus* than the other two species (*O. aureus* and *O. mossambicus*) they studied. However the technique used in these studies had limitations in that it had low discriminating loci and was only really suitable for the simplest two species management system. The system was to some extent unreliable as *O. aureus* and *O. niloticus* also shared alleles at the esterase loci and it could not distinguish hybrids past the F_1. A number of data (unpublished) on red tilapia strains which have been tested by Dr McAndrew (pers. comm.) at the Institute of Aquaculture suggest that in general they are multispecies gene pools containing varying number of alleles from *O. mossambicus, O. niloticus, O. aureus* and *O. u. hornorum*. He also recommends that the characteristics of any given individual strain need to be assessed because of their different histories -- some of the stock cultured at Stirling are red despite being pure *O. niloticus*.

CHROMOSOME MANIPULATION

Chromosome manipulation techniques, gynogenesis and polyploidy inductions, are applicable to nearly all fish species. These techniques are expected to become increasingly important in the analysis of genetic traits and various commercial applications. The development of these techniques will however rely on the identification of all of the new genotypes produced in order to optimize the various manipulations. Electrophoretic and visible markers are

required to identify maternal inheritance in gynogenetics and ensure that the sperm irradiation has been efficient and has not allowed any paternal inheritance (Thompson *et al.*, 1981; Thorgaard, 1983). While electrophoretic markers allow identification of gynogenetics, they also permit detection of polyploids (Romana, 1988). Electrophoretic studies on induced gynogenetic diploids and triploids in tilapias by Romana (1988) applied enzyme polymorphism at three loci, adenosine deaminase (*ADA**), aminopeptidase (*AP**) and malic enzyme (*MEP**), as the markers in genotypically typed tilapia broodstocks of both *O. niloticus* and *O. aureus*. The results of manipulations to induce diploid gynogenetic and triploid broods from heterozygous females were assessed by electrophoretic analysis of offspring. The electrophoretic analysis of triploids revealed banding patterns different from those observed in normal and gynogenetic diploids, and such banding phenotypes, peculiar only to triploids, denoted success in triploidy induction. This same technique has also been used in the similar genetic study on *O. niloticus* by Hussain (1992) to identify meiotic and mitotic gynogenetic offsprings.

3.2.2 Genetic Variation

An understanding of the amount and pattern of genetic variation within and between populations can be obtained by an electrophoretic analysis of variation at a range of enzyme loci. This has been a useful means of inferring the genetic structures of natural populations (Allendorf & Phelps, 1981; Utter *et al.*, 1987) and for delineating taxonomic relationships (Ferguson, 1980; Moritz & Hillis, 1990).

A variety of statistic measures can be used to denote the amount of genetic variation in populations and species; i.e. the expected heterozygosity (*He*), the proportion of polymorphic loci in a population or species (*P*), the average number of alleles per locus, and the effective number of alleles per locus (Ferguson, 1980). Among these measures, Ferguson (1980) regards heterozygosity, which is the calculated or expected frequency of heterozygotes and is normally expressed as the mean frequency of heterozygotes per locus, as the most informative measure. In tilapiine cichlids, several papers (e.g. Kornfield *et al.*, 1979; McAndrew & Majumdar, 1983; Basiao & Taniguchi, 1984; Van der Bank *et al.*, 1989) have presented average heterozygosity values as the measures of genetic variability among species. The heterozygosity levels of most tilapiine fishes in general appear to be less than or within the limits of those for other wild fishes (Kornfield & Koehn, 1975; Kornfield *et al.*, 1979; McAndrew & Majumdar, 1983; Van der Bank *et al.*, 1989), however in some tilapiine species/stocks higher than average heterozygosities appear (Basiao & Taniguchi, 1984; Sodsuk *et al.*, in preparation). Van der Bank *et al.* (1989) found that the extent of genetic variation in the southern African cichlids appeared to be less than that of other cichlids as outlined by Kornfield & Koehn (1975), Kornfield *et al.* (1979) and McAndrew & Majumdar (1983), and the values were also less than the mean value of a range of other fish species. McAndrew & Majumdar (1983) reported that the low heterozygosity observed in *O. mossambicus* in their study was probably due to severe bottlenecking of this population at the time of capture from the wild and its use as an aquarist stock which had probably caused further inbreeding. Low levels of heterozygosity possibly caused by bottleneck effects were also observed in the Japanese stock of *Tilapia zillii*, as reported by Basiao & Taniguchi (1984). Nevertheless, these authors reported a high level of heterozygosity in the cultured stock of *O. niloticus* used in Japan and suggested

that this was because the Japanese *O. niloticus* cultured stock was maintained in such a way as ensure no loss in genetic variability in its population since its introduction from Egypt in 1962, when compared to other similar stocks from Egypt. The *T. zillii* heterozygosities appearing in the cultured stock (Basiao & Taniguchi, 1984) and wild stock (McAndrew & Majumdar, 1983) were at the same level, whereas the Japanese *O. niloticus* cultured stock (Basiao & Taniguchi, 1984) appeared to have a higher heterozygosity level than the wild stock (McAndrew & Majumdar, 1983). The different loci and number of loci studied between the two studies probably resulted in these different heterozygosities (McAndrew & Majumdar, 1983, 25 loci; Basiao & Taniguchi, 1984, 35 loci). However Basiao & Taniguchi (1984) recorded polymorphism in some of the same loci studied (e.g. *IDHP**) that McAndrew & Majumdar (1983) did not find. This could also be due to different subpopulations/stocks used, although both of the two samples originally came from Egypt. A recent study by Sodsuk *et al.* (in preparation) showed the level of heterozygosity found in the chambo species of Lake Malawi (Lake Malawi *Nyasalapia*) was considerably higher than in other wild tilapia species studied. This may be a result of the long-term stability of large population size in the lacustrine environment.

3.2.3 Genetic Distances & Evolutionary Relationships

Molecular variation data have proved very useful in tackling systematic problems in many groups of organisms (Moritz & Hillis, 1990). The molecular data obtained from electrophoretic analyses as allele frequencies can be analysed to give a range of genetic distance measurements which can be used to derive dendrograms (phylogenetic trees) for the measure of similarities or distances

between species or populations and to compare their evolutionary relationships (Ferguson, 1980). It is generally found that phylogenetic trees derived from molecular data are similar to those derived from anatomical morphological characteristics (Mickevich & Johnson, 1976). A number of workers have used electrophoretically derived molecular data from tilapiines to compare genetic distances and evolutionary relationships between species in this group (e.g. Kornfield *et al.*, 1979; McAndrew & Majumdar, 1984; Sodsuk & McAndrew, 1991).

The study on six species in four cichlid genera (*Tristramella, Haplochromis, Tilapia* and *Sarotherodon*), which included three tilapiine genera, by Kornfield *et al.* (1979) reported that the species pairs within *Sarotherodon* and *Tristramella* exhibited a high degree of similarity which suggested very recent divergence; the estimates of similarity among genera indicated varied and prolonged periods of independent phyletic evolution. They also asserted that their electrophoretic findings closely approximated the formal taxonomic relationships established independently from morphology and were compatible with the elevation of *Sarotherodon* to generic status by Trewavas (1973). Though there was substantial divergence among tilapiine species in their study, they claimed evidence of a *Tilapia-Haplochromis* dichotomy.

McAndrew & Majumdar (1984) considered the evolutionary trees produced by three different dendrograms construction methods [the UPGMA method of cluster analysis and the Fitch-Margoliash method of phylogenetic tree construction, using genetic distance data; and the Wagner tree procedure of Farris (1970), using allele freqency data and the presence or absence of alleles coded as '1' or'0']. They used 25 enzyme loci in nine different tilapiine species [*T.*

zillii, S. galilaeus, O. (O.) andersonii, O. (O.) aureus, O. (O.) jipe, O. (O.) mossambicus, O. (O.) niloticus, O. (O.) spilurus and *O. (O.) macrochir*] from the three genera, *Tilapia, Sarotherodon* and *Oreochromis*, proposed by Trewavas (1982, 1983). These authors reported that their results were equivocal as to which of the two main hypotheses on the evolution of this group (Trewavas, 1980; or Peters & Berns, 1978, 1982) was correct. This was caused by *O. (O.) jipe* being consistently placed outside the maternal mouthbrooding clade which possibly suggested polyphyletic origin of this group. This study only included one species each from the *Tilapia* and *Sarotherodon* genera and they suggested that further work should be undertaken on additional *Tilapia* and *Sarotherodon* species. A molecular systematic study using allozyme data from 44 different loci examining 15 tilapiine species, including six additional species [*T. buttikoferi, T. mariae, T. rendalli, T. tholloni, O. (O.) mortimeri* and *O. (O.) urolepis hornorum*] to the McAndrew & Majumdar (1984) study, was recently published by Sodsuk & McAndrew (1991). This paper has demonstrated that the substrate spawning *Tilapia* are consistently separated from the two mouthbrooding genera *Sarotherodon* and *Oreochromis* by the dendrograms constructed using both genetic distance and binary coded data. In addition, the *O. (O.) jipe* was now consistently placed with the other *Oreochromis* species possibly due to the inclusion of more closely related species.

IV. MOLECULAR APPROACH TO SYSTEMATIC STUDIES

4.1 Molecular, Morphological, and Combined Approaches

In the past two decades, molecular investigations of systematic problems have progressed to a standard means of elucidating phylogenetic history. This sudden rise of biochemical systematics has precipitated debates between the traditional morphological and the new molecular camps. However, as stated by Hillis (1987), systematic studies of any set of genetically determined characters should be congruent with other such studies based on different sets of characters in the same organisms. Consequently, congruence between studies is strong evidence that the underlying historical pattern has been discovered (Mickevich & Johnson, 1976; Mickevich & Farris, 1981; Miyamoto, 1981); conflict may indicate theoretical or procedural problems in one or both analyses, or it may indicate that additional data are needed to resolve the phylogenetic relationships in question (Hillis, 1987).

A major conflict of the two approaches is that the histories of the application of the two techniques to systematic problems differ to a large extent. Molecular systematics grew mostly out of population genetics, whereas morphological systematics stemmed largely from comparative anatomy. Although this difference in background has presented numerous problems in comparing many past studies, recent advances in systematic theory have transcended traditional boundaries and have been applied with equal success to both morphological and molecular data sets (Goodman *et al.*, 1979; Wiley, 1981; Buth, 1984).

Many systematists are realizing the value of multidisciplinary studies and are combining as many sources of information as possible in order to maximize information, explanation, and stability. No single systematic data set can be expected to be informative at all phylogenetic levels simultaneously (Hillis, 1987). Some techniques are useful for resolving questions of phylogeny among closely related species, whereas others are useful across ancient time spans (Hillis & Davis, 1986). Often, several different techniques are required to maximize phylogenetic resolution within a group of interest (Hillis, 1987).

As reviewed by Hillis (1987), morphological and molecular systematic techniques each have distinct advantages for phylogenetic reconstruction. On one hand, morphological techniques are applicable to an enormous range of museum and fossil material, a large portion of the Earth's organisms continuing to be studied primarily or exclusively from morphological information. On the other hand, the potential molecular data set is incredibly extensive and, when fully utilized, should provide a detailed record of the history of life. Studies that combine the two approaches can thereby maximize both information content and usefulness. However, Hillis (1987) notes that it is important to select methods of analysis that are as assumption-free as possible and also are amenable to a combination of data sets (i.e. the use of network construction with outgroup comparison method, as well as the use of character-state coded data). Such combinations of molecular and morphological studies should provide a truly comprehensive view of biotic evolution.

4.2 Electrophoretic Application in Molecular Systematics

4.2.1 Electrophoretic data & approach to systematics

Electrophoretic data is widely acknowledged to be of value to systematics (Avise, 1974; Baverstock *et al.*, 1979; Shaklee *et al.*, 1982; Buth, 1984; Swofford & Olsen, 1990). Although starch-gel electrophoresis of enzymes has become the established method of generating the data, the analysis of electrophoretic data has remained varied and at times openly contested (Mickevich & Johnson, 1976; Prager & Wilson, 1978; Farris, 1981; Felsenstein, 1981; Swofford, 1981; Tateno *et al.*, 1982; Nei *et al.*, 1983; Patton & Avise, 1983). Many studies purporting to compare systematic treatments of electrophoretic data actually confuse the issue by simultaneously varying procedures at several levels, e.g. data transformation and coding, as well as methods of analyses.

ISOZYMES & ALLOZYMES

Markert & Moller (1959) introduced the concept of *isozymes*, which they defined as *'the different molecular forms in which proteins may exist with the same enzymatic specificity'*. The field of population genetics developed rapidly as a primary consumer of isozyme technology. However, only the relevant *allozyme* subset, which was defined by Prakash *et al.*(1969) as *'the variant proteins produced by allelic forms of the same locus'*, has been used. In a review of the systematic value of electrophoretic data, Avise (1974) recognized the difference between isozyme and allozyme data sets but limited the discussion to allozymes only. Swofford & Olsen (1990) stated that allozyme (allelic isozyme) data represent the only type of isozyme data routinely used in phylogenetic analysis. However, some workers (e.g. Buth, 1984) have also suggested that isozyme data could be useful in systematic studies.

APPROACH TO SYSTEMATICS

Bush & Kitto (1978) evaluated several molecular methods for estimating the levels of genetic divergence between taxa (based on the degree of sensitivity and ease of analysis) and concluded that gel electrophoresis was the best method for comparing races, species, and closely related genera. Avise (1974), however, noted that it was doubtful that overall genic similarities determined by electrophoresis would be of great systematic value much beyond the level of the genus. Avise's (1974) perspective was based on the observation that the levels of genetic similarities among conspecific populations are high, whereas comparative values among species are, in general, much lower. He therefore deduced that closely related species may be arranged according to the percentages of shared alleles or genotypes. Avise (1974) also stated that many readers would recognize the electrophoretic technique as a phenetic, as opposed to a phyletic, approach to systematics. But Mickevich & Johnson (1976) asserted that Avise had thoroughly confused the important distinction between types of analysis (i.e. approaches to systematics) and sources of data (i.e. techniques of data collection); electrophoretic methods yield data, thus they are not an approach to systematics. Buth (1984) noted that in subsequent allozyme studies, Avise and his colleagues employed phyletic (i.e. phylogenetic) methods. These and numerous other studies have used electrophoretic data to infer the phylogenies of a broad array of organisms.

4.2.2 Types of data used in molecular systematics

Electrophoretic data used in molecular systematics fall into two broad categories : discrete characters and similarities or distances. The most recent

explanation on the use of molecular data in systematic studies is presented by Swofford & Olsen (1990). The following is a brief overview of the types of molecular data.

Discrete character data Discrete character data are those for which a data matrix **X** assigns a **character state** x_{ij} to each taxon i for each character j. Although systematists sometimes disagree regarding the terminological distinction between 'character' and 'character-state', Swofford & Olsen (1990) prefer to think of characters as independent variables whose possible values are collections of mutually exclusive character states. A discrete character provides data about an individual species or taxon.

A classification of character types In general, character data are either **qualitative**, in which the possible states are two or more discrete values, or **quantitative**, in which the characters vary continuously and are measured on an interval scale. Qualitative characters may be further subdivided into **binary** (two possible states) and **multistate** (three or more possible states). Binary characters typically represent the presence or absence of some item, such as the recognition sequence for a restriction endonuclease at a certain map location (restriction site) or a particular allele at an isozyme locus.

Quantitative characters are less commonly used as character data in molecular systematics, the prominent exception occurring when polymorphic characters such as allozymes or mtDNA haplotypes are coded as frequencies.

Distance or similarity data A similarity or distance value is a quantitative comparison of two species or sequences. Unlike character data, in which values are assigned to individual taxa, distance or similarity data specifies

a relationship between *pairs* of taxa or molecules. Allozyme data can be treated using distance methods following an appropriate transformation. In some cases, the use of distances may be preferable, even though alternative character-based methods are available.

4.2.3 Forms of allozyme data commonly used

As stated by Swofford & Olsen (1990), electrophoretic data routinely used in phylogenetic analysis are represented by allozymes. These data are usually presented as a three-dimensional array that specifies the frequency of each allele at each locus in each population or taxon. Allozymes used in systematic study will be in three different forms of data: genetic distances (quantitative comparisons between taxa which describe pairwise relationships), character-state or presence/absence coded data (discrete characters), and allele frequencies (continuous characters).

GENETIC DISTANCES

A large number of measures have been proposed for transforming allelic and genotypic frequency data to genetic distances (Wright, 1978). The commonly used measures recently reviewed and demonstrated by Swofford & Olsen (1990) are those of Nei (1972, 1978), Rogers (1972), Cavalli-Sforza & Edwards (1967), and Wright (1978).

(a) ***Nei's distance.*** Historically, the most frequently used genetic distance has been that of Nei (1972, 1978). Nei's distances (in either their original form or as modified by Hillis, 1984) are non-metric in that they frequently violate the triangle inequality (see additive trees & the distance

Wagner procedure and Fig. 1 below). Farris (1981) has heavily critized it for this reason, arguing that when a distance measure is non-metric, it is meaningless to fit branch lengths under an **additive tree** model in which branch lengths are interpreted as amounts of evolutionary change. Felsenstein (1984) countered that if branch lengths were interpreted as expected rather than actual amounts of change, Farris's objections were moot (see also Farris, 1985, 1986; Felsenstein, 1986). However, as Nei's model of evolution has been put in doubt, routine usage of Nei's distance is not recommended by some workers (e.g. Swofford & Olsen, 1990).

(b) ***Roger's distance*** Another widely used distance measure is that of Rogers (1972). Rogers's measure has the virtues of simplicity and an easily interpretable geometric basis. It is the Euclidean distance between the allele frequency vectors for each locus of the two taxa being compared. However, Rogers's coefficient shares with Nei's the undesirable property of being too heavily influenced by within-taxon heterozygosity (Wright, 1978; Hillis, 1984); the distance between two taxa that are fixed for alternate alleles exceeds that between two taxa in which one or both are heteroallelic but have no alleles in common (Swofford & Olsen, 1990).

(c) ***Cavalli-Sforza & Edwards's arc and chord distances*** An alternative Euclidean measure that overcomes the limitation of Roger's distance measure is the arc distance of Cavalli-Sforza & Edwards (1967). More importantly, this distance incorporates an angular transformation of gene frequencies in an attempt to make the variances of the transformed frequencies independent of the ranges in which they fall (Swofford & Olsen, 1990). This transformation has the effect of standardizing the distance with respect to random drift, so that the rate of increase in genetic distance under drift is nearly independent of the initial gene

frequencies. The Cavalli-Sforza & Edwards (1967) arc distance and its relative, the chord distance, thus incorporate some realistic assumptions about the nature of evolutionary change in gene frequencies without the undesirable properties of the Nei (1972, 1978) and Rogers (1972) measures (Swofford & Olsen, 1990).

(d) ***Wright's Prevosti distance*** The simplest distance of all is the Manhattan distance, which Swofford & Olsen (1990) said was attributed to Prevosti by Wright (1978). Unlike the Cavalli-Sforza & Edwards (1967) distances, as stated by Swofford & Olsen (1990), this method gives equal weight to a given frequency difference regardless of where it occurs on the scale from zero to one, so it is not sensitive to intrataxon variability.

CHARACTER-STATE & PRESENCE/ABSENCE CODED DATA

The use of allozymic data in the form of discrete character or character-state data in systematic study is prefered by some systematists, who have argued because of its suitability it should be employed in phylogenetic reconstruction (e.g. Mickevich & Johnson, 1976; Buth, 1984; Hillis, 1987). Mickevich & Johnson (1976) preferred allozyme data to be used in the recast presence/absence form (discrete character) rather than as allele frequency data because they believed that the presence or absence of an allele was of more fundamental evolutionary importance than its frequency. Buth (1984) and Hillis (1987) have also recommended the use of discrete character data. Buth's (1984) preference was to use allozyme as well as isozyme data in particulate fashion, encoding these data as characters and states. He mentioned that the method was preferable because it yielded the maximal information content. Hillis (1987) has suggested the use of molecular data as qualitative character-states in systematic study because it is the appropriate method for the combination of molecular and

morphological approaches, which can maximize the phylogenetic information and provide a comprehensive evolutionary view. However, this form of allozymic character data is not recommended by some workers, e.g. Swofford & Olsen (1990) who believed that methods which require recoding of allele frequency arrays into discrete states should only be used when levels of polymorphism are extremely low, with problematical loci being excluded from the data set.

ALLELE FREQUENCIES

Rogers (1984, 1986) and Swofford & Berlocher (1987) developed methods of analysis that used the observed frequencies directly in character-based analyses rather than requiring their recoding as discrete character states. Swofford & Olsen (1990) mentioned that the Felsentein's (1981) maximum likelihood method for continuous characters evolving under a Brownian motion process could also be applied to gene frequency data (after an appropriate transformation). However, the use of allozyme data in the form of allele frequencies in systematic study has caused much controversy (e.g. Mickevich & Johnson, 1976; Farris, 1981, 1985; Crother, 1990; cf. Swofford & Olsen, 1990). Numerous arguments have been presented against the use of allele frequencies in phylogenetic reconstruction, focusing on the flaws of not only the particular analytical methods employed for frequency data (Farris, 1981, 1985), but the *nature* of allele frequencies themselves (Crother, 1990). Farris (1981) found, from his survey of empirical data, that frequency arrays were either similar between the pair of taxa or were essentially fixed for different alleles, and consequently suggested that allele frequencies were unnecessary in phylogenetic analysis because alleles appeared not to possess information that would alter a set of relationships based only on fixed differences. Moreover Crother (1990) has recently criticized in his essay that it is the *nature* of allele frequencies (i.e. the absence of synapomorphy,

non-metricity and a clock assumption) that renders the data inappropriate for phylogenetic analysis. Among these natural problems of allele frequencies, the absence of synapomorphy seems to be the most severe criticism. He (Crother, 1990) has pointed out that allele frequencies vary temporally over the span of a few years, and therefore cannot, by definition, be synapomorphic. The fact is that **synapomorphies** are **character states** hypothesized to delineate unique geneological groups that share a common ancestral taxon (Wiley, 1981). The synapomorphic condition is relative to hierarchical level, thus synapomorphy reflects descent with modification within a cladogram. A phylogeny represents development and relationships of lineages and clades through time, so information (apomorphies) used to construct a phylogeny should be temporally stable to result in a historical pattern (Crother, 1990). Mickevich & Johnson (1976) also believe that allele frequencies are too easily modified by random drift and/or selection, and therefore do not provide reliable information for phylogenetic analysis. These problems, therefore, have been used to question the relevance of allele frequencies to phylogeny. Swofford & Olsen (1990) would argue, however, that even if the information contained in allele frequencies is somewhat unreliable, the frequencies at least provide a way to weight the presence or absence of particular alleles.

4.3 Methodology of Data Analyses for Inferring Phylogenies

Two recent reviews on the methods for inferring phylogeny from molecular data are by Felsenstein (1988) and Swofford & Olsen (1990). The following are the methods commonly used at present, some of which are believed to give the least erroneous and problematic results in phylogenetic analysis, and therefore have been used in this thesis.

4.3.1 Methods based on pairwise distances

In Felsenstein's (1988) review, distance methods fit a tree to a matrix of pairwise distances between species. The phylogeny makes a prediction of the distance for each pair as the sum of branch lengths in the path from one species to another through the tree. A measure of goodness-of-fit of the observed distances to the expected distances is used. Consequently, that phylogeny which minimizes the discrepancy between them as evaluated by this measure is preferred. Felsenstein has noted that the concept of that distance methods assume a molecular clock is a widespread misunderstanding. He has emphasized that it is possible to either assume or not assume a molecular clock when using distance methods.

The most commonly used methods for pairwise distance data probably are the cluster analysis (with ultrametric distances) and the additive tree technique (with the distance Wagner algorithm). These methods are well described and demonstrated by Swofford & Olsen (1990), and are summarized below.

ULTRAMETRIC DISTANCES & THE USE OF CLUSTER ANALYSIS

Ultrametric distances Mathematically, ultrametric distances are defined by satisfaction of the three-point condition. The ultrametric inequality requires the satisfaction of the three-point condition for any three taxa (A, B, and C) to establish that the distances are ultrametric:

$$d_{AC} \leq \max (d_{AB}, d_{BC})$$

or:

$$\max (d_{AB}, d_{BC}, d_{AC}) = \text{mid } (d_{AB}, d_{BC}, d_{AC})$$

'max' = the maximum value function

'mid' = the middle value (median) function

Phylogenetically (Fig. 1 b), ultrametric distances will precisely fit a tree so that the distance between any two taxa is equal to the sum of the branches joining them, and the tree can be rooted so that all of the taxa are equidistant from the root (a constant molecular clock). If distance data are ultrametric, then the use of *cluster analysis* to infer a branching pattern is valid.

Cluster analysis Cluster analysis is a family of related techniques for representing similarity or distance data (distance is more preferable) in the form of an ultrametric tree. If the data themselves are ultrametric, then the representation on the tree will be exact. It should be obvious that if the distance data themselves are not ultrametric, then they *cannot* be fit exactly to such a tree, and therefore errors might be introduced.

The most widely used clustering method is **UPGMA** (**U**nweighted **P**air **G**roup **M**ethod using **A**rithmetic average), or 'average linkage method', which is defined by Felsenstein (1988) as the result of applying a certain algorithm, and that algorithm would work perfectly only if the data were generated by a clocklike evolution: if the data were an exact fit to a non clock-like tree the UPGMA method could give erroneous results.

Cluster analysis has drawbacks which have been emphasized by Swofford & Olsen (1990). Whereas ultrametric distances are the most constrained and the likelihood of obtaining ultrametric data is small, the algorithm of the method has no objective definition of what constitutes an optimal tree when the data are not ideal. In particular, because genes do not diverge uniformly in all organisms or organelles, systematic errors are likely to be introduced into cluster analysis reconstructions. Alternatively more rapid methods, such as the distance Wagner procedure, are applicable to work for all additive trees with no requirement of such ultrametric distances.

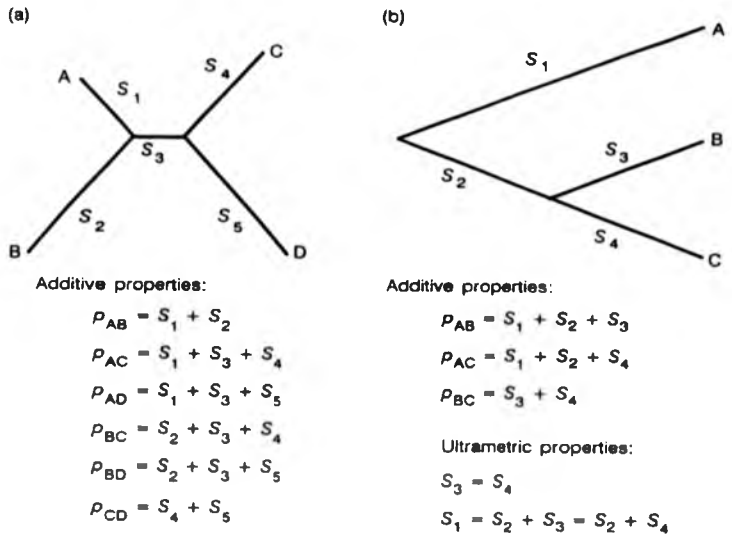

Fig. 1 Additive and ultrametric trees.

(a) An additive tree relating four taxa: A, B, C, and D. Also shown are the relationships between the six taxon-to-taxon distances (p_{AB} - p_{CD}) and the five branch lengths (S_1 - S_5). Additive distances and trees do not make any assumption about the rooting, hence the relationships are displayed in an unrooted format. All sets of pairwise distances that satisfy the four-point condition can be represented as a unique additive tree.

(b) An ultrametric tree relating three taxa: A, B, and C. In addition to having additive properties (all taxon-to-taxon distances are the total of the branch lengths joining them), every common ancestor is equidistant from all its descendants. Thus the most recent common ancestor of B and C is S_3 from B and S_4 from C, therefore $S_3 = S_4$. Likewise the common ancestor of A and B is S_1 from A and $S_2 + S_3$ from B, therefore $S_1 = S_2 + S_3$. (From Swofford & Olsen, 1990)

ADDITIVE TREES & THE DISTANCE WAGNER

Additive distances satisfy the four-point condition, specifically for any four taxa A, B, C, and D:

$$\max (d_{AB}+d_{CD},\ d_{AC}+d_{BD},\ d_{AD}+d_{BC}) = \text{mid } (d_{AB}+d_{CD},\ d_{AC}+d_{BD},\ d_{AD}+d_{BC})$$

Additive tree techniques (Fig. 1 a) comprise a relatively broad class of methods that operate under the assumption that the lengths of the branches lying on the path between any pair of taxa can be summed to yield a meaningful quantity (e.g. amount of evolution).

A variety of algorithmic methods related to cluster analysis have been proposed that will correctly reconstruct additive trees, whether the data are ultrametric or not. In an analogy to character-based parsimony, the desired tree is the one that minimizes the total of all branch lengths in the tree, while using the pairwise distances as lower bounds of the paths. In this case, the distance Wagner algorithm of Farris (1972), which builds an additive representation of the tree by sequential additional taxa, is an effective heuristic. The distance function defined in the distance Wagner procedure is a metric, usually referred to as the Manhattan metric, that has a property known as ***triangle inequality*** (Farris, 1972).

For any three points, *A*, *B* and *C*, the triangle inequality is:

$$D\ (A,C) \leq D\ (A,B) + D\ (B,C)$$

Unlike cluster analysis, additive-tree methods yield unrooted trees, which are adequate for some purposes. If a root is to be placed, however, it must be based on an ancillary criterion. Usually, one or more taxa (outgroup taxa) that are assumed to lie outside a monophyletic group of interest are included in the analysis. The location at which these taxa join the tree defines the root with respect to the ingroup. (See 'ROOTING WITH OUTGROUP' below.)

4.3.2 Parsimony methods for discrete character data

Of the existing numerical approaches for inferring phylogenies directly from character data, methods based on the principle of **maximum parsimony** (the principle of minimizing the amount of evolutionary change needed to explain data) have been the most widely used (Swofford & Olsen, 1990).

In general, parsimony methods for inferring phylogenies operate by selecting trees that minimize the total **tree length**: the total amount of change or the number of evolutionary 'steps' (transformations from one character state to another) required to explain a given set of data. Any parsimony methods must be within the conceptual framework of two distinguished sections, the optimality criterion (minimal tree length under a specified set of restrictions on permissible character-state changes) and the actual algorithm used to search for optimal trees. Swofford & Olsen (1990) have assumed that every possible tree can be evaluated, optimizing each one according to the chosen criterion and ranking them according to that criterion.

WAGNER PARSIMONY

Wagner parsimony is one of the simplest parsimony methods, besides Fitch parsimony, and is widely used. The method imposes minimal constraints on permissible character-state changes. The Wagner method, formalized by Eck & Dayhoff (1966), Kluge & Farris (1969) and Farris (1970), assumes that characters are measured on an interval scale. Thus, it is appropriate for binary, ordered multistate (multistate characters for which the changes between states are constrained; not all states can be reached directly from any other), and continuous

characters. Wagner parsimony assumes that any transformation from one character state to another also implies a transformation through any intervening states, as defined by the ordering relationship. In addition the Wagner method permits free **reversibility**; change of character states in either direction is assumed to be equally probable, and character states may transform from one state to another and back again. A consequence of reversibility is that the tree may be rooted at any point with no change in the tree length.

ROOTING WITH OUTGROUP

Swofford & Olsen (1990) stated that a common misconception regarding the use of the parsimony method was the method requiring a determination of character *polarities* (the direction of character evolution). In morphologically based studies, character polarity is often inferred using the method of **outgroup comparison**, and the resulting polarized characters form the basis of the analysis. Furthermore, since the 'hypothetical ancestor' is implied by the polarity assignments, the output of an analysis of polarized characters is a rooted tree.

Most of the methods discussed above do not specify the location of the root. If a rooted tree is desired, as is generally the case, the root must be located using extrinsic information. The most commonly used method is to include one or more of the 'outgroup' taxa that are assumed to lie cladistically outside a presumed monophyletic group. The location at which the outgroup joins the unrooted tree implies a root with respect to the ingroup. However, it is emphasized (Swofford & Olsen, 1990) that the assignment of taxa to the outgroup constitutes an assumption that the remaining taxa (the **ingroup** taxa) are **monophyletic**; if this assumption is wrong, the tree will be rooted incorrectly.

4.3.3 Compatibility methods

In some cases, characters would be classified into two groups, one comprising characters that are all equally reliable, the other containing characters that are worthless. If it is believed that characters behaved in that way, a method of analysis known as **character compatibility** (Felsenstein, 1981), would be used (Swofford & Olsen, 1990). Compatibility methods are considered to be closely related to parsimony methods (e.g. Felsenstein, 1988), but use a different criterion for resolving conflict among characters. A compatibility method searches for the **largest 'clique'**, which is a set of mutually compatible characters that can all evolve without homoplasy (i.e. convergence, parallelism, and reversal) on the same evolutionary tree, so that each character state arises only once (Felsenstein, 1988; Swofford & Olsen, 1990). Compatibility methods are no longer in widespread use, probably because of their implicit adherence to an unrealistic model that asserts that once a character has been excluded from the largest clique, it no longer conveys any useful information whatsoever (Swofford & Olsen, 1990).

4.3.4 Maximum likelihood methods

Maximum likelihood methods have been put forward for estimating phylogenies (i.e. Felsenstein, 1973, 1981). The primary assumption of Felsenstein's (1981) maximum likelihood method for continuous characters (gene frequencies) is that each character evolves independently according to a Brownian motion process (the mean phenotype in the population undergoing a random

diffusion on an infinite linear scale). Therefore random genetic drift will be well approximated by Brownian motion, except that the rate of diffusion will differ in different parts of the gene frequency scale (Felsenstein, 1981). A maximum likelihood method was also proposed by Felsenstein (1973) for data in which there are a number of discrete states for each characters. In this case the details of the method would depend on the details of the assumed probabilistic model of evolution. For a few of the simpler models, the maximum likelihood tree would be the same as the 'most parsimonious' (or minimum steps) tree if the probability of change during the evolution of the group was assumed to be very small. Felsenstein (1973), however, noted that most sets of data required too many assumed state changes per character to be compatible with this assumption. In a review of maximum likelihood phylogenies for nucleotide sequences, Swofford & Olsen (1990) could see that a major objection to apply a maximum likelihood approach is that a concrete model of the evolutionary process that converts one sequence into another must be specified. Accordingly, it was inferred by these authors that this model may contain many parameters that are to be estimated from the data, although it may be fully defined. Swofford & Olsen (1990) realized that a maximum likelihood approach to phylogenetic inferrence evaluates the net likelihood that the given evolutionary model will yield the observed sequences; the inferred phylogenies are those with the highest likelihood.

Likelihood methods are not as widely known as they ought to be, because the computation of the likelihood frequently involves taking products of a large number of quantities or sum of logarithms, even though maximum likelihood is

the most general method of deriving statistical estimates (Felsenstein, 1988). Swofford & Olsen (1990) said that several areas of biological research, notably genetic mapping and clinical testing, routinely use maximum likelihood methods for testing hypotheses. They, however, suggest that the perceived and actual complexities of obtaining maximum likelihood solutions to problems that involve numerous alternative hypotheses have inhibited the more general use of these techniques.

4.3.5 Jackknifes & bootstraps: the resampling methods

Even if evolution works exactly in the way that meets the assumptions for a particular analytical method, with finite data an incorrect tree may be inferred due to chance events (Swofford & Olsen, 1990). The best way to prevent this is to avoid random errors by using the methods known as 'resampling methods' (Felsenstein, 1988; Swofford & Olsen, 1990). Felsenstein (1988) has remarked that these methods, notably the jackknife and bootstrap, which have been applied to phylogenies only recently, provide a powerful way of escaping from some of the restrictive assumptions of other methods. The methods are called 'resampling methods' because they operate by estimating the form of the sampling distribution by repeatedly resampling data from the original data set (Swofford & Olsen, 1990); under certain reasonable assumptions (Efron, 1982) the distribution of the statistic of interest can be approximated from the distribution of the sample estimate over replications of the resampling process. The jackknife was first used in a phylogenetic context by Mueller & Ayala (1982). Felsenstein (1985) discussed the potential application of the bootstrap to the estimation of confidence intervals for phylogenies.

In an overview of the two techniques by Swofford & Olsen (1990), the bootstrap and jacknife differ in the way in which resampling is performed. In the bootstrap, 'data points' are sampled randomly, with replacement, from the original data set until a new data set of the same size as the original (same number of observations) is obtained. Thus, some data points will not be included at all in a given bootstrap replication, others will be included once, and still others twice or more. The jackknife, on the other hand, resamples the original data set by dropping k data points at a time and recomputing the estimate from the remaining n - k observations. Typically, k is set to 1, so that *each* of the n data points are dropped, in turn, and a 'pseudoestimate' is computed from the remaining n - 1 points. Thus, in the jacknife, we can estimate the variance of the estimate by extrapolating from the pseudoestimates, whereas, in the bootstrap, the estimates made from the resampled data set need not be extrapolated in any way. The confidence interval associated with the 'statistic of interest' for each replication in the bootstrap can be constructed by the 'percentile method' of simply discarding the upper and lower 2.5% of the data distribution to obtain a 95% bootstrap confidence limit.

As applied by Felsenstein (1985), the 'data points' are characters (columns of the data matrix) and the 'statistic of interest' is a binary variable representing the presence or absence of a prespecified monophyletic group on the tree(s) resulting from each replication. Thus, characters are weighted according to the number of times they appear in each replicate sample; if a particular group occurs in 95% or more of the trees resulting from these replicates, one can conclude that the group is significantly supported at the 95% level (Swofford & Olsen, 1990).

V. AIMS OF THE STUDY

This thesis will focus on the molecular genetics and systematics of the Cichlid fishes, tribe Tilapiini. The work will expand the number of species and enzyme loci examined to date and utilize the genetic data to draw conclusions about the population genetics and evolution of this group. The analysis of the evolutionary relationships will use the molecular data in a variety of forms and using a number of different types of algorithmic methods. The results from the molecular data will be compared with the analysis of a range of behavioural, biogeographical and morphological (e.g. meristic, morphometric, colouration, etc.) characters deemed to be of evolutionary importance by taxonomists. These data will be coded and analysed using the same phylogenetic techniques. The phylogenies generated by the two different sets of characters will be compared and concluded as a consensus phylogeny. The results of this study will be compared to previous work in these and related species, and the importance of the results for the genetics of the group will be discussed.

****

CHAPTER 2

GENETIC INTERPRETATION, VARIATION & DIFFERENTIATION

I. INTRODUCTION

The increasing popularity of the tilapiines in aquaculture and capture fisheries in both African and Asian countries has resulted in the widespread movement of tilapia species and the mixing of previously isolated stocks (Thys van den Audenaerde, 1988). Many incidences of hybridization have been noted in natural waterbodies after introductions of non-indigenous species (McAndrew & Majumdar, 1983). Introgressive hybridizations have been reported in both natural and cultured tilapia species (e.g. Daget & Moreau, 1981; Taniguchi *et al.*, 1985; Macaranas *et al.*, 1986). This has caused great difficulties for aquaculturists and fisheries biologists who have to accurately identify the specific status of individual stocks in culture and in natural waterbodies. Morphological charateristics are often useless because of the intermediate nature of hybrids and the great overall similarities between species (Fryer & Iles, 1972; Trewavas, 1983). It is because of these problems that biochemical techniques have come to the fore for species identification, population genetics, taxonomic studies and the documentation of genetic resources in this group.

A biochemical technique known as protein electrophoresis has revolutionized the studies of the population genetics of fish (for recent reviews

see Allendorf & Utter, 1979; Shaklee *et al.*, 1982, 1990b; Utter *et al.*, 1987). Electrophoretic techniques can be used as a strategic tool to provide estimates of the level of molecular variation in allozymes. The allele frequency data obtained from electrophoretic studies provides information about the breeding structure of a particular species; single large panmictic population (species) or reproductively isolated and genetically differentiated subpopulations can be identified. Such knowledge contains important information for the management of wild stocks and the initial selection and long-term management of potential stocks for aquaculture. The allozyme data can be used in a wide range of different ways: unique allozymic genotypes can be used as genetic tags for wild and cultured stocks, enabling interactions between different strains to be assessed, e.g. restocking. Allozymes are useful for species identification and monitoring hybridization in wild and farmed stocks. Overall levels of genetic variation enable the effects of inbreeding caused by poor management or selection programmes to be monitored, identifying potential problems in longterm viability. Allozymes are now also important in many genetic studies, as fish generally have few visible markers, particularly genomic manipulations in which the fate of the paternal or maternal genome are of some consequence (Seeb & Miller, 1990). Recently female allozyme heterozygotes were also reported to be used significantly for the determination of gene-centromere recombination frequencies in gynogens (Thorgarrd *et al.*, 1983; Seeb & Seeb, 1986).

In tilapias, despite being a major genetic resource in Africa and becoming highly important in aquaculture in most tropical countries, few detailed studies on population genetics of these species have been published. The majority of the published work has concentrated on the use of allozymes for species identification mainly in cultured populations (see Chapter 1 for ref.) and

increasingly for evolutionary and taxonomic purposes (Kornfield *et al.*, 1979; McAndrew & Majumdar, 1984; Sodsuk & McAndrew, 1991). Many of the studies to date have been limited in some way either because of the low number of species, or loci used. This study was hoped to generate a significant data base from a large number of allozyme loci (40+) on a wide range of species from each of the main grouping from wild or recently captured stocks of tilapia. It was hoped that this approach would give a clear picture of the level of genetic variation in tilapia and its degree of differentiation within and between species. It was then hoped to utilize this data base to study the evolutionary relationships within this group and to compare it with the classically derived hypotheses for these species. The classically derived data would itself be assessed independently using numerical techniques, not previously used on this data, and the various results compared. This chapter will concentrate on the first of these goals, the collection and assessment of the allozyme data.

II. MATERIALS & METHODS

2.1 Species Used in the Study

Twenty-three different tilapiine species in the three genera of Trewavas (1983), *Tilapia*, *Sarotherodon* and *Oreochromis*, were used in this study. Their sources, and the number of individuals of each species used are shown in Table 2. These species were examined for 43 enzyme loci using starch gel electrophoresis. *O. (O.) placidus* and *O. (Neotilapia) tanganicae* could not be completely examined and resolved for all 43 loci because of the limited numbers

Table 2. Species used in the study, number of indidviduals used in each species, and their sources.

Species	Number of individuals	Source
Pelvicachromis pulcher (Boulenger)	2	Aquarium stock
Tilapia (Heterotilapia)		
T. (H.) buttikoferi (Hubrecht)	3	Liberia
Tilapia (Pelmatolapia)		
T. (P.) mariae Boulenger	5	Various aquarium stocks
Tilapia (Coptodon)		
T. (C.) rendalli (Boulenger)	10	Zimbabwe
T. (C.) tholloni (Sauvage)	10	Aquarium stock
T. (C.) zillii (Gervais)	10	Egypt (Lake Manzala)
Sarotherodon		
S. melanotheron Rüppel	10	Aquarium stock
S. melanotheron Rüppel	8	Ivory Coast stock
S. galilaeus (Linnaeus)	10	Kenya (Lake Turkana) (Bamburi)

Species	Number of individuals	Source
Oreochromis (Oreochromis)		
O. (O.) niloticus niloticus (Linnaeus)	60	Egypt (Lake Manzala)
O. (O.) aureus (Steindachner)	15	Egypt (Lake Manzala)
O. (O.) spilurus spilurus (Günther)	15	Kenya (River Tana) (Bamburi)
O. (O.) urolepis hornorum (Trewavas)	10	Israel (Zanzibar)
O. (O.) mossambicus (Peters)	20	Zimbabwe (Lower Zambezi)
O. (O.) mortimeri (Trewavas)	10	Zimbabwe (Upper Zambazi)
O. (O.) andersonii (Castelnau)	15	Zambia (Kafue)
O. (O.) placidus placidus (Trewavas)	3	Zimbabwe (Masingo)
O. (O.) shiranus shiranus (Boulenger)	20	Lake Malawi
O. (O.) jipe (Lowe)	15	Kenya (Lake Jipe) (Bamburi)
Oreochromis (Nyasalapia)		
O.(Ny.) macrochir macrochir (Boulenger)	10	Botswana (Okavango) (Bamburi)
O.(Ny.) karongae (Trewavas)	42	Lake Malawi
O.(Ny.) lidole (Trewavas)	33	Lake Malawi
O.(Ny.) squamipinnis (Günther)	25	Lake Malawi
Oreochromis (Neotilapia)		
O. (Ne.) tanganicae (Günther)	1	Direct import from Lake Tanganyika

of individuals available for analysis. *O. (O.) placidus* could only be examined for 36 loci, and *O. (Ne.) tanganicae* for 16 enzyme loci. *Pelvicachromis pulcher* (Boulenger) was analysed as a presumed outgroup species in the systematic study for the same enzyme loci. (The allozyme results of *P. pulcher* compared with those of the other species will be shown but will not be discussed in the details, due to its small sample size.)

Most species used in this study were live specimens from the Tilapia Reference Collection which is maintained in the recirculated warm water systems at the Institute of Aquaculture. These species came from various sources in Africa (Table 2) and have been maintained for a number of generations at Stirling. Some species, i.e. *T. mariae, T. tholloni* and *S. melanotheron*, were collected from aquarist stocks directly imported from Africa. The three chambo species and *O. (O.) shiranus* were collected from Lake Malawi in 1991 and were transported on ice to the Institute for analysis.

2.2 Electrophoretic Analysis

The electrophoretic procedures used in this study are mostly based on the methods of Harris & Hopkinson (1976), Aebersold *et al.* (1987), and Murphy *et al.* (1990). The electrophoretic conditions and all buffer systems followed those used by McAndrew & Majumdar (1983) and Sodsuk & McAndrew (1991). Table 3 gives a summary of all enzymes investigated, their relevant buffers, and number of loci observed. The procedure may be separated into six different stages: (i) sample preparation, (ii) gel preparation, (iii) sample application (gel loading), (iv) gel running (electrophoresis), (v) gel slicing, and (vi) staining for various enzymes.

Table 3. 43 enzyme loci examined, the buffer system used, subunit structure of the enzymes, and the tissue sources for each locus.

Enzyme	Enzyme no.	Buffer	Subunit structure	Locus	Tissue source
Aspartate aminotransferase	2.6.1.1	TBE	Dimeric	*AAT-1**	M
				*AAT-2**	L
				*AAT-3**	M, K, Sp, F
Acid phosphatase	3.1.3.2	TBE	Dimeric	*ACP**	M
Adenosine deaminase	3.5.4.4	TBE	Monomeric	*ADA**	M, F
Alcohol dehydrogenase	1.1.1.1	TBE	Dimeric	*ADH**	L
Aconitate hydratase	4.2.1.3	TBE	Monomeric	*AH-1**	H
				*AH-2**	L, K
Adenylate kinase	2.7.4.3	TC	Monomeric	*AK**	M
Alanine aminotransferase	2.6.1.2	TBE	Dimeric	*ALAT**	L
Creatine kinase	2.7.3.2	TC	Dimeric	*CK**	M
Dihydrolipoamide dehydrogenase	1.8.1.4	TBE	Monomeric	*DDH-1**	M
			(?)	*DDH-2**	M
Esterase	3.1.1.-	TBE	Monomeric	*EST-1**	M
				*EST-2**	M, B

Enzyme	Enzyme no.	Buffer	Subunit structure	Locus	Tissue source
Esterase-D	3.1.-.-	TC	Dimeric	*ESTD**	M
Fructose-biphosphate aldolase	4.1.2.13	AC	Tetrameric	*FBALD-1**	L
				*FBALD-2**	M
Fumarate hydratase	4.2.1.2	TC	Tetrameric	*FH-1**	M
				*FH-2**	M
Glycerol-3-phosphate dehydrogenase	1.1.1.8	TBE	Dimeric	*G3PDH-1**	M
				*G3PDH-2**	L
Glucose-6-phosphate dehydrogenase	1.1.1.49	TCB	Dimeric	*G6PDH-1**	L
				*G6PDH-2**	L
Glucose-6-phosphate isomerase	5.3.1.9	TC	Dimeric	*GPI-1**	M
				*GPI-2**	M, L
Guanine deaminase	3.5.4.3	TC	Dimeric	*GDA**	L
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	TC	Dimeric	*IDHP-1**	L
				*IDHP-2**	M
L-Iditol dehydrogenase	1.1.1.14	TC	Tetrameric	*IDDH**	L
L-Lactate dehydrogenase	1.1.1.27	TBE	Tetrameric	*LDH-1**	E, M
				*LDH-2**	E, M, L
				*LDH-3**	E

Enzyme	Enzyme no.	Buffer	Subunit structure	Locus	Tissue source
Malate dehydrogenase	1.1.1.37	TBE	Dimeric	*MDH-1**	M
				*MDH-2**	M, L
				*MDH-3**	M
Malic enzyme ($NADP^+$)	1.1.1.40	TC	Tetrameric	*MEP-1**	M
				*MEP-2**	M, L
Mannose-6-phosphate isomerase	5.3.1.8	TBE	Monomeric	*MPI**	M, L
Peptidase-C	3.4.-.-	TBE	Monomeric	*PEPC**	M
Phosphogluconate dehydrogenase	1.1.1.44	TBE	Dimeric	*PGDH**	M
Phosphoglucomutase	5.4.2.2	TC	Monomeric	*PGM**	M
Superoxide dismutase	1.15.1.1	TBE	Dimeric	*SOD**	M, L

Tissue abbreviations: B=blood, E=eye, F=fin, H=heart, K=kidney, L=liver, M=muscle, Sp=spleen. Electrode buffers: AC = Amine-citrate (Morpholine) pH 6.5, TBE = Tris-borate-EDTA pH 8.5, TC = Tris-citrate pH 8, TCB = Tris-citrate/Lithium-borate pH 8.5 (McAndrew & Majumdar, 1983; Sodsuk & McAndrew, 1991).

(i) Sample Preparation Small pieces of the various tissues, i.e. blood, eye, fin, heart, kidney, liver, muscle and spleen, were dissected from individual fish and were put separately into Eppendorf tubes to be kept for further analyses. (These tissue samples were always stored at -25°C or lower.) A range of tissues from an individual fish would be analysed to determine the number and tissue specificity of the various enzymes.

Cytoplasm released from the tissue samples by homogenization or freeze thaw was absorbed onto rectangular pieces of filter paper (Whatman No. 1), or ***sample wicks*** (3 x 7 mm). Samples were absorbed onto the wicks immediately prior to being loaded onto the gel to avoid concentrating samples at the wick edges as a result of drying.

(ii) Gel Preparation For a single gel (11.5%), 25 g of hydrolysed potato starch was mixed with 220 mls of the appropriate buffer solution (Table 3 and Appendix 1) in a Buchner flask. With constant rotation of the flask, the mixture was heated until the starch became gelatinous. It was then quickly degassed using a vacuum water pump and poured into a ***gel mould*** (a 15 x 18 x 0.55 cm^3 perspex frame placed on a glass plate), and was then immediately covered with a thin glass plate. The gel was allowed to set and cool overnight. The mould was then removed, and the gel placed on a clean glass plate and cooled in a refrigerator prior to the application of samples.

(iii) Gel Loading (Sample Application) To load the samples (already absorbed onto sample wicks) on to the gel, a cut was made parallel to the longest side and 5 cm from the inner face of the mould. The gel was slid apart at the cut and the sample wicks were placed on the cut face of the larger slice: 2 - 3 mm was left between adjacent sample wicks. When all samples had

been loaded, the gel was pushed back together and the mould replaced. An extra spacer was also added between the gel and the mould parallel to the cut edge to ensure that the cut did not open during the run.

(iv) Gel Running (Electrophoresis) The gel containing sample wicks was placed in an electrophoretic bath filled with the appropriate buffer (Table 3 and Appendix 1). Two soft cloths were used as bridges to complete the electrical circuit between the gel and buffer wells. The gel was then covered with a thin polythene sheet to minimise evaporation and gel shrinkage. Gels were run at 4°C in a refrigerator using a voltage of 200-250 V for 3-4 hrs.

(v) Gel Slicing After running (electrophoresis had been completed), the gel (5.5 mm deep) was sliced horizontally by placing the gel between two glass plates of the same thickness. The gel (together with the two glass plates) was then placed on a gel slicer (a taut wire set 2 mm above the thickness of the glass), and then was pushed forward through the taut wire, turned over and pushed back again, thus producing three slices.

(vi) Staining for Various Enzymes Each of the three slices from a gel could be stained for a different enzyme. To stain for an enzyme, an appropriate staining mixture dissolved in its relevant buffer and mixed with 2% agar (Appendix 1) was poured onto a gel slice in a polythene stain tray, and then incubated at 37°C until distinct bands became visible on the gel slice.

Any particular enzyme loci is visualized using a highly specific histochemical stain in which the enzyme is supplied with its usual substrate. The action of that enzyme in then linked into a biochemical pathway which initially

results in a band being visualized on the gel either as the precipitate of a dye or the production of band which visible under UV light. The various stains and their mode of action have been extensively described by a number of authors (e.g. Shaw & Prasad, 1970; Harris & Hopkinson, 1976).

2.3 Interpretation of Electrophoretic Patterns

The following system was used for locus and allele designation. Loci were designated numerically beginning from the cathodal end of a gel; the locus with the least anodal migration was designated one, the next two, and so on. Alleles were designated according to their mobilities relative to the most common allele in all species, which was designated 100; allelic variants were given numbers that indicate the mobility of their products faster or slower than that of the common allele. Alleles migrating cathodally from the origin were given a minus sign. Locus and allele nomenclature follow Shaklee *et al.* (1990a).

2.4 Data Analysis

Allelic (gene) frequencies were estimated from genotypic frequencies by gene counting, since all protein variants observed in this study were interpreted to reflect products coded by codominant alleles. Deviations from expected Hardy-Weinberg proportions for each locus were not tested in all species used, except for the three chambo species and *O. (O.) shiranus*, because firstly their

sample sizes were rather too small, and secondly the samples used were not originally collected from the natural or wild populations; they were descendants of the original stocks that came from the different sources shown in Table 2.

The deviations from expected Hardy-Weinberg proportions for each locus were tested (in the chambo species and *O. (O.) shiranus*) (Appendix 3) using the chi-square test for goodness-of-fit (Sokal & Rohlf, 1969). Expected frequencies were calculated using Levene's (1949) formula for small samples. The use of chi-square test, however, is suspect in cases where expected frequencies of some classes are low (Sokal & Rohlf, 1969). So, when more than two alleles were observed at a locus, genotypes were pooled into three classes (homozygotes for the most common allele, heterozygotes for the most common allele and one of the other alleles, and all other genotypes) and the tests were repeated, using the resulting chi-square value with one degree of freedom. The calculation of exact significance probabilities was also performed to avoid the difficulties encountered in using the chi-square distribution for small samples (Haldane, 1954; Vithayasai, 1973; Elston & Forthofer, 1977).

Expected heterozygosities (unbiased estimate of Nei, 1978) and percentage of polymorphic loci were calculated for genetic variability measures in each species. Genetic differentiations were observed using the F-statistics of Wright (1978). The calculating formulas employed are shown in Appendix 2. The software package **BIOSYS-1** release 1.7 (Swofford & Selander, 1989) was used for all calculations in this chapter.

III. RESULTS & DISCUSSION

3.1 Genetic Interpretation & Description of Enzyme Banding Patterns

The protein structure of all the various enzymes investigated, the tissues specific to multilocus isozymes observed, and the number of encoding loci are shown in Table 3. Of the 43 loci examined, 37 loci showed allelic differences between the species studied and six loci (*AAT-1**, *AH-1**, *DDH-2**, *FBALD-1**, *IDHP-2**, and *MDH-1**) were always expressed as single invariant bands of the same mobility. The 37 variable loci, the mobility of the various alleles and their frequencies in each species are shown in Table 4. The tissue specificity of the various enzymes did not appear to vary in any of the species studied.

ASPARTATE AMINOTRANSFERASE (AAT)

[GLUTAMIC-OXALOACETIC TRANSAMINASE (GOT)]

Three different loci, *AAT-1**, *AAT-2** and *AAT-3**, were scored. The products of *AAT-1** which was observed in muscle tissue appeared at the origin. *AAT-1** was monomorphic, a single invariant bands in all the species studied. The products of *AAT-2** and *AAT-3** appeared at the anodal zone. Whereas *AAT-2** was only detected in liver tissue, *AAT-3** could be detected in a variety of different tissues including muscle, kidney, spleen and fin. *AAT-2** and *AAT-3** were fixed for alternate alleles in some species, but in other species they were polymorphic for either the same or different variant alleles. *AAT-2** was polymorphic in *T. rendalli, T. tholloni, T. zillii, O. (O.) niloticus, O. (O.) aureus, O. (O.) mossambicus, O. (O.) jipe, O. (Ny.) macrochir, O. (Ny.) karongae, O. (Ny.) lidole,* and *O. (Ny.) squamipinnis*; *AAT-3** in *O. (O.) niloticus, O. (O.)*

Table 4. 37 variable loci and allele frequencies from the 43 loci examined in all species studied.

Locus	All.	*Pelv.*	*Tilapia*					*Sarotherodon*			*Oreochromis*														
			Het.	*Pel.*	*Coptodon*						*Oreochromis*										*Nyasalapia*				*Neo.*
		PUL	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
*1. AAT-2**	*a 126*																								
	b 118							1.000	1.000																1.000
	c 112				0.929						0.167	0.100													
	d 100		1.000	1.000		0.278	0.500			1.000	0.666	0.900	1.000	1.000	0.857			1.000	1.000	0.300 0.700	0.250 0.125	0.919	0.758	0.750	
	e 90	1.000																			0.625	0.081	0.242	0.250	
	f 80				0.071	0.111	0.100				0.167				0.143	1.000	1.000								
	g 42					0.611	0.400																		
*2. AAT-3**	*a 117*																								
	b 110																	1.000							
	c 100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.080 0.920	1.000	1.000	1.000	1.000	1.000	1.000		0.357 0.643	1.000	0.500 0.500	1.000	1.000	1.000	1.000
	d 77																								
*3. ACP**	*a 150*	1.000			1.000	1.000	1.000																		
	b 100		1.000	1.000				1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-
*4. ADA**	*a 145*																								-
	b 142											0.813													
	c 138										0.005														
	d 135											0.187													
	e 121										0.553														
	f 118					0.625	0.318				0.068														
	g 113									1.000															
	h 108				1.000	0.375	0.682				0.374														
	i 104	1.000	0.167					1.000	1.000								0.300				1.000	0.125	0.074	0.250	
	j 100		0.833	1.000																			0.537	0.200	
	k 97													0.950	0.783	1.000	0.300					0.359		0.450	
	l 93																					0.516	0.389	0.100	
	m 91																0.400								
	n 82													0.050											
	o 80												1.000		0.217										
	p 77																			0.500					
	q 75																								1.000
	r 66																	1.000	1.000						
	s 46																			0.500					
*5. ADH**	*a 100*	1.000																							
	b -40					0.100	0.100																		-
	c -83		1.000	1.000						0.167		1.000							0.364				0.061	0.167	-
	d -100				0.909	0.900	0.900	1.000	1.000	0.833	1.000		1.000	1.000	1.000	1.000	1.000	1.000	0.636		0.125 0.875	0.976	0.894	0.833	-
	e -134				0.091																	0.024	0.045		-
	f -180																			1.000					-

Locus	All.	Pelv.	Tilapia					Sarotherodon			Oreochromis										Nyasalapia				Neo.
			Het.	Pel.	Coptodon						Oreochromis														
		PUL	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
6. AH-2*	a 112	1.000																-							-
	b 108				0.278													-							-
	c 100		1.000		0.722		0.063	0.625	0.550		0.100	1.000			0.167		0.500	-	0.214	0.300		0.136	0.232	0.182	-
	d 95			0.182						0.750	0.067		0.500		0.333	0.750		-				0.546	0.339	0.454	-
	e 86			0.818				0.375	0.450	0.250	0.533				0.500		0.500	-	0.572	0.700	0.167	0.076	0.304		-
	f 79					1.000	0.937						0.500			0.250		-							-
	g 73										0.300							-	0.214		0.833	0.242	0.125	0.364	-
	h 66													1.000				-							-
7. AK*	a 100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	-
	b 87																1.000								-
8. ALAT*	a 100	1.000	1.000	1.000	1.000					1.000	1.000	1.000	0.833	1.000			1.000	1.000	1.000	1.000					1.000
	b 87					1.000	1.000	1.000	1.000				0.167		1.000	1.000					1.000	1.000	1.000	1.000	
9. CK*	a 100		1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000				1.000	1.000	1.000	1.000				-
	b 89																1.000								-
	c 85	1.000			1.000										1.000	1.000						1.000	1.000	1.000	-
10. DDH-1*	a 180	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000									0.750						-
	b 110														1.000	1.000		1.000	0.250						-
	c 100										1.000	1.000	1.000	1.000			1.000			1.000	1.000	1.000	1.000	1.000	-
11. EST-1*	a 115							1.000	1.000	0.100				1.000		1.000		1.000	0.214						-
	b 100	1.000			1.000	1.000	1.000			0.900	1.000	1.000	1.000		1.000		1.000		0.786	1.000	1.000	1.000	1.000	1.000	-
	c 85		1.000	1.000																					-
12. EST-2*	a 120	1.000																							-
	b 109											0.086								0.375					-
	c 105		1.000								0.119														-
	d 100							1.000	1.000	1.000	0.881	0.914	1.000	1.000	0.938	1.000		1.000	1.000	0.625	1.000	0.833	1.000	0.971	-
	e 93			1.000	1.000	1.000	1.000								0.062		1.000					0.167		0.029	-
13. ESTD*	a 125	1.000																							-
	b 115			1.000																					-
	c 107		1.000																						-
	d 100				1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-
	e 92							1.000																	-
14. FBALD-2*	a 30	1.000																-							-
	b -47										1.000	0.900	1.000					-							-
	c -100							1.000	1.000	1.000		0.100		1.000	1.000	1.000	1.000	-	1.000	1.000		1.000	1.000	1.000	-
	d -115		1.000	1.000	1.000	1.000	1.000											-			1.000				-

Locus	All.	Pelv.	Tilapia					Sarotherodon			Oreochromis														
			Het.	Pel.	Coptodon						Oreochromis										Nyasalapia				Neo.
		PUL	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
15. FH-1*	a 107										1.000														
	b 100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000
	c 86																			1.000					
16. FH-2*	a 150										0.467														
	b 100	1.000	1.000	1.000	1.000	1.000	1.000			1.000	0.533	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c 65							1.000	1.000																
17. GDA*	a 113				0.222			0.750	0.250	0.400								-							-
	b 100			1.000	0.778	1.000	1.000	0.250	0.750	0.600	0.100			1.000	0.750	1.000	1.000	-	1.000	1.000	1.000	0.472	0.530	0.235	-
	c 95										0.900		0.875		0.250			-				0.528	0.470	0.647	-
	d 90		1.000									1.000	0.125					-						0.118	-
	e 50	1.000																-							-
18. GPI-1*	a 200	1.000																							-
	b 150									0.140															-
	c 100		1.000	1.000				1.000	1.000	0.860	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.060			-
	d 33										0.050											0.940	1.000	1.000	-
	e -100				1.000	1.000	1.000																		-
19. GPI-2*	a 115						0.100							0.100	0.857	0.625	1.000								-
	b 103									0.060															-
	c 100		1.000		1.000	1.000	0.900	1.000	1.000	0.940	1.000	1.000	1.000	0.900	0.143	0.375		0.750	1.000	1.000	1.000	1.000	1.000	1.000	-
	d 89	1.000		1.000														0.250							-
20. G3PDH-1*	a 120		1.000	1.000	1.000	1.000	1.000		0.375																-
	b 100	1.000						1.000	0.625	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-
21. G3PDH-2*	a 110																			0.016					-
	b 100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.971	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.984	1.000	1.000	1.000	1.000	-
	c 76											0.029													-
22. G6PDH-1*	a 111			1.000		1.000	1.000		1.000	1.000				1.000				-				0.438	0.333	0.324	-
	b 100		1.000		1.000			1.000			1.000	1.000	1.000		1.000	1.000	1.000	-	1.000	1.000	1.000	0.562	0.667	0.529	-
	c 82																	-						0.147	-
	d 60	1.000																-							-
23. G6PDH-2*	a 108				1.000	1.000	1.000	1.000	1.000		1.000		1.000	1.000				-		1.000				0.364	-
	b 100									1.000		1.000			1.000	1.000	1.000	-	1.000		1.000	1.000	1.000	0.636	-
	c 80			1.000														-							-
	d 60	1.000																-							-
	e 55		1.000															-							-

Locus	All.	Pelv.	Tilapia					Sarotherodon			Oreochromis										Nyasalapia				Neo.
			Het.	Pel.	Coptodon						Oreochromis														
		PUL	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
24. IDDH*	a 158	1.000													1.000	1.000	1.000	1.000	1.000	0.200	1.000				
	b 112												0.188												
	c 100		1.000	1.000				1.000	1.000	1.000	1.000	1.000	0.812	1.000						0.800		0.821	1.000	1.000	1.000
	d 50																					0.179			
	e 38					1.000	1.000																		
	f 19				1.000																				
25. IDHP-1*	a 113							1.000	1.000	0.040								1.000							-
	b 100	1.000	1.000	1.000		1.000	1.000			0.960	1.000	1.000	1.000	0.313	1.000	1.000	0.057		1.000	1.000	1.000	1.000	0.833	1.000	-
	c 90				1.000																				-
	d 86													0.687			0.943						0.167		-
26. LDH-1*	a 150	1.000																							
	b 125		1.000	1.000		1.000	1.000																		
	c 100				1.000			1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.560	0.446	0.588	1.000
	d -100																					0.440	0.554	0.412	
27. LDH-2*	a 180	1.000	1.000	1.000	1.000	1.000	1.000												0.464						
	b 100							1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.536	1.000	1.000	1.000	1.000	1.000	1.000
28. LDH-3*	a 100							1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b 90		1.000		1.000	1.000	1.000																		
	c 87	1.000		1.000																					
29. MDH-2*	a 135									1.000															-
	b 100							1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	-
	c 98																			1.000					-
	d 82	1.000	1.000	1.000	1.000	1.000	1.000																		-
30. MDH-3*	a 116	1.000						0.500																	-
	b 100		1.000	1.000	1.000	1.000	1.000	0.500	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	-
	c 92																			1.000					-
	d 85										1.000														-
31. MEP-1*	a 100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.444	1.000	1.000	1.000	1.000	1.000
	b 90																			0.556					
32. MEP-2*	a 110										0.286														
	b 106										0.286									0.875					
	c 100	1.000						1.000	1.000	1.000	0.428	1.000	1.000	0.417	1.000	1.000	1.000			0.125	1.000				
	d 95				1.000	1.000	1.000															0.092	0.333	0.269	
	e 90		1.000	1.000										0.583				1.000	0.714			0.645	0.515	0.538	1.000
	f 85																		0.286			0.263	0.152	0.193	
33. MPI*	a 100		1.000	1.000	1.000	1.000	1.000	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b 80	1.000						0.071																	

Locus	All.	*Pelv.*	*Tilapia*					*Sarotherodon*			*Oreochromis*														
			Het.	*Pel.*	*Coptodon*						*Oreochromis*										*Nyasalapia*				*Neo.*
		PUL	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
*34. PEPC**	*a 106*							1.000	0.750																-
	b 100		1.000			1.000	1.000		0.250	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-
	c 84				1.000																				-
	d 81			1.000																					-
	e 60	1.000																							-
*35. PGDH**	*a 180*	1.000																							
	b 150		1.000	0.250																					
	c 125			0.750	1.000	1.000	1.000																		
	d 116												1.000									0.214			
	e 100							1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.786	1.000	1.000	1.000
*36. PGM**	*a 120*	1.000																							
	b 100		1.000	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000	1.000	0.955	1.000	1.000	1.000	1.000	1.000	1.000	0.750	1.000	0.964	0.985	0.933	1.000
	c 85																			0.250					
	d 75							0.250					0.045									0.036	0.015	0.067	
*37. SOD**	*a 125*	1.000																							
	b 100			1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.812			0.375	0.875		0.500	1.000	0.875	0.152	0.361	0.200	1.000
	c 25		1.000										0.188	1.000	0.975	0.625	0.125	1.000	0.500		0.125	0.848	0.639	0.800	
	d 10														0.025										

Abbreviations: All. = Allele; *Pelv.* = *Pelvicachromis* (genus); *Het.* = *Heterotilapia* (subgenus); *Pel.* = *Pelmatolapia* (subgenus); *Neo.* = *Neotilapia* (subgenus). PUL = *P. pulcher*; BUT = *T. buttikoferi*; MAR = *T. mariae*; REN = *T. rendalli*; THO = *T. tholloni*; ZIL = *T. zillii*; MEL = *S. melanotheron* (aquarium stock); MIC = *S. melanotheron* (Ivory Coast stock); GAL = *S. galilaeus*; NIL = *O. niloticus*; AUR = *O. aureus*; SPI = *O. spilurus*; UHN = *O. u. hornorum*; MOS = *O. mossambicus*; MOR = *O. mortimeri*; AND = *O. andersonii*; PLA = *O. placidus*; SHI = *O. shiranus*; JIP = *O.jipe*; MAC = *O.macrochir*; KAR = *O. karongae*; LID = *O. lidole*; SQU = *O. squamipinnis*; and TAN = *O. tanganicae*.

shiranus, and *O. (O.) macrochir*. Although the enzyme is scored as dimer in tilapiines, both in this and other studies (Basiao & Taniguchi, 1984; Macaranas *et al.*, 1986), the three-banded heterozygote sometimes could not always be resolved in some species because of the very similar mobilities of the alleles. However, the three-banded pattern could be recognised as a consistently longer band than the homozygote.

Basiao & Taniguchi (1984) previously reported only two loci encoding for AAT; *AAT-1** being the anodal form which was polymorphic in *O. (O.) niloticus* and *T. zillii*; and *AAT-2** producing the products migrating to the cathodal region and being monomorphic in these species. It may be possible that *AAT-1** reported by Basiao & Taniguchi (1984) and *AAT-2** reported in this study are the same locus; being polymorphic in the same species at the anodal migration region, and being detectable in liver tissue. In the same way, *AAT-2** reported by Basiao & Taniguchi is probably *AAT-1** in this study because it is monomorphic in the same species.

ACID PHOSPHATASE (ACP)

A single locus *ACP** was scored. The enzyme which was observed in muscle tissue appeared in the anodal zone. Only single-banded homozygotes were observed with the fixation for alternate alleles between the groups of *Tilapia (Coptodon)* and the other species. ACP has been reported as a dimeric enzyme in fishes (Aebersold *et al.*, 1987; Morizot & Schmidt, 1990).

ADENOSINE DEAMINASE (ADA)

Activity reflecting a single *ADA** locus encoding for this monomeric enzyme was detected. The enzyme could be observed in muscle and fin tissues, but fin gave the stronger and clearer activity. Homozygotes were single-banded,

heterozygotes double-banded. This system was highly polymorphic with two to four alleles expressed in the majority of species studied; three alleles were detected in *O. (O.) andersonii*, *O. (Ny.) karongae* and *O. (Ny.) lidole*, and four alleles in *O. (O.) niloticus* and *O. (Ny.) squamipinnis*. Even though the system was monomorphic in a number of species, the locus was fixed for alternate alleles between such species. This monomeric enzyme has a wide range of different allozyme mobilities. The results are mainly consistent to those of McAndrew & Majumdar (1983) study.

ALCOHOL DEHYDROGENASE (ADH)

A single *ADH** locus encoding the dimeric enzyme ADH was suggested. With the buffer systems used in this study, the enzyme observed in liver tissue appeared in the cathodal zone, agreeing with the previous studies of McAndrew & Majumdar (1983), Basiao & Taniguchi (1984), Macaranas *et al.* (1986), and Van der Bank *et al.* (1989). Three-banded patterns were observed in heterozygotes, confirming the dimeric structure of the enzyme. Polymorphisms were detected in all four species used in the subgenus *O. (Nyasalapia)*, all three species in the *T. (Coptodon)*, *S. galilaeus*, and *O. (O.) shiranus*, whereas the locus was fixed for alternate alleles between some other species. Cruz *et al.* (1982) reported that *ADH** was not polymorphic in *T. (C.) zillii*. McAndrew & Majumdar (1983) reported that the locus was not polymorphic in any species used in their study. Basiao & Taniguchi (1984) reported that the locus was polymorphic in *O. (O.) niloticus* but monomorphic in *T. (C.) zillii*. However, *ADH** polymophism has recently been reported in *T. (C.) rendalli* by Van der Bank *et al.* (1989), agreeing with the polymorphisms detected in *T. (Coptodon)* from this study.

ACONITATE HYDRATASE (AH)

[ACONITASE (ACO)]

Two loci, *AH-1** and *AH-2**, were detected. The products of the two loci appeared in the anodal zone. The *AH-1** locus was observed in heart tissue expressed as single invariant band between all species studied, no interspecific variation was detected. The products of *AH-2**, which was polymorphic in many species, were observed in liver and kidney tissues. Homozygotes were single-banded, heterozygotes double-banded, suggesting the monomeric structure of the enzyme (Fig. 2 a). In addition to *ADA**, this system was highly polymorphic with two to four alleles expressed in nearly all species. Three alleles were detected in *O. (O.) mossambicus*, *O. (O.) shiranus*, and *O. (Ny.) squamipinnis*; four alleles in *O. (O.) niloticus*, *O. (O.) karongae*, and *O. (Ny.) lidole*.

Cruz *et al.* (1982) also observed *AH-1** as a monomorphic heart specific enzyme in *T. (C.) zillii*. They also observed that it was very labile and could only be detected using fresh heart tissue, an observation confirmed in this study.

ADENYLATE KINASE (AK)

Activity reflecting a single anodally migrating *AK** locus was detected in muscle tissue. No polymorphisms were observed in any species during these studies, but the locus was fixed for alternate alleles between *O. (O.) andersonii* and the other species. However double-banded heterozygotes observed by Van der Bank *et al.* (1989) in wild *O. (O.) andersonii*. McAndrew & Majumdar (1983) also observed double-banded heterozygotes in hybrid crosses between *O. (O.) niloticus* x *O. (O.) mossambicus*. These results confirm the monomeric structure of this enzyme in tilapiines.

Cruz *et al.* (1982) detected four different AK loci (*AK-1**, *AK-2**, *AK-3** and *AK-4**) distributed in liver, gonad, brain and eye, and three loci (*AK-1**,

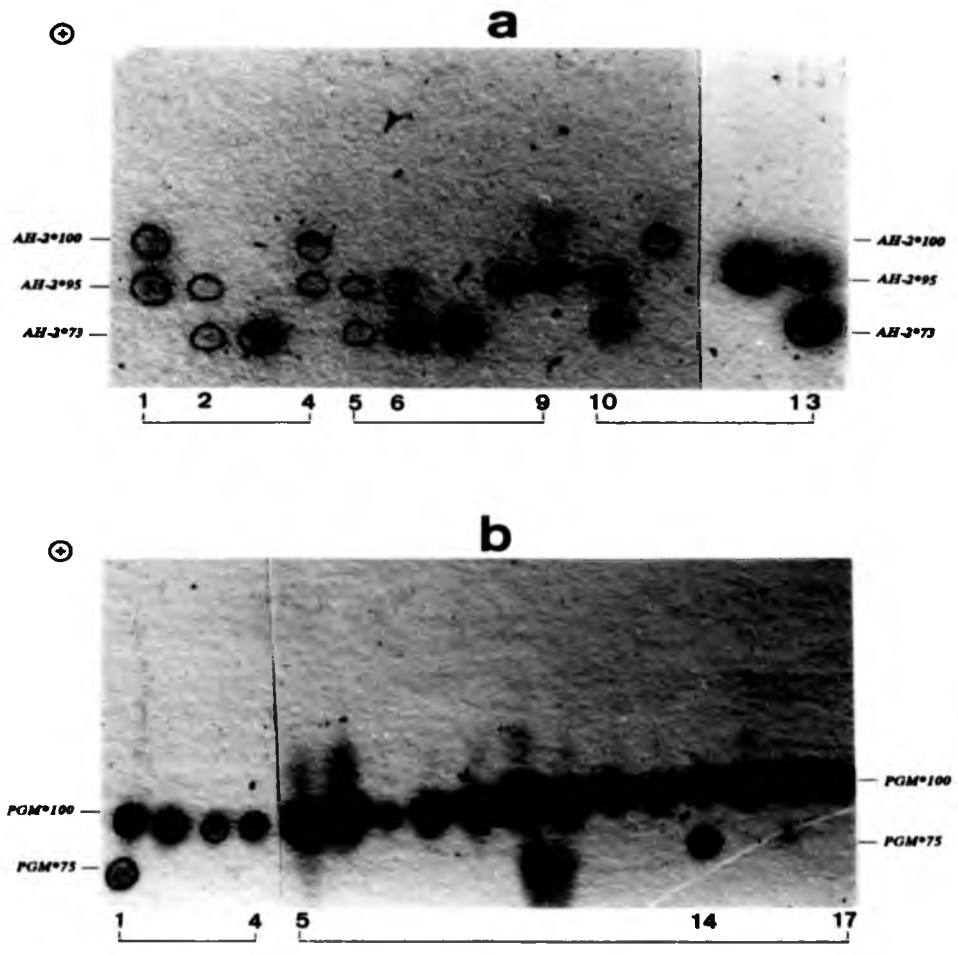

Fig. 2. (a) *AH-2**, monomeric, observed in liver tissue of *O. (Ny.) karongae*, 1-4; *O. (Ny.) lidole*, 5-9; and *O. (Ny.) squamipinnis*, 10-13. The specimens 1, 4 and 9 are *AH-2*100/95* heterozygotes; 2, 5, 6, 10 and 13 are *AH-2*95/73* heterozygotes.

(b) *PGM**, monomeric, observed in muscle tissue of *O. (Ny.) squamipinnis*, 1-4; and *O. (Ny.) karongae*, 5-17. The specimens 1 and 14 are *PGM*100/75* heterozygotes.

*AK-2** and *AK-3**) in white muscle of *T. (C.) zillii*. McAndrew & Majumdar (1983) reported only a single AK locus in muscle tissues in nine tilapia species. Van der Bank *et al.* (1989) reported three AK loci, *AK-1** in liver, *AK-2** in muscle and heart, and *AK-3** in heart and liver, in 15 cichlid species. The single locus detected in muscle tissue from this study was consistent to that reported by McAndrew & Majumdar (1983). Van der Bank *et al.* (1989) assumed that the *AK-2** they found in muscle tissue corresponded with the *AK-3** in muscle tissue as found by Cruz *et al.* (1982), and therefore probably corresponded with the locus detected in muscle tissue from this study. The other tissue-specific loci were detected in the other studies but were not detected in McAndrew & Majumdar's (1983) and this study, probably because of the different electrophoretic conditions employed among studies. The interspecific mobility differences in AK observed in the three studies are different for some of the species comparisons. This may be due to the use of different populations representing the same species in each study.

ALANINE AMINOTRANSFERASE (ALAT)

[GLUTAMIC-PYRUVATE TRANSAMINASE (GPT)]

Activity reflecting a single anodally migrating locus was detected in liver. *ALAT** was fixed for alternate alleles between some species and polymorphic in *O. (O.) spilurus*. Although the enzyme structure has been reported as a dimeric isozyme system in fishes (Aebersold *et al.*, 1987; Morizot & Schmidt, 1990), the three-banded heterozygous patterns could only be observed as longer bands in this study; the UV-light detectable stain for this enzyme (Appendix 1) giving better resolution than the positive dye stains.

CREATINE KINASE (CK)

A single anodally migrating locus was observed in muscle tissue for *CK**. The locus showed single band differences fixed for alternate alleles between a number of species. Cruz *et al.* (1982) similarly reported only a single strong *CK** locus in white muscle of *T. (C.) zillii*. McAndrew & Majumdar (1983) and Van der Bank *et al.* (1989), however, reported two loci for CK; one expressed as single invariant band with no interspecific difference, and the other one expressed as a single-band with mobility differences observed between a number of species. Although CK is known as a dimer, CK isozymes act as monomers in skeletal muscle of fish (Aebersold *et al.*, 1987). This was evident in the study of Scopes & Hamoir (1971) who observed polymorphisms for CK in *O. (O.) mossambicus* in which two bands of CK activity were recorded in some individuals.

DIHYDROLIPOAMIDE DEHYDROGENASE (DDH)

[DIAPHORASE (DIA)]

Two anodally migrating loci, *DDH-1** and *DDH-2**, were observed for this enzyme. *DDH-1** was polymorphic in *O. (O.) shiranus* and was fixed for alternate alleles between a number of species. No interspecific mobility differences were observed at the *DDH-2** locus. This is a very difficult isozyme system to work with; the activity was sometimes very weak, the electrophoretic patterns appeared confused, and the genetic interpretation was poorly resolved. This enzyme protein has been said by Morizot & Schmidt (1990) to be a very poorly understood isozyme system in fishes. They have occasionally observed two zones of activity in poeciliid livers, similar to these observed in *O. (O.) shiranus* in this study. The enzyme is monomeric in man (Harris & Hopkinson,

1977). However, to define this enzyme system in fishes, a careful study using all alternative staining systems is recommended (Morizot & Schmidt, 1990).

ESTERASE (EST)

α-Naphthyl acetate was used as the enzyme substrate in the staining mixture (Appendix 1). Two anodal loci, *EST-1** and *EST-2**, were scored for the EST isozyme system. The *EST-1** and *EST-2** products were observed in muscle tissue, whereas the *EST-2** products could also be observed in blood serum. The *EST-1** band did not resolve as well as that for *EST-2**. Both loci were seen to be polymorphic and in a number of cases they were fixed for alternate alleles in a number of the species studied. Single-banded homozygotes and double-banded heterozygotes were observed confirming the monomeric structure of this enzyme. This agrees with the findings of McAndrew & Majumdar (1983), Basiao & Taniguchi (1984) and Van der Bank *et al.* (1989).

ESTERASE-D (ESTD)

A single monomorphic locus was resolved for ESTD. A single anodally migrating monomorphic band was observed in muscle tissue. *ESTD** was expressed as single-banded homozygotes with the interspecific allelic fixation differences between a number of species of *Tilapia* and *Sarotherodon*. (Note that the *EST-2** and *ESTD** products in *T. (H.) buttikoferi* were observed at the same mobility.)

This is a fast migrating system specific to 4-methyl umbelliferyl acetate (McAndrew & Majumdar, 1983). ESTD in mammals is unambiguously characterized as a dimeric enzyme that preferentially hydrolyses 4-methyl umbelliferyl and fluorescein esters (Harris & Hopkinson, 1977). In fishes, these

esters often are cleaved by monomeric carboxylesterases (EST) with broad substrate specificities, including hydrolysis of naphthyl esters (Morizot & Schmidt, 1990). The dimeric ESTD, however, has been identified in some fishes (Shaklee & Keenan, 1986; Aebersold *et al.*, 1987).

FRUCTOSE-BIPHOSPHATE ALDOLASE (FBALD)

[ALDOLASE (ALDO)]

Two loci, *FBALD-1** and *FBALD-2**, were observed. *FBALD-1** was monomorphic expressed as single invariant bands in all species. *FBALD-2** was polymorphic in *O. (O.) aureus* and fixed for alternate alleles in a number of the other species. Under the buffer system used in this study, both *FBALD-1**, which was observed in liver tissue, and *FBALD-2**, which was observed in muscle tissue, moved to the cathodal zone. Cruz *et al.* (1982) observed two FBALD loci in *T. (C.) zillii* which migrated anodally under a different buffer system. These authors also reported the five-banded heterotetrameric patterns of the isozymes produced by the two loci. These heterotetrameric bands were not observed clearly in this study, but the presumed multiple-banded heterozygotes were recorded for *FBALD-2** in *O. (O.) aureus.* The tetrameric FBALD isozyme system has also been recorded in some other fishes (Aebersold *et al.*, 1987; Morizot & Schmidt, 1990), but typically they showed poorly resolved bands under most buffer systems (Morizot & Schmidt, 1990).

FUMARATE HYDRATASE (FH)

[FUMARASE]

Two loci, *FH-1** and *FH-2**, were scored. The products of both loci were detected in muscle tissue and migrated anodally. *FH-1** was monomorphic in all

species studied with the interspecific allelic fixation of three different alleles between *O. (O.) niloticus*, *O. (O.) jipe*, and the other species. *FH-2** was polymorphic in *O. (O.) niloticus* with the interspecific allelic fixation of three different alleles between *S. melanotheron*, *O. (O.) tanganicae*, and the other species. [Note that for *O. (O.) niloticus* the faster homozygote of *FH-1** (*FH-1*107*) and the slower homozygote of *FH-2** (*FH-2*100*) were observed at (or nearly at) the same place.] The tetrameric subunit structure of the enzyme has been recorded in fishes (Aebersold *et al.*, 1987; Morizot & Schmidt, 1990). In this study presumed multiple-banded patterns of the isozymes produced by the two different loci and alleles were observed in *O. (O.) niloticus*, although the bands were not clearly separate. Cruz *et al.* (1982) also recorded that the expected hybrid bands between loci were occasionally absent. The three-banded patterns with equal spacing they observed in muscle tissue of *T. (C.) zillii* probably indicated two FH loci with a single intermediate hybrid zone.

GUANINE DEAMINASE (GDA)

Activity reflecting a single *GDA** locus encoding for the dimeric GDA was detected. The *GDA** products were observed in liver tissue and moved to the anodal zone. Polymorphisms were detected in some species with fixed interspecific mobility differences between a number of others. Van der Bank *et al.* (1989) recorded two GDA loci in 15 cichlid species; *GDA-1** in muscle tissue was monomorphic in all species they studied, and *GDA-2** in muscle and liver tissues was polymorphic in a number of those species. The locus found in this study probably corresponds with the *GDA-2** found by Van der Bank *et al.* (1989), who also recorded the three-banded heterozygotes confirming the dimeric structure of the enzyme.

GLUCOSE-6-PHOSPHATE ISOMERASE (GPI)

[PHOSPHOGLUCOSE ISOMERASE (PGI)]

Two anodal loci, *GPI-1** and *GPI-2**, were scored for this enzyme in most species. In *T. (Coptodon)* species the *GPI-1** products moved to the cathodal region. Both loci were observed in muscle tissue, whereas the *GPI-2** locus alone was observed in liver tissue at better levels of activity. Polymorphisms and fixed interspecific allelic mobility differences were observed in both loci. The three-banded heterozygotes, as well as heterodimeric hybrid bands between loci were observed (Fig. 3), indicating the dimeric structure of this enzyme molecule. Two GPI loci have been consistently recorded in tilapiines by Cruz *et al.* (1982), McAndrew & Majumdar (1983) and Van der Bank *et al.* (1987).

GLYCEROL-3-PHOSPHATE DEHYDROGENASE (G3PDH)

[A-GLYCEROPHOSPHATE DEHYDROGENASE (α-GPDH, GPD)]

Two anodal loci, *G3PDH-1** and *G3PDH-2**, were scored for the enzyme G3PDH. The products of *G3PDH-1** and *G3PDH-2** were observed in muscle and liver tissues respectively. *G3PDH-1** was fixed for alternate alleles in the *Tilapia* (**120*) and the two mouthbrooding genera (**100*), whereas *S. melanotheron* from the Ivory Coast was polymorphic for both these alleles. This suggests that the common alleles in these different genera are homologously related. *G3PDH-2** was polymorphic in two species; *O. (O.) aureus* had two alleles a slower **76* and the common **100*, and in *O. (O.) jipe* a faster **110* and the common **100*. Three-banded heterozygotes were observed in both these species, indicating the dimeric structure of the enzyme. The observation of two G3PDH loci in the tilapiines is consistent with the findings of McAndrew & Majumdar (1983) and Basiao & Taniguchi (1984), even though three loci rather than two loci were claimed for this enzyme by Cruz *et al.* (1982) and Van der Bank *et al.* (1989).

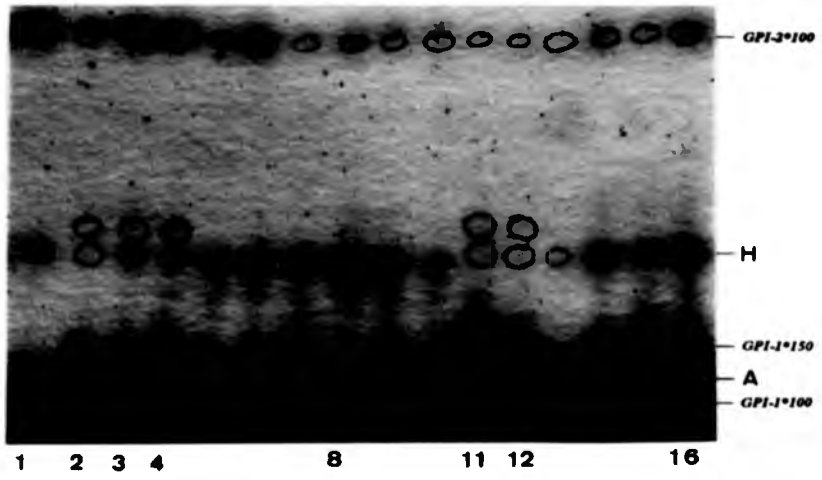

Fig. 3. *GPI-1** and *GPI-2**, dimeric, observed in muscle tissue of *O. (Ny.) karongae*. H represents heteromeric bands between *GPI-1** and *GPI-2**. Artefacts (A) in *GPI-1** easily occur and are frequently observed as bands migrating in front of the *GPI-1*100*. Banding patterns are always expressed as three bands at the bottom and two bands in the middle (H) where *GPI-1** heterozygotes (**100/150*) and *GPI-2** homozygotes are observed (the specimens 2, 3, 4, 11 and 12).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH)

Two presumed loci, *G6PDH-1** and *G6PDH-2**, were observed for this enzyme. The products of the two anodally migrating loci were observed in liver tissue. Interspecific allelic fixation differences were observed between a number of the species studied. *G6PDH-1** was polymorphic in the three chambo species, whereas *G6PDH-2** was polymorphic only in *O. (Ny.) squamipinnis*. Multiple-banded heterozygote look-alikes were observed. It has been suggested that the enzyme structure is dimeric (Aebersold *et al.*, 1987) or perhaps a mixture of dimeric and tetrameric isozyme systems (Morizot & Schmidt, 1990) in fishes. The banding patterns observed in *T. (C.) zillii* by Cruz *et al.* (1982) also suggests two G6PDH loci; *G6PDH-1** the slower band observed in only liver, and *G6PDH-2** the faster band observed in a variety of tissues including liver. These results agree with the two loci found in this study.

ISOCITRATE DEHYDROGENASE ($NADP^+$) (IDHP)

Two anodally migrating loci, *IDHP-1** and *IDHP-2** were observed in liver and muscle tissues respectively. Polymorphisms as well as interspecific allelic differences were observed at *IDHP-1** in a number of species studied. *IDHP-2** was monomorphic in all species; no interspecific differences were found. Heterozygotes were observed as three-banded patterns, indicating the dimeric structure of the enzyme. The liver *IDHP-1** observed in this study is consistent to the one observed by McAndrew & Majumdar (1983), and probably corresponds with the liver *IDHP-2** observed by Basiao & Taniguchi (1984). However, Basiao & Taniguchi (1984) recorded a polymorphism in *O. (O.) niloticus* and interallelic difference between *O. (O.) niloticus* and *T. (C.) zillii* for the liver specific locus which were not observed by McAndrew & Majumdar (1983) or this study. The two spcies sampled by Basiao & Taniguchi (1984)

were Japanese stocks that were introduced into Japan in 1962 from Egypt, where also were the source of the two species used in this and McAndrew & Majumdar (1983) studies. However, the different observations are probably due to different subpopulations/stocks of the two species used in these studies.

L-IDITOL DEHYDROGENASE (IDDH)

[SORBITOL DEHYDROGENASE (SDH, SORD)]

A single anodal locus *IDDH** was scored for this enzyme in liver tissue. Polymorphisms were observed in *O. (O.) spilurus*, *O. (O.) jipe* and *O. (Ny.) karongae*, whereas fixed allelic differences between other species were observed. A tetrameric structure has been observed for this enzyme system in fish (Basiao & Taniguchi, 1984; Aebersold *et al.*, 1987; Morizot & Schmidt, 1990). Five-banded heterotetrameric patterns were also observed in this study, confirming the tetrameric structure of this enzyme system. Basiao & Taniguchi (1984) also observed a single locus of IDDH, whereas Cruz *et al.* (1982) and Van der Bank *et al.* (1989) recorded two loci for this enzyme in liver tissue.

L-LACTATE DEHYDROGENASE (LDH)

Three loci, *LDH-1**, *LDH-2** and *LDH-3** (Figs. 4 & 5), were detected to encode for this enzyme. The three LDH loci showed tissue specific activity. Eye tissue could be used to observe all three loci, whereas muscle tissue was only active for *LDH-1** and *LDH-2** but *LDH-2** was the predominant locus in liver tissue. All three loci products migrated anodally in most species, except for *LDH-1** in the three chambo species (Fig. 4 b). The chambo *LDH-1** was polymorphic for the common anodal **100* and a cathodal **-100* variant. Heterozygotes were recognised as dense multiple-banded patterns across the origin. *LDH-2** was polymorphic in *O. (O.) shiranus* (Fig. 5 b), with the typical

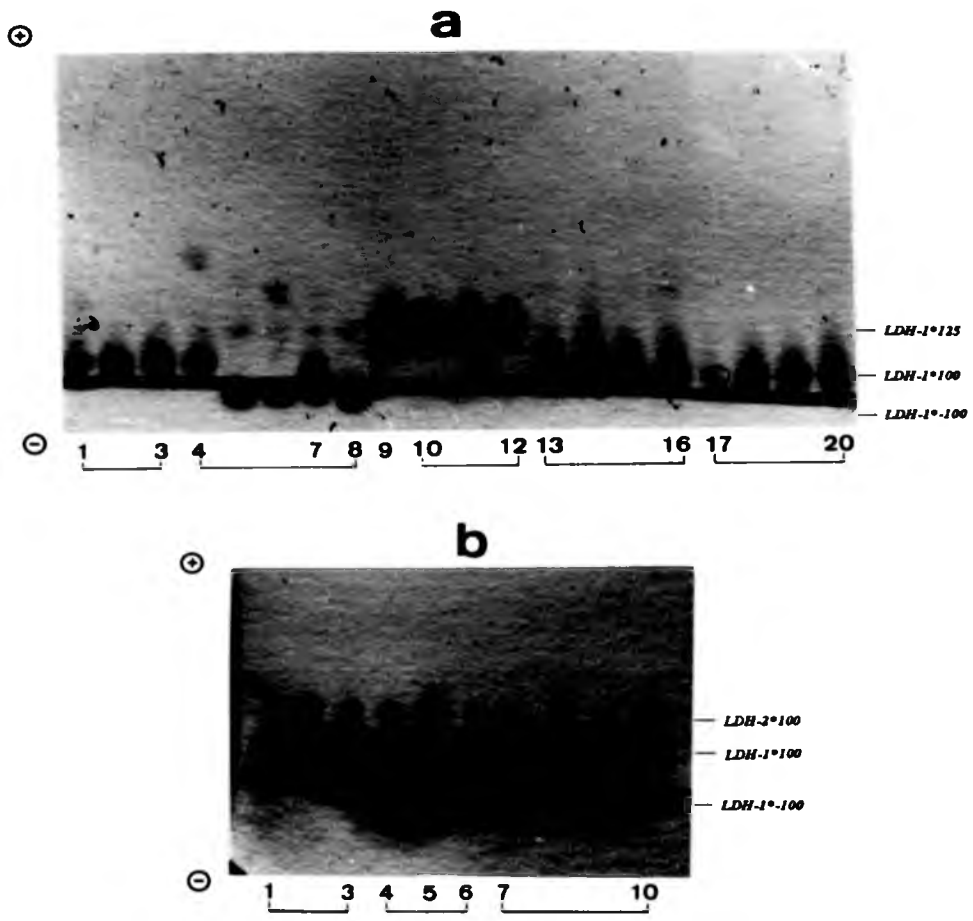

Fig. 4. *LDH-1**, tetrameric, observed in muscle tissue of

(a) *O. (O.) aureus*, 1-3; *O. (Ny.) squamipinnis*, 4-8; *T. (H.) buttikoferi*, 9; *T. (P.) mariae*, 10-12; *O. (Ny.) macrochir*, 13-16 and *S. galilaeus*, 17-20; and

(b) *O. (Ny.) karongae*, 1-3; *O. (Ny.) lidole*, 4-6; *O. (Ny.) squamipinnis*, 7-10.

The specimens 7 in (a) and 1, 4, 5 and 7 in (b) are five-banded *LDH-1*100/-100* heterozygotes. *LDH-2** can be also observed in muscle tissue but is very faint.

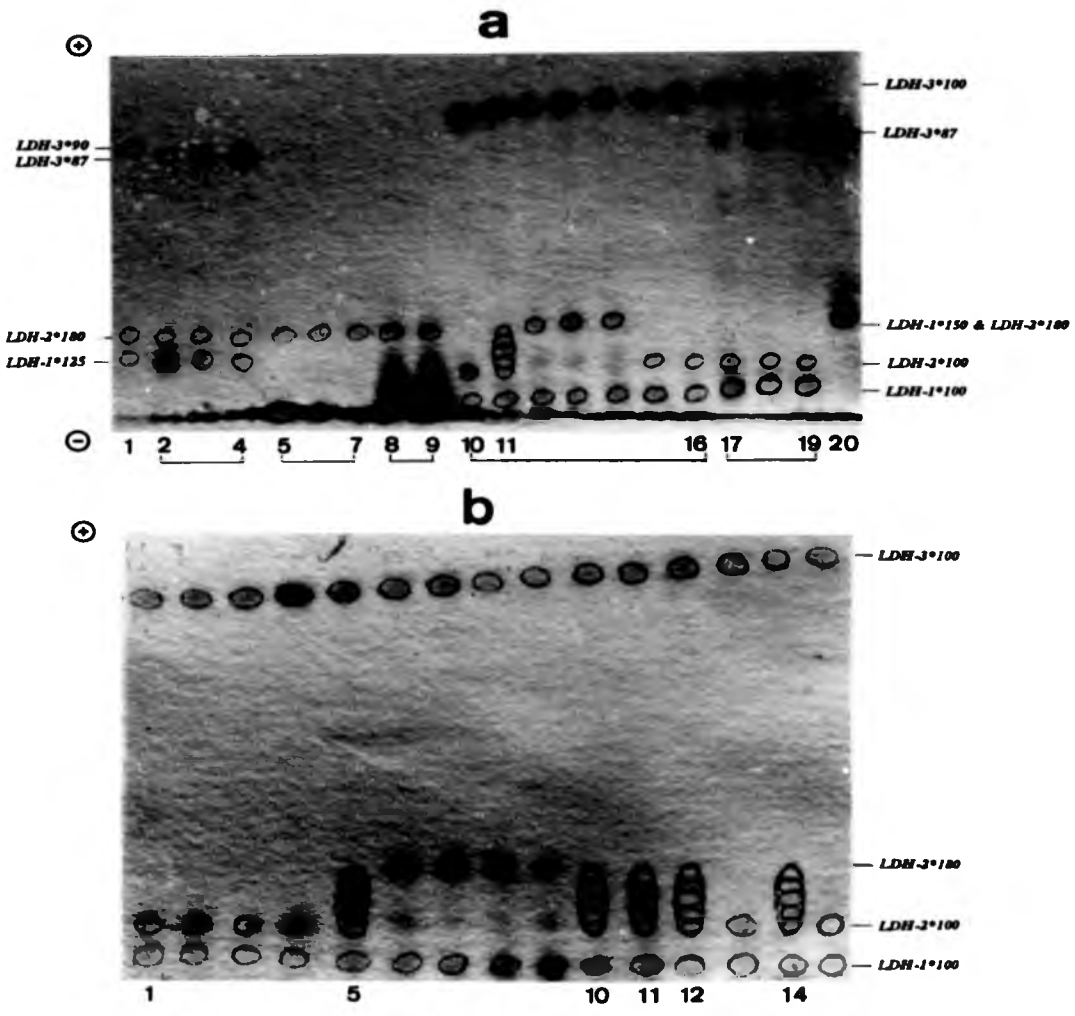

Fig. 5. *LDH-1**, *LDH-2**, and *LDH-3**, tetrameric, observed in a variety of tissues and species:

(a) 1, eye of *T. (H.) buttikoferi*; 2-4, eyes of *T. (P.) mariae*; 5-7, livers of *T. (P.) mariae*; 8-9, muscles of *T. (C.) rendalli*; 10-16, eyes of *O. (O.) shiranus*; 17-19, eyes of *O. (O.) mossambicus*; and 20, eye of *P. pulcher*.

(b) all samples, eyes of *O. (O.) shiranus*

The numbers 11 in (a) and 5, 10, 11, 12 and 14 in (b) are *LDH-2*100/180* heterozygotes observed only in *O. shiranus*.

five-banded heterozygotes. The tetrameric structure of all three loci was confirmed by the presence of five-banded heterozygotes at one or other locus in a range of species. Interspecific allelic fixation differences were observed between some species at all three loci, while intergeneric allelic fixation differences were observed at *LDH-2** and *LDH-3** between the genus *Tilapia* and the two mouthbrooding genera. The three loci found in this study are consistent with the observations of McAndrew & Majumdar (1983) and Basiao & Taniguchi (1984) and possibly correspond with three of the five loci (*LDH-1**, *LDH-2** and *LDH-5**) observed by Cruz *et al.* (1982). In addition to the three loci found in tilapiines, Van der Bank *et al.* (1989) recorded one more locus from various tissues including brain in 15 cichlids.

MALATE DEHYDROGENASE (MDH)

Three anodally migratory loci, *MDH-1**, *MDH-2** and *MDH-3**, were observed (Fig 6). Tissue specific activity was observed; *MDH-2** was predominantly active in liver tissue, while all three loci could be observed in muscle tissue. No species differences were observed at the *MDH-1** locus. Single fixed monomorphic allelic difference were observed between a number of species for *MDH-2** and *MDH-3**. A *MDH-3** polymorphism was observed in *S. melanotheron* (aquarium stock). In all species except for *S. galilaeus*, *O. (O.) niloticus* and *O. (O.) jipe*, a three-banded patterns representing the expression of two homodimeric and one intermediate heterodimeric isozymes produced by *MDH-2** and *MDH-3** were observed (Fig. 6 a & b). The *MDH-2** and *MDH-3** three-banded patterns observed in *Tilapia* species are wider spaced than those observed in the other species, because the *MDH-2*82* allele in *Tilapia* overlies the *MDH-1** band in these species. Where heterozygotes were observed at *MDH-3** in *S. melanotheron*, a five-banded pattern is observed because of the formation

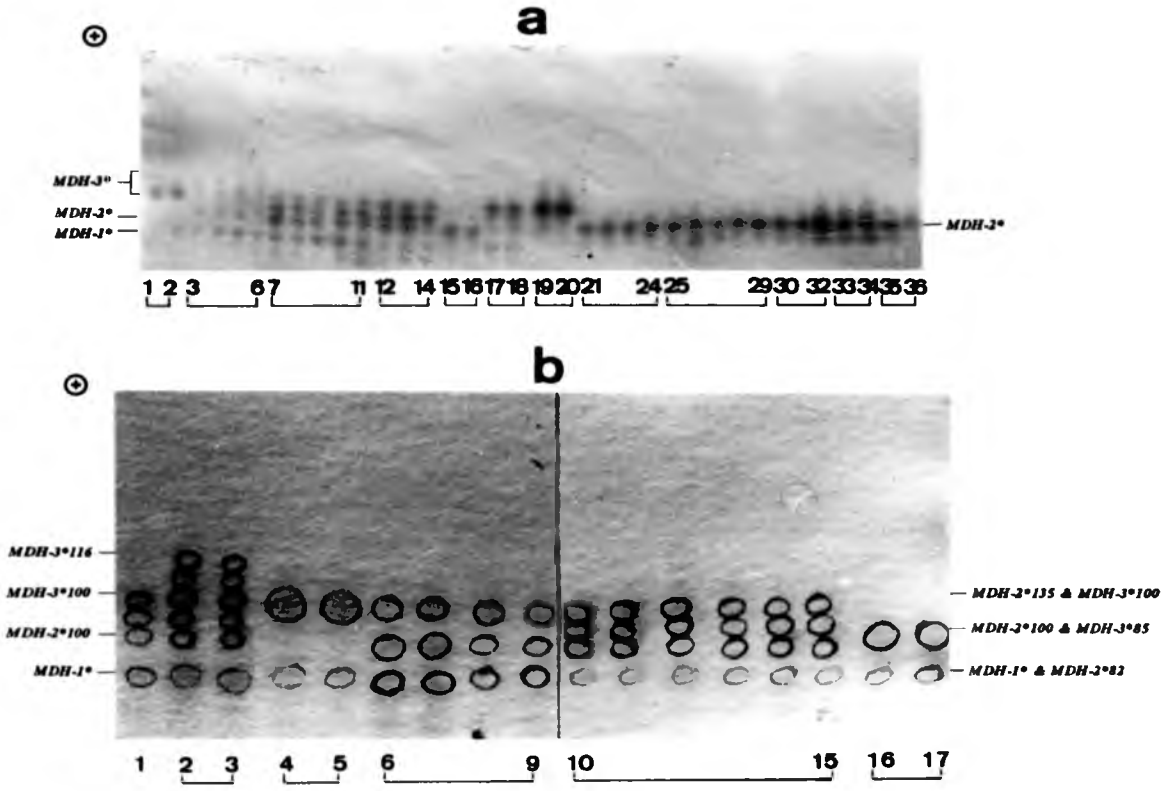

Fig. 6. (a) The numbers 1-18 show *MDH-1**, *MDH-2** and *MDH-3** observed in muscle tissue, and the numbers 19-36 show the liver-specific *MDH-2** observed in the same individuals [1-2 & 19-20, *S. galilaeus*; 3-6 & 21-24, *S. melanotheron* (aquarium stock); 7-11 & 25-29, *O. (O.) karongae*; 12-14 & 30-32, *O. (O.) lidole*; 15-16 & 33-34, *O. (O.) niloticus*; and 17-18 & 35-36, *O. (O.) mossambicus*]. In general the patterns of *MDH-2** and *MDH-3** were expressed as three bands (7-14). When *MDH-3** is heterozygous, the patterns of the *MDH-3** heterozygotes and the fixed *MDH-2** are expressed as five bands (3-6). The numbers 1-2 & 19-20 confirm the same mobility of *MDH-2** and *MDH-3** observed in *S. galilaeus*, and 15-16 & 33-34 in *O. (O.) niloticus*.

(b) The dimeric *MDH-1**, *MDH-2** and *MDH-3** observed in muscle tissue in a range of species. The three-banded and five-banded patterns of *MDH-2** and *MDH-3** are more clearly shown. 1, three-banded pattern in *S. melanotheron* (Ivory Coast); 2-3, the five-banded patterns in *S. melanotheron* (aquarium stock); 4-5, *MDH-2** and *MDH-3** at the same mobility in *S. galilaeus*; 6-9, three-banded patterns of *MDH-1**, *MDH-2** and *MDH-3** in *Tilapia* spp, with *MDH-1** and *MDH-2** observed at the same mobility; 10-15, three-banded patterns of *MDH-2** and *MDH-3** in most *Oreochromis* spp; and 16-17, *MDH-2** and *MDH-3** at the same mobility in *O. (O.) niloticus*.

of heterodimers between the fixed *MDH-2** (*MDH-2*100*) locus and the alleles at the *MDH-3** (*MDH-3*100* and *MDH-3*116*) locus. The three-banded patterns seen in the other species were not observed in *S. galilaeus*, *O. (O.) niloticus* and *O. (O.) jipe* because it appears that the alleles at these loci have not diverged in two of the three species [*MDH-2*135 & MDH-3*100* in *S. galilaeus*; *MDH-2*100 & MDH-3*85* in *O. (O.) niloticus*] and are very similar in the other [*MDH-2*98 & MDH-3*92* in *O. (O.) jipe*]. Consequently, only two bands were observed [the invariant *MDH-1** and a single faster band in *S. galilaeus*, a single slower band in *O. (O.) niloticus*, and a dense multiple band in *O. (O.) jipe*]. McAndrew & Majumdar (1983) scored two MDH loci which probably corresponds with *MDH-2** and *MDH-3** in this study.

MALIC ENZYME ($NADP^+$) (MEP)

[MALATE DEHYDROGENASE ($NADP^+$) (MDHp)]

Two anodal loci, *MEP-1** and *MEP-2**, were scored for this enzyme. Being detectable in muscle tissue, both *MEP-1** and *MEP-2** migrate close to each other on the gel, but tissue specific activity showed *MEP-2** to be liver specific and enabled the pattern to be analysed. Except for *O. (O.) jipe* which was polymorphic for two alleles between the common *MEP-1*100* and the *MEP-1*90* variant, no interspecific and intraspecific variations between and within the other species studied were observed in *MEP-1**. *MEP-2** was polymorphic for two or three different alleles in a number of species, as well as being fixed mobility differences between some other species. *MEP-2** was polymorphic for three alleles in the three chambo species and *O. (O.) niloticus*. The findings in the latter species agreed with McAndrew & Majumdar (1983). The closely packed multiple-band patterns observed in the heterozygotes showed a probable tetrameric structure, although the band separation was not clear enough to

confirm. The enzymes tetrameric structure has been confirmed in fishes by other studies (Aebersold *et al.*, 1987; Morizot & Schmidt, 1990).

MANNOSE-6-PHOSPHATE ISOMERASE (MPI)

A single anodal *MPI** locus was detectable in muscle and liver tissues. Apart from *Pelvicachromis pulcher* and *S. melanotheron* (aquarium stock), the enzyme was monomorphic and no interspecific mobility differences were observed. An *MPI** polymorphism was observed in *S. melanotheron*. Double-banded heterozygotes were observed which confirmed the monomeric structure of this enzyme (Shaklee & Keenan, 1986; Aebersold *et al.*, 1987; Morizot & Schmidt, 1990).

PEPTIDASE-C (PEPC)

[PEPTIDE HYDROLASE OR PEPTIDASE (PEP)]

A single locus with double-banded heterozygotes was observed using glycyl-L-leucine as the substrate which corresponded to the *PEPC** type enzyme (Morizot & Schmidt, 1990; Murphy *et al.*, 1990).

PHOSPHOGLUCONATE DEHYDROGENASE (PGDH)

Activity reflecting a single anodal *PGDH** locus was observed in muscle tissue in all species. Polymorphisms was observed in *T. (P.) mariae* and *O. (O.) karongae* with three-banded heterozygotes, confirming the dimeric structure of the enzyme. Interspecific mobility differences were observed between a number of species, while consistent intergeneric mobility differences were observed between the groups of the *Tilapia* species and the *Sarotherodon & Oreochromis* species. These results of PGDH in tilapiines in this study agrees with those in McAndrew & Majumdar (1983) study.

PHOSPHOGLUCOMUTASE (PGM)

Only a single anodal *PGM** locus was detected in muscle tissue. Polymorphisms with two or three different alleles were observed in a number of species studied. The monomeric structure of the enzyme in tilapiines (McAndrew & Majumdar, 1983; Basiao & Taniguchi, 1984; Van der Bank, 1989) was confirmed with the expressions of double-banded heterozygotes (Fig. 2 b). McAndrew & Majumdar (1983) found a single PGM locus in muscle tissue, consistent with this study, whereas Cruz *et al.* (1982), Basiao & Taniguchi (1984) and Van der Bank *et al.* (1989) recorded another locus for PGM in a range of various tissues.

SUPEROXIDE DISMUTASE (SOD)

Activity reflecting only a single locus *SOD** was observed. The enzyme which was detectable in liver and muscle tissue appeared in the anodal zone. Polymorphisms were observed in a number of species, the three-banded heterozygotes confirming the dimeric structure of the enzyme in tilapiines recorded by Cruz *et al.*, (1982), McAndrew & Majumdar (1983) and Van der Bank *et al.* (1989).

3.2 Allozymic Differences & Discriminating Loci

Allozymic differences and discriminating loci (Table 5) were observed and can be recorded in three categories: between genera (*Tilapia, Sarotherodon* and *Oreochromis*), between subgenera [the *T. (Coptodon)* and the other *Tilapia*, and the *Nyasalapia* chambo and the other *Oreochromis*], and between all species studied.

Table 5. A matrix of pairwise differences of enzyme loci between all species studied. Figures above the diagonal are diagnostic loci (fixed for alternate alleles) found for each species pair, represented by the locus numbers as shown in Table 4. Figures below diagonal are total numbers of these diagnostic loci. (Species abbreviation same as in Table 4.)

Taxa/ Species	*Tilapia*					*Sarotherodon*			*Oreochromis*														
	Het.	*Pel.*	*Coptodon*						*Oreochromis*									*Nyasalapia*				*Neo.*
	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
BUT	***	6,12 13,17 19,22 23,28 34,37	1,3,4,5,9 11,12,13,17 18,23,24,25 26,32,34,35 37	3,4,5,6,8 11,12,13 17,18,22 23,24,32 35,37	3,4,5,8 11,12,13 17,18,22 23,24,32 35,37	1,5,8,11,12 13,14,16,17 20,23,25,26 27,28,29,32 34,35,37	1,5,8,11,12 13,14,16,17 22,23,25,26 27,28,29,32 35,37	4,6,11,12,13 14,17,20,22 23,26,27,28 29,32,35,37	4,5,10,11 13,14,15,17 20,23,26,27 28,29,30,32 35,37	2,4,10,11 12,13,14 20,23,26 27,28,29 32,35,37	4,5,6,10 11,12,13 14,20,23 26,27,28 29,32,35	5,6,10,12 13,14,17 20,22,23 26,27,28 29,32,35	5,8,9,10,11 12,13,14 20,23,24 26,27,28 29,32,35	1,2,5,6,8,9 10,11,12,13 14,17,20,23 24,26,27,28 29,32,35	1,2,5,6,7,9 10,11,12,13 14,17,19,20 23,24,26,27 28,29,32,35	2,4,5,10 11,12,13 20,24,25 26,28,29 32,35	4,5,12,13 14,17,20 23,24,26 28,29,35	4,5,10,11 12,13,14,15 17,20,23,26 27,28,29,30 32,35,37	6,8,10,11 12,13,17 20,23,24 26,27,28 29,32,35	5,8,9,10 11,12,13 14,17,20 23,26,27 28,29,35	5,8,9,10 11,12,13 14,17,20 23,26,27 28,29,35	5,8,9,10 11,12,13 14,20,23 26,27,28 29,35	1,4,16 26,27 28,35 37
MAR	10	***	1,3,4,5,6,9 11,13,18,19 22,23,24,25 26,28,32,34	3,4,5,6,8 11,13,18 19,23,24 28,32,34	3,4,5,6,8 11,13,18 19,23,24 28,32,34	1,4,5,8,11 12,13,14,16 19,20,22,23 25,26,27,28 29,32,34,35	1,4,5,8,11 12,13,14,16 19,23,25,26 27,28,29,32 34,35	4,11,12,13 14,19,20,23 26,27,28,29 32,34,35	4,5,10,11,12 13,14,15,19 20,22,23,26 27,28,29,30 32,34,35	2,4,6,10,11 12,13,14,17 19,20,22,23 26,27,28,29 32,34,35	4,5,10,11 12,13,14 17,19,20 22,23,26 27,28,29 32,34,35	5,6,10,11 12,13,14,19 20,23,26,27 28,29,34,35 37	5,8,9,10,11 13,14,19 20,22,23 24,26,27 28,29,32 34,35,37	1,2,5,8,9,10 11,12,13,14 19,20,22,23 24,26,27,28 29,32,34,35	1,2,5,7,9,10 11,13,14,19 20,22,23,24 26,27,28,29 32,34,35	2,4,5,10 11,12,13 20,24,25 26,27,28 29,34,35 37	4,5,11,12 13,14,19 20,22,23 24,26,28 29,34,35	4,5,10,11,12 13,14,15,19 20,22,23,26 27,28,29,30 32,34,35	4,8,10,11 12,13,19 20,22,23 24,26,27 28,29,32 34,35	4,5,8,9 10,11,13 14,19,20 23,26,27 28,29,34 35	5,8,9,10 11,12,13 14,19,20 23,26,27 28,29,34 35	5,8,9,10 11,13,14 19,20,23 26,27,28 29,34,35	1,4,16 26,27 28,35
REN	18	18	***	6,8,9,22 24,25,26 34	8,9,22 24,25,26 34	1,3,4,8,9,11 12,13,14,16 18,20,24,25 27,28,29,32 34,35	1,3,4,8,9,11 12,14,16,18 22,24,25,27 28,29,32,34 35	1,3,9,12,14 18,20,22,23 24,25,27,28 29,32,34,35	3,4,9,10,12 14,15,18,20 24,25,27,28 29,30,32,34 35	2,3,4,5,9,10 12,14,17,18 20,23,24,25 27,28,29,32 34,35	1,3,4,6,9 10,12,14 17,18,20 24,25,27 28,29,32 34,35	1,3,4,6,9,10 11,12,14,18 20,22,24,25 27,28,29,32 34,35,37	3,4,8,10,14 18,20,23 24,25,27 28,29,32 34,35,37	2,3,4,6,8,10 11,12,14,18 20,23,24,25 27,28,29,32 34,35	2,3,7,9,10 14,18,19,20 23,24,25,27 28,29,32,34 35	1,2,3,4,9 10,11,12 18,20,24 25,27,28 29,32,34 35,37	1,3,4,9 12,14,18 20,23,24 25,28,29 32,34,35	3,4,5,9,10 12,14,15,18 20,24,25,27 28,29,30,32 34,35	3,4,6,8,9 10,12,18 20,23,24 25,27,28 29,32,34 35	1,3,4,8 10,14,18 20,23,24 25,26,27 28,29,34 35	1,3,4,8 10,12,14 18,20,23 24,25,26 27,28,29 34,35	1,3,4,8 10,14,18 20,24,25 26,27,28 29,34,35	1,4,16 24,27 28,35
THO	16	14	8	***	NO	1,3,4,6,11 12,13,14,16 18,20,22,24 25,26,27,28 29,32,34,35	1,3,4,6,11 12,14,16,18 24,25,26,27 28,29,32,35	3,4,6,8,12 14,18,20,23 24,26,27,28 29,32,35	3,4,6,8,10 12,14,15,18 20,22,24,26 27,28,29,30 32,35	2,3,4,5,6,8 10,12,14,17 18,20,22,23 24,26,27,28 29,32,35	3,4,10,12 14,17,18 20,22,24 26,27,28 29,32,35	3,4,6,8,10 11,12,14,18 20,24,26,27 28,29,32,35 37	3,4,6,9 10,14,18 20,22,23 24,26,27 28,29,32 35,37	2,3,4,9,10 11,12,14,18 20,22,23,24 26,27,28,29 32,35	2,3,6,7,8,9 10,14,18,19 20,22,23,24 26,27,28,29 32,35	2,3,4,8 10,11,12 18,20,24 25,26,27 28,29,32 35,37	3,4,6,8 12,14,18 20,22,23 24,26,28 29,32,35	3,4,5,6,8,10 12,14,15,18 20,22,24,26 27,28,29,30 32,35	3,4,6,10 12,18,20 22,23,24 26,27,28 29,32,35	3,4,6,9 10,14,18 20,23,24 26,27,28 29,35	3,4,6,9 10,12,14 18,20,23 24,26,27 28,29,35	3,4,6,9 10,14,18 20,24,26 27,28,29 35	1,4,8 16,24 26,27 28,35
ZIL	15	14	7	NO	***	1,3,4,6,11 12,13,14,16 18,19,20,22 24,25,26,27 28,29,32,34 35	1,3,4,6,11 12,14,16,18 19,24,25,26 27,28,29,32 35	3,6,8,12,14 18,19,20,23 24,26,27,28 29,32,35	3,4,6,8,10 12,14,15,18 19,20,22,24 26,27,28,29 30,32,35	2,3,4,5,6,8 10,12,14,17 18,19,20,22 23,24,26,27 28,29,32,35	3,4,10,12 14,17,18 19,20,22 24,26,27 28,29,32 35	3,4,6,8,10 11,12,14,18 20,24,26,27 28,29,32,35 37	3,4,6,9 10,14,18 20,22,23 24,26,27 28,29,32 35,37	2,3,4,9,10 11,12,14,18 20,22,23,24 26,27,28,29 32,35	2,3,6,7,8,9 10,14,18,20 22,23,24,26 27,28,29,32 35	2,3,4,8 10,11,12 18,19,20 24,25,26 27,28,29 32,35,37	3,4,5,8 12,14,18 19,20,22 23,24,26 28,29,32 35	3,4,8,10,12 14,15,18,19 20,22,24,26 27,28,29,30 32,35	3,4,6,10 12,18,19 20,22,23 24,26,27 28,29,32 35	3,4,9,10 14,18,19 20,23,24 26,27,28 29,35	3,4,9,10 12,14,18 19,20,23 24,26,27 28,29,35	3,4,9,10 14,18,19 20,24,26 27,28,29 35	1,4,8 16,24 26,27 28,29 35
MEL	20	21	20	21	22	***	13,22	1,4,8,13,16 22,23,29,34	1,4,8,10,11 13,14,15,16 25,30,34	1,2,4,5,8,10 11,13,16,17 23,25,34	1,4,6,10 11,13,14 16,17,25 34,35	1,4,6,8,10 13,16,22,25 34,37	1,4,10,11 13,16,23 24,25,34 37	1,2,4,6,10 13,16,23,24 25,34	1,2,4,7,8 10,11,12,13 16,19,23,24 25,34	1,2,4,8 10,13,16 24,32,34 37	1,4,8,13 16,23,24 25,32,34	1,4,5,8,10 11,13,15,16 25,29,30,34	1,10,11 13,14,16 23,24,25 34	1,10,11 13,16,23 25,26,32 34	1,10,11 13,16,23 25,26,32 34	1,10,11 13,16,23 25,26,32 34	1,8,16 32

Taxa/ Species	*Tilapia*					*Sarotherodon*			*Oreochromis*														
	Het.	*Pel.*	*Coptodon*						*Oreochromis*										*Nyasalapia*				*Neo.*
	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
MIC	19	19	19	17	18	2	***	1,4,8,16,23 29	1,4,8,10,11 14,15,16,22 25,30	1,2,4,5,8,10 11,16,17,22 23,25	1,4,6,10 11,14,16 17,22,25 35	1,4,6,8,10 16,25,37	1,4,10,11 16,22,23 24,25,37	1,2,4,6,10 16,22,23,24 25	1,2,4,7,8,10 11,12,16,19 22,23,24,25	1,2,4,8 10,16,24 32,37	1,4,8,16 22,23,24 25,32	1,4,5,8,10 11,15,16,22 25,29,30	1,10,11 14,16,22 23,24,25	1,10,11 16,23,25 26,32	1,10,11 16,23,25 26,32	1,10,11 16,25,26 32	1,16,32
GAL	17	15	17	16	16	9	6	***	4,10,14,15 22,23,29	2,4,10,17 22,29,30	4,10,14 17,22,23 29,35	4,10,11,23 29,37	1,4,8,9,10 22,24,29 37	2,4,8,9,10 11,22,24,29	1,2,4,7,9,10 12,19,22,24 29	2,4,10,11 24,25,29 32,37	4,22,24 29,32	4,5,10,15,22 23,29,30	4,8,10,14 22,24,29	4,8,9,10 26,29,32	4,8,9,10 26,29,32	4,8,9,10 26,29,32	1,4,16 32
NIL	18	20	18	19	20	12	11	7	***	2,4,5,15 23,30	4,15,17 30,35	4,6,11,14,15 22,30,37	4,9,10,14 15,23,24 30,37	2,4,9,10,11 14,15,23,24 30	4,7,9,12,14 15,19,23,24 30	2,4,10,11 15,24,25 30,32,37	4,10,14 15,23,24 30,32	4,5,14,15 29,30	4,14,15 23,24,30	4,9,14,15 23,26,30 32	4,9,14,15 23,26,30 32	4,9,14 15,26,30 32	1,4,15
AUR	16	20	20	21	22	13	12	7	6	***	2,4,5,6 23,35	2,4,5,6,11 17,22,23,37	2,4,5,8,9 10,17,24 37	1,2,4,5,6,8 9,10,11,17 24	1,4,5,6,7,9 12,17,19,24	2,4,5,10 11,24,25 32,37	4,5,17 24,32	2,4,5,15,17 23,29,30	4,6,8,13 17,24	2,4,5,8,9 17,26,32	2,4,5,8,9 17,26,32	2,4,5,8 9,26,32	1,2,4 16,32
SPI	16	19	19	16	17	12	11	8	5	6	***	4,6,11,14,17 22,35	9,10,14,23 24,35	1,2,4,9,10 11,14,17,23 24,35	1,2,4,6,7,9 12,14,17,19 23,24,35	2,4,10,11 24,25,32 35	4,6,10,14 17,23,24 32,35	4,5,6,14,15 17,29,30,35	4,6,14,17 23,24,35	4,9,14 23,26,32	4,9,14 23,26,32	4,9,14 26,32,35	1,4,16 32,35
UHN	16	17	21	18	18	11	8	6	8	9	7	***	6,8,9,10,11 22,23,24	1,2,6,8,9,10 22,23,24	1,2,6,7,9,11 12,22,23,24	2,4,10,24 25	4,6,10,22 23,24	4,5,6,11,15 22,29,30,37	4,6,8,11 14,22,23 24	4,6,8,9 11,23,26	6,8,9,11 23,26	6,8,9,11 26	1,16,37
MOS	17	20	17	18	18	11	10	9	9	9	6	8	***	2,11	2,7,8,9,10	2,4,8,9 11,25,32	4,8,9,32	4,5,8,9,10 15,23,29,30 37	4,9,10,14	4,10,24 26,32	10,24,32 26	10,24,32 26	1,8,16 24,32 37
MOR	21	22	20	19	19	11	10	9	10	11	11	9	2	***	2,6,7,8,9,10 11,12,19	1,2,4,8,9 25,32	1,2,4,6,8 9,32	1,2,4,5,6,8,9 10,11,15,23 29,30	1,2,4,6,9 10,11,14	1,2,4,10 11,24,32 26	1,2,10,11 24,32,26	1,2,10 11,24,32 26	1,2,8 16,24 32
AND	22	21	18	20	19	15	14	11	10	10	13	10	5	9	***	1,2,4,7,9 10,11,12 19,25,32	1,4,7,9 10,12,19 32	1,2,4,5,7,9 12,15,19,23 29,30	1,4,7,8,9 12,14,19	1,2,4,7,8 9,19,24 26,32	1,2,7,8,9 12,19,24 26,32	1,2,6,7,8 9,19,24 26,32	1,2,16 24,32
PLA	15	17	19	18	19	11	9	9	10	9	8	5	7	7	11	***	2,25	2,4,5,10,11 15,25,29,30 32,37	2,4,8,10 11,25,32	2,4,8,9 10,11,24 25,26	2,4,8,9 10,11,24 25,26	2,4,8,9 10,11,24 25,26	1,2,4 16,24 37
SHI	13	16	16	16	17	10	9	5	8	5	9	6	4	7	8	2	***	4,5,10,15,23 29,30,32	4,8,10,14 32	4,8,9,10 24,26	4,8,9,10 24,26	4,8,9,10 24,26	1,4,16 24,32

Taxa/ Species	*Tilapia*					*Sarotherodon*			*Oreochromis*													
	Het.	*Pel.*	*Coptodon*						*Oreochromis*										*Nyasalapia*				*Neo.*
	BUT	MAR	REN	THO	ZIL	MEL	MIC	GAL	NIL	AUR	SPI	UHN	MOS	MOR	AND	PLA	SHI	JIP	MAC	KAR	LID	SQU	TAN
JIP	19	20	19	20	19	13	12	8	6	8	9	9	10	13	12	11	8	***	4,5,8,14 15,23,29 30	4,5,8,9 15,23,26 29,30,32	4,5,8,9 15,23,26 29,30,32	4,5,8,9 15,26,29 30,32	1,4,15 32
MAC	16	18	18	16	17	10	9	7	6	6	7	8	4	8	8	7	5	8	***	9,14,24 26,32	9,14,24 26,32	9,14,24 26,32	1,4,8 16,24 32
KAR	16	17	16	15	15	9	7	6	7	7	5	6	4	7	9	8	5	9	4	***	NO	NO	1,4,8 16
LID	16	17	17	16	16	9	7	6	7	7	6	5	3	6	9	8	5	9	4	NO	***	NO	1,4,8 16
SQU	15	16	15	14	14	9	6	6	6	6	5	4	3	6	9	8	5	8	4	NO	NO	***	1,4,8 16
TAN	8	7	7	9	10	4	3	4	3	5	5	3	6	6	5	6	5	4	6	4	4	4	***

'*' No comparisons.**
'NO' No fixed allele differences at that locus found for that species pair.

Loci 28, 29 and 35 are intergeneric discriminating loci between the *Tilapia* and *Sarotherodon-Oreochromis*, and locus 10 between the *Sarotherodon* and *Oreochromis*.

Loci 3, 4, 5, 11, 13, 18, 24 and 32 are intersubgeneric discriminating loci between the *T. (Coptodon)* and the other *Tilapia*, loci 1 and 4 between the *O. (Neotilapia)* and the other *Oreochromis*, and locus 26 between the *Nyasalapia* chambo and the other *Oreochromis* (only polymorphic in the chambo).

The specific discriminating loci are the loci 13 and 23 observed for BUT; 13, 23, 28 and 34 for MAR; 24, 25 and 34 for REN; 24 for THO & ZIL; 1 and 16 for MEL & MIC; 29 for GAL; 15 and 30 for NIL; 4 for AUR; 35 for SPI; 6 for UHN; 2 for MOR; 7 and 9 for AND; 2 for PLA; 4 for PLA & SHI; 5, 15, 29 and 30 for JIP; 26 for the three chambo species; and 1 and 4 for TAN.

3.2.1 Intergeneric Allozymic Differences & Discriminating Loci between Genera

Allozyme differences between the three tilapiine genera were observed in a number of loci discriminating between the *Tilapia* and the *Sarotherodon-Oreochromis*, and between the *Sarotherodon* and the *Oreochromis*, as following. A single discriminating locus for distinguishing the three genera from each other was not observed within the number of enzyme loci examined in this study.

(i) *Tilapia* & *Sarotherodon-Oreochromis*

	Tilapia	*Sarotherodon-Oreochromis*
*LDH-3**	**87, *90*	**100*
*MDH-2**	**82*	**98, *100, *135*
*PGDH**	**125, *150*	**100, *116*

(ii) *Sarotherodon* & *Oreochromis*

	Sarotherodon	*Oreochromis*
*DDH-1**	**180*	**100, *110* *(O. shiranus *110/180)*

Allozyme differences between *Tilapia* and *Sarotherodon-Oreochromis* were observed at three discriminating loci, *LDH-3**, *MDH-2** and *PGDH**, whereas only one locus *DDH-1** could be recorded to discriminate between *Sarotherodon* and *Oreochromis*. [Due to an observation of *DDH-1** polymorphism with the very poor resolution in *O. (O.) shiranus* (**110/180*), as mentioned earlier (see '**DDH**'), this locus may not be able to be counted as a good discriminating locus.] This means that the *Tilapia* species are unambiguously

different from the species in the other two genera, which are quite close to each other. This result is possibly unsurprising because usually *Tilapia* species can be separated from the species in the other two genera quite easily because of their distinct morphological characters of the genus (Trewavas, pers. comm.). *Sarotherodon* and *Oreochromis* are not completely distinguishable from each other since *S. galilaeus* was shown to be sharing a range of allozymes with some *Oreochromis* species. This finding is comparable to a number of their morphological characters (Trewavas, 1983) which are much alike. Trewavas (1983) has grouped *S. galilaeus* with the type-species of the genus, *S. melanotheron*, on the reproductive behaviour (paternal mouthbrooder) they share. In non-reproductive features *S. galilaeus* is certainly more like *Oreochromis* than the type-species of *Sarotherodon* (Trewavas, pers. comm.).

3.2.2 Intersubgeneric Allozymic Differences & Discriminating Loci between Subgenera

Two *Tilapia* subgenera, *T. (Heterotilapia)* and *T. (Pelmatolapia)*, were represented by single species, *T. (H.) buttikoferi* and *T. (P.) mariae* respectively. So, allozyme differences observed between these two subgenera were specific differences between the two species. Consistent allozyme differences between the three subgenera of *Oreochromis* were not recorded; the *O. (Neotilapia)* comparisons were not used as it was felt to be unreliable because of the difference in the number of loci examined in the three subgenera [16 in the *O. (Neotilapia)*, 43 in the other two]. Within the 43 loci examined, no discriminating alleles were detected between the two subgenera *O. (Oreochromis)* and *O. (Nyasalapia)* (Table 4). However, some variation between the chambo,

the Lake Malawi *O. (Nyasalapia)*, and the other *Oreochromis* were detected. The discriminatory loci and allele differences observed between subgenera are listed below.

T. (Coptodon)* & the other *Tilapia

	T. (Coptodon)	The other *Tilapia*
*ACP**	**150*	**100*
*ADA**	**108, *118*	**100, *104*
*ADH**	**-40,*-100,*-134*	**-83*
*EST-1**	**85*	**100*
*ESTD**	**100*	**107, *115*
*GPI-1**	**-100*	**100*
*IDDH**	**19, *38*	**100*
*MEP-2**	**95*	**90*

Allozyme differences between *T. (Coptodon)* and the other *Tilapia* were observed at eight loci. With a large number of these possible discriminating loci, *T. (Coptodon)* was shown to be very different from the other *Tilapia*. These eight loci, in addition to the previous three loci which discriminate between the *Tilapia* and *Sarotherodon-Oreochromis*, imply that the *Tilapia* are not only different from the species outwith the genus, but the *Tilapia* subgenera are also very different to each other. Among the *Oreochromis* subgenera only one locus *LDH-1** had an allele which was totally unique of a given subgenus. *LDH-1*-100*, although it was at a resonable frequency, not all individuals carried it, so it could not be said to be totally discriminating. The results indicate that there are close relationships between the *Oreochromis* subgenera, i.e. *O. (Oreochromis)* and *O. (Nyasalapia)*, and that the chambo (Malawian *Nyasalapia*) are a distinct and related group.

3.2.3 Interspecific Allozymic Differences & Specific Discriminating Loci

Species specific alleles were observed at a number of loci for most of the species studied (Table 5). These are summarized below.

Species	**Species discriminating locus and allele mobility**
T. buttikoferi	*ESTD*107, G6PDH-2*55*
T. mariae	*ESTD*115, G6PDH-2*80, LDH-3*87, PEPC*81*
T. rendalli	*IDDH*19, IDHP-1*90, PEPC*84*
T. tholloni	-
T. zillii	-
(T. tholloni & T. zillii)	*(IDDH*38)*
S. melanotheron (Aquarium & Ivory Coast stocks)	*AAT-2*118, FH-2*65*
S. galilaeus	*MDH-2*135*
O. niloticus	*FH-1*107, MDH-3*85*
O. aureus	*ADA*145, ADA*138*
O. spilurus	*PGDH*116* (also shown as the variant in *O. karongae* polymorphism)
O. u. hornorum	*AH-2*66*
O. mossambicus	-
O. mortimeri	*AAT-3*77*
O. andersonii	*AK*87, CK*89*
O. placidus	*AAT-3*117*
O. shiranus	-

Species	Species discriminating locus and allele mobility
(O. placidus & O. shiranus)	*(ADA*66)*
O. jipe	*ADH*-180, FH-1*86, MDH-2*98, MDH-3*92*
O. macrochir	-
O. karongae	-
O. lidole	-
O. squamipinnis	-
(O. karongae, O. lidole, & O. squamipinnis)	*(LDH-1** polymorphism with the common **100* and the variant **-100)* **[NOT FIXED DIFFERENCE]**
O. tanganicae	*AAT-2*126, ADA*75*

Along with the species specific loci for any particular species pair in Table 5 the above list gives details of single loci which will unequivocally identify any given species from all the others in this study. Some species, i.e. *T. tholloni, T. zillii, O. mossambicus, O. shiranus, O. macrochir,* and the three chambo, did not show any single discriminatory locus. However it is possible to unequivocally identify these species if a combination of loci are used. For example, a single discriminating locus was not observed for *O. shiranus*, but *O. shiranus* as well as *O. placidus* could be distinguished from all other species by the variant allele *ADA*66*. Then, *O. placidus* could be distinguished from *O. shiranus* by the variant *AAT-3*117*. Similarly, *O. mossambicus* and *O. macrochir* could be identified using discriminating loci of particular species, as shown in the following possible identifications.

(i) *O. shiranus* identification

Fixed at the *ADA*66* . . . . . *O. shiranus & O. placidus*

1) Fixed at the variant *AAT-3*117* . . . . . *O. placidus*

2) Polymorphic at *AAT-3* (*100 & *110)* . . . . . *O. shiranus*

(ii) *O. mossambicus* identification

(a) Fixed at the *CK*85* . . . . . *O. mossambicus, O. mortimeri, T. rendalli,* and the chambo

1) Fixed at *IDDH*19, IDHP-1*90* or *PEPC*84* . . . . *T. rendalli*

2) Polymorphic at *LDH-1* (*100 & *-100)* . . . . . the chambo

3) Fixed at the variant *AAT-3*77* . . . . . *O. mortimeri*

4) None of the above . . . . . *O. mossambicus*

or (b) Fixed at the *DDH-1*110* . . . . . *O. mossambicus, O. mortimeri, O. placidus & O. shiranus*

1) Fixed at the *ADA*66* . . . . . *O. placidus & O. shiranus*

2) Fixed at the *AAT-3*77* . . . . . *O. mortimeri*

3) None of the above . . . . . *O. mossambicus*

(iii) *O. macrochir* identification

Fixed at the *FBALD-2*-115* . . . . . *O. macrochir & Tilapia* species

1) Fixed at *LDH-2*180, LDH-3*90, LDH-3*87* . *Tilapia* species

2) Fixed at *LDH-2*100, LDH-3*100* . . . . . *O. macrochir*

Despite the large number of loci observed in this study it was not possible to distinguish between *T. tholloni* and *T. zillii*, and between the chambo species. On the other hand, our inability to distinguish these species from each other also implies that the species have close genetic relationships to each other. The *T.*

tholloni used in this study was an aquarium strain of uncertain origin. It looked very much like *T. zillii* and was not morphologically distinguishable from that species although adult colour patterns suggested it was *T. tholloni*. Trewavas (pers. comm.) was aware of the very similar morphology of these two species and regarded them as very close relatives of each other.

The results from the chambo suggest a high level of genetic integrity as no fixed alleles were found in any of the shared polymorphic loci (Table 5). Morphologically, the chambo have a number of unique characters (Trewavas, 1983) which are so uniform in all species that these fish have been classified as closely related endemic species of Lake Malawi (Thys van den Audenaerde, 1968b; Trewavas, 1983). It is notable that the fish species can hardly identified from each other by morphology or allozyme electrophoresis. This is not the case in any of the other tilapia species or genera studied.

3.3 Genetic Variability

Average heterozygosities and percentages of polymorphic loci estimated in each species are shown in Table 6. The range of expected heterozygosities (*He*, 0.008 - 0.122) observed in this study compare well with previous electrophoretic studies on the tilapia (*Ho* = 0.002 - 0.058, McAndrew & Majumdar, 1983; *He* = 0.054 - 0.073, Kornfield, 1984; and *He* = 0.013 - 0.035, Van der Bank *et al.*, 1989) and the general level observed in many group of fishes (0.058, Powell, 1975; 0.08, Selander, 1976). The levels of *He* observed could arbitrarily be devided into a number of groups. The group

Table 6. Percentage of polymorphic loci and average heterozygosities (unbiased estimate, Nei, 1978) of tilapia species studied.

Species	Percentage of polymorphic loci	Expected heterozygosity
T. (H.) buttikoferi	2.3	0.008 (0.008)
T. (P.) mariae	4.7	0.017 (0.012)
T. (C.) rendalli	9.3	0.026 (0.014)
T. (C.) tholloni	7.0	0.029 (0.018)
T. (C.) zillii	11.6	0.037 (0.018)
S. melanotheron (Aquarium stock)	11.6	0.045 (0.021)
S. melanotheron (Ivory Coast)	9.3	0.043 (0.021)
S. galilaeus	16.3	0.043 (0.017)
O. (O.) niloticus	20.9	0.081 (0.028)
O. (O.) aureus	11.6	0.021 (0.010)
O. (O.) spilurus	14.0	0.041 (0.017)
O. (O.) u. hornorum	9.3	0.029 (0.016)
O. (O.) andersonii	9.3	0.036 (0.020)
O. (O.) mortimeri	7.0	0.032 (0.018)
O. (O.) mossambicus	14.0	0.047 (0.020)
O. (O.) placidus	2.8	0.012 (0.012)
O. (O.) shiranus	18.6	0.086 (0.028)
O. (O.) jipe	20.9	0.078 (0.026)
O. (Ny.) macrochir	11.6	0.043 (0.020)
O. (Ny.) karongae	27.9	0.112 (0.030)
O. (Ny.) lidole	23.3	0.110 (0.032)
O. (Ny.) squamipinnis	25.6	0.122 (0.034)

Standard errors in parentheses

showing low heterozygosities are *T. buttikoferi* (0.008), *T. mariae* (0.017) and *O. placidus* (0.012). In the case of these species their levels appear to be related to the severe bottlenecking (*T. mariae* from various aquarist stocks) or small sample size (*T. buttikoferi* and *O. placidus*, 3 individuals) of the particular populations used. McAndrew & Majumdar (1983) observed a similar low level in an *O. mossambicus* stock (0.002) whereas the level 0.047 was observed for another conspecific population obtained directly in this study. The majority of the species are around the average for fish in general and seem to be representative of the species, as they compare well with previous studies (Table 7) within the margin of errors associated with the use of electrophoretic techniques (different electrophoretic conditions, buffer systems, number and choice of loci used between studies, Sarich, 1977). The third group show relatively high *He* and are all Lake Malawi species, *O. shiranus* (0.086), *O. lidole* (0.110), *O. karongae* (0.112) and *O. squamipinnis* (0.122). The possible reasons for these high levels compared to other tilapiines will be discussed in more detail in the last section.

3.4 Genetic Differentiation

The fixation index F_{ST}, Wright's (1978) F-statistics, serves as a convenient and widely used measure of genetic differentiation among populations, interpreted in terms of random genetic drift in an ideal population with no mutation, migration, or selection (Hartl, 1988). In this study the F_{ST} serves as the amounts of genetic divergence among tilapia species observed at different taxonomic levels in the tilapiine classification of Trewavas (1983), as shown in Table 8. Wright (1978) suggests the following qualitative guidelines for the interpretation of F_{ST}:

Table 7. Summary of genetic variability of the same tilapia species estimated between studies.

	Kornfield *et al.* (1979)	McAndrew & Majumdar (1983)	Basiao & Taniguchi (1984)	Van der Bank *et al.* (1989)	The present study
T. (C.) rendalli					
No. of loci examined				40	43
No. of polymorphic loci	-	-	-	10	4
Proportion of polymorphic loci				0.25	0.093
Heterozygosity				*He* = 0.031	*He* = 0.026
T. (C.) zillii					
No. of loci examined	21	25	35		43
No. of polymorphic loci	0	1	2	-	5
Proportion of polymorphic loci	0	0.04	0.057		0.116
Heterozygosity	*He* = 0	*Ho* = 0.021	*He* = 0.022		*He* = 0.037
S. galilaeus					
No. of loci examined	21	25			43
No. of polymorphic loci	2	3	-	-	7
Proportion of polymorphic loci	0.095	0.12			0.163
Heterozygosity	*He* = 0.027	*Ho* = 0.043			*He* = 0.043
O. (O.) niloticus					
No. of loci examined		25	35		43
No. of polymorphic loci	-	4	12	-	9
Proportion of polymorphic loci		0.16	0.343		0.209
Heterozygosity		*Ho* = 0.058	*He* = 0.088		*He* = 0.081
O. (O.) aureus					
No. of loci examined	21	25			43
No. of polymorphic loci	5	3	-	-	5
Proportion of polymorphic loci	0.238	0.12			0.116
Heterozygosity	*He* = 0.078	*Ho* = 0.023			*He* = 0.021

	Kornfield *et al.* (1979)	McAndrew & Majumdar (1983)	Basiao & Taniguchi (1984)	Van der Bank *et al.* (1989)	The present study
O. (O.) spilurus					
No. of loci examined		25			43
No. of polymorphic loci	-	3	-	-	6
Proportion of polymorphic loci		0.12			0.140
Heterozygosity		*Ho* = 0.021			*He* = 0.041
O. (O.) mossambicus					
No. of loci examined		25		40	43
No. of polymorphic loci	-	1	-	6	6
Proportion of polymorphic loci		0.04		0.15	0.140
Heterozygosity		*Ho* = 0.002		*He* = 0.019	*He* = 0.047
O. (O.) andersonii					
No. of loci examined		25		40	43
No. of polymorphic loci	-	2	-	6	4
Proportion of polymorphic loci		0.08		0.15	0.093
Heterozygosity		*Ho* = 0.020		*He* = 0.020	*He* = 0.036
O. (O.) jipe					
No. of loci examined		25			43
No. of polymorphic loci	-	4	-	-	9
Proportion of polymorphic loci		0.16			0.209
Heterozygosity		*Ho* = 0.050			*He* = 0.078
O. (Ny.) macrochir					
No. of loci examined		25		40	43
No. of polymorphic loci	-	2	-	9	5
Proportion of polymorphic loci		0.08		0.225	0.116
Heterozygosity		*Ho* = 0.031		*He* = 0.031	*He* = 0.043

Ho = Observed heterozygosity
He = Expected heterozygosity

Table 8. Observed F-statistics (F_{ST}) within different levels of taxa (genera & subgenera) of tilapiines.

Taxa	Number of populations (species)	F_{ST}
Genus *Tilapia*	5	0.907
Subgenus *T. (Coptodon)*	3	0.749
Genus *Sarotherodon*	3	0.734
Genus *Oreochromis*	13	0.734
Subgenus *O. (Oreochromis)*	9	0.798
Subgenus *O. (Nyasalapia)*	4	0.378
The chambo	3	0.051

1) The range 0 to 0.05 may be considered as indicating *little* genetic differentiation.
2) The range 0.05 to 0.15 indicates *moderate* genetic differentiation.
3) The range 0.15 to 0.25 indicates *great* genetic differentiation.
4) Values of F_{ST} above 0.25 indicate *very great* genetic differentiation.

Following these guidelines of Wright (1978), despite its normal use for different subpopulations, the results in Table 8 suggest that genetic differentiation among tilapia species is great at all taxa levels. From the different subpopulations point of view, the observed maximum of F_{ST} is usually much less than 1 (Hartl, 1988), although F_{ST} has theoretical values from 0 (indicating no genetic divergence) to 1 (indicating fixation for alternative alleles in the subpopulations). But the large F_{ST} values observed in this study seem to be fine for the interspecies differences, suggesting these species have been isolated for long time.

The F_{ST} values of the chambo species, which are examples of intralacustrine speciation, indicate that genetic divergence among chambo species is rather small. Of the total genetic variation found in the three chambo species, only 0.05 (5 %) is ascribable to genetic differences in allele frequency among species, which means that 95 % of the total genetic variation is found within any single species. These results may imply that each chambo species represents (in a certain sense) a separate species (5 %), whereas the chambo as a whole also represents a distinct species (95 %), which may indicate a shared common ancestor among them.

The allozyme data show greater levels of genetic variation within rather than between the species. This however may be misleading as the morphological characteristics used to discriminate between the species obviously have a genetic basis. However it is impossible to say how many genes are involved with these discriminating morphological characters and how variable they may be. (This point has been well discussed by Hartl, 1988). Any set of involved genes of allozymes or morphological characteristics in nature may or may not be representative of the genome as a whole, so the combination of as many different sources of variation as possible is essential before any firm conclusions are made.

3.5 The Lake Malawi Species

As mentioned earlier it was not appropriate to use the information on allele frequency to make assumptions about the populations of species used in this survey because the fish had come from various artificially maintained populations in which many of the assumptions underlying Hardy-Weinberg expectations

would have been invalid. This was not the case of the Malawian sample, as these were samples of fish collected in relatively large numbers from the wild. Most of the observed genotypic frequencies in the four Malawian species, i.e. *O. shiranus* and the three chambo species, did not significantly deviate from expected Hardy-Weinberg proportions ($\chi^2_{P \geq 0.05}$; Appendix 3). Some deviations ($\chi^2_{P < 0.05}$) were detected at *EST-2** ($\chi^2_{P = 0.031}$) in *O. karongae*, and at *AAT-2** ($\chi^2_{P = 0.039}$) and *ADH** ($\chi^2_{P = 0.020}$) in *O. lidole*, however the chi-square test with pooling genotypes and significance test using exact probabilities (Appendix 3) did not show any of these to be significant. Also, Cooper's (1968) correction for multiple simultaneous tests would not have made these significant [0.05 ÷ by number of χ-tests to get new significant level for each species; *O. shiranus* (0.006), *O. karongae* (0.004), *O. lidole* (0.005) and *O. squamipinnis* (0.004)]. Accordingly this means that there are no significant differences within a species. So these species are in Hardy-Weinberg equilibrium, therefore large random mating populations in each species can be assumed. The contingency chi-square analyses of heterogeneity of the three chambo (Appendix 3) show significant differences in allele frequencies between these species, although all of them came from the same area (the south) of the lake. This means that good species identity appears even in a restricted area. These two findings suggest that there are a number of reproductively isolated species in Lake Malawi. Morphological variation in the lower jaw in *O. karongae* (Trewavas, 1983) also suggests isolation in this species. Future work should be done focusing on different conspecific populations (same species collected in different areas) to see if there is substructuring within a species.

It is clear from the allele frequency data (Table 4) that *O. shiranus* is genetically very different from the three chambo species, showing different fixed

alleles at a number of loci (see earlier section). Among the tilapia species studied the three chambo species are very unusual in that they do not display any species specific bands and share at least 10 polymorphic loci, this being reflected in the high *He* of these species. This would strongly suggest that these three species must have shared a common ancestor. The high heterozygosity in the chambo is unusual in the tilapiines and may well be a result of the great stability in Lake Malawi enabling the chambo to remain at a large population size for long periods so that genetic variation could be accumulated in the species. This is different from the case of the droughts which would seriously bottleneck populations in rivers or smaller shallow lakes. Large fluctuations in Lake Malawi have been recorded (Fig. 17.1 of Beadle, 1981; Owen *et al.*, 1990) but the lake is not believed to have dried, as in the case of Lake Victoria. The stability of the lacustrine environment therefore enabled these species to maintain large effective sizes. It is possible that some of the variation observed may have been in response to environmental fluctuation such as water temperature, but at present no adaptive role for any allozyme has been identified.

It appears from the allozyme data that the morphometric characters used to identify the three species do reflect reproductively isolated populations and 'good' species (no mixing between the different morphological forms). The allozyme data suggest a common ancestor because of the number of shared polymorphic loci and unique morphological characteristics. The level of genetic differentiation between these species as measured by Wright's (1978) fixation index F_{ST} is very low (0.051), which would suggest very little genetic differentiation between subpopulations (species) of a single distinct species (the whole chambo). The levels observed in the other taxa (Table 8) indicate large levels of differentiation even within the other subgenera. The main difference

between the chambo and other tilapia is that most riverine species have probably evolved allopatrically by some form of geographic barrier or geological upheavals in the African rift valley. For the chambo it appears that they have evolved sympatrically, the only other good example of this being in Lake Barombi Mbo in Cameroon.

Intralacustrine speciation is however the norm for another cichlid tribe, the Haplochromini. In total, the three East African Great Lakes (Malawi, Victoria and Tanganyika) contain many hundreds of species in this tribe. The data on allozyme variation in the haplochromines (McKaye, 1982, 1984; Sage *et al.*, 1984) are not as detailed as that in tilapia but do suggest a similar pattern in that many of the haplochromines have shared polymorphic loci, even in morphologically very distinct groups. The overall level of genetic distance is very low (0.006) at the allozyme level (Sage *et al.*, 1984). Recent studies on mtDNA (Meyer *et al.*, 1990) and sequenced proteins such as MHC (major histocompatibility complex, Klein *et al.*, 1993) show a high level of genetic variation which appears to be ancestral as it is spread over many different species. However, whether the evolution of the chambo have any resemblance to the explosive speciation that has characterized the haplochromines in these lakes needs a thorough consideration. The fact is that there are hundreds of different haplochromines in many different genera in Lake Malawi whereas at most seven species are of the tilapiine lineage (Ribbink, 1984) with only three (Turner & Robinson, 1991; Turner *et al.*, 1991) to five species (Trewavas, 1983) in the chambo group. So obviously these tilapias have not undergone such speciation. This may be because they have not had the same actual amount of time to evolve, although there is no evidence that they occurred in the lake any later than the haplochromines. The chambo may not have been preadapted to

such rapid speciation because they are not only pelagic fish (Lowe, 1952) but their sizes also relatively big; they could disperse over the whole lake both as adults and young. With this ability to disperse, the chambo should not have been affected by ecological barriers such as the trophic specialization and habitat restriction which theoretically are the main causes of the rapid speciation in haplochromines (Greenwood, 1981; Witte, 1984). The allozyme data suggest that the chambo speciation event(s), when it occurred, was not accompanied by a dramatic reduction in the size of the population causing a bottleneck and an overall reduction in the level of heterozygosity in the different species.

One suggestion put forward has been the fluctuations in water depth and its effect on spawning behaviour which may be major factors in the speciation of Lake Malawi species (Lowe, 1953; Trewavas, 1983). A polymorphism for spawning depth or a strong preference for spawning site in times of fluctuating water levels may have been enough to establish some form of assortative mating (for depth or colour pattern) within the ancestral population which has resulted in the species we see today.

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CHAPTER 3

PHYLOGENIES & EVOLUTIONARY RELATIONSHIPS FROM ALLOZYMES

I. INTRODUCTION

The increasing importance of tilapia in aquaculture worldwide and therefore the greater ease by which they could be studied has led some biologists to realise the real biological differences in behaviour and feeding. This encouraged Trewavas (1973; 1981; 1982a,b; 1983) to rethink the classification of this cichlid tribe and reclassify them into separate generic taxa based on differences in their breeding and dietary features. In her classification she subdivided the broad genus *Tilapia* into four genera by retaining the substrate spawners as the genus *Tilapia* and raising the other three *Tilapia* mouthbrooding subgenera, *Sarotherodon*, *Oreochromis* and *Danakilia* (Thys van den Audenaerde, 1968b, 1971; Chapter 1), to the generic levels. In addition she (Trewavas, 1983) reclassified all species with tasselled male genital papillae as belonging to the subgenus *O. (Nyasalapia)*. Originally Thys van den Audenaerde (1968b) restricted the *Nyasalapia* to a *Tilapia* subgenus confined to the closely related endemic species in Lake Malawi (formerly Lake Nyasa), but he proposed a separate subgenus *Loruwiala* for the other tasselled species.

The present reclassification (Trewavas, 1983) of the three major tilapia genera, *Tilapia*, *Sarotherodon* and *Oreochromis*, and the two *Oreochromis*

subgenera, *O. (Oreochromis)* and *O. (Nyasalapia)*, has not been accepted by all taxonomists and other workers. A number of hypotheses on how the species should be ranked (Thys van den Audenaerde, 1978, 1980) and on the evolution of the group (Peters & Berns, 1978, 1982; Trewavas, 1980, 1983) have been proposed (as reviewed in Chapter 1). However the generic and subgeneric characteristics Trewavas (1983) defined in her reclassification are mainly based on behavioural and morphological differences. None of the workers who have proposed the various hypotheses on the evolution of these group have drawn cladograms based on the various meristic, morphological and behavioural traits they believed to be of evolutionary significance.

As reviewed by Moritz & Hillis (1990), the most agreeable hierarchical system of taxonomy should be based on evolutionary theory and phylogenetic relationships, particularly if it combines the mutual skills of systematists estimating phylogeny and population geneticists looking for microevolutionary change. It has also been generally agreed that systematics based largely on analysis of morphological and behavioural variation will still be the main method used, albeit continuing with increasing sophistication. With the elucidation of the molecular basis of inheritance, biological macromolecules have assumed an increasingly important role in evolutionary studies. Molecular data such as that obtained from studies on nucleic acids (DNA and RNA), proteins, and chromosomes can provide a broadly applicable set of heritable markers to examine the genetic structure of populations or to estimate relationships among taxa (Moritz & Hillis, 1990). The molecular studies have also provided important insights into the evolution of the molecules themselves (reviewed by MacIntyre, 1985; Nei, 1987; Ward *et al.*, 1992). Practically enzymatic protein electrophoresis is among the most cost-efficient methods of investigating genetic

phenomena at the molecular level (Murphy *et al.*, 1990). This biochemical technique has generated a massive comparative data base which has proved very useful in tackling systematic problems in many groups of organisms (Avise, 1974; Buth, 1984; Moritz & Hillis, 1990).

Molecular techniques such as cytogenetics, protein electrophoresis and mtDNA analysis, have been applied to the tilapias principally in order to solve the problems of species-stock identification (see review in Chapter 1 for cytogenetics and protein electrophoresis; and Seyoum & Kornfield, 1992, for mtDNA analysis). Among the molecular data obtained from these techniques, allozyme data have been most commonly used for further evolutionary study in tilapias (e.g. Kornfield *et al.*, 1979; McAndrew & Majumdar, 1984; Sodsuk & McAndrew, 1991). However due either to the inadequate number of enzyme loci investigated or the difficulties in obtaining some species in all generic and subgeneric levels, no study could have hoped to give a definitive phylogeny particularly at the subgeneric level. Nevertheless it is clear that the investigation of a larger number of loci and species could give a general idea of the evolution of the group suggesting monophyletic rather than polyphyletic origin (Sodsuk & McAndrew, 1991). In the study of Sodsuk & McAndrew (1991), the substrate spawning *Tilapia* were consistently separated from the two mouthbrooding genera, *Sarotherodon* and *Oreochromis*, although the single *Sarotherodon* species used (*S. galilaeus*) could not be clearly separated from the *Oreochromis* species.

The study in this chapter is a molecular systematic study using allozyme data generated from electrophoretic analyses on the large numbers of different tilapiine species (22 species including a presumed outgroup species) and enzyme loci (43 loci) investigated in the last chapter. Because of the present controversy

on the correct methodology for the analysis of allozyme data in systematic studies and the need to compare the results of this study with earlier work on these species, a wide range of different techniques has been used. The various arguments for the appropriateness of the various ways of handling data and the correct analysis for each given data set are presented in Chapter 1.

II. MATERIALS & METHODS

2.1 Forms of Data Used in the Study

The allozyme data obtained from electrophoretic analyses of 43 enzyme loci examined in 22 different tilapiine species [excluding *O. (Ne.) tanganicae*, as only 16 loci resolved from the limited material available for this species, compared to the 36 and 43 loci resolved in the other species] in the last chapter were used to calculate various ***genetic distances*** and a number of ***character*** sets (binary codes and allele frequencies), which could be used in various computer programs available for specifying relationships and constructiing dendrograms (see 'SOFTWARE PACKAGES USED' below).

GENETIC DISTANCES

Genetic distances were calculated from the allele frequencies in Table 4. The two distance measures used in this study are the unbiased distance of Nei (1978) which is the most frequently used distance in fish studies and therefore

interesting for comparisons, and the arc distance of Cavalli-Sforza & Edwards (1967) which incorporates some realistic assumptions about the nature of evolutionary change in gene frequencies without the undesirable properties of the Nei (1972, 1978) and Rogers (1972) measures (as reviewed in Chapter 1 and by Swofford & Olsen, 1990).

CHARACTERS

The allozyme data, as characters, were used either qualitatively in which two possible discrete values were coded in a binary system as the presence (1) or absence (0) of a given allele at an isozyme locus, and quantitatively in which the characters varied continuously and were measured on an interval scale such as allele frequencies.

2.2 Data Analytical Approaches for Inferring Phylogenies

The analytical approaches used for inferring phylogenies in this study are the methods based on pairwise distances, maximum parsimony, and maximum likelihood.

2.2.1 Methods based on pairwise distances

Two algorithmic methods based on pairwise distances, the cluster analysis (Sneath & Sokal, 1973) and distance Wagner procedure (Farris, 1972), were implemented for dendrogram reconstruction using pairwise distance data.

CLUSTER ANALYSIS

The most widely used clustering method is the UPGMA (Unweighted Pair Group Method using Arithmetic average). This was used on the unbiased distances of Nei (1978) which were treated as being ultrametric data, an underlying assumption of the method (see Swofford & Olsen, 1990, and 'ULTRAMETRIC DISTANCE' in Chapter 1 for details). The tree was finished as a rooted tree in which all of the taxa were equidistant from the root.

DISTANCE WAGNER PROCEDURE

The distance Wagner algorithm of Farris (1972), is effectively a heuristic method of building a tree by the sequential addition of taxa (Swofford & Olsen, 1990). This analysis was used on the arc distances of Cavalli-Sforza & Edwards (1967) as the data input. The tree was rooted at the point where the presumed outgroup taxa (*P. pulcher*) joined, as it was assumed to lie cladistically outside the presumed monophyletic ingroup (Swofford & Olsen, 1990). Nei's standard distances, both biased (Nei, 1972) and unbiased (Nei, 1978), are not appropriate data sets for the distance Wagner procedure because they are nonmetric in that they frequently violate the triangle inequality (Swofford & Selander, 1989; Swofford & Olsen, 1990; and see Chapter 1 for 'triangle inequality').

2.2.2 Parsimony method

Wagner parsimony (Eck & Dayhoff, 1966; Kluge & Farris, 1969; Farris, 1970) is one of the simplest parsimony methods based on the principle of **maximum parsimony** (see Chapter 1 and Swofford & Olsen, 1990) and is

widely used on binary coded data (1 or 0 at presence or absence of alleles at a locus). The tree was rooted with the presumed outgroup *P. pulcher* as in the distance Wagner method mentioned above.

One of **the resampling methods** known as **the bootstrap method** (Felsenstein, 1985, 1990) was implemented in the Wagner parsimony algorithm to avoid random errors; sampling the data input, drawing characters with replacement, and creating a new data table.

2.2.3 Maximum likelihood method

The Felsenstein's (1981) maximum likelihood method for continuous characters (gene frequencies) evolving under the primary assumption of a Brownian motion process (see Chapter 1) was performed using the allele frequencies in Table 4 as the data input directly. The tree was also rooted with the presumed outgroup *P. pulcher*.

2.3 Software Packages Used

Two software packages, the BIOSYS-1 release 1.7 (Swofford & Selander, 1989) and the PHYLIP version 3.3 (Felsenstein, 1990), were used in this study. The BIOSYS-1 was used for computing all genetic distances and the two algorithmic methods, the UPGMA and the distance Wagner, based on pairwise distances. The PHYLIP package was used for computing all procedures in the parsimony, bootstrap, and maximum likelihood methods.

III. RESULTS & DISCUSSION

3.1 Genetic Distances & Distribution of Loci in Various Degrees of Distances

The pairwise comparisons of the unbiased genetic distances of Nei (1978) and the arc distances of Cavalli-Sforsa & Edwards (1967) calculated between all species studied are shown in Table 9. Tables 10 and 11 show the intra- and inter-subgeneric (including the chambo group) and generic averages of distance respectively. The two distances show relationships between the tilapiines from the most similar to the least similar at the same pairs of taxa in all three taxonomic levels (Tables 9, 10 and 11), although both values in each pair are a little bit different. Excluding *P. pulcher*, the most similar species are *T. (C) tholloni* and *T. (C.) zillii* and the least similar are *T. (C.) rendalli* and *S. melanotheron* (aquarium stocks) (Table 9). However, the closest intra-subgeneric distance within a subgenus or group is the distance within the chambo, and the most divergent intersubgeneric distance is the distance between the subgenera *T. (Coptodon)* and *O. (Oreochromis)* (Table 10). Overall, the intergeneric distances between the genera *Tilapia* and the two mouthbrooding genera are more divergent than those between the *Sarotherodon* and *Oreochromis* (Table 11). This shows that the *Tilapia* are more distantly related to the *Sarotherodon* and *Oreochromis*, which are more closely related to each other. However, the intrageneric distances within the *Sarotherodon* and the *Oreochromis* are less divergent than the intergeneric distance between them (Table 11). This suggests that the congeneric species within the *Sarotherodon* and the *Oreochromis* are more closely related to the species within their own genus than they are to the species in the other genus. The subgeneric distances within and between the *Tilapia* subgenera,

Table 9. Pairwise comparisons of genetic distances between all tilapiine species studied. The figures above the diagonal are the Cavalli-Sforza & Edwards (1967) arc distances, and below the diagonal are the Nei (1978) unbiased distances.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*P. pulcher*	-	0.755	0.726	0.726	0.745	0.745	0.781	0.802	0.746	0.792	0.782	0.765	0.794	0.794	0.782	0.745	0.739	0.807	0.789	0.735	0.768	0.767	0.762
2	*T. (H.) buttikoferi*	0.860	-	0.425	0.667	0.587	0.584	0.706	0.673	0.592	0.667	0.625	0.616	0.571	0.741	0.699	0.662	0.576	0.648	0.680	0.658	0.628	0.619	0.619
3	*T. (P.) mariae*	0.744	0.203	-	0.648	0.590	0.586	0.715	0.694	0.615	0.696	0.647	0.651	0.637	0.730	0.733	0.704	0.622	0.696	0.700	0.697	0.675	0.667	0.670
4	*T. (C.) rendalli*	0.746	0.579	0.541	-	0.450	0.446	0.735	0.695	0.649	0.702	0.700	0.671	0.709	0.691	0.715	0.691	0.648	0.729	0.723	0.684	0.666	0.667	0.668
5	*T. (C.) tholloni*	0.793	0.414	0.423	0.222	-	0.054	0.716	0.663	0.596	0.681	0.676	0.626	0.685	0.708	0.694	0.659	0.609	0.718	0.705	0.627	0.629	0.630	0.629
6	*T. (C.) zillii*	0.790	0.406	0.411	0.212	0.002	-	0.716	0.664	0.598	0.679	0.674	0.623	0.681	0.699	0.690	0.651	0.606	0.716	0.703	0.627	0.626	0.628	0.626
7	*S. melanotheron (AQ)*	0.961	0.711	0.719	0.786	0.713	0.716	-	0.216	0.498	0.566	0.564	0.528	0.520	0.655	0.554	0.577	0.548	0.565	0.632	0.493	0.552	0.545	0.541
8	*S. melanotheron (IC)*	1.035	0.633	0.654	0.660	0.582	0.584	0.043	-	0.446	0.528	0.518	0.481	0.470	0.616	0.508	0.533	0.501	0.520	0.598	0.440	0.508	0.500	0.496
9	*S. galilaeus*	0.807	0.433	0.474	0.541	0.441	0.446	0.299	0.238	-	0.408	0.364	0.352	0.392	0.551	0.513	0.467	0.376	0.490	0.458	0.410	0.448	0.443	0.445
10	*O. (O.) niloticus*	0.989	0.586	0.645	0.677	0.606	0.601	0.358	0.311	0.169	-	0.387	0.374	0.423	0.541	0.548	0.508	0.449	0.542	0.418	0.417	0.459	0.451	0.453
11	*O. (O.) aureus*	0.936	0.484	0.529	0.675	0.600	0.592	0.378	0.312	0.139	0.154	-	0.347	0.402	0.494	0.520	0.493	0.409	0.506	0.446	0.349	0.446	0.438	0.439
12	*O. (O.) spilurus*	0.872	0.483	0.547	0.594	0.502	0.493	0.338	0.275	0.125	0.137	0.119	-	0.350	0.522	0.499	0.415	0.401	0.492	0.461	0.352	0.363	0.381	0.380
13	*O. (O.) u. hornorum*	1.022	0.400	0.523	0.682	0.636	0.626	0.312	0.250	0.173	0.183	0.174	0.137	-	0.500	0.442	0.415	0.374	0.385	0.499	0.416	0.386	0.350	0.366
14	*O. (O.) andersonii*	1.000	0.828	0.776	0.666	0.715	0.702	0.556	0.477	0.356	0.349	0.267	0.313	0.309	-	0.483	0.475	0.517	0.567	0.607	0.472	0.550	0.536	0.546
15	*O. (O.) mortimeri*	0.931	0.684	0.768	0.748	0.675	0.671	0.380	0.313	0.319	0.365	0.316	0.300	0.230	0.282	-	0.284	0.461	0.459	0.609	0.425	0.465	0.440	0.446
16	*O. (O.) mossambicus*	0.797	0.588	0.690	0.668	0.588	0.572	0.407	0.339	0.242	0.296	0.273	0.206	0.203	0.279	0.088	-	0.409	0.463	0.564	0.377	0.376	0.359	0.360
17	*O. (O.) shiranus*	0.788	0.395	0.485	0.552	0.475	0.466	0.368	0.301	0.142	0.200	0.162	0.161	0.148	0.310	0.247	0.182	-	0.325	0.499	0.380	0.416	0.415	0.409
18	*O. (O.) placidus*	1.072	0.540	0.668	0.760	0.731	0.720	0.388	0.321	0.286	0.329	0.289	0.278	0.150	0.380	0.235	0.236	0.111	-	0.585	0.482	0.495	0.499	0.496
19	*O. (O.) jipe*	0.995	0.609	0.663	0.736	0.669	0.663	0.507	0.456	0.229	0.179	0.223	0.235	0.282	0.462	0.489	0.399	0.270	0.424	-	0.482	0.524	0.517	0.517
20	*O. (Ny.) macrochir*	0.781	0.610	0.686	0.645	0.490	0.491	0.272	0.212	0.184	0.185	0.133	0.134	0.203	0.243	0.192	0.157	0.149	0.265	0.274	-	0.372	0.364	0.355
21	*O. (Ny.) karongae*	0.884	0.481	0.608	0.598	0.499	0.491	0.372	0.305	0.211	0.205	0.203	0.139	0.144	0.358	0.227	0.129	0.173	0.265	0.300	0.149	-	0.155	0.120
22	*O. (Ny.) lidole*	0.890	0.481	0.590	0.594	0.500	0.494	0.349	0.283	0.202	0.194	0.190	0.149	0.127	0.344	0.208	0.126	0.181	0.276	0.288	0.141	0.014	-	0.110
23	*O. (Ny.) squamipinnis*	0.866	0.480	0.609	0.603	0.507	0.501	0.348	0.283	0.202	0.194	0.189	0.148	0.135	0.357	0.213	0.125	0.172	0.270	0.285	0.134	0.008	0.008	-

Table 10. Mean intra- and intersubgeneric distances (averaged by SUBGENUS or GROUP) of tilapiines. The Cavalli-Sforza & Edwards (1967) arc distances are above the diagonal, and the Nei (1978) unbiased distances below the diagonal.

Subgenus (or group)	No. of pops.	1	2	3	4	5	6	7	No. of pops.	Subgenus (or group)
		***	0.425 (0.425 - 0.425)	0.613 (0.584 - 0.667)	0.657 (0.592 - 0.706)	0.648 (0.571 - 0.741)	0.631 (0.619 - 0.658)	0.622 (0.619 - 0.628)	1	1 *T. (Heterotilapia)*
1 *T. (Heterotilapia)*	1	***	***	0.608 (0.586 - 0.648)	0.675 (0.615 - 0.715)	0.682 (0.622 - 0.733)	0.677 (0.677 - 0.697)	0.671 (0.667 - 0.675	1	2 *T. (Pelmatolapia)*
2 *T. (Pelmatolapia)*	1	0.203 (0.203 - 0.203)	***	0.317 (0.054 - 0.450)	0.670 (0.596 - 0.735)	0.682 (0.606 - 0.729)	0.642 (0.626 - 0.684)	0.641 (0.626 - 0.668)	3	3 *T. (Coptodon)*
3 *T. (Coptodon)*	3	0.466 (0.406 - 0.579)	0.458 (0.411 - 0.541)	0.146 (0.002 - 0.222)	0.387 (0.126 - 0.498)	0.512 (0.352 - 0.655)	0.485 (0.410 - 0.552)	0.498 (0.443 - 0.552)	3	4 *Sarotherodon*
4 *Sarotherodon*	3	0.592 (0.433 - 0.711)	0.616 (0.474 - 0.719)	0.608 (0.441 - 0.786)	0.193 (0.043 - 0.299)	0.464 (0.284 - 0.609)	0.436 (0.349 - 0.550)	0.443 (0.350 - 0.550)	10	5 *O. (Oreochromis)*
5 *O. (Oreochromis)*	10	0.560 (0.395 - 0.828)	0.629 (0.485 - 0.776)	0.635 (0.466 - 0.760)	0.318 (0.125 - 0.556)	0.252 (0.088 - 0.489)	0.246 (0.110 - 0.372)	0.364 (0.355 - 0.372)	4	6 *O. (Nyasalapia)*
6 *O. (Nyasalapia)*	4	0.513 (0.480 - 0.610)	0.623 (0.590 - 0.686)	0.534 (0.490 - 0.645)	0.269 (0.184 - 0.372)	0.206 (0.125 - 0.358)	0.076 (0.008 - 0.149)	0.128 (0.110 - 0.155)	3	7 *The chambo*
7 *The chambo*	3	0.480 (0.480 - 0.481)	0.602 (0.590 - 0.609)	0.532 (0.491 - 0.603)	0.284 (0.202 - 0.372)	0.204 (0.125 - 0.358)	0.141 (0.134 - 0.149)	0.010 (0.008 - 0.014)		

'***' No comparisons

Table 11. Within and between generic averages of genetic distance of tilapiines. The arc distances of Cavalli-Sforza & Edwards (1967) are above the diagonal, and the unbiased distances of Nei (1978) below the diagonal.

Genus	No. of pops.	*Tilapia*	*Sarotherodon*	*Oreochromis*	No. of pops.	Genus
		0.504 (0.054 - 0.667)	0.668 (0.592 - 0.735)	0.667 (0.571 - 0.741)	5	*Tilapia*
Tilapia	5	0.341 (0.002 - 0.579)				
			0.387 (0.216 - 0.498)	0.504 (0.352 - 0.655)	3	*Sarotherodon*
Sarotherodon	3	0.606 (0.433 - 0.786)	0.193 (0.043 - 0.299)			
				0.437 (0.110 - 0.609)	14	*Oreochromis*
Oreochromis	14	0.599 (0.395 - 0.828)	0.304 (0.125 - 0.556)	0.220 (0.008 - 0.489)		

between *Tilapia* subgenera and the mouthbrooding subgenera (Table 10) and the distances between the *Tilapia* species (Table 9) show that the *Tilapia* species are not only much diverged from the species in the other two genera, but are also quite different from each other.

The genetic distances between each chambo species and the other *Nyasalapia*, *O. (Ny.) macrochir* (Table 9), and within the chambo (Table 10) show that the chambo species are closer to each other than they are to *O. (Ny.) macrochir*. Morphologically, *O. (Ny.) macrochir* is more similar to some species in the *O. (Oreochromis)* subgenus. Trewavas (1983) reclassified this species into the subgenus *O. (Nyasalapia)* together with the chambo species because of the tasselled genital papillae in the male fish, the feature she defined as the distinctive subgeneric character for the *O. (Nyasalapia)*. Electrophoretically, the allozyme results in the last chapter (Tables 4 and 5) show that *O. (Ny.) macrochir* has affinities to some *O. (Oreochromis)* species with a number of shared alleles. However, the subgeneric distances within the *O. (Nyasalapia)* and between the *O. (Nyasalapia)* and *O. (Oreochromis)* (Table 10) show that the *O. (Nyasalapia)* consubgeneric species are related to each other more closely than they are to the species in the *O. (Oreochromis)* subgenus. Among all species studied the smallest genetic distances shown in the chambo (Table 10) also suggest the recent times of separation in these species.

Three distributions of single-locus distance coefficients [the Cavalli-Sforza & Edwards (1967) arc distances] at three different taxonomic levels, species, subgenus and genus, are shown by Figs. 7 a, b and c respectively. The unbiased distances of Nei (1978) were not used because Nei distances (Nei, 1972, 1978) do not have finite possible ranges and therefore are not suitable for such

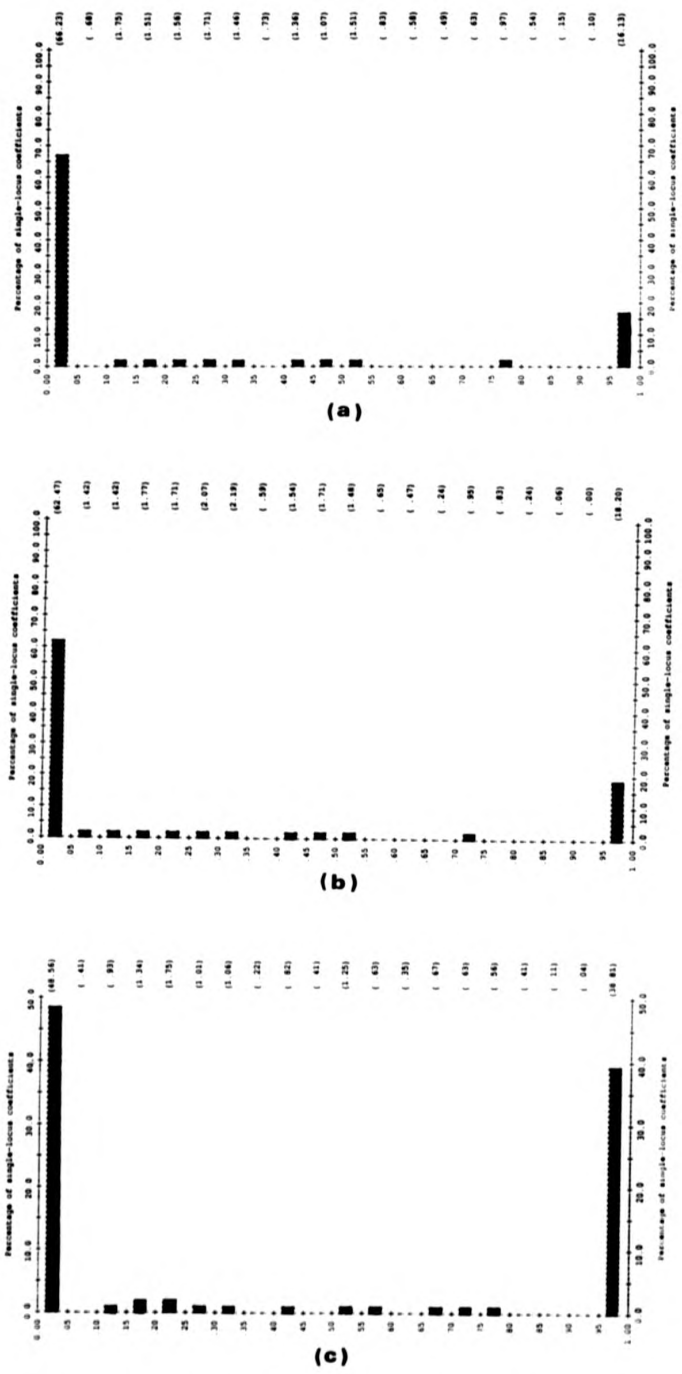


Fig 7. Distributions of single-locus distance coefficients [the Cavalli-Sforza & Edwards (1967) arc distances] shown at three levels of divergences, (a) species, (b) subgenus, and (c) genus. At each level of divergence the possible range of values of the distance measure (0 to 1) was divided into 20 equal intervals. Then the percentages of loci falling in the various intervals were plotted as a histogram.

comparisons (Swofford & Selander, 1989) although they had been commonly used in previous studies. Despite using different distance estimates, the U-shaped patterns typical for studies at the sibling species, species and genera level in most outcrossing sexual organisms are seen (Avise & Smith, 1977; Ferguson, 1980; McAndrew & Majumdar, 1984). The bimodal distribution of genetic distances or similarities does make it important that as many loci as possible are sampled as the systematic information lies in the number of loci studied (Ferguson, 1980). Therefore more information will be gained from increasing the number of loci rather than the number of individuals within a species. With use of the relatively large number (43) of enzyme loci, this study produced the typical U-shaped distribution of loci, clearly showing that in any level of comparison (species, subgenera, or genera) the majority of loci are identical in allelic composition or completely distinct with unique alleles at the majority of loci studied.

3.2 Comparison of Dendrograms Constructed Using Different Methods and Forms of Data Sets

The four dendrograms constructed by the different methods outlined [the UPGMA using Nei (1978) unbiased distances, the maximum likelihood using allele frequencies, the distance Wagner using Cavalli-Sforza & Edwards (1967) arc distances, and the Wagner parsimony with the bootstrap resampling method using binary (1 or 0) coded data] are presented by Figs. 8, 9, 10, and 11 respectively. Differences in branching pattern and species grouping between the four dendrograms are shown. Overall, it can be seen that the *Tilapia* species are always separated from the *Sarotherodon* and *Oreochromis* in all dendrograms.

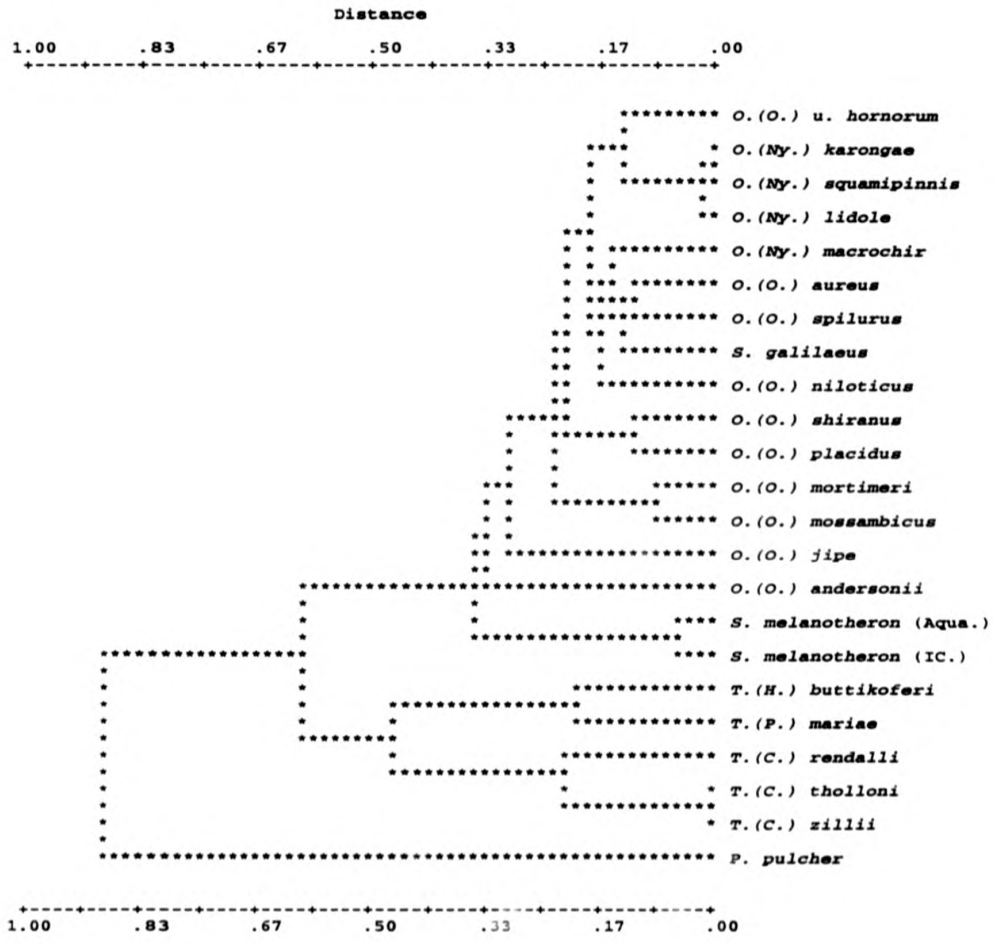
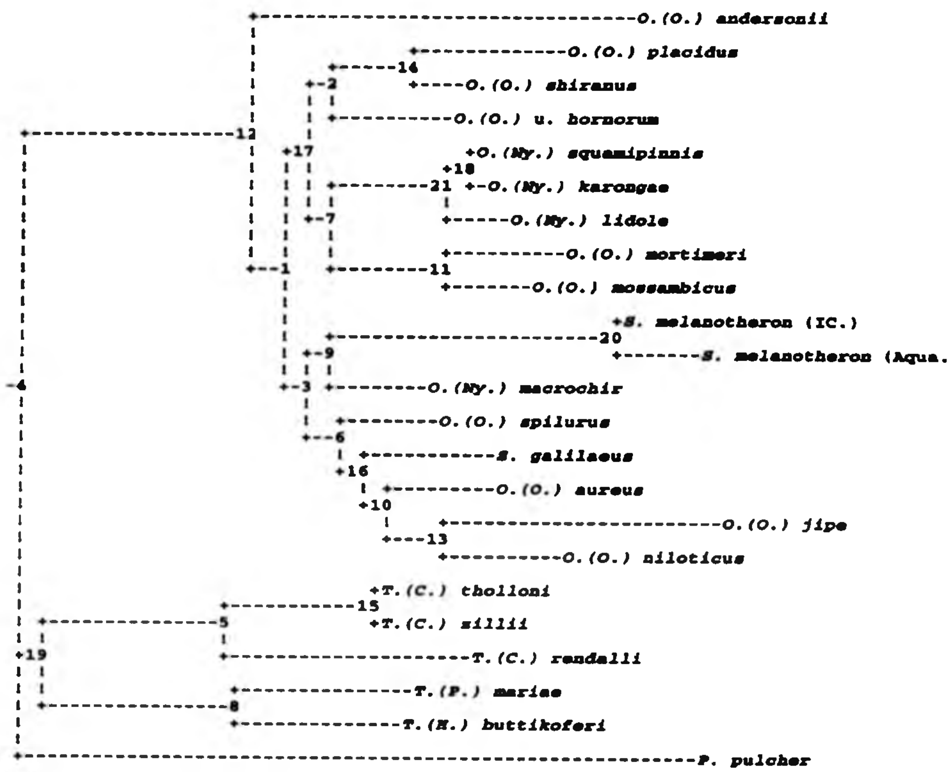


Fig. 8 Dendrogram constructed using the UPGMA clustering method and unbiased distance data (Nei, 1978). The software package BIOSYS-1 (Swofford & Selander, 1989) was used to produce the dendrogram.



Between	And	Length	Approx. Confidence Limits	
4	12	0.08148	(0.04600,	0.12477)
12	OOAN	0.13023	(0.09797,	0.16436)
12	1	0.01284	(-0.00996,	0.03896)
1	17	0.00373	(-0.00942,	0.01621)
17	2	0.00633	(-0.00409,	0.01860)
2	14	0.02950	(0.01440,	0.04608)
14	OOPL	0.05703	(0.03939,	0.07903)
14	OOSH	0.02237	(0.01036,	0.03613)
2	OOUH	0.04238	(0.02001,	0.06000)
17	7	0.00091	(-0.00300,	0.02360)
7	21	0.04117	(0.02861,	0.05928)
21	18	0.00340	(-0.00362,	0.01069)
18	ONYS	0.00434	(0.00169,	0.00718)
18	ONYK	0.00798	(0.00503,	0.01113)
21	ONYL	0.02467	(0.01691,	0.03387)
7	11	0.04100	(0.03367,	0.06300)
11	OOMR	0.04491	(0.02972,	0.06660)
11	OOMS	0.03200	(0.01738,	0.04604)
1	3	0.00818	(-0.00300,	0.01673)
3	9	0.00796	(-0.00398,	0.02159)
9	20	0.10100	(0.07236,	0.13871)
20	SMIC	0.00104	(-0.00898,	0.00900)
20	SMEL	0.03438	(0.02333,	0.04474)
9	ONYM	0.03894	(0.02333,	0.05283)
3	6	0.01077	(0.00110,	0.02176)
6	OOSP	0.03870	(0.02369,	0.04946)
6	16	0.00383	(-0.00500,	0.01307)
16	SGAL	0.04003	(0.03364,	0.06643)
16	10	0.00433	(-0.00611,	0.01030)
10	OOAR	0.04270	(0.03851,	0.05013)
10	13	0.03063	(0.00436,	0.03030)
13	OOJP	0.10331	(0.07107,	0.13900)
13	OONL	0.06460	(0.03673,	0.06401)
4	19	0.00653	(-0.02631,	0.06613)
19	5	0.06397	(0.03933,	0.10796)
5	15	0.03313	(0.01699,	0.00500)
15	TCTH	0.00340	(0.00108,	0.00332)
15	TCZL	0.00000	(-0.00063,	0.00071)
5	TCRN	0.00077	(0.03493,	0.12583)
19	8	0.06934	(0.03220,	0.11107)
8	TPMR	0.06673	(0.03940,	0.09901)
8	THBT	0.06366	(0.03876,	0.09390)
4	PPUL	0.34700	(0.17464,	0.38998)

Fig. 9 Dendrogram constructed using the maximum likelihood method (Felsenstein, 1981) and allele frequency data. The branch lengths, shown (in the table) by the numbers between two nodes or a node and a species, are amounts of expected accumulated variance. The software package PHYLIP (Felsenstein, 1990) was used to produce the dendrogram.

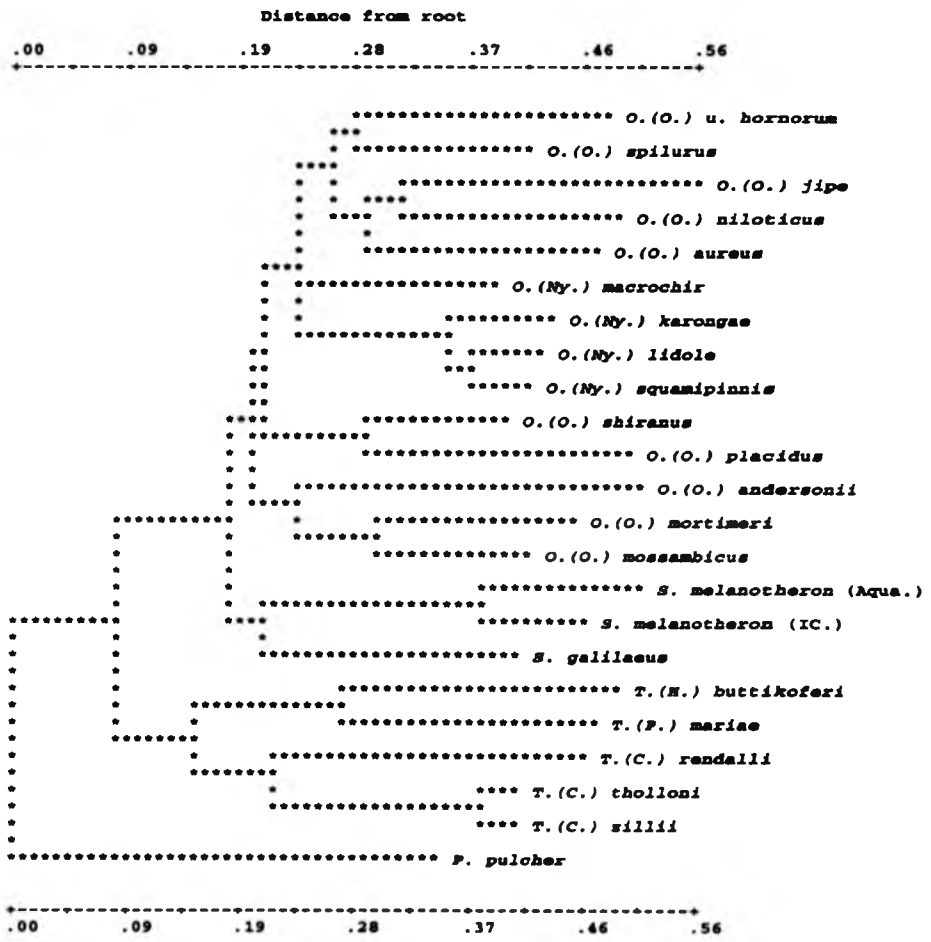


Fig. 10 Dendrogram constructed using the distance Wagner procedure of Farris (1972) and the arc distance data of Cavalli-Sforza & Edwards (1967). The software package BIOSYS-1 (Swofford & Selander, 1989) was used to produce the dendrogram.

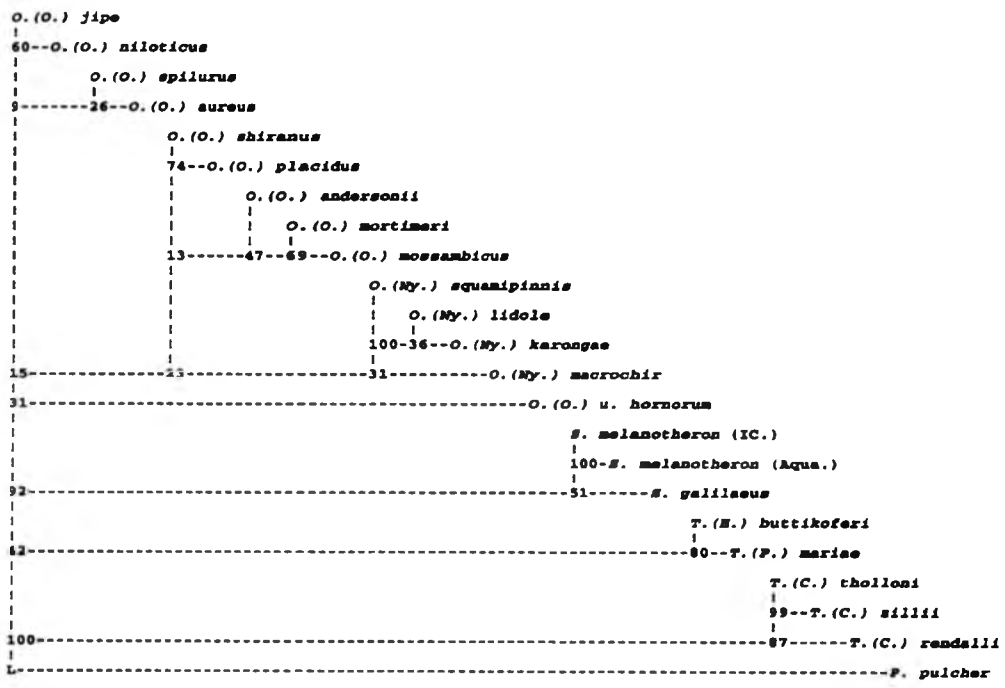


Fig. 11 Dendrogram constructed using the Wagner parsimony method (Eck & Dayhoff, 1966; Kluge & Farris, 1969; Farris, 1970) and binary coded data as '1' or '0' (presence or absence of alleles at a locus), and carrying the bootstrap resampling data method (Felsenstein, 1985, 1990) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the species above and to the right of that fork occurred among the 100 bootstrap replicates. No branch lengths available. The software package PHYLIP (Felsenstein, 1990) was used to produce the dendrogram.

In Figs. 8 and 9, the dendrograms not only show *O. (Ny.) macrochir* separated from the other consubgeneric species, the chambo, but also show the *Sarotherodon* species in the *Oreochromis* clade. Generally, dendrograms constructed by clustering methods such as the UPGMA (Fig. 8) are likely to give erroneous results (Felsenstein, 1988; Swofford & Olsen, 1990). Basically, the ultrametric distances which are required in clustering methods (see Chapter 1) are the most constrained and extremely unlikely to be obtained in allozyme data sets (as emphasized by Swofford & Olsen, 1990). Accordingly the species will be grouped incorrectly as the distance data cannot be exactly fitted into an ultrametric tree. Felsenstein (1988) has pointed out that the UPGMA method is an application of a certain algorithm which will work perfectly only if the data are generated by a clocklike evolution. The evidence is that molecular distances obtained from allozyme data are not purely clocklike in nature (Farris, 1981; Thorpe, 1982; Felsenstein, 1990).

The dendrogram produced by the maximum likelihood method using allele frequency data (Fig. 9) gave a number of unexpected associations, particularly the placings of *S. melanotheron* (Ivory Coast and aquarium stocks), *S. galilaeus* and *O. (Ny.) macrochir*. Although Felsenstein (1988) regards the maximum likelihood method as the most appropriate method for generating statistical estimates in systematics, its use with allozyme data in the form of allele frequencies has been severely criticised. The use of allele frequency data in systematic studies has been said by many workers to be inappropriate both because of the level of accuracy by which they can be calculated and their lability in natural populations. These workers (e.g. Mickevich & Johnson, 1976; Crother, 1990) believe that allele frequencies are of unreliable systematic information because they are shown to fluctuate over relatively short periods in

natural populations, and therefore do not yield true synapomorphic information which is the most important in phylogenetic analysis (see 'ALLELE FREQUENCIES' in Chapter 1 for details). Swofford & Olsen (1990) agree that they are unreliable but suggest that the frequency of a given allele is at least a form of weighting for its presence or absence. Ferguson (1980) pointed out that it is the number of loci per individual and not the number of individuals which provides the most informative systematic data therefore the number sampled are likely to be small and any frequency very unreliable. Despite the widespread use of this method and data form, it is theoretically flawed and results in inaccuracies and erroneous assortments.

The dendrograms in Figs. 10 and 11 present the *Sarotherodon* species grouped together on the same branch separated from the *Oreochromis*, and placed *O. (Ny.) macrochir* on the branch close to the other *Nyasalapia* (the chambo). These two dendrograms (Figs. 4 and 5) give phylogenetic results which reflect the tilapiine evolutionary relationships in line with the ideas of Trewavas's (1982a, 1983) reclassification. The distance Wagner procedure (Farris, 1972) used with the Cavalli-Sforza & Edwards (1967) arc distances generated the dendrogram in Fig. 10, and the Wagner parsimony method (Eck & Dayhoff, 1966; Kluge & Farris, 1969; Farris, 1970) with the binary coded data generated the dendrogram in Fig. 11. From a variety of dendrogram construction techniques, as reviewed in Chapter 1, these two techniques together with the use of outgroup rooting seem to be the most effective and least controversial (Buth, 1984; Hillis, 1987; Swofford & Olsen, 1990). Certainly the dendrograms generated by these two techniques are consistent with each other and most ideas about the evolution of this group based on more classical approaches.

The results shown by the two dendrograms (Figs. 10 and 11) are similar to each other if *O. (O.) u. hornorum* was excluded. The bootstrap resampling method which was also implemented to avoid random errors by resampling data in the production of the dendrogram (Fig. 11) might have caused the position of *O. (O.) u. hornorum* to be in the different place from the dendrogram in Fig. 10 of which the production was not involved with the bootstrap. Generally, any parsimony methods must operate under the conceptual framework of two different criteria, the optimality criterion (minimal tree length under a specified set of restrictions on permissable character-state changes) and the actual algorithm used to search for optimal trees. Additionally, the Wagner parsimony permits free reversibility, in which character-state changes in either direction are assumed to be equally probable and the character-states may transform from one state to another and back again (Swofford & Olsen, 1990). The free reversibility assumption probably makes this method potentially effective and appropriate for the binary characters. Also, this may be another effect causing differences in branching patterns between the two dendrograms, since Fig. 11 is based on character-states whereas the other (Fig. 10) on distance data.

With thorough consideration of all these various advantages and disadvantages of the methods and data forms used for dendrogram construction and the results from this study, the dendrograms in Figs. 10 and 11 seem the most acceptable. Not only do they give similar evolutionary patterns (species groupings) but these patterns are also strikingly consistent with the evolutionary idea of the tilapiine reclassification put forward by Trewavas (1973, 1982a, 1983).

3.3 Generic Groupings & Evolutionary Relationships between the Three Tilapiine Genera

Three main groupings *Tilapia, Sarotherodon* and *Oreochromis*, have been shown to exist in the allozyme based data set (Figs. 10 and 11), although there are slight rearrangements at the bases of the two dendrograms. In Fig. 10 the three genera split off from each other by two ancient dichotomies, consisting of three monophyletic groups. Lying outside the whole group, the presumed outgroup species (*P. pulcher*) gave rise to the first dichotomy which was the split into the *Tilapia* and the *Sarotherodon-Oreochromis*. The latter (*Sarotherodon-Oreochromis*) became the second dichotomy splitting into two, giving rise to the *Sarotherodon* and *Oreochromis* lineages. In Fig. 11 the split between the *Tilapia* and the *Sarotherodon-Oreochromis* was slightly different to Fig. 10; the *Tilapia* species consists of two separate groups rather than a single branch. With branch lengths (interpreted as amounts of evolutionary change) available in Fig. 10, the *Sarotherodon* appear to be more closely related to the *Oreochromis* rather than to the *Tilapia*. Previous studies could not separate *S. galilaeus* from the *Oreochromis* as it was the only species of the genus to be studied (McAndrew & Majumdar, 1984; Sodsuk & McAndrew, 1991). This study shows that it has much closer affinities with other *Sarotherodon* species and they can be separated from the *Oreochromis*.

The genera *Tilapia, Sarotherodon,* and *Oreochromis* were reclassified basically on their breeding habits by Trewavas (1973, 1980, 1982a, 1983), consisting of substrate spawners, paternal and biparental mouthbrooders, and maternal mouthbrooders respectively. This reclassification clashes with both the ideas of Thys van den Audenaerde (1968b), who gave a different rank to the

groups by leaving *Tilapia* as the genus and *Sarotherodon* and *Oreochromis* as subgenera, and Peters & Berns (1978, 1982), who believe that any of these subdivisions are not justified and that the various forms should all be called *Tilapia* based on their ideas of the evolution of the group. In general, it is agreed that mouthbrooding species have evolved from substrate spawning ancestors. *Sarotherodon* species also exhibit characteristics intermediate between substrate spawners and maternal mouthbrooders, particularly in their reproductive behavior and in the regression of substrate-spawning characteristics in their larvae such as the adhesive layer on the eggs and adhesive glands on the larval head (Peters, 1965; Peters & Berns, 1978, 1982). Peters & Berns (1978, 1982) believe that mouthbrooding has evolved a number of times from substrate spawners possibly from different ancestors and at different times. With breeding characters, these authors demonstrate that *Sarotherodon* are closer to *Tilapia* than *Oreochromis* and propose that *Sarotherodon* have only recently split from the *Tilapia* ancestor (Fig. 1A in Trewavas, 1980). Trewavas (1980) put forward an alternative theory in which a *Tilapia*-like ancestor gave rise to a mouthbrooding branch which soon divided into two: one which retained some conservative breeding behavior (but not other characteristics) and which led to the *Sarotherodon*, and a more progressive branch which led to the *Oreochromis* (Fig. 1B in Trewavas, 1980).

It appears from the overall figures of genetic distances obtained in this study (Table 11) that the *Sarotherodon* are closer to the *Oreochromis* than the *Tilapia*, which might be against Peters & Berns' (1978, 1980) hypothesis in this case. More significantly, phylogenetic results from the dendrograms in Figs. 10 and 11 show exactly the branching patterns of the three genera *Tilapia*, *Sarotherodon* and *Oreochromis*, with the overall picture supporting the general hypothesis that the *Sarotherodon-Oreochromis* (mouthbrooders) evolved from the

Tilapia (substrate spawners). In particular, Fig. 10 shows that the separate branches of *Sarotherodon* and *Oreochromis*, which had evolved from a *Tilapia*-like ancestor, quickly and nearly simultaneously began to develop into a range of new species. Therefore it is possible that some of the early species, such as *S. galilaeus* and some *Oreochromis* species, would still retain a level of similarity, although they are not expected to be congeneric. Monophyly of the tilapiines has not been established in this work. Results suggest that the evolution of the mouthbrooders (*Sarotherodon* & *Oreochromis*) is monophyletic but the substrate spawners (*Tilapia*) paraphyletic: i.e. all the descendants are not included on the same branch in Fig. 11, but the results are consistent with the monophyly of *T. (Coptodon)*. The allozyme data consistently supports Trewavas's (1980, 1982a) ideas not only on her reclassification of the three tilapia genera but also the evolution of this group, even though the work has been done on very different sets of characters.

3.4 Subgeneric Groupings & Evolutionary Relationships between Subgenera within the *Tilapia* and *Oreochromis*

3.4.1 Genus *Tilapia*

All dendrograms show that the *Tilapia* consist of two main groups: one is the three species in the subgenus *T. (Coptodon)* and the other are the species *T. (H.) buttikofferi* and *T. (P.) mariae*. The three *T. (Coptodon)* species being grouped together agrees with the classification of Thys van den Audenaerde (1968b) for this subgenus, one of six subgenera of substrate-brooding *Tilapia* he proposed. The grouping of the other two species from different subgenera, *T.*

(Heterotilapia) and *T. (Pelmatolapia)*, was probably because only one species from each subgenus was used, more species from these and other *Tilapia* subgenera being needed for a true grouping. The branch lengths between the two monophyletic groups and between *T. (H.) buttikofferi* and *T. (P.) mariae* (Fig. 10), and the genetic distances between these pairs (Table 10), show that the *T. (Heterotilapia)* and *T. (Pelmatolapia)* are rather closer to each other than they are to the *T. (Coptodon)* which appears to be clearly distinct.

3.4.2 Genus *Oreochromis*

The two dendrograms (Figs. 10 and 11) show minor rearrangements in branching patterns within the *Oreochromis*, however a number of main groups are always found. One of the most interesting things to appear from the two dendrograms is that *O. (Nyasalapia)* consubgeneric species were closely placed together with the *O. (Oreochromis)*. The grouping of species in the subgenus *O. (Nyasalapia)* resulting from this study (Figs. 10 and 11) agrees with the reclassification of Trewavas (1983) in that all maternal mouthbrooding species having tasselled male genital papillae should be in the same subgenus *Nyasalapia*. However, it is too soon to say whether all the *Nyasalapia* are a monophyletic grouping as only four of the 14 potential species were studied, three of these (the chambo) being sympatric. Other consubgeneric species are needed to confirm whether this is a monophyletic grouping.

Trewavas's (1983) sole definition of the *Nyasalapia* is the presence of the male genital papillae. She herself speculated about its origin and mentioned that the only other species which show this trait are *T. sparrmanii* and *T. margaritacea*. Both these species have bifid male papillae similar to the incipient

stages in the *Nyasalapia* species. She speculated whether this was a form of parallelism or an indication of the possible ancestor for the *Nyasalapia*. The range of distribution of *T. sparrmanii* and *O. (Ny.) macrochir* are almost identical. *T. sparrmanii* was not included in this study so discussion on the possible ancestor for the *Nyasalapia* and presumably the *Oreochromis* is not possible.

3.5 Interspecific Relationships between Species within the *Oreochromis*

A number of minor groupings within the *Oreochromis* consistently appear in the two dendrograms (Figs. 4 and 5), although there were slight differences in branching pattern and position. However, three main groups of species, one subgenus *O. (Nyasalapia)* and two groups in the subgeneus *O. (Oreochromis)* [with an inconsistent position of *O. (O.) u. hornorum*], will be described in more details.

3.5.1 Relationships between *O. (Nyasalapia)* species

O. (NY.) MACROCHIR & THE CHAMBO

Considering the position of *O. (Ny.) macrochir* in Fig. 10 and 11, it seems to show that this species shares intermediate relationship between the Malawian *Nyasalapia* chambo and the other *O. (Oreochromis)* species. Morphologically, geographically (Trewavas, 1983) and electrophoretically (Tables 4 and 5 in Chapter 2; Sodsuk *et al.*, in prep.), it has become evident that Lake Malawi chambo species have come about by some form of intralacustrine speciation,

sharing a number of synapomorphic characters unique in tilapia. However, which species, external to Lake Malawi, is most related to the chambo species-flock is still open to discussion. The chambo species all have a less deep body, longer and thinner caudal peduncle with a length/depth ratio equal to or less than 1, higher modal numbers of vertebrae and lateral line scales, lower modal numbers of gill-rakers, and a wider interorbital region than other species. Only the subgeneric characters, the genital tassel, the dentition, nonenlargement of the jaws in mature fishes, and possibly a common pattern of mating pits, are left to unite the Malawi flock with species outside the lake. Geographically, the Rukwa basin is part of the same section of the Rift Valley as Lake Malawi, from which it is now separated by the Rungwe volcanic mountains. This has brought Trewavas (1983) to the assumption that the Rukwa basin endemic species, *O. (Ny.) rukwaensis*, and its close relative, *O. (Ny.) macrochir*, or their common ancestor would seem to be the most likely sister-species of the Malawi flock. A possible implication from the dendrograms is that the *Oreochromis* split off a branch developing into the *Nyasalapia*, in which the first group of the subgenus such as *O. (Ny.) macrochir* and *O. (Ny.) rukwaensis*, or some other related species outside Lake Malawi still remained conservative with a number of *Oreochromis* characters, while the others such as the Malawi chambo had developed the more progressively unique characters.

Among the *Nyasalapia* subgeneric characters, Trewavas (1983) described the male genital tassel as being the most distinctive character of the subgenus and this was her main reason in regrouping *O. (Ny.) macrochir* and all other tasselled species, which used to be in the subgenus *Loruwiala* of Thys van den Audenaerde (1968b), into the same subgenus *Nyasalapia* as the chambo. Without the tassel, *O. (Ny.) macrochir*, perhaps nearly all tasselled species outwith Lake

Malawi, would seem to resemble *O. (Oreochromis)* species rather than the chambo. However, averaged genetic distances between *O. (Ny.) macrochir* and *O. (Oreochromis)* species (0.194, Nei, 1978; 0.415, Cavalli-Sforza & Edwards, 1967) and *O. (Ny.) macrochir* and the three chambo (0.141, Nei, 1978; 0.364, Cavalli-Sforza & Edwards, 1967) would confirm that *O. (Ny.) macrochir* is closer to the consubgeneric species than the others.

THE CHAMBO SPECIES

As mentioned above the chambo species of Lake Malawi appear to have come about by some form of intralacustrine speciation. Trewavas (1983) comments that the Lake Malawi chambo have so much in common (synapomorphies) that they must have had a common ancestor. Sharing a common ancestor between the chambo is even more evident when the allozyme data is taken into account (Table 4). In this chapter, phylogenetic results from the two dendrograms (Figs. 10 and 11) do show that they actually evolved from a common ancestor. The short and nearly equal branch length of each species (Fig. 10) not only confirms that all three species have a very close evolutionary relationship, but also suggests that their speciation began recently and at nearly the same time, or perhaps simultaneously. All this supports the idea of intralacustrine speciation of these species.

3.5.2 Relationships between *O. (Oreochromis)* species

THE GROUP OF *O. (O.) SHIRANUS, O. (O.) PLACIDUS, O. (O.) ANDERSONII, O. (O.) MORTIMERI* AND *O. (O.) MOSSAMBICUS*

These five species are consistently shown (Figs. 10 and 11) as two clades: one consists of *O. (O.) shiranus* and *O. (O.) placidus*, and the other one includes

the other three species, *O. (O.) andersonii*, *O. (O.) mortimeri* and *O. (O.) mossambicus*. Relationships within the clade of three species has been reported by Sodsuk & McAndrew (1991). Trewavas (1983) had placed these three species in separate groups of the subgenus *O. (Oreochromis)*, *O. (O.) mossambicus* in group-V and the two species *O. (O.) andersonii* and *O. (O.) mortimeri* in group-VI. Balon (1974) had suggested that *O. (O.) mortimeri* should be a subspecies of *O. (O.) mossambicus*. Trewavas (1983) however kept the specific rank for *O. (O.) mortimeri* because of the major difference in the breeding colouration, she notes that *O. (O.) mossambicus* and *O. (O.) mortimeri* have great similarity and actually occupy adjacent geographical areas. Extending Sodsuk & McAndrew's (1991) study by adding more *O. (Oreochromis)* species in this study, the three species still form a clade, and *O. (O.) mortimeri* and *O. (O.) mossambicus* still remain as two sister-taxa. This extended study also shows the close grouping of *O. (O.) shiranus* and *O. (O.) placidus* suggesting that they should be considered as closely related or possibly subspecies.

A convincing explanation on allopatric relationships among these five species has been proposed by Trewavas (1983). Geographically, two similar species *O. (O.) mossambicus* and *O. (O.) placidus* occur together from the Lower Zambezi southwards to Sodwana, but *O. (O.) placidus* extends further northwards and *O. (O.) mossambicus* further southwards, a distribution that has the appearance of an allopatric origin with secondary overlap. Each of these two has an allopatric related species. *O. (O.) mortimeri* represents *O. (O.) mossambicus* in the Middle Zambezi, and *O. (O.) shiranus* replaces *O. (O.) placidus* in the Upper Shiré, Lakes Malawi, Chilwa and Chiuta. Structurally *O. (O.) andersonii* seems to continue the *mossambicus-mortimeri* series in the Upper Zambezi, and extends also to the Ngami region and the Cunene in Angola. Furthermore, the

distant relationship between *O. (O.) shiranus* and the chambo shown by the dendrograms (Figs. 10 and 11) is consistent with the proposal of Trewavas (1983) that *O. (O.) shiranus* probably entered Lake Malawi from the south and became sympatric with the species-flock of chambo only when Lake Malawi expanded southwards in the Pleistocene.

THE GROUP OF *O. (O.) SPILURUS, O. (O.) AUREUS, O. (O.) NILOTICUS* AND *O. (O.) JIPE*

Relationships between species within this group are less clear as the two dendrograms (Figs. 10 and 11) have grouped one of the East African '*mossambicus* complex' members *O. (O.) spilurus* with the two species *O. (O.) aureus* and *O. (O.) niloticus* that Trewavas (1983) described as being geographically isolated from the eastern group of species. A further complication is a species from the Pangani system (Lake Jipe), *O. (O.) jipe*, which has been grouped with these species as an *O. (O.) niloticus* sister-species (three dendrograms).

Using colour features, Trewavas (1983) defined *O. (O.) aureus* as being closer to the eastern members such as *O. (O.) spilurus* rather than *O. (O.) niloticus*. However, she also proposed an alternative hypothesis that *O. (O.) niloticus* and *O. (O.) aureus* might possibly be related as sympatric sister-species because they live mostly together and both share the characters which differ from the eastern species, namely the narrow preorbital bone and the non-enlargement of the jaws in mature fishes. The dendrogram (Fig. 11) shows *O. (O.) spilurus* and *O. (O.) aureus* relationship agreeing with Trewavas in the former case, whereas the Fig. 10 would agree with the latter case if *O. (O.) jipe* was excluded. The overall relationship of the whole group, including *O. (O.) jipe*, is not

impossible as the series of species in the lower reaches of the eastern rivers from *O. (O.) mossambicus* in the south (South Africa) are linked with the species of the Pangani system through to *O. (O.) spilurus* in the north (Kenya and Somalia) (Trewavas, 1983). But the presence of unexpected relationship between *O. (O.) jipe* and *O. (O.) niloticus* as sister-species may be caused by the absence of their other close relatives in the dendrograms. Since *O. (O.) jipe* was the only species from the Pangani system used and no close relative of *O. (O.) niloticus* such as *O. (O.) esculentus* of Lake Victoria (Trewavas, 1983) was assessed, this relationship should not be given too much credibility. The uncertainty about the position of *O. (O.) jipe* based on allozyme data had also been recorded in previous studies (McAndrew & Majumdar, 1984; Seyoum & Kornfield, pers. comm.). But an extended study incorporating additional taxa and a larger number of allozyme loci examined (Sodsuk & McAndrew, 1991) and a comparative study using mitochondrial DNA (Seyoum, 1989) placed *O. (O.) jipe* unambiguously among other congeneric species.

Notably, the *O. (O.) jipe* relationship involved with the other three species in this study does not change from the previous study of Sodsuk & McAndrew (1991), although more consubgeneric species have been added. However, it must also be noted that the clustering of this group as a clade shows a very low bootstrap confident level (9 %) in Fig. 11. A clearer phylogenetic relationship between the four species in this group may probably be obtained, if the close relatives of *O. (O.) niloticus* and *O. (O.) jipe* are added.

POSITION OF *O. (O.) U. HORNORUM*

The relationship of *O. (O.) u. hornorum* to other species of the subgenus *O. (Oreochromis)* is presently ambiguous since its position is inconsistent in the

two accepted dendrograms (Figs. 10 and 11). The species has been placed as the sister-species of *O. (O.) spilurus* in Fig. 10, whereas it is shown at the bottommost (earliest stage) of the *Oreochromis* evolution in Fig. 11. Trewavas (1983) has grouped *O. (O.) u. hornorum* into the Eastern African '*mossambicus* complex' (the group of species inhabiting the lower parts of the eastward-flowing rivers, from the Webi Shebeli and Juba in Somalia, to Algoa Bay in South Africa) as being one of the five members, including from north to south *O. (O.) spilurus, O. (O.) korogwe, O. (O.) urolepis* (and *u. hornorum*), *O. (O.) placidus*, and *O. (O.) mossambicus*. According to this, the relationship between *O. (O.) u. hornorum* and *O. (O.) spilurus* should be sister-species as shown by the Fig. 10. However, its position at the earliest stage of the *Oreochromis* evolution shown in the Fig. 11 is still consistent with the previous study of Sodsuk & McAndrew (1991) despite the use of different algorithmic methods for the dendrogram construction and incorporating more consubgeneric species. The position of *O. (O.) u. hornorum* in Fig. 11 may also be caused by the absence of the *O. (O.) niloticus* and *O. (O.) jipe* close relatives. Consequently, the evolutionary position within the group of these species, as well as the overall *O. (Oreochromis)*, would require further work being undertaken on a few more relevant species. The most likely probably are *O. (O.) esculentus* as the *O. (O.) niloticus* close relative and another species from the Pangani system as the relative of *O. (O.) jipe*, such as *O. (O.) pangani girigan*.

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CHAPTER 4

EVOLUTIONARY RELATIONSHIPS FROM MORPHOLOGICAL CHARACTERS

I. INTRODUCTION

In the last Chapter a systematic study of the three major tilapiine genera, *Tilapia*, *Sarotherodon* and *Oreochromis*, using molecular variation revealed a phylogenetic relationship very much in line with the hypothesis of Trewavas (1973, 1980, 1981, 1982, 1983). This and all previous hypotheses on the evolution of this group have been based on a classical approach and the morphological, behavioural and biogeographical characters used have never been subjected to a numerical taxonomic approach.

Generally, systematic studies of any set of genetically determined characters should be congruent with other such studies based on different sets of characters in the same organisms. Multidisciplinary systematic studies are of great value, and combining as many sources of information as possible maximizes information and reliability, because no single systematic data set can be expected to be informative at all phylogenetic levels simultaneously (Hillis, 1987). Some techniques are useful for resolving questions of phylogeny among closely related species, whereas others are useful across ancient time spans (Hillis & Davis, 1986). Recent advances in systematic theory have transcended traditional boundaries and have been applied with equal success to both

morphological and molecular data sets (Goodman *et al.*, 1979; Wiley, 1981; Buth, 1984). Therefore, it is interesting to know whether or not biological and morphological (morphometric and meristic) characters which have been described as distinctive generic, subgeneric and specific differences in tilapiines will also result in phylogenetic relationships congruent with the molecular approach. This requires further comparative studies based on such characters of both generic and specific significance from the same taxa used in the last study.

In addition to the three major genera, there are a number of other related tilapiine genera, i.e. *Pelmatochromis, Danakilia, Iranocichla* and *Tristramella* (see Table 1 in Chapter 1). The genus *Pelmatochromis* Steindachner 1895 was proposed by Thys van den Audenaerde (1968b) as a subgenus of *Tilapia*, but Trewavas (1973) disputed this and revised its rank back to the generic level. Trewavas (1982, 1983) also upgraded *Danakilia* to a separate genus from a subgenus (Thys van den Audenaerde, 1968b), defining its close affinities with the Iranian genus *Iranocichla* Coad 1982. This relationship conflicted with the suggestion by Coad (1982) that *Iranocichla* had close affinities with the Jordanian and Syrian genus *Tristramella* Trewavas 1942. Therefore the relative ranking and relationships using more classical characters are still uncertain in many cases. The tilapiine genera mentioned will be studied and will be compared with the three major genera in order to shed light on tilapiine evolution at the generic level and then compare this with the evolutionary scheme resulting from the other study at the species level.

II. MATERIALS & METHODS

2.1 Data

Two different morphological data sets were collected from various published works one for generic and the others for species level data for two different phylogenetic analyses. Data for seven related tilapiine genera, *Pelmatochromis, Tilapia, Sarotherodon, Oreochromis, Danakilia, Iranocichla* and *Tristramella* were collected for the generic level analysis, taking *Pelmatochromis* as an outgroup due to the suggestion of Trewavas (1983) that it is the least specialized genus among the others. For the species level analysis, the data for all the species included in the last molecular study were collected except that data for *Pelmatochromis nigrofasciatus* (Pellegrin) were collected instead of *Pelvicachromis pulcher* (the presumed outgroup in the last study). This was because it has also been suggested by Trewavas (1983) that it is more representative of the ancestral tilapiines.

2.1.1 Sources of Data

Both generic and specific data were collected from published works on tilapiine taxonomy by several authors i.e. Boulenger (1915), Thys van den Audenaerde (1968a), Trewavas (1942, 1973, 1983) and Coad (1982). The followings are sources from which data have been collected.

GENERIC DATA

Genus	Source of Data
Pelmatochromis	Thys van den Audenaerde (1968a); Trewavas (1973)
Tilapia	Trewavas (1973, 1983)
Sarotherodon	Trewavas (1973, 1983)
Oreochromis	Trewavas (1973, 1983)
Danakilia	Trewavas (1983)
Iranocichla	Coad (1982); Trewavas (1983)
Tristramella	Trewavas (1942, 1983); Coad (1982)

SPECIES DATA

Species	Source of Data
P. nigrofasciatus	Boulenger (1915); Trewavas (1973)
T. (H.) buttikoferi	Boulenger (1915); Trewavas (1983)
T. (P.) mariae	Boulenger (1915); Trewavas (1983)
T. (C.) rendalli	Boulenger (1915); Trewavas (1983)
T. (C.) tholloni	Boulenger (1915); Trewavas (1983)
T. (C.) zillii	Boulenger (1915); Trewavas (1983)
S. m. melanotheron	Trewavas (1983)
S. g. galilaeus	Trewavas (1983)
O. (O.) n. niloticus	Trewavas (1983)
O. (O.) aureus	Trewavas (1983)
O. (O.) s. spilurus	Trewavas (1983)
O. (O.) u. hornorum	Trewavas (1983)
O. (O.) mossambicus	Trewavas (1983)
O. (O.) mortimeri	Trewavas (1983)

Species	Source of Data
O. (O.) andersonii	Trewavas (1983)
O. (O.) p. placidus	Trewavas (1983)
O. (O.) s. shiranus	Trewavas (1983)
O. (O.) jipe	Trewavas (1983)
O. (Ny.) macrochir	Trewavas (1983)
O. (Ny.) karongae	Trewavas (1983)
O. (Ny.) lidole	Trewavas (1983)
O. (Ny.) squamipinnis	Trewavas (1983)
O. (Ne.) tanganicae	Trewavas (1983)

2.1.2 Data Collection Management

The data collection was arranged in three steps: (i) collecting and coding data as multistate characters by coding each character into different states, (ii) connecting and linearly ordering the different states of each character, and (iii) recoding the linearly ordered character states into the binary characters (0 or 1).

(i) ***Multistate character data and the different states coded.***

The data were collected as multistate characters because they varied either qualitatively or quantitatively in appearance between different taxa. Different ***character states*** were coded (scored) for the different observed levels of each character as a linear sequential number (...., -1, 0, 1, 2, ...). By using the technique of Camin & Sokal (1965) as well as the outgroup comparison (Wiley, 1981), the presumed primitive states (the ancestral character states appearing in the outgroup) were conveniently coded as zero (0), derived states being positively or negatively coded, as required.

(ii) ***Character state connection and the linear order.***

Connections among the states within characters need to be informative concerning the hypotheses on character state therefore the ***order*** and ***polarity*** need to be defined (Mabee, 1989). Generally character state order refers to the evolutionary connections among character states, whereas polarity refers to the direction of evolution along hypothesized connections (i.e. which character conditions are primitive and which are derived). However it is the information on order and not polarity, that is used in the undirected tree (unrooted tree or network) construction (Mabee, 1989), which has been suggested as being probably the most appropriate for reconstructing phylogenies in systematic studies (Hillis, 1987). There are three classes of character state order, **reticulate**, **branched** and **linear**. Although the **linearly ordered** character states are probably the most commonly proposed type of ordered character in phylogenetic analyses, it generally is not appreciated that this character state order requires the most restrictive assumptions about the evolutionary process (Mabee, 1989). The order of different states within each multistate character collected in this study are mostly based on the linear order technique of Camin & Sokal (1965), however a branched order was also used for some characters in which the ordered states would be more informative about the presumed origins of these characters.

(iii) ***Recoding ordered character states into binary (0 or 1) characters***

All the ordered character states were then recoded into binary forms, the two-state characters to be used in the phylogeny reconstruction method. The recoding program FACTOR of Meacham (1990) in the PHYLIP package (Felsenstein, 1990), which is based on the recoding methods of Sneath & Sokal (1963) and Kluge & Farris (1969), was used to recode these data.

2.2 Methods Used in Phylogeny Reconstruction

The rate-independent (most assumption-free) methods of unrooted network construction and outgroup rooting are the most appropriate for maximizing phylogenetic information from the morphological and molecular approaches used in systematic studies (Hillis, 1987). The dendrogram construction techniques which use binary coded data are equally applicable to molecular and morphology based studies. Therefore, the Wagner parsimony (Eck & Dayhoff, 1966; Kluge & Farris, 1969; Farris, 1970) and the bootstrap resampling data methods (Felsenstein, 1985) which avoid random errors have been used in this study. The trees were finished as outgroup rooted trees.

Morphologically based character sets require some form of differential weighting as not all are of equal evolutionary importance. For instance, comparing characters at three taxonomic levels, genus, subgenus and species, the characters that are defined as being generically distinctive should have the highest degree of importance, followed by the subgeneric and then specific characters. Farris (1969) also suggested a way of weighting the value of a character that the characters that more frequently change their states are more unreliable a guide to relationships. The fact is that most specific characters are morphometric, meristic or colour pattern differences which usually have a wider range of variable states than the generic and subgeneric characters, so they were given an unweighted simple specific level of '1'. In the classification of the three major tilapiine genera, Trewavas (1983) has given greater weight to the the reproductive features at both generic and subgeneric levels: the differences in breeding behaviour (biparental, paternal or maternal) have been used as the generically distinctive characters, and the male tasselled genital papillae as a subgeneric character. In

addition, she also used biogeography as another character in differentiating or relating groups of species. Therefore in the interspecific study of the three major genera these generic, subgeneric and biogeographical characters were simply and respectively weighted as '4', '3' and '2' times the simple specific level '1'. In order to determine the effect of the extra weighting given to breeding and biogeographical characters, a number of analyses without these characters were performed in both the generic and species level studies. These analyses included the following:

i) At the generic level study, analysis with biogeographical character removed from the original data set.

ii) At the generic level study, analysis with biogeographical and breeding characters removed from the original data set.

ii) At the species level study, analysis with biogeographical character removed from the original data set, and with unweighted breeding character data.

iii) At the species level study, analysis with biogeographical and breeding characters removed from the original data set.

2.3 The Software Package Used

The PHYLIP package (Felsenstein, 1990) was used for all computations in this study. The subprogram BOOT carrying the Wagner parsimony (Eck & Dayhoff, 1966; Kluge & Farris, 1969; Farris, 1970) with the bootstrap resampling data method (Felsenstein, 1985) was used for dendrogram construction. The subprogram FACTOR (Meacham, 1990) was used for recoding multistate characters into the binary characters.

III. RESULTS & DISCUSSION

3.1 Generic Level Study

3.1.1 Collected Multistates, Character State Order & Binary Recoded Characters

The character details collected for seven tilapiine genera are presented in Table 12. Although the '?' symbols have been given in some places where the character details for the genus was unknown, some other characters (despite being important) had to be omitted because the information could not be found for the outgroup, which is important for phylogenetic analysis using outgroup comparison (Camin & Sokal, 1965; Wiley, 1981). Consequently some interesting details on important characters such as sexual dichromatism and size of eggs (Trewavas, pers. comn.), which show significant differences between the three major genera, have had to be omitted. However, the most important of the breeding characters, i.e. the information on whether they are substrate spawners or mouth brooders (the character no. 4 in the Table), still remains. The pattern (roman or gothic) of the circuli rearrangement on scales (the character no. 8 in the Table) was suggested by Trewavas (1973) as being a significant difference between *Pelmatochromis* (gothic) and *Tilapia* (roman). In roman scales the circuli in the posterior field are parallel to the edge, whereas in gothic they meet at an angle (see Fig. 10 of Trewavas, 1973 for more details). This pattern definition has also been used for the other genera.

Table 12. Morphological and biogeographical character details of seven related tilapiine genera. Data collected from Trewavas (1942, 1973, 1983), Thys van den Audenaerde (1968b) and Coad (1982).

Character	*Pelmatochromis* (Outgroup)	*Tilapia*	*Sarotherodon*	*Oreochromis*	*Danakilia*	*Iranocichla*	*Tristramella*
1. Food and trophic structural features							
1.1 Diet	Small invertebrates and plankton	Macrophytes, phytoplankton and organic detritus	Epiphytes, algae, phytoplankton and organic detritus	Epiphytes, algae, phytoplankton and organic detritus	Green algae (tricuspid teeth suggest epilithic)	Algae, diatoms and bottom deposits	Vegetarian
1.2 Dentition							
1.2.1 Jaw teeth	Unicuspid teeth, but one species *P. nigrofasciatus* showing outer notched or shouldered in the very young individuals.	Pluricuspid, bicuspid outer and tricuspid inner, but some of *T. ruweti* showing pluricuspid teeth at first and then becoming pointed in adult; coarse	Pluricuspid, bicuspid outermost, but in *S. linnelli* becoming unicuspid in mature males; fine	Pluricuspid, bicuspid outermost, but becoming unicuspid in adult or mature males of various subgenera; coarse to fine	Pluricuspid, all tricuspid	Pluricuspid, bicuspid outer and tricuspid inner	Pluricuspid, bicuspid outer and tricuspid inner, but conical in one species *Tr. sacra*

Character	*Pelmatochromis* (Outgroup)	*Tilapia*	*Sarotherodon*	*Oreochromis*	*Danakilia*	*Iranocichla*	*Tristramella*
1.2.2 Pharyngeal teeth	With enlarged teeth at the middle-posterior and anterolateral regions; coarse or relatively coarse	No enlarged or flattened teeth (except in *T. buttikoferi*); coarse or relatively coarse	No enlarged or flattened teeth; very fine	No enlarged or flattened teeth; relatively coarse to very fine	With enlarged teeth; fine	No medially enlarged teeth but replaced by large flattened teeth forming a pavement; fine	With enlarged teeth; fine
1.3 Gill rakers							
1.3.1 Series of epibranchial gill rakers	Long and slender	Short or relatively short and blunt	Long and very fine	Long and slender	Long and slender	Short	?
1.3.2 Number of lower gill rakers	6-14	6-17	14-27	9-27	10-12	14-19	9-12
1.4 Length of intestine (in times Standard Length)	2.3	2.5 in one species *T. busamana* but usually much longer (7-14)	7-14	7-14	Very long intestine	6.8-8.3	Long
2. Lower pharyngeal bone	With heartshaped dentigerous area	With heartshaped dentigerous area	With heartshaped dentigerous area	With heartshaped dentigerous area	With heartshaped dentigerous area	With nearly circular dentigerous area	With heartshaped dentigerous area
3. Microbranchio-spines	Present	Present	Present	Present or absent	Absent	Present	?

Character	*Pelmatochromis* (Outgroup)	*Tilapia*	*Sarotherodon*	*Oreochromis*	*Danakilia*	*Iranocichla*	*Tristramella*
4. Breeding behaviour	Substrate spawner	Substrate spawner; larvae having three pairs of adhesive glands on the top of the head	Mouth brooders, paternal or biparental; larvae having vestigial adhesive glands	Mouth brooders, maternal; no adhesive glands on larvae	Mouth brooders (no details)	Mouth brooders (no details)	Mouth brooders; biparental
5. Ossification of the ethmoid	The ethmoid cartilage is well covered in bone, with the lateral ethmoid and supraethmoid meeting the vomer so then leaving only a narrow cartilaginous surface between	The ethmoid cartilage is well covered in bone; or the supra-ethmoid ends freely from the vomer	Supraethmoid is free from the vomer	Supraethmoid is free from the vomer	Supraethmoid is free from the vomer	Supraethmoid is free from the vomer	Supraethmoid meeting the vomer
6. Vertebrae (modal number)	26-27 (26)	26-30 (28)	26-31 (29)	26-34 (30)	27	28-30 (29)	29
7. Lateral line system							
7.1 Openings in the preorbital bone	4	5	5	5,4	5	1	5

Character	*Pelmatochromis* (Outgroup)	*Tilapia*	*Sarotherodon*	*Oreochromis*	*Danakilia*	*Iranocichla*	*Tristramella*
7.2 Lateral line scales	27-28	27-31	27-32	28-38	27-28	28-40	30-31
8. Circuli rearrangement on scales	Roman or gothic, a mixture of roman and gothic but conforming to a more gothic pattern	Roman or gothic, in one species *T. ruweti* showing gothic but usually roman	Roman	Roman	Roman	Roman	Roman
9. The typical ringed tilapia-mark (a black spot) on the dorsal fin	Present in adult; or present in the young but absent in adult; or a homologous mark present in the young and then moving to below the fin in older fish	Present throughout life	Present in the young but absent in older fish	Present in the young but absent in older fish	Present in the young	Present in juvenile and still remained in adult	Details unknown (?)
10. Depth of pre orbital bone (in % head length)	16.0-22.5	?	20-33, positively allometric	12.5-30, positively, allometric	20.5-26.3	approximately in the range of 20-25	approximately in the range of 20-25
11. Pectoral fins	Short or long	Long	Long	Short or long	Short in the lake fish but longer in the swamp fish	Short, not reaching the vent	Long, reaching the vent

Character	*Pelmatochromis* (Outgroup)	*Tilapia*	*Sarotherodon*	*Oreochromis*	*Danakilia*	*Iranocichla*	*Tristramella*
12. Anal spines	III	III; >III	III	III; >III; rarely II	III	III	III
13. Biogeography	Liberia to Sierra Leone; Congo. (Western Africa)	West Africa from the Zaire to Senegal and the Draa in the south of the Atlas Mountains; the Soudanian region and Lake Chad; the Nile system, the Levant and the Jordan Valley; and the Zambezi basin and southwards, excluding the rivers flowing to the Indian Ocean in the north of the Zambezi.	West Africa from the Zaire to Senegal and the Draa in the south of the Atlas Mountains; the Soudanian region and Lake Chad; and extending to the Nile system, the Levant and the Jordan Valley.	East and Central Africa including the Nile system, the Levant and the Jordan Valley; the Soudanian region and Lake Chad; the western rivers only in Angola; and the Zambezi basin and southwards, including the rivers flowing to the Indian Ocean.	Lake Afrera (Giulietti) and the adjoining swamps in the Danakil depression, between the Dancalian Mountains and the Ethiopian highlands, near the shore of the Red Sea. (Eastern Africa)	Southwestern Iran	Jordan Valley and Damascus waters in Syria

The different states of each character collected and the character state order are presented by Table 13. The outgroup was given the state '0' for each character as this was assumed to be ancestral. All other character states were therefore 'derived' and were compared accordingly. Most of the character states have been linearly ordered in which the largest conditions are separated from the smallest by states of intermediate size (Mabee, 1989). However some characters, i.e. the breeding behaviour and biogeography (character nos. 4 and 13 in Table 13), could not be definitely ordered, so a branched order was used to make the character states as reliable as the original information on the characters allowed. Considering the breeding character state (no. 4), the lack of details on breeding behaviour in two mouthbrooding genera, *Danakilia* and *Iranocichla*, means they should possibly be coded and ordered in three ways: (1) coded as '1' and linearly ordered as the general state 1 of the paternal-biparental mouthbrooders, (2) coded as '2' and linearly ordered as the more restrictive state 2 of the maternal mouthbrooders, and (3) coded as another separate state '3' and having a branched connection with the intermediate condition of the paternal-biparental mouthbrooders state 1 (same as the maternal mouthbrooders state 2). These different coded states and orders of this character were applied separately to generate comparative dendrograms. The biogeographical character (no. 13) states 2, 3 and 4 have all been ordered (branched) adjacent to state 1 because the large size of state 1 would make it more likely to be the large intermediate condition between the state 0 of the outgroup distribution (which would be presumed as the original area of tilapiine distribution) and all the other three states. However, comparative dendrograms were generated using and not using these biogeographical data separately.

Table 13. Collected multistate characters of the seven tilapiine genera. The different states are coded as sequential numerical numbers with ancestral states (the states present in the outgroup) being 0. The character states are ordered in linear and branched series.

Character	States	State order
1. Food and trophic structure features		
1.1 Diet		
Small invertebrates and planktons	0	0 1 2
Macrophytes, phytoplanktons and organic detritus	1	
Epiphytes, epilithic algae, phytoplanktons and organic detritus (rarely or not eating macrophytes)	2	
1.2 Dentition		
1.2.1 Jaw teeth		
Unicuspid teeth	0	0 1 2 3 4
Pluricuspid (bicuspid outer and tricuspid inner); coarse	1	
Pluricuspid (bicuspid outermost); coarse to fine	2	
Pluricuspid (bicuspid outermost); fine	3	
Pluricuspid (all tricuspid)	4	
1.2.2 Pharyngeal teeth		
With enlarged or flattened teeth; fine	-1	-1 0 1 2 3
With enlarged or flattened teeth; coarse or relatively coarse	0	
No enlarged or flattened teeth; coarse or relatively coarse	1	
No enlarged or flattened teeth; relatively coarse to very fine	2	
No enlarged or flattened teeth; very fine	3	
1.3 Gill rakers		
1.3.1 Series of epibranchial gill rakers		
Long and slender or very fine	0	0 1
Short or relatively short and blunt	1	
1.3.2 Number of lower gill rakers		
6-17 6 17	0	0 1 2
9-19 9 19	1	
9-27 9 27	2	
1.4 Length of intestine (in times SL)		
Short (2.3)	0	0 1 2
Short or long (2.5 or 7-14)	1	
Long (7-14)	2	

Character	States	State order
2. Lower pharyngeal bone		
With heart-shaped dentigerous area	0	0—1
With nearly circular dentigerous area	1	
3. Microbranchiospine		
Present	0	0—1—2
Present or absent	1	
Absent	2	
4. Breeding behaviour		
Substrate spawner	0	0—1—2
Mouth brooders, paternal or biparental	1	|
Mouth brooders, maternal	2	3
Mouth brooders (no details)	3(1)(2)	0—1—2
5. Ossification of the ethmoid		
The ethmoid cartilage is well covered in bone (the lateral ethmoid and supraethmoid meeting the vomer)	0	0—1—2
The ethmoid cartilage is well covered in bone; or the supraethmoid ends freely from the vomer	1	
The supraethmoid is free from the vomer	2	
6. Vertebrae (modal number)		
26	0	0—1—2—3—4
27	1	
28	2	
29	3	
30	4	
7. Lateral line system		
7.1 Openings in the preorbital bone		
1	-1	-1—0—1—2
4	0	
5	1	
5, 4	2	
7.2 Lateral line scales		
27-28 27,28	0	0—1—2
27-32 27———32	1	
28-40 28————40	2	
8. Circuli rearrangement on scales		
Roman or gothic, or a mixture of both but confirming to a more gothic pattern	0	0—1—2
Roman or gothic, but usually roman	1	
Roman	2	

Character	States	State order
9. The typical ringed tilapia-mark (a black spot) on the dorsal fin		
Present in the young but absent in adult	-1	-1–0–1
Present in adult, or present in the young but absent in adult, or a homologous mark present in the young and then moving to below the fin in older fish	0	
Present throughout life	1	
10. Depth of preorbital bone (in % head length)		
13-23 13——23	0	0–1–2–3
13-33, positively allometric 13————33	1	
20-33, positively allometric 20———33	2	
20-26 20—26	3	
11. Pectoral fins		
Short, not reaching the vent	-1	-1–0–1
Short or long	0	
Long	1	
12. Anal spines		
III	0	0–1
III or >III	1	
13. Biogeography		
Western Africa (Liberia to Sierra Leone and the Congo basin)	0	2 | 0—1—3 | 4
Western, Central, Southern, Eastern and Northeastern Africa; the Levant and the Jordan Valley	1	
Eastern Africa (Lake Afrera and the adjoining swamp in the Danakil depression near the shore of the Red Sea, Ethiopia)	2	
The Jordan Valley and Damascus, Syria	3	
Southwestern Iran	4	

In the character of the number of preorbital bone openings (character 7.1), the state of having five or four openings in the preorbital bone has been scored as state 2 and linearly ordered as terminal end of the series rather than an intermediate condition between the two states of having four (state 0) and five (state 1) openings. There has been an awareness (Trewavas, 1983) that in cichlids the state of having four openings in the preorbital bone is primitive (ancestral) and the state of having five openings is derived. However among the *Oreochromis* species there are both four and five openings present. The presence of four openings, which is found in certain specialized species of *Oreochromis*, has been suggested as being derived reversal or secondarily derived state. Therefore it is more likely that the state of having five or four openings is closer to the state of having five openings. For the state of having only one single opening (state -1) although it is rather constrained, the presence of this state has been found in *Iranocichla* by Coad (1982). There is some doubt whether this state should be connected to the ancestral state of having four openings or the derived state of having five/four openings, since both states would seem to be connectable with the smaller number of openings. The principle of the linear order technique is that character states are ordered according to their sequential degree of similarity so that the largest condition is separated from the smallest by states of intermediate size (Mabee, 1989). Accordingly the state of single preorbital bone opening seems more likely connected to the state of four openings, becoming the smallest derived state.

The different states of each multitistate character collected in seven tilapiine genera and the new binary character data are shown in the Table 14. All the ordered character states have been recoded into binary characters which were used in the dendrogram construction.

Table 14. Different states of each character in seven tilapiine genera and their new binary recoded characters. Each character number represents each character in the same order as Table 12 & 13. These multistate characters have been recoded into binary characters under the state order shown in Table 13. The factors indicate the correspondences of which characters have been recoded into the new binary characters. In the character no. 4, the character states 1, in '()', and 2, in '[]', are also given to *Danakilia* and *Iranocichla* separately from the character state 3, so this multistate character with the new inclusion of only three states (0, 1, 2) and two factors has been recoded into only the binary character which are respectively presented in the '()' and '[]' brackets.

Character no.	Different states of each character in seven genera						
	Pel.	*Til.*	*Sar.*	*Ore.*	*Dan.*	*Ira.*	*Tri.*
1.1	0	1	2	2	2	2	2
1.2.1	0	1	3	2	4	1	1
1.2.2	0	1	3	2	-1	-1	-1
1.3.1	0	1	0	0	0	1	?
1.3.2	0	0	2	2	1	1	1
1.4	0	1	2	2	2	2	2
2	0	0	0	0	0	1	0
3	0	0	0	1	2	0	?
4	0	0	1	2	3(1)[2]	3(1)[2]	1
5	0	1	2	2	2	2	0
6	0	2	3	4	1	3	3
7.1	0	1	1	2	1	-1	1

Factors	New data recoded into binary (0, 1) form						
	Pel.	*Til.*	*Sar.*	*Ore.*	*Dan.*	*Ira.*	*Tri.*
1.1	0	1	1	1	1	1	1
1.1	0	0	1	1	1	1	1
1.2.1	0	1	1	1	1	1	1
1.2.1	0	0	1	1	1	0	0
1.2.1	0	0	1	0	1	0	0
1.2.1	0	0	0	0	1	0	0
1.2.2	0	1	1	1	0	0	0
1.2.2	0	0	1	1	0	0	0
1.2.2	0	0	1	0	0	0	0
1.2.2	0	0	0	0	1	1	1
1.3.1	0	1	0	0	0	1	?
1.3.2	0	0	1	1	1	1	1
1.3.2	0	0	1	1	0	0	0
1.4	0	1	1	1	1	1	1
1.4	0	0	1	1	1	1	1
2	0	0	0	0	0	1	0
3	0	0	0	1	1	0	?
3	0	0	0	0	1	0	?
(4)	(0)	(0)	(1)	(1)	(1)	(1)	(1)
[(4)]	[(0)]	[(0)]	[(0)]	[(1)]	[(0)]	[(0)]	[(0)]
[4]	[0]	[0]	[0]	[1]	[1]	[1]	[0]

Character no.	Different states of each character in seven genera						
	Pel.	*Til.*	*Sar.*	*Ore.*	*Dan.*	*Ira.*	*Tri.*
7.2	0	1	1	2	0	2	1
8	0	1	2	2	2	2	2
9	0	1	-1	-1	-1	1	?
10	0	?	2	1	3	3	3
11	0	1	1	0	0	-1	1
12	0	1	0	1	0	0	0
13	0	1	1	1	2	4	3

Factors	New data recoded into binary (0, 1) form						
	Pel.	*Til.*	*Sar.*	*Ore.*	*Dan.*	*Ira.*	*Tri.*
4	0	0	0	0	1	1	0
5	0	1	1	1	1	1	0
5	0	0	1	1	1	1	0
6	0	1	1	1	1	1	1
6	0	1	1	1	0	1	1
6	0	0	1	1	0	1	1
6	0	0	0	1	0	0	0
7.1	0	1	1	1	1	0	1
7.1	0	0	0	1	0	0	0
7.1	0	0	0	0	0	1	0
7.2	0	1	1	1	0	1	1
7.2	0	0	0	1	0	1	0
8	0	1	1	1	1	1	1
8	0	0	1	1	1	1	1
9	0	1	0	0	0	1	?
9	0	0	1	1	1	0	?
10	0	?	1	1	1	1	1
10	0	?	1	0	1	1	1
10	0	?	0	0	1	1	1
11	0	1	1	0	0	0	1
11	0	0	0	0	0	1	0
12	0	1	0	1	0	0	0
13	0	1	1	1	1	1	1
13	0	0	0	0	1	0	0
13	0	0	0	0	0	0	1
13	0	0	0	0	0	1	0

3.1.2 Tilapiine Intergeneric Relationships from the Dendrogram Constructed Using Generic Characters

The dendrogram constructed from the generic character data of the seven tilapiine genera (*Pelmatochromis, Tilapia, Sarotherodon, Oreochromis, Danakilia, Iranocichla* and *Tristramella*) with the inclusion of morphology, biogeography and the breeding character state in *Danakilia* and *Iranocichla* being coded as state 3 is shown by Fig. 12, with the inclusion of morphology, biogeography and breeding character state in *Danakilia* and *Iranocichla* being coded as state 1 by Fig. 13, with the inclusion of morphology, biogeography and breeding character state in *Danakilia* and *Iranocichla* being coded as state 2 by Fig. 14, with the inclusion of morphology and breeding character states in *Danakilia* and *Iranocichla* being coded as state 1 (excluding biogeographical data) by Fig. 15, with the inclusion of morphology and breeding character states in *Danakilia* and *Iranocichla* being coded as state 2 (excluding biogeographical data) by Fig. 16, and with the morphological characters only (excluding biogeographical and breeding behavioural data) by Fig. 17. From all dendrograms, *Tilapia* is the genus closest to *Pelmatochromis* (the presumed outgroup) giving rise to a monophyletic group of the other genera. This is because *Tilapia* shares a larger number of characters (0) with *Pelmatochromis* (Table 14) whose characters mostly are expected to be ancestral. A consequence of this is that the theory that the substrate spawners gave rise to all the mouthbrooding genera (Peters & Berns, 1978, 1982; Trewavas, 1980, 1982) is supported. Within the mouthbrooding monophyletic group, the close relationship between *Sarotherodon* and *Oreochromis* is shown as being sister-taxa in all dendrograms. Trewavas (1973) raised these two taxa from the different subgeneric ranks into the same genus *Sarotherodon* because of this close relationship. However, she (Trewavas, 1981,

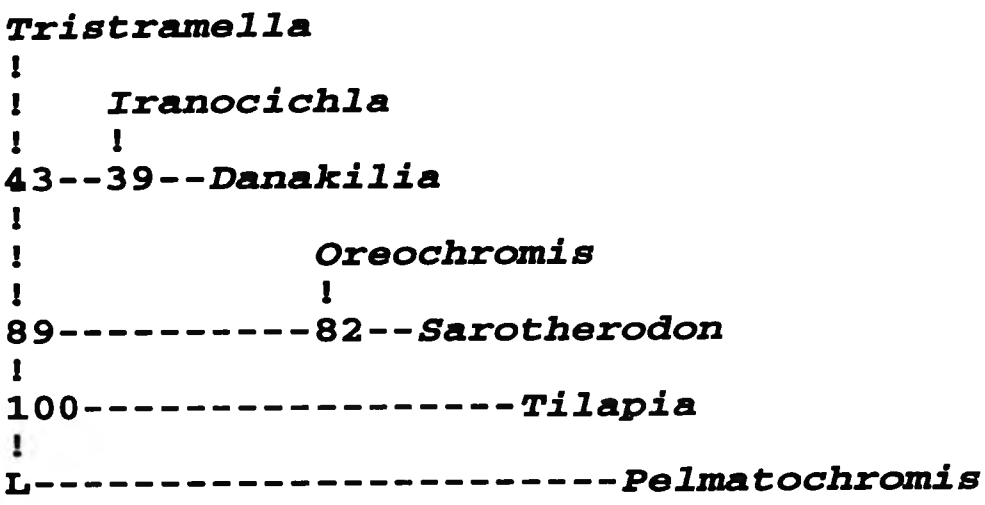

Fig. 12 Dendrogram constructed from multistate characters (recoded into the form of binary characters) of seven tilapiine genera, using the methods of the Wagner parsimony (Eck & Dayhoff, 1966; Kludge & Farris, 1969; Farris, 1970) and the bootstrap resampling data (Felsenstein, 1985) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the genera above and to the right of that fork occurred among 100 bootstrap replicates.

The data used include morphology, biogeography, and breeding behaviour with the character state in *Danakilia* and *Iranocichla* being coded as state 3.

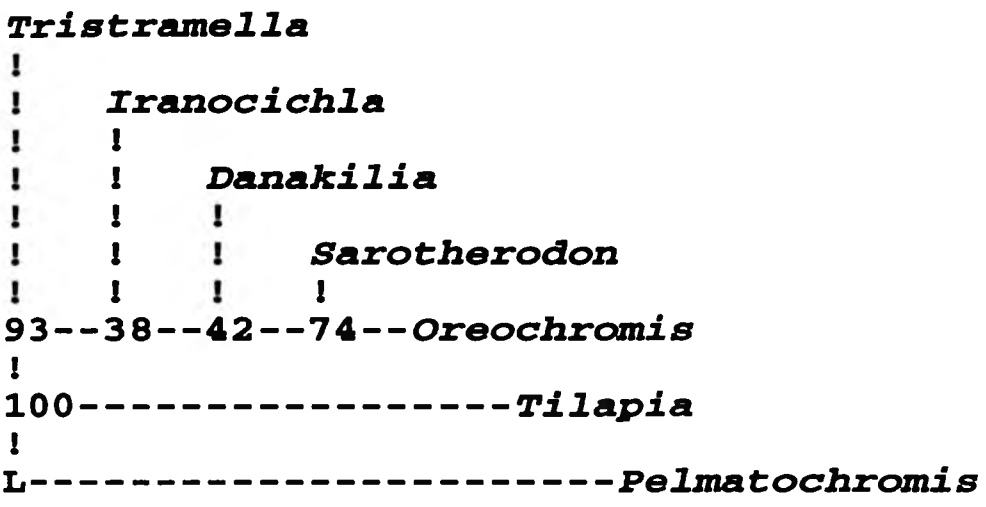

Fig. 13 Dendrogram constructed from multistate characters (recoded into the form of binary characters) of seven tilapiine genera, using the methods of the Wagner parsimony (Eck & Dayhoff, 1966; Kludge & Farris, 1969; Farris, 1970) and the bootstrap resampling data (Felsenstein, 1985) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the genera above and to the right of that fork occurred among 100 bootstrap replicates.

The data used include morphology, biogeography, and breeding behaviour with the character state in *Danakilia* and *Iranocichla* being coded as state 1.

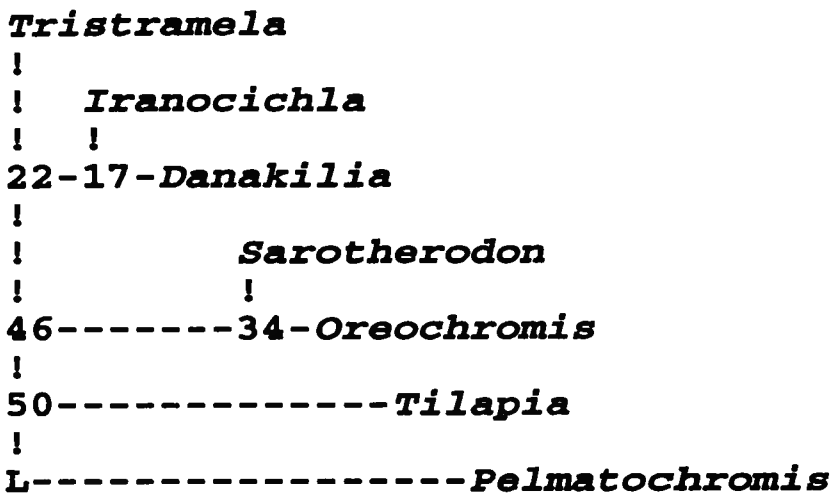

Fig. 14 Dendrogram constructed from multistate characters (recoded into the form of binary characters) of seven tilapiine genera, using the methods of the Wagner parsimony (Eck & Dayhoff, 1966; Kludge & Farris, 1969; Farris, 1970) and the bootstrap resampling data (Felsenstein, 1985) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the genera above and to the right of that fork occurred among 50 bootstrap replicates.

The data used include morphology, biogeography, and breeding behaviour with the character state in *Danakilia* and *Iranocichla* being coded as state 2.

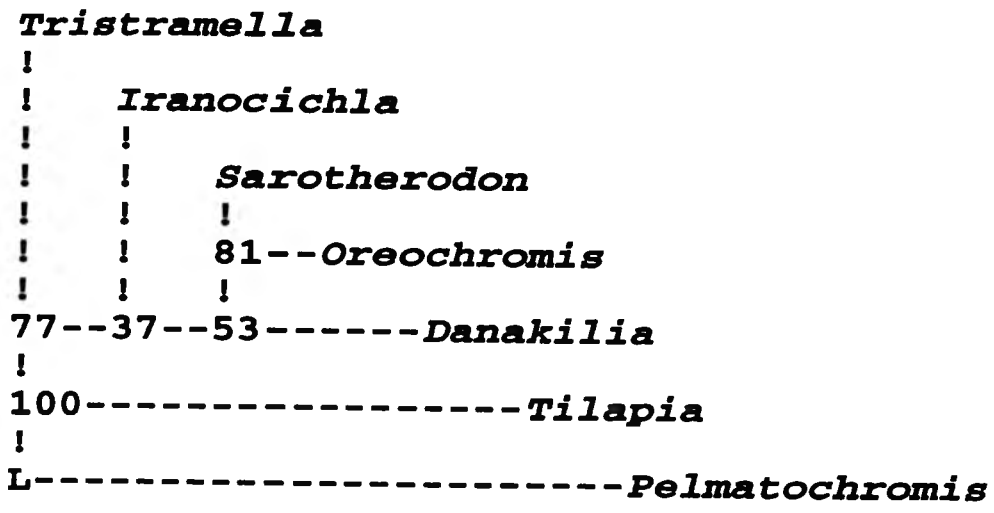

Fig. 15 Dendrogram constructed from multistate characters (recoded into the form of binary characters) of seven tilapiine genera, using the methods of the Wagner parsimony (Eck & Dayhoff, 1966; Kludge & Farris, 1969; Farris, 1970) and the bootstrap resampling data (Felsenstein, 1985) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the genera above and to the right of that fork occurred among 100 bootstrap replicates.

The data used include morphology and breeding behaviour with the character state in *Danakilia* and *Iranocichla* being coded as state 1, but excluding biogeography.

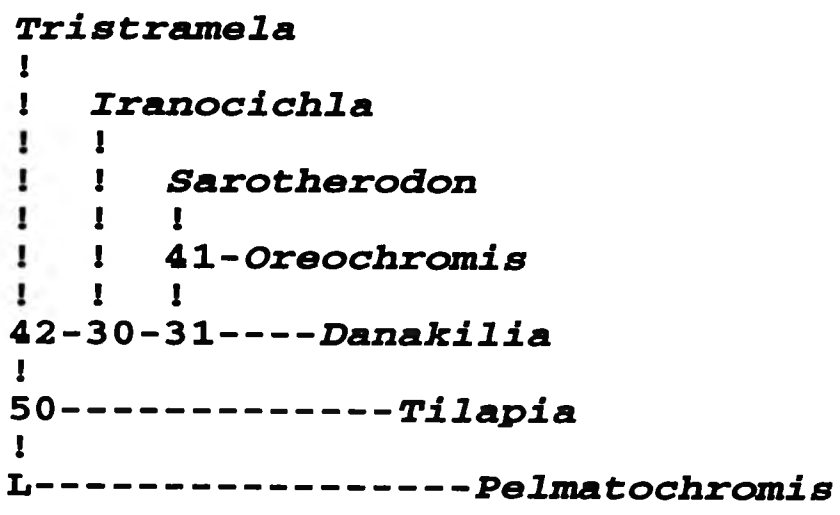

Fig. 16 Dendrogram constructed from multistate characters (recoded into the form of binary characters) of seven tilapiine genera, using the methods of the Wagner parsimony (Eck & Dayhoff, 1966; Kludge & Farris, 1969; Farris, 1970) and the bootstrap resampling data (Felsenstein, 1985) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the genera above and to the right of that fork occurred among 50 bootstrap replicates.

The data used include morphology and breeding behaviour with the character state in *Danakilia* and *Iranocichla* being coded as state 2, but excluding biogeography.

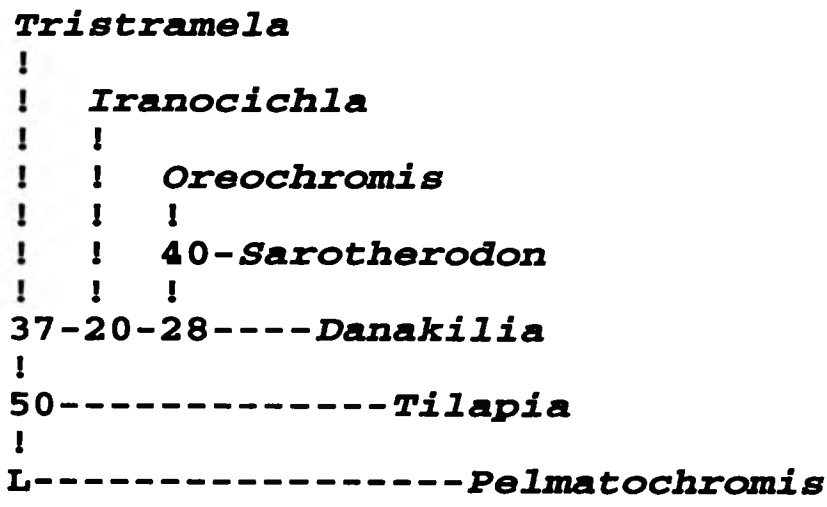

Fig. 17 Dendrogram constructed from multistate characters (recoded into the form of binary characters) of seven tilapiine genera, using the methods of the Wagner parsimony (Eck & Dayhoff, 1966; Kludge & Farris, 1969; Farris, 1970) and the bootstrap resampling data (Felsenstein, 1985) to avoid random errors. The numbers at the forks indicate the number of times the group consisting of the genera above and to the right of that fork occurred among 50 bootstrap replicates.

The data used include morphological data only, excluding breeding behaviour and biogeography.

1982a, 1983) finally classified them as separate genera basically on differences in breeding behaviour.

The other three mouthbrooding genera *Tristramella, Iranocichla* and *Danakilia* have been grouped together with *Iranocichla* and *Danakilia* adjoining each other in two dendrograms (Figs. 12 & 14), but in the other four dendrograms (Figs. 13, 15, 16 and 14) similarly *Danakilia* has been grouped closely to *Sarotherodon* and *Oreochromis*. This implies that the use of biogeography together with the breeding character state in *Danakilia* and *Iranocichla* both being coded as another separate state 3 and as maternal mouthbrooding state 2 in the dendrogram construction in Figs. 12 & 14 has affected the clustering of *Tristramella, Iranocichla* and *Danakilia* in these two dendrograms. Interesting relationships between these three genera have been suggested and proposed by Coad (1982) and Trewavas (1983). Whereas *Tristramella* comprises three congeneric species *Tr. sacra, Tr. simonis* and *Tr. magdalenae*, both *Iranocichla* and *Danakilia* comprise only single species, *I. hormuzensis* and *D. franchettii* respectively. *Iranocichla* has been discovered in southwestern Iran by Coad (1982). With insufficient knowledge of *Danakilia*, the genus of the Danakil Depression, between the Ethiopian Highlands and the Red Sea, Coad (1982) suggested a relationship between *Iranocichla* and *Tristramella*, a genus of the Jordan Valley and the waters of Damascus. But later Trewavas (1983) described a number of similarities between *Danakilia* and *Iranocichla* and proposed that they should be seen as close relatives. This would seem to agree with the relationship shown by the dendrograms (Figs. 12 and 14). This is particularly interesting because the habitats of the two genera are separated by the whole Arabian Peninsula, where no cichlids are found today. Coad (1982) assumed that the present distribution of *Iranocichla* in southwestern

Iran could be a result of a once wider distribution across the Arabian Peninsula and/or the Tigris-Euphrates basin of Mesopotamia, the headwater of this system being closer to the headwaters of the Levant rivers at various times during the late Pliocene and Pleistocene. Further, Trewavas (1983) has pointed out that the survival of the two related cichlids on the opposite sides of the once habitable desert is evidence of more widespread occurrence of their ancestor(s) in Arabia in the Late Pliocene/early Pleistocene, which is considered to be the last long humid period in what is now the Arabian desert. The relationship of the two genera to *Tristramella* could be explained by one of two possible scenarios, the link between *Tristramella* and *Iranocichla* by connections between the Tigris-Euphrates headwater system confining *Iranocichla* and Syrian waters harbouring *Tristramella* (Coad, 1982), or a link between *Tristramella* and *Danakilia* via the Red Sea basin and the Jordan Rift (Trewavas, 1983).

However, a close relationship of *Danakilia* to *Tristramella* and *Iranocichla* in Fig. 12, as well as its relationship to *Sarotherodon* and *Oreochromis* in Figs. 13, 15, 16 and 17, is still unresolved in this study because the bootstrap confidence level shown for the clustering as a clade is too low to support their relationships.

3.2 Species Level Study

3.2.1 Collected Multistates, Character State Order & Binary Recoded Characters

The details of characters collected for 23 tilapiine species are summarized by Tables 15.a, 15.b, 15.c and 15.d. The different sequential states and the

Morphological and biogegraphical character details of 23 tilapiine species. Data were collected from Boulenger (1915) and Trewavas (1983).

Table 15.a The character informations of *Pelmatochromis nigrofasciatus* (the outgroup) and five species in the genus *Tilapia, T. (H.) buttikoferi, T. (P.) mariae, T. (C.) rendalli, T. (C.) tholloni* and *T. (C.) zillii*.

Character	*P. nigrofasciatus*	*T. (H.) buttikoferi*	*T. (P.) mariae*	*T. (C.) rendalli*	*T. (C.) tholloni*	*T. (C.) zillii*
1. Body depth	41.1-47.5 %SL	44.44-50 %TL, ≈ 47-53 %SL	46.67-55.56 %TL, ≈ 49-58 %SL	42.86-44.44 %TL, ≈ 45-47 %SL	38.46-41.67 %TL, ≈ 40-43 %SL	37.5-46.15 %TL, ≈ 40-47 %SL
2. Head length	35-39 %SL	29.41-33.33 %TL, ≈ 32-36 %SL	33.33-36.36 %TL, ≈ 36-39 %SL	29.41-30 %TL, ≈ 32-33 %SL	33.33 %TL, ≈ 35-36 %SL	30-37.5 %TL, ≈ 33-40 %SL
3. Eye diameter	Larger than preorbital depth	Equal to preorbital depth	Exceeding preorbital depth	Equal to preorbital depth	Equal to preorbital depth	Less than preorbital depth in adult
4. Mouth (length of lower jaw in %HL)	Moderately large, lower jaw 35-42 %HL	Large	Rather small	Small	Large	Rather large
5. Jaw teeth (number of rows or series)	4 rows	3 or 4 series	3 to 5 series	4 series	4 or 5 series	3 to 6, more or less, regular series
6. Gill rakers (number on lower part of the first arch)	8-14	11 or 12	13-15	9 or 10	10	8-11

Character	*P. nigrofasciatus*	*T. (H.) buttikoferi*	*T. (P.) mariae*	*T. (C.) rendalli*	*T. (C.) tholloni*	*T. (C.) zillii*
7. Scales						
7.a Scales on cheek (number of rows or series)	3 rows	5 or 6 series	3 or 4 series	3 or 4 series	3 or 4 series	3 or 4 series
7.b Lateral lines	27-28	28-35	29-37	31-37	28-31	28-37
8. Vertebrae	26 (26-27)	28	29 (28-29)	29 (28-30)	?	28 (27-29)
9. Dorsal fin						
9.a Spines	XIII-XV	XIV-XV	XVI (rarely XV)	XVI-XVII	XV-XVI	XIV-XVI
9.b Soft rays	10-12	14-16	12-13	12-13	8-10	10-13
10. Anal fin						
10.a Spines	III	III	III	III	III	III
10.b Soft rays	9-10	10-11	10-11	9-10	8-9	7-10 (usually 8-9)
11. Pectoral fins (the fin length compared with length of head)	About the same length or a little shorter than head	As long as or a little shorter than head	As long as head	As long as or a little longer than head	As long as head	As long as head or a little shorter

Character	*P. nigrofasciatus*	*T. (H.) buttikoferi*	*T. (P.) mariae*	*T. (C.) rendalli*	*T. (C.) tholloni*	*T. (C.) zillii*
12. Ventral fins	Produced into a long black filament	Produced into a long filament	Produced, reaching vent or anal	Produced into a filament	Not produced	Not produced
13. Caudal fin	Bluntly rounded or truncate in the middle and rounded at the corners	Rounded	Rounded	Rounded	Rounded	Truncate or rounded-subtruncate, rounder in old specimens
14. Caudal peduncle	Very short, its depth 0.5-0.8 of its depth	Deeper than long	Deeper than long	Much deeper than long	As long as deep or a little deeper than long	As long as deep or a little deeper than long
15. Upper profile of snout	Straight or very slightly concave	Concave	Straight	Convex	Straight	Straight or slightly concave
16. Male genital papilla	Simple	Simple	Simple	Simple	Simple	Simple
17. Breeding behaviour	Substrate spawners	Substrate spawners	Substrate spawners	Substrate spawners	Substrate spawners	Substrate spawners
18. Melanin patterns on the body						
18.a A black opercular spot	Present	Absent	Present	Present	Present	Present

Character	*P. nigrofasciatus*	*T. (H.) buttikoferi*	*T. (P.) mariae*	*T. (C.) rendalli*	*T. (C.) tholloni*	*T. (C.) zillii*
18.b A black spot on the base of anterior soft dorsal rays	Absent	Absent	Present	Present	Present	Present
18.c Dark vertical markings on the caudal fin	Absent	Absent	Absent	Absent	Absent	Absent
18.d Vertical bands or cross-bars and midlateral or dorsal parallel series	6 dark vertical bands of varying intensity, these bearing up to six large and round blotches in series from the first one behind operculum to the last one at the end of caudal peduncle	7-8 distinct black vertical bars, beginning from the first one passing through the eye to the last one on caudal peduncle	7-8 very regular dark cross-bars, beginning from the first one behind operculum to the last one on caudal peduncle	6 rather indistinct dark cross-bars on the body	An interrupted dark band along side the body; rather indistinct dark cross-bars may be present	Usually with 6-8 dark cross bars, distinct or indistinct
19. Biogeography	Throughout the central Congo basin	Western coastal rivers from Ivory Coast to Guinea	Lowland coastal forest and lagoons from Cameroon to Ghana, Ivory Coast and Guinea (Cross River to Volta)	The Lualaba and Katanga system in the middle of Southern Africa, Lakes Tanganyika and Bangweulu and the Zambezi in the east through to the Cunene in the west	Congo, Gabon and Cameroon	West and Central Africa; extending to the East in the Nile system from south to north through to the Levant and Jordan Valley

Table 15.b The character informations of *Sarotherodon* species, *S. melanotheron* and *S. galilaeus*, and four species in the *O. (Oreochromis)*, *O. (O.) niloticus, O. (O.) aureus, O. (O.) spilurus* and *O. (O.) u. hornorum*.

Character	***S. melanotheron***	***S. galilaeus***	***O. (O.) niloticus***	***O. (O.) aureus***	***O. (O.) spilurus***	***O. (O.) u. hornorum***
1. Body depth	37-48.6 %SL	43-56.5 %SL, usually more than 45 (Deep-bodied species)	40-47 %SL, usually over 42	35-49 %SL, usually over 40	37-47.5 %SL	38-45.5 %SL
2. Head length	36-41.5 %SL	32.5-39 %SL	31.5-40.5 %SL	33-37.2 %SL	33.4-38 %SL	30.5-37.7 %SL
3. Eye diameter (compared with the depth of preorbital bone	Less than preorbital depth	A little less to greater than preorbital depth (the bigger size, the smaller eyes)	Greater than preorbital depth in the young, but approximately equal in adult	Greater than preorbital depth in the young, but approximately equal in adult	Greater than preorbital depth or approximately equal	Greater, equal or less than preorbital depth (the bigger size, the smaller eyes)
4. Mouth	Very small, lower jaw 28.8-34 %HL	Small, lower jaw 28-32.5 %HL	Rather small, lower jaw 29.6-35.6 %HL	Rather small, lower jaw 29.5-36.8 %HL, usually 31-35	Moderate, lower jaw 33-43.3 %HL	Lower jaw : ♀ 32.8-36.7 %HL ♂ 36.5-49.7 %HL
5. Jaw teeth (number of rows or series)	3-6 series	4-8 rows	3-5 series	3-5 rows	3 or 4 (5) series	4-7 rows
6. Gill rakers	16 (13-19)	24 (20-25)	21 (19-26)	18-26	16 or 17 (15-19)	21 or 22 (19-26)

Character	*S. melanotheron*	*S. galilaeus*	*O. (O.) niloticus*	*O. (O.) aureus*	*O. (O.) spilurus*	*O. (O.) u. hornorum*
7. Scales						
7.a Cheek (number of rows)	2 series, rarely with 2 or 3 additional scales	in 2 rows	Usually 2, rarely 3	2 or 3 horizontal series	2-3 series	2-3, usually 3 rows
7.b Lateral lines	28 (27-30)	31 (28-32)	32 or 33 (31-34)	32 (30-33)	29-31	30-32
8. Vertebrae	27 (26-28)	29 (27-31)	31 (30-32)	30 (28-31)	29 (28-30)	30 (29 or 30)
9. Dorsal fin						
9.a Spines	XV (XIV-XVII)	XVI (XV-XVII)	XVII (XVI-XVIII)	XVI (XIV-XVII)	XVI (XIV-XVII)	XVII (XVI-XVIII)
9.b Soft rays	11 (10-12)	13 (12-13)	13 (13-14)	12-15	11 (10-13)	12
10. Anal fin						
10.a Spines	III	III	III	III	III, rarely IV	III
10.b Soft rays	9 (8-10)	11 (9-12)	9-11	9-11	9-10	10 or 11 (9-12)
11. Pectoral fins (the fin length compared with length of head)	Greater than length of head	Greater than length of head, usually extending to above vent or spinous anal fin	Greater than length of head	Approximately equal to/or a little greater than length of head	A little greater than length of head	Equal or approximately equal to length of head

Character	*S. melanotheron*	*S. galilaeus*	*O. (O.) niloticus*	*O. (O.) aureus*	*O. (O.) spilurus*	*O. (O.) u. hornorum*
12. Ventral fins	Produced to a short, white filament	Produced to a short, white filament reaching the vent	Not produced to a filament	Minutely produced	Produced, not much	Not produced
13. Caudal fin	Slightly emarginate	Slightly emarginate	Truncate, unless the corners are worn away	Truncate, often with rounded corners	Truncate	Truncate in the young, with two rounded lobes in larger fishes
14. Caudal peduncle	Short, its length less than its depth (l/d = 0.6-0.85)	Short, its length less than its depth (l/d = 0.6-0.9)	Short, its length less than its depth (l/d = 0.5-0.9)	Short, its length less than its depth (l/d usually 0.7, rarely 0.5 or 1.0)	Its length less than its depth or slightly the same (l/d = 0.7-1)	Short, its length less than its depth (l/d = 0.6-0.8)
15. Upper profile of snout	Straight	Straight	Straight	Straight	Straight	Straight
16. Male genital papilla	Small and simple	Small and simple	Shortly and conically developed or bluntly bifid at tip.	Mostly developed with a narrow flange.	Conical or club-shaped, with a subterminal pore, bluntly bifurcate beyond the pore in precociously ripe male.	Large, conical and white, without appendages, the pore rim being red with full capillaries.
17. Breeding behaviour	Paternal mouthbrooders	Biparental mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders

Character	*S. melanotheron*	*S. galilaeus*	*O. (O.) niloticus*	*O. (O.) aureus*	*O. (O.) spilurus*	*O. (O.) u. hornorum*
18. Melanin patterns on the body						
18.a A black opercular spot	Present	Present	Present	Present	Present	Present
18.b A black spot on the base of anterior soft dorsal rays	Absent	Absent	Absent	Absent	Absent	Absent
18.c Dark vertical markings on the caudal fin	Absent	Absent	Present, regular dark vertical stripes on the caudal fin	Caudal may bear vague vertical markings in the young	Small indistinct vertical stripes appeared at the upper half of the caudal	Upper half or two-third of caudal with narrow grey vertical stripes or reticulations on a paler ground
18.d Vertical bands or cross-bars and midlateral or dorsal parallel series	Some individuals have the broken vertical bands common in more western populations, and others a midlateral horizontal series of contiguous blotches. Vertical	Black vertical bars on the body may be formed of a discontinuous array of spots or become continuous or entirely switched off in the same individual.	The basic pattern of dark vertical bars and two horizontal dark bands often represented by two or three midlateral dark blotches, one of which is on the	The young have the usual grey vertical bars on the body. In adults dark vertical bars appear on the body in some emotional states with midlateral blotches on the	With a midlateral series of blotches and a more dorsal parallel series, usually having a dorsal blotch on the caudal peduncle.	2-4 midlateral blotches usually evident; the young with the usual vertical bars on the body.

Character	*S. melanotheron*	*S. galilaeus*	*O. (O.) niloticus*	*O. (O.) aureus*	*O. (O.) spilurus*	*O. (O.) u. hornorum*
18.d Continued	bars appear in juvenile or maybe at higher temperature which is under physiological and emotional control.		top of the caudal peduncle.	bars and a blotch on the top of the caudal peduncle.		
19. Biogeography	Brackish estuaries and lagoons from Zaire to Senegal.	Widely distributed in the West and Central Africa; extending to the East in the Nile system through to the Levant and Jordan Valley.	Widely distributed in the West and Central Africa; extending to the East in the Nile system, Lake Tanganyika and tropical lakes, through to the Levant and Jordan Valley in the Northeast.	Sharing much of the area of distribution with *O. (O.) niloticus* including the West and Central Africa, the Nile system through to the Levant and Jordan Valley	A member of a series of species inhabiting the lower parts of the eastward rivers flowing to the Indian Ocean. Within the group *O. (O.) spilurus* is the northernmost distributing in Ethiopia, Somalia (the Webi Shebeli and Juba) and Kenya.	Being a member of the species of the eastward-flowing rivers, *O. (O.) u. hornorum* inhabits in the Tanzanian rivers.

Table 15.c The character details of another six *O. (Oreochromis)* species, *O. (O.) mossambicus, O. (O.) mortimeri, O. (O.) andersonii, O. (O.) placidus, O. (O.) shiranus* and *O. (O.) jipe*.

Character	*O. (O.) mossambicus*	*O. (O.) mortimeri*	*O. (O.) andersonii*	*O. (O.) placidus*	*O. (O.) shiranus*	*O. (O.) jipe*
1. Body depth	36-49.5 %SL, usually over 40	39-46 %SL, deeper in the big size of male individuals	40.5-50.5 %SL	37.0-53.5 %SL, usually 41-47 %SL	36.5-45 %SL	34.4-40.7 %SL
2. Head length	♀ 32.3-37 %SL ♂ 34-39 %SL	34-39 %SL	32.0-37 %SL	♀ 32.4-34.7 %SL ♂ 32.2-36.8 %SL	32-35.5 %SL	32-36.5 %SL
3. Eye diameter (compared with preorbital depth)	Equal to preorbital depth in the size < 150 mmSL, but slightly less than preorbital depth in the bigger size	The bigger size the smaller eyes; the bigger size the deeper preorbital bone	A little more or less than preorbital depth, reaching equality at approximately 125-130 mmSL (Same as in *O. mortimeri*)	The bigger size the smaller eyes, less than or slightly the same length as preorbital depth, reaching equality at 150 mmSL	The bigger size the smaller eyes, slightly greater than or equal to or less than preorbital depth	About the same length as the depth of preorbital bone
4. Mouth	Moderate, lower jaw 32-45.5 %HL, mature males jaw enlarged	Moderate, lower jaw enlarged in mature males ♀ 32.8-38.5 %HL ♂ 39.0-44.0 %HL	Rather small to moderate, lower jaw 29-42 %HL	Moderate, lower jaw : ♀ 30.5-38.5 %HL ♂ 33.5-42.0 %HL	Moderate, lower jaw 29-44.6 %HL	Small to moderate, lower jaw 28.6-33.4 %HL, jaw not enlarged
5. Jaw teeth (rows or series)	3-5 series	3-5 series	3-8 series	3-6 series	4-5 rows	5-7 series

Character	*O. (O.) mossambicus*	*O. (O.) mortimeri*	*O. (O.) andersonii*	*O. (O.) placidus*	*O. (O.) shiranus*	*O. (O.) jipe*
6. Gill rakers	**17,18 (14-20)**	18 (16-20)	12 (20-27)	18 (16-20)	19 (17-21)	18-22, usually 18-20
7. Scales						
7.a Cheek (number of rows)	**3, occasionally 2**	2-3 rows	3 full rows (2-4)	2 or 3 series	2-3 rows	2
7.b Lateral lines	**31 (30-32)**	31 (30-32)	32 or 33 (31-35)	30-32	31 or 32 (31-33)	34 (33-36)
8. Vertebrae	**30 (28-31)**	29 or 30	31 (30-32)	29 or 30 (29-31)	30	32 or 33
9. Dorsal fin						
9.a Spines	**XVI (XV-XVII)**	XVI or XVII	XVI or XVII (XVI-XVIII)	XVI or XVII (XV-XVIII)	XVI or XVII (XV-XVIII)	XVIII (XVII-XIX)
9.b Soft rays	**12 (10-13)**	12 (10-13)	13 (11-15)	11 or 12	11 or 12 (10-13)	12
10. Anal fin						
10.a Spines	**III**	III	III	IV, rarely III	IV, rarely III	IV, rarely III
10.b Soft rays	**10 or 11 (9-12)**	11 (10-12)	12 (11-13)	10 (8-11)	9-11	11 (10-12)

Character	*O. (O.) mossambicus*	*O. (O.) mortimeri*	*O. (O.) andersonii*	*O. (O.) placidus*	*O. (O.) shiranus*	*O. (O.) jipe*
11. Pectoral fins (the fin length compared with length of head)	The same or a little greater than length of head	The same or a little greater than length of head	The same or a little greater than length of head	Greater or slightly greater than length of head	The same or a little greater than length of head	A little greater than length of head
12. Ventral fins	Minutely produced	Not produced	Minutely produced	Not produced	Not produced	Produced, not much
13. Caudal fin	Truncate, often with rounded corners	Truncate, occasionally with rounded corners	Truncate	Truncate, very slightly emarginate in the young	Truncate with rounded corners	Truncate with rounded corners
14. Caudal peduncle	The length less than or the same as the depth (l/d = 0.7-1)	The length less than the depth (l/d = 0.5-0.8)	The length less than or the same as the depth (l/d = 0.65-1)	The length less than the depth (l/d = 0.5-0.88)	The length slightly less than or equal to the depth (l/d = 0.75-1)	The length slightly less than to greater than the depth (l/d = 0.8-1.1)
15. Upper profile of snout	Straight	Concave	Concave	Straight	Straight	Straight
16. Male genital papilla	Deleloped with a shallow distal notch	Developed	Bluntly conical with a narrow flange slightly notched in the middle	Developed to a simple cone with terminal pore	Not tasselled, but in some individuals with a thick, scalloped narrow flange	Not tasselled, but prominently developed
17. Breeding behaviour	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders

Character	*O. (O.) mossambicus*	*O. (O.) mortimeri*	*O. (O.) andersonii*	*O. (O.) placidus*	*O. (O.) shiranus*	*O. (O.) jipe*
18. Melanin patterns on body						
18.a A black opercular spot	Present	Present	Present	Present, with grey/black markings	Present	Absent
18.b A black spot on the base of anterior soft doral rays	Absent	Absent	Absent	Absent	Absent	Absent
18.c Dark vertical markings on the caudal fin	Absent	Caudal usually with a grey reticulum on proximal two-thirds with pale or iridescent interstices	Absent	Absent	Absent	Caudal with vertical dark bars proximally
18.d Vertical bands or cross-bars and midlateral or dorsal parallel	Females and non-breeding males silvery with 2-5 midlateral blotches and some of a more dorsal series; breeding males black.	Males and females often with 1-3 midlateral dark blotches, which may appear only as the fish dies. Breeding males have predominantly light blue colour.	Non-breeding fish and females always having 3-4 conspicuous mid-lateral blotches. Breeding males masking the lateral blotches with iridescent purplish-brown colour.	Three lateral spots and faint vertical bars appearing in the young under some conditions.	A dark midlateral band may be present, entire or broken. Breeding males masked by the general dark green to black colour.	7-9 vertical bars on the body with a midlateral longitudinal band generally formed by emphasis of spots; mature males masked with green or purple colour sheen on body.

Character	*O. (O.) mossambicus*	*O. (O.) mortimeri*	*O. (O.) andersonii*	*O. (O.) placidus*	*O. (O.) shiranus*	*O. (O.) jipe*
19. Biogeography	Being a member of the series of species of the eastward-flowing rivers, *O. (O.) mossambicus* is the southernmost inhabiting in the Lower Zambezi to Sodwana, Lower Shiré, and Limpopo in South Africa.	Being a member of the species of the eastward-flowing rivers, *O. (O.) mortimeri* replaces *O. (O.) mosambicus* in the Middle Zmabezi.	Continuing the *mossambicus-mortimeri* series in the Upper Zambezi, and extending to the Ngami region and the Cunene in Angola	Being a member of the species of the eastward-flowing rivers, *O. (O.) placidus* is present with *O. (O.) mossambicus*, sharing almost the same habitat.	Being a member of the species of the eastward-flowing rivers, *O. (O.) shiranus* replaces *O. (O.) placidus* in the Upper Shiré, Lakes Malawi and Chilwa.	A member of the Upper Pangani system

Table 15.d The character informations of the other five species in the subgenera *O. (Nyasalapia)* and *O. (Neotilapia)* including one *Nyasalapia* species outside Lake Malawi *O. (Ny.) macrochir* and three Malawi species *O. (Ny.) karongae, O. (Ny.) squamipinnis* and *O. (Ny.) lidole*, and the other one species *O. (Ne.) tanganicae*.

Character	***O. (Ny.) macrochir***	***O. (Ny.) karongae***	***O. (Ny.) squamipinnis***	***O. (Ny.) lidole***	***O. (Ne.) tanganicae***
1. Body depth	42.5-55.7 %SL, a notably deep-bodied fish	35.3-42.8 %SL	36.5-43.7 %SL	35.7-43.1 %SL	43.4-51.1 %SL, deep-bodied fish
2. Head length	31.2-38.4 %SL	30.8-36.0 %SL	35.6-38.6 %SL	35.5-40.5 %SL	31.7-36.0 %SL
3. Eye diameter (compared with preorbital depth)	Less than to slightly greater than preorbital depth	Less than to approximately equal to preorbital depth	Less than to approximately equal to preorbital depth	Less than preorbital depth	The bigger size the smaller eyes, less than preorbital depth in the size of 120 mmSL and over
4. Mouth (length of lower jaw in %HL)	Rather small, lower jaw 27.3-35.8 %HL	Rather small, lower jaw 30-33.7 %HL	Rather small, lower jaw 31.4-34.9 %HL	Rather small to moderate, lower jaw 31.3-39.5 %HL	Rather small, lower jaw 31-38 %HL
5. Jaw teeth (number of rows or series)	4-7 series	4-6 rows	3-5 rows	3-4 rows	Up to 10 irregular rows
6. Gill rakers (number on lower part of the first arch)	Usually 22-24 (22-26) in individuals of 90 mmSL or more	19 or 20 (17-22)	19 or 20 (17-22)	19 or 20 (17-22)	22-26

Character	***O. (Ny.) macrochir***	***O. (Ny.) karongae***	***O. (Ny.) squamipinnis***	***O. (Ny.) lidole***	***O. (Ne.) tanganicae***
7. Scales					
7.a Cheek (number of rows)	2 or 3 rows	3 rows	3 rows	2 rows	3 rows
7.b Lateral lines	Usually 31 or 32	33 or 34 (33-36)	34 (33-35)	34 (32-35)	33 (32-33)
8. Vertebrae	30 (29-31)	32 (31-33)	32 (31 or 32)	31 or 32	31 (30-31)
9. Dorsal fin					
9.a Spines	XVI (XV-XVII)	XVI (XV-XVII)	XVI (XVI-XVII)	XVI (XIV-XVII)	XVI (XV-XVII)
9.b Soft rays	12 or 13	11 (10-11)	11 (10-11)	11	13 (11-15)
10. Anal fin					
10.a Spines	III	III	III	III	III
10.b Soft rays	10 or 11 (9-12)	9 (8-10)	9 (8-10)	9 (8-10)	9
11. Pectoral fins (the fin length compared with length of head)	Longer than head length. The long pectoral fin, which gave its name, reaches to a vertical above some part of the anal fin-base.	Longer than head length	Longer than head length	As long as to longer than head length	Longer than head length, another long pectoral fin fish
12. Ventral fins	Minutely produced	Not produced	Not produced	Not produced	Minutely produced

Character	*O. (Ny.) macrochir*	*O. (Ny.) karongae*	*O. (Ny.) squamipinnis*	*O. (Ny.) lidole*	*O. (Ne.) tanganicae*
13. Caudal fin	Truncate in adults, slightly emargiante in the young	Emarginate, in adults both upper and lower lobes rounded	Emarginate, in adults both upper and lower lobes rounded	Emarginate, in adults both upper and lower lobes rounded	Slightly emarginate
14. Caudal peduncle	Deep and short, its length always less than its depth (l/d = 0.5-0.84)	Long, its length equal to or longer than its depth (l/d = 1.0-1.4)	Long, its length longer than its depth (l/d = 1.05-1.3)	Long, its length always longer than its depth (l/d = 1.36-1.5)	Moderate, its length slightly less than or equal to its depth (l/d = 0.7-1.0)
15. Upper profile of snout	Concave	Straight	Straight	Straight	Straight
16. Male genital papilla	Prolonged into a double tassel which reaches a length of 25 mm or more	Developed to a prominent and bifid tassel with each branch bearing tubercles and filaments	Developed to a prominent and bifid tassel with each branch bearing tubercles and filaments	Developed to a prominent and bifid tassel with each branch bearing tubercles and filaments	Small and simple
17. Breeding behaviour	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders	Maternal mouthbrooders
18. Melanin patterns on body					
18.a A black opercular spot	Present	Present	Present	Present	Present

Character	*O. (Ny.) macrochir*	*O. (Ny.) karongae*	*O. (Ny.) squamipinnis*	*O. (Ny.) lidole*	*O. (Ne.) tanganicae*
18.b A black spot on the base of anterior soft dorsal rays	Absent, only a darker part of whatever pattern (barred or reticulate) decorating the dorsal fin.	Absent	Absent	Absent	Absent
18.c Dark vertical markings on the caudal fin	Absent	Absent	Absent	Absent	Absent, only spotted not striped or reticulate
18.d Vertical bands or cross-bars and midlateral or dorsal parallel series	Silvery with 8-10 dark vertical bars on the body in the young; no midlateral blotches in adult but in some individuals with dark centers on scales giving the effect of longitudinal stripes.	With black vertical bars of unequal length from the dorsum to mid-flanks; masked with black body in breeding male.	Black vertical bars of unequal length on body	Black vertical bars of unequal length on body, with black body in breeding male.	Facultative vertical bands of constant width, not extending to the ventral surface, usually invisible in large preserved fishes.
19. Biogeography	Inhabiting South African lakes i.e Lakes Mweru and Bangweulu in the middle of the southeast, and also distributing in the Upper Zambezi, Okovango and Cunene in the west.	Only in Lake Malawi	Only in Lake Malawi	Only in Lake Malawi	Distributing in Lake Tanganyika, the eastern coastal areas and river mouths.

Table 16. Collected multistate characters of the 23 tilapiine species. The different states coded as sequential numbers with ancestral states being 0, and the character state orders are shown.

Character	States	State order
1. Body depth (% SL)		
35-45 35——45	-1	-1–0–1
40-50 40——50	0	
45-55 45——55	1	
2. Head length (% SL)		
35-40 35——40	0	0–1–2
30-40 30——40	1	
30-35 30——35	2	
3. Eye diameter (compared with the depth of preorbital bone)		
Greater (than the depth of preorbital bone)	0	0–1–2–3–4
Greater -, or, equal	1	
Greater - equal - less	2	
Equal - less	3	
Less	4	
less equal greater		
4. Mouth (length of lower jaw in % head length)		
Large to extra large (35-50) 35——50	-1	-1–0–1–2–3
Large (35-45) 35—45	0	
Moderate to large (30-45) 30——45	1	
Moderate (30-35) 30—35	2	
Small to moderate (25-35) 25——35	3	
5. Jaw teeth (number of rows or series)		
3-5	0	0–1–2
3-8	1	
>8	2	
6. Gill-rakers (number on lower part of the first arch)		
8-16 8——16	0	0–1–2
12-20 12——20	1	
16-24 or more 16——24 or more	2	
7. Scales		
7.a Cheek (number of rows)		
2	-2	-2–-1–0–1–2
2-3	-1	
3	0	
3-4	1	
>4	2	

Character		States	State order
7.b Lateral lines			
27-30	27—30	0	0–1–2–3
27-33	27———33	1	
30-37	30———37	2	
33-37	33—37	3	
8. Vertebrae			
26-28	26—28	0	0–1–2–3
26-30	26———30	1	
28-30	28—30	2	
30-33	30—33	3	
9. Dorsal fin			
9.a Spines			
XIII-XV	13—15	0	0–1–2–3
XIV-XVII	14—17	1	
XV-XVIII	15—18	2	
XVII-XIX	17—19	3	
9.b Soft rays			
8-10	8——10	-1	-1–0–1–2
10-12	10—12	0	
12-15	12—15	1	
15-16	15—16	2	
10. Anal fin			
10.a Spines			
III		0	0–1–2
III, rarely IV		1	
IV, rarely III		2	
10.b Soft rays			
7-11	7———11	-1	-1–0–1
9-11	9——11	0	
9-13	9———13	1	
11. Pectoral fin (its length compared with length of head)			
A little shorter to as long as, or approximately as long as		**0**	**0–1–2**
As long as, or approximately as long as, to a little longer		**1**	
Longer		**2**	

Character	States	State order
12. Ventral fin		
Produced into a filament	0	0–1–2–3
Produced into a short, white filament	1	
Minutely produced	2	
Not produced	3	
13. Caudal fin		
Rounded	-1	-1–0–1–2
Bluntly rounded or truncate in the middle with rounded corners	0	
Truncate or slightly emarginate	1	
Emarginate in the middle with two rounded or oval lobes	2	
14. Caudal peduncle		
Short l/d (length/depth) < 1	0	0–1–2–3
Short to moderate l/d ≤ 1	1	
Moderate to long l/d ≥ 1	2	
Long l/d > 1	3	
15. Upper profile of snout		
Convex	-1	-1–0–1
Straight or very slightly concave	0	
Concave	1	
16. Male genital papilla		
Simple	0	0–1–2
Developed but not tasselled	1	
Developed to an elongate tassel in mature fish	2	
17. Breeding behaviour		
Substrate spawners; larvae having three pairs of adhesive glands on the top of head	0	0–1–2
Paternal or biparental mouth brooders; larvae having vestigial adhesive glands	1	
Maternal mouth brooders; no adhesive glands on larvae	2	
18. Melanin patterns on the body		
18.a A black opercular spot		
Present in adult	0	0–1
Absent in adult	1	
18.b A black spot on the base of anterior soft dorsal rays		
Absent in adult	0	0–1
Present in adult	1	

Character	States	State order
18.c Dark vertical markings on the caudal fin		
Absent	0	0–1–2–3
Present in the young, absent in adult	1	
Present in adult, rather distinct or just a reticulum only at the proximal part of the caudal	2	
Present in adult, regular dark vertical stripes on the caudal	3	
18.d Vertical bands or cross-bars, and midlateral or dorsal parallel series of blotches on the body		
Dark vertical bars or 1-2 horizontal dark bands represented by some midlateral dark blotches, or maybe dark vertical bars presented only in the young or in some emotional states	-1	-1–0–1–2
Dark vertical bars of varying intensity, these bearing up to six, or more, large and round blotches in series from the first one behind operculum to the last one at the end of caudal peduncle	0	
Distinct black vertical bars, beginning from the first one behind operculum to the last one on caudal peduncle	1	
Distinct black vertical bars, beginning from the first one passing through the eye to the last one on caudal peduncle	2	
19. Biogeography		
The Congo and Zaire in the South-Western Africa (Centre of the Western Africa)	0	4 | 0–2–3–5–6 | | 1 7–8
Distributing in the Lualaba system in the middle, Lakes Tanganyika and Bangweulu and the Zambezi in the east, through to the Cunene in the west of Southern Africa. Except the eastward rivers flowing to the Indian Ocean in the north of Zambezi	1	
West and West Coast of Africa from Zaire to Senegal	2	
West and Central Africa; extending to the East in the Nile system from south to north through to the Levant and Jordan Valley	3	
Inhabiting in Lake Tankanyika, the coastal areas and river mouths	4	
Distributing in the southern eastward rivers flowing to the Indian Ocean from Somalia in the north through the Zambezi to Cunene in the west and to the Limpopo in South Africa	5	
Inhabiting in the Upper Pangani system	6	
Distributing from Lakes Mweru and Bangweulu in the middle to the Upper Zambezi, Okavango and Cunene in the west of Southern Africa	7	
Restricted to Lake Malawi	8	

character state order of each of the multistate characters collected are presented in Table 16. All the character states have been scored as sequential numbers with ancestral states being 0, and ordered in linear series, except the biogeographical character states (character 19) that have been branch ordered. The size and shape of the mouth, e.g. the enlarged lower jaw in mature males of some *Oreochromis* species (character 4, Table 15.c), has been widely used as a character for species identification. It was not possible to obtain this data for all species in a quantitative way. So a qualitative state was generated from variable sources to ensure its inclusion in the data set. Similarly in some other species eye size was effectively a continuous character, negatively related to the body size (character 3 in Tables 15.b, 15.c, 15.d). So a variety of states of eye diameter compared with the depth of preorbital bone (<, ≤, <=>, ≥, >) was generated (character 3 in Table 16).

The branched order of the biogeographical character recognises eight different area of distribution states in a branched series as shown in Table 16. The order begins with the area of the outgroup distribution, which is the Congo and Zaire (the centre) in South-West Africa, as the ancestral state 0. From the ancestral state (0) two different states of distribution (1 & 2) spread out in opposite directions, state (1) spreads southward from the Lualaba system to the Cunene in the west of Southern Africa without extending into the rivers flowing eastward into the Indian Ocean to the north of Zambezi, and the other (state 2) spreading northwards to the West and West Coast of Africa from Zaire to Senegal. State 3 is continuous with state 2 widening the area of distribution in the West and Central Africa with extentions to the East and the Nile system through to the Levant and Jordan Valley. This area now becomes a focus for other expansions of the distribution. One is the single state 4 in Lake

Tankanyika, its coastal areas and river mouths. States 5 and 6 are in East Africa, with state 5 including the southern eastward rivers flowing to the Indian Ocean from Somalia in the north through the Zambezi, Cunene in the west and to Limpopo in South Africa. State 6 includes the rivers and lakes in the Upper Pangani system. The last two groups in states 7 and 8 are Lakes Mweru and Bangweulu in the middle to the Upper Zambezi, Okavango and Cunene in the west of Southern Africa, and Lake Malawi to the north respectively.

The different states of each multitistate character collected for the 23 tilapiine species are shown in Table 17, and the new binary character data are shown in the Table 18. These multistate characters have been recoded into binary characters which were used in the dendrogram construction. [Comparative dendrograms were constructed both using and not using the biogeographical data.]

3.2.2 Tilapiine Interrelationships from the Dendrogram Constructed Using Specific Characters

The dendrogram constructed from multistate characters of 23 tilapiine species with the inclusion of morphology and weighted breeding and biogeographical data is shown by Fig. 18, with the inclusion of morphology and unweighted breeding characters (excluding biogeography) by Fig. 19, and with the inclusion of morphological data only (excluding breeding behaviour and biogeography) by Fig. 20. Three main groupings of tilapiine congeneric species which correspond to the three main genera, *Tilapia*, *Sorotherodon* and *Oreochromis*, could be seen in the dendrograms (Figs. 18 & 19) produced from the data set that contained breeding behaviour data. Three possible implications from this result could be derived: 1) breeding behaviour is the real generically

Table 17. Different states of each multistate character in the 23 tilapiine species. The character numbers represent the multistate characters as same order as in Tables 15 and 16.

Species	Character states of each multistate character (characters represented by nos.)																							
	1	2	3	4	5	6	7a	7b	8	9a	9b	10a	10b	11	12	13	14	15	16	17	18a	18b	18c	18d	19
1. *P. nigrofasciatus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2. *T. (H.) buttikoferi*	1	1	1	0	0	0	2	1	0	0	2	0	0	0	0	-1	0	1	0	0	1	0	0	2	2
3. *T. (P.) mariae*	1	0	0	3	0	0	1	2	1	1	1	0	0	0	0	-1	0	0	0	0	0	1	0	1	2
4. *T. (C.) rendalli*	0	2	1	3	0	0	1	2	1	1	1	0	0	1	0	-1	0	-1	0	0	0	1	0	0	1
5. *T. (C.) tholloni*	0	0	1	0	0	0	1	1	?	1	-1	0	-1	0	3	-1	1	0	0	0	0	1	0	-1	0
6. *T. (C.) zillii*	0	1	4	0	1	0	1	2	1	1	0	0	-1	0	3	0	1	0	0	0	0	1	0	0	3
7. *S. melanotheron*	0	0	4	3	1	1	-2	0	0	1	0	0	-1	2	1	1	0	0	0	1	0	0	0	-1	2
8. *S. galilaeus*	1	1	2	3	1	2	-2	1	1	1	1	0	1	2	1	1	0	0	0	1	0	0	0	-1	3
9. *O. (O.) niloticus*	0	1	1	2	0	2	-1	2	3	2	1	0	0	2	3	0	0	0	1	2	0	0	3	-1	3
10. *O. (O.) aureus*	0	1	1	2	0	2	-1	2	2	1	1	0	0	1	2	0	0	0	1	2	0	0	1	-1	3
11. *O. (O.) spilurus*	-1	1	1	1	0	1	-1	1	2	1	0	1	0	1	2	0	1	0	1	2	0	0	2	-1	5
12. *O. (O.) u. hornorum*	-1	1	2	-1	1	2	-1	2	2	2	1	0	1	1	3	2	0	0	1	2	0	0	2	-1	5
13. *O. (O.) mossambicus*	0	1	3	1	0	1	-1	2	2	1	0	0	1	1	2	0	1	0	1	2	0	0	0	-1	5
14. *O. (O.) mortimeri*	-1	0	2	1	0	2	-1	2	2	1	0	0	1	1	3	0	0	1	1	2	0	0	2	-1	5
15. *O. (O.) andersonii*	0	1	2	1	1	2	0	2	3	2	1	0	1	1	2	0	1	1	1	2	0	0	0	-1	5
16. *O. (O.) placidus*	0	2	2	1	1	2	-1	2	2	2	0	2	-1	2	3	1	0	0	1	2	0	0	0	-1	5
17. *O. (O.) shiranus*	-1	2	2	1	0	2	-1	2	3	2	0	2	0	1	3	0	1	0	1	2	0	0	0	-1	5
18. *O. (O.) jipe*	-1	2	1	3	1	2	-2	3	3	3	1	2	1	1	2	0	1	0	1	2	1	0	2	-1	6
19. *O. (Ny.) macrochir*	1	1	2	3	1	2	-1	2	2	1	1	0	1	2	2	1	0	1	2	2	0	0	0	-1	7
20. *O. (Ny.) karongae*	-1	2	3	2	1	2	0	3	3	1	0	0	-1	2	3	2	2	0	2	2	0	0	0	-1	8
21. *O. (Ny.) squamipinnis*	-1	0	3	2	0	2	0	3	3	1	0	0	-1	2	3	2	2	0	2	2	0	0	0	-1	8
22. *O. (Ny.) lidole*	-1	0	4	1	0	2	-2	3	3	1	0	0	-1	1	3	2	3	0	2	2	0	0	0	-1	8
23. *O. (Ne.) tanganicae*	1	2	3	1	2	2	0	2	3	1	1	0	0	2	2	1	1	0	0	2	0	0	0	-1	4

Table 18. Binary characters of the 23 tilapiine species recoded from the Table 17 with the character states being ordered as shown in Table 16. The species are represented by numbers as same numerical order as in the Table 17. The factors in the first column indicate the correspondences of which characters in the Table 17 have been recoded into the new binary characters.

Factors	New binary recoded characters of the 23 tilapiine species (species represented by nos.)																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	1	1	1	0
2	0	1	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1
2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	1
3	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1
3	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1
3	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
4	0	0	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
4	0	0	1	1	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0
4	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
5	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	0	1	1	1	0	0	1
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
6	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
7a	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	0
7a	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
7a	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7a	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7b	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7b	0	0	1	1	0	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
7b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0
8	0	0	1	1	?	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	0	0	0	0	?	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	0	0	0	0	?	0	0	0	1	0	0	0	0	0	1	0	1	1	0	1	1	1	1
9a	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9a	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	1	0	0	0	0	0
9a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
9b	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9b	0	1	1	1	0	0	0	1	1	1	0	1	0	0	1	0	0	1	1	0	0	0	1
9b	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
10a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
10a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
10b	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0

Factors	New binary recoded characters of the 23 tilapiine species (species represented by nos.)																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
10b	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	1	1	0	0	0	0
11	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	1
12	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	0	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	0	0	0	0	1	1	0	0	1	0	0	1	0	1	0	1	1	0	0	1	1	1	0
13	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	1	1	1	1
13	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0
14	0	0	0	0	1	1	0	0	0	0	1	0	1	0	1	0	1	1	0	1	1	1	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
15	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0
16	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
17	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18a	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
18b	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18c	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0
18c	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0
18c	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18d	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18d	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18d	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
19	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0

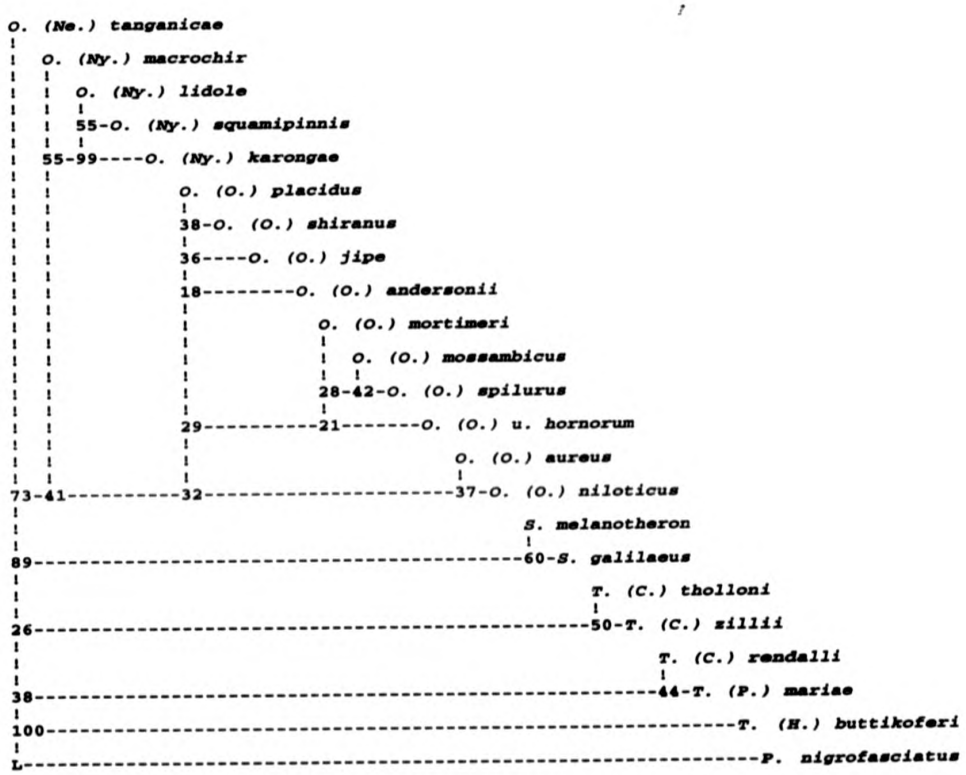

Fig. 18 Dendrogram consructed from multistate characters of 23 tilapiine species. The numbers at the forks indicate the number of times the group consisting of the species above and to the right of that fork occurred among the 100 bootstrap replicates.

The data used include morphology and weighted breeding and biogeographical character data

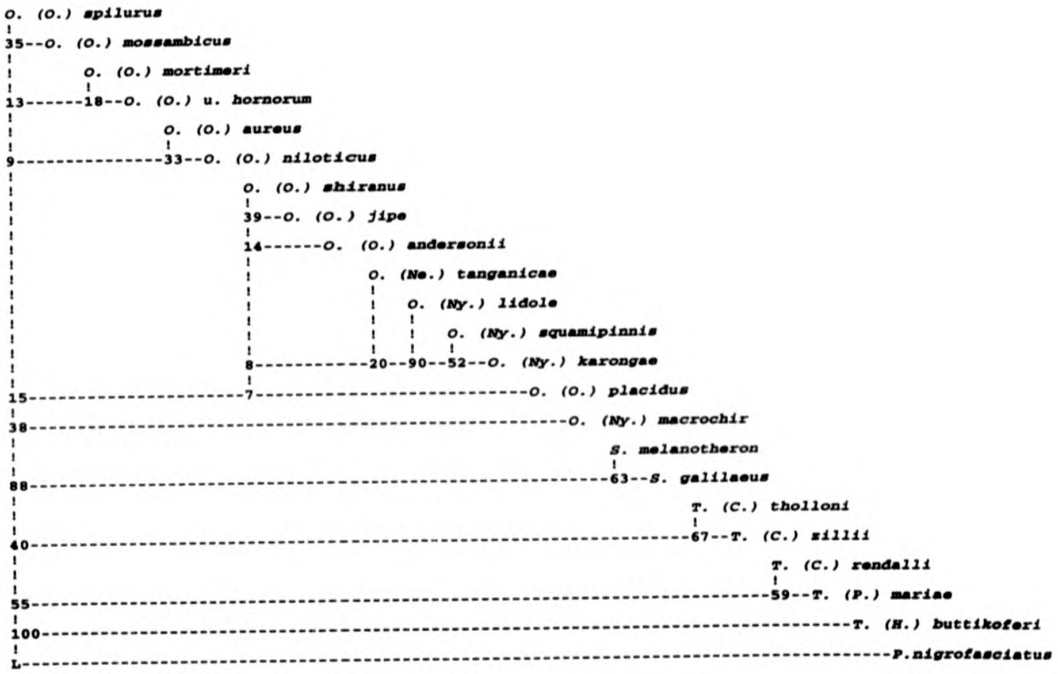

Fig. 19 Dendrogram consructed from multistate characters of 23 tilapiine species. The numbers at the forks indicate the number of times the group consisting of the species above and to the right of that fork occurred among the 100 bootstrap replicates.

The data used include morphology and unweighted breeding character data, but excluding biogeography.

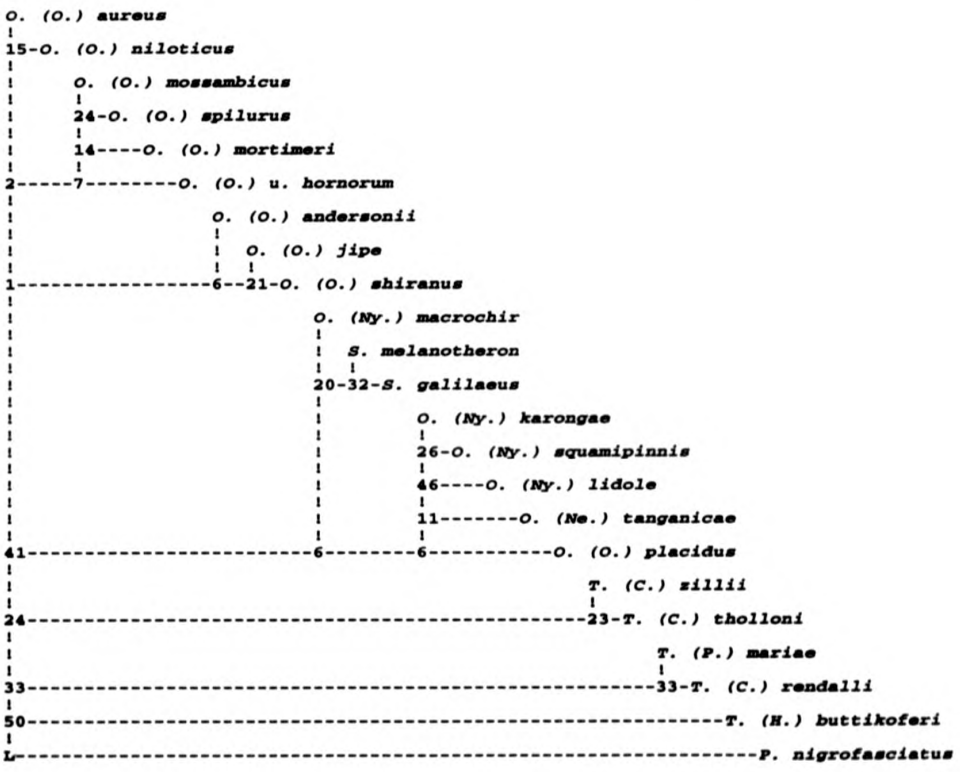

Fig. 20 Dendrogram consructed from multistate characters of 23 tilapiine species. The numbers at the forks indicate the number of times the group consisting of the species above and to the right of that fork occurred among the 50 bootstrap replicates.

The data used include morphological data only, excluding biogeography and breeding behaviour.

informative character to discriminate tilapia into different genera, so its information is needed for the phylogenetic classification at the generic level of these species. 2) breeding behaviour may not be the real generically discriminating character but the number of other morphological characters used were not enough to establish their divergence as an ideally congeneric phylogeny. 3) the two mouthbrooding genera, *Sarotherodon* and *Oreochromis*, may not be so divergent that they could be separated as different genera.

GENUS *TILAPIA*

All *Tilapia* species have been grouped together next to the outgroup species *P. nigrofasciatus*, which again supports the general idea of Peters & Berns (1978, 1982) and Trewavas (1980, 1982b) that the substrate spawners gave rise to the mouthbrooding genera. The close relationship between the sister-species *T. (C.) tholloni* and *T. (C.) zillii* is seen in this analysis as well as the previous allozyme based dendrogram, however the bootstrap confident level shown for this clade in this analysis is not very high. The grouping of *T. (C.) rendalli* with *T. (P.) mariae* in this analysis was unexpected considering the previous allozyme results, however it was unsupported by the bootstrap value. This may suggest that some important subgeneric characters may have been omitted for the *T. (Coptodon)* in this analysis. The results in this analysis suggest that the evolution of *Tilapia* seems to be paraphyletic, as suggested by the allozyme results (Fig. 11).

GENUS *SAROTHERODON*

From the substrate spawners a large monophyletic group of the mouthbrooding genera is exhibited (all dendrograms). Within this group, as mentioned above, two main clades of species, *Sarotherodon* and *Oreochromis*,

would be congenerically branched, if the breeding behaviour data were included. However, the clustering together as a clade of *Sarotherodon* congeneric species can be seen in all dendrograms.

GENUS *OREOCHROMIS*

On the genus *Oreochromis* branch, the species have been congenerically grouped together in all dendrograms, although the bootstrap values shown for the branch are not very high in Fig. 19. The *O. (Nyasalapia)* species have been grouped such that the three chambo species of Lake Malawi are more closely grouped to each other than they are to *O. (Ny.) macrochir*. Within the subgenus *O. (Oreochromis)* two major clades (the eastern *mossambicus* members and the *O. (O.) niloticus & O. (O.) aureus*) can be observed, but again the bootstrap value shown cannot support their relationships.

***Oreochromis* Intersubgeneric Relationships** The overall relationship among the three subgenera [*O. (Oreochromis), O. (Nyasalapia)* and *O. (Neotilapia)*] within the genus *Oreochromis* is shown by the dendrograms in Figs. 19 & 20 as being subgenerically similar. Fig. 18 shows that they could be subgenerically separated if breeding behaviour and biogeography are taken into account. Morphologically the relationship of *O. (Neotilapia)* to other tilapias is still uncertain. Trewavas (1983) included the single species of the *Neotilapia* into the genus *Oreochromis*, based on the fact that it is a maternal mouthbrooder. Other details on reproductive and parental habits are unknown, yet she retained its subgeneric status.

***O. (Oreochromis)* Interspecific Relationships** The close relationship between *O. (O.) niloticus* and *O. (O.) aureus* observed by all

dendrograms supports one of the two hypotheses of Trewavas (1983) that they are sympatric sister-species sharing characters in which both differ from the eastern species by having a narrow preorbital bone and by non-enlargement of the jaws in mature fishes. The other hypothesis that *O.'(O.) niloticus* is more closely related to *O. (O.) esculentus* (a Lake Victoria endemic), and *O. (O.) aureus* is more closely related to the eastern species than they are to each other based on the sexual dichromatism still has not been fully tested until sample of *O. (O.) esculentus* can be analysed.

The relationships of the other *O. (Oreochromis)* species are poorly resolved. Use of biogeographic data supports Trewavas's idea of an East African '*mossambicus* complex', but the biogeographic coding is itself influenced by Trewavas's evolutionary ideas and thus cannot be an independent test of these same theories.

Both molecular and non-molecular analyses consistently support Trewavas's (1973, 1980) idea that mouthbrooders arose but once from a substrate brooding *Tilapia* ancestor, although incomplete information about some lineages (e.g. the three smaller mouthbrooding genera) means that this must still be accepted with reservation. The two *Sarotherodon* species studied are clearly closely related, but there are conflicting indications about the position of the clade in relation to *Oreochromis*.

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CHAPTER 5

SUMMARY & GENERAL DISCUSSION

The studies presented in this thesis are mainly aimed at the genetics and systematics of the tilapiines. Phylogenetically 22 species in the three major genera, *Tilapia*, *Sarotherodon* and *Oreochromis*, were studied using both molecular and morphological approaches in order to maximize the systematic information and provide a comprehensive view of biotic evolution. The followings are a summary of the main results obtained from these studies.

I. Tilapiine Species Identification Based on Allozymes

Of the 43 enzyme loci examined, 37 loci have shown allelic differences between the 22 tilapiine species studied. All enzyme banding patterns have been described. The 37 enzyme loci have been interpreted as genetic markers, based on the allozymic differences, for the 22 tilapiine species (Tables 4 and 5, Chapter 2). These markers are of great potential to all tilapia aquaculture and fisheries workers. They can be used as an unequivocal aid to species identification in both pure and hybrid populations of wild and cultured stocks, an aid to assess levels of genetic variation in exploited populations and as genetic markers for various experimental and genetic manipulations. Among these 37 loci, the intergeneric, intersubgeneric and interspecific discriminating loci have been indicated and recorded respectively at the different taxonomic levels.

II. Heterozygosities & Genetic Differentiation of the Tilapiines

Estimated heterozygosities (*He*) of the tilapiines have fallen into two groups: the group of the low level, or relatively low, heterozygosities (≤ 0.08) which include much of the riverine species, and the high level heterozygosities (≥ 0.1) found in the Lake Malawi chambo (Table 6, Chapter 2). The differences between *He* in the chambo and other tilapias is probably due to the lacustrine environment which has remained stable for long periods, and so has not caused any serious bottlenecks in the populations of the chambo which has resulted in the gradual accumulation of genetic variation within these species. Speciation is usually accompanied by a reduction of variation. This does not appear to have been the case in the chambo, possibly suggesting that the founder populations of each species remained relatively large. This has to be seen in contrast to the severe bottlenecking in many savannah rivers and lakes. Present droughts in southern Africa are an example - Lake Malombe dried up about 100 years ago. The next three highest *He* also came from lake populations - 0.086 in *O. (O.) shiranus* (Lake Malawi), 0.081 in *O. (O.) niloticus* (Lake Manzala) and 0.078 in *O. (O.) jipe* (Lake Jipe). A more detailed analysis of lacustrine and riverine populations would be of great interest, as it would provide obvious implications for the selection of fish stocks for aquaculture.

The fixation indices F_{ST}, F-statistics (Wright, 1978), observed at different tilapiine taxonomic levels (Table 8, Chapter 2) indicate that there is little genetic differentiation among the chambo species, but a large amount among the other tilapiines. The implication is that speciation in the chambo species within Lake Malawi was recent, and the other species outwith the lake evolved allopatrically.

This is clearly shown in the groupings of *O. (O.) mossambicus* & *O. (O.) mortimeri* and *O. (O.) shiranus* & *O. (O.) placidus* (Figs. 10 & 11, Chapter 3) which are allopatric but with contiguous ranges and probably diverged after geographic isolation.

III. Tilapiine Genetic Distances & Relationships

The genetic distances (Cavalli-Sforza & Edwards, 1967; Nei, 1978) calculated for the tilapiines (Table 11, Chapter 3), generally show that the substrate spawners *Tilapia* are more distantly related to the two mouthbrooding genera *Sarotherodon* and *Oreochromis*, which are more closely related to each other. The *Sarotherodon* and *Oreochromis* split shown by dendrograms in Chapter 3 is not clearly resolved: it is weakly supported by the bootstrap value in Fig. 11, unseparated in Figs. 8 & 9, and very close in Fig. 10. Some of the genetic distances between *Sarotherodon* and *Oreochromis* are actually very small e.g. *S. galilaeus* and *O. niloticus*. However, the intra-generic distances averaged within the *Sarotherodon* and *Oreochromis* are less than the averaged inter-generic distance between them, which may suggest that generally most congeneric species in the *Sarotherodon* and the *Oreochromis* are rather more closely related to the species of their own genus than they are to the species in the other genus.

The chambo species show a very much closer genetic relationship to each other (Table 10, Chapter 3) than they do to the other *Nyasalapia, O. (Ny.) macrochir* (Table 9, Chapter 3). Morphologically (Trewavas, 1983) and electrophoretically (Tables 4 and 5, Chapter 2) *O. (Ny.) macrochir* has been shown to have affinities to some species in the subgenus *O. (Oreochromis).*

However, the averaged intra-subgeneric distance within the *O. (Nyasalapia)* is less than the averaged inter-subgeneric distance between the *O. (Nyasalapia)* and *O. (Oreochromis)* (Table 10, Chapter 3). This suggests that some *O. (Oreochromis)* species are very distant from the *O. (Nyasalapia)*.

IV. Appropriate Algorithmic Method for Use with the Morphological and Molecular Data

In this study a number of analytical approaches have been used. The UPGMA (Sneath & Sokal, 1973) and maximum likelihood (Felsenstein, 1981) methods have been included for historic reasons of comparisons. However, Hillis (1987) has shown that these rate-dependent methods are unlikely to give reliable relationships because many of their underlying assumptions could not be met from allozyme data. The assumption free methods, such as the distance Wagner (Farris, 1972) and Wagner parsimony (Eck & Dayhoff, 1966; Kluge & Farris, 1969; Farris, 1970), are suggested to be more appropriate, and in this particular study can be used equally well on molecular and morphological data sets. Any conflicts in the dendrograms produced under these circumstances are much more likely to be real and consensus or combination methods can be adopted to reconcile any differences.

V. Systematic Results from the Two Approaches

The tilapiine phylogenies resulting from the two studies (molecular and morphological approaches) have generally shown the three major groupings

which coincide with the three separate genera (Figs. 10 & 11, Chapter 3; Figs. 18 & 19, Chapter 4) of the Trewavas's (1973, 1982a, 1983) classification. Some minor rearrangements were observed between the two studies, however they were real conflicts and amenable to be reconciled. Consensus and combination techniques have been suggested for reconciling conflicts between molecular and morphological studies (Hillis, 1987). Combination techniques emphasize descriptive power and global parsimony (Miyamoto, 1985), whereas consensus techniques emphasize stability and common information among multiple data sets (Hillis, 1987). In this study the consensus tree program carrying the strict and majority consensus technique (Margush & McMorris, 1981) available in the PHYLIP package (Felsenstein, 1990) was used. A consensus dendrogram (Fig. 21) of the two tilapiine phylogenies (Fig. 11, Chapter 3; and Figs. 18, 19 and 20, Chapter 4) was obtained.

RESOLVED PHYLOGENETIC RESULTS

As shown by the consensus dendrogram (Fig. 21), both the morphological and molecular data sets give the same major groupings which also support the generic and subgeneric divisions of the tilapiines. Within these groups of congeneric and consubgeneric species, the following phylogenetic data conflicts between the morphological and molecular studies are resolved; the common information between the two studies are summarized and retained in the consensus dendrogram.

a) The group of *Tilapia* species Both studies showed the *Tilapia* species congenerically grouped together. However the interspecific relationships between species within the group were shown differently between the two studies. Summarizing the information common to both studies, the consensus dendrogram

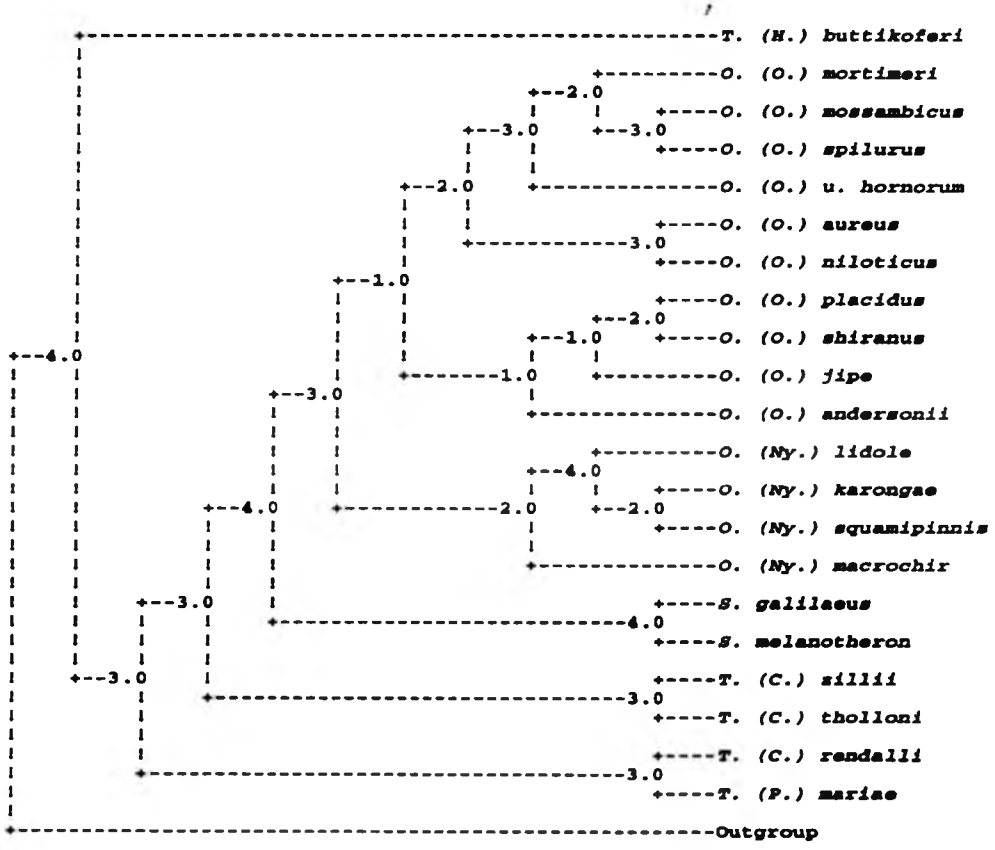

Fig. 21 CONSENSUS TREE produced by the PHYLIP (Felsenstein, 1990), following the technique of Margush & McMorris (1981). The numbers at the forks indicate the number of times the group consisting of the species which are to the right of that fork occurred among the trees, out of 4 trees.

resolves this group in favour of the morphological information which grouped *T. (P.) mariae* with *T. (C.) rendalli*. However, this relationship is still unconfirmed as long as more species in the subgenus *T. (Pelmatolapia)* have not been tested.

b) The group of *Sarotherodon* species Two Sarotherodon species *S. melanotheron* and *S. galilaeus* are resolved in favour of the molecular information as a separate clade congenerically grouped together.

c) The group of *Oreochromis* species Excluding *O. (Ne.) tanganicae*, the intersubgeneric relationship between the other two *Oreochromis* subgenera [the *O. (Oreochromis)* and *O. (Nyasalapia)*] is resolved in favour of the molecular information as two separate monophyletic groups, each of which consists of its own consubgeneric species.

d) Small groups within the subgenus *O. (Oreochromis)* Within the subgenus *O. (Oreochromis)*, the conflicts of the different minor rearrangements between the two studies are reconciled. The three small groupings each of which resulted differently from the molecular and morphological data sets are resolved by the consensus dendrogram:

The grouping of *O. (O.) placidus* and *O. (O.) shiranus*
(resolved in favour of the molecular information)

The grouping of *O. (O.) niloticus* and *O. (O.) aureus*
(resolved in favour of the morphological information)

The grouping of *O. (O.) spilurus*, *O. (O.) mossambicus*, *O. (O.) mortimeri* and *O. (O.) u. hornorum*
(resolved in favour of the morphological information)

However it must be noted that the groupings of these species, as well as the other *O. (Oreochromis)* species, are still uncertain because of the incomplete sampling of all possible intermediate species. Further work needs to be undertaken on a

few more relevant consubgeneric species to confirm these groupings. *O. (O.) esculentus* and *O. (O.) pangani*, as the *O. (O.) niloticus* and *O. (O.) jipe* close relatives, and *O. (O.) korogwe*, as the Lower Pangani species which links members of the Upper Pangani and the '*mossambicus* complex', should be obtained for analysis to finalize these relationships.

VI. The Chambo and Lake Malawi

A high level of genetic integrity is apparent in the chambo. The electrophoretic results showed no loci fixed for alternate alleles and that the three species shared the same 10 polymorphic loci (Table 4, Chapter 2). Morphologically they share a number of unique characters not found in other tilapiines (Trewavas, 1983). It is therefore very difficult to distinguish among these three species using either morphology or allozyme electrophoresis. This fits with the results obtained from mtDNA analysis by Kornfield (pers. comm.). With a number of 20+ restriction endonucleases used, he could find only two discriminating restriction enzymes (*Apa*I and *Ava*II) between *O. (Ny.) lidole* and *O. (Ny.) squamipinnis*, and in a phenotype of many bands only one band distinguished the two species.

Lake Malawi is probably better known for the work that has been done on the many haplochromines. These species have been extensively studied using biological, behavioural, coloural, allozyme, mtDNA and gene sequencing informations (McKaye *et al.*, 1982, 1984; Reinthal *et al.*, 1989; McElroy & Kornfield, 1990; Meyer *et al.*, 1990; Klein *et al.*, 1993). The evidence from all

of these studies is that there has been extensive adaptive radiation which has resulted in many colour variants and adaptions to various feeding structures and habitats within the lake but very little underlying genetic variation between the species. This is presumed to have been the result of 'explosive' adaptive radiation from a common ancestor within the lake. In contrast the evidence from the chambo suggest that they have not undergone such rapid evolution. The evidence is:

1) The number of the chambo species is very small (Trewavas, 1983; Turner & Robinson, 1991; Turner *et al.*, 1991) compared with the several hundreds of species of haplochromines (Ribbink, 1984) in the lake.

2) The allozyme data with the high heterozygosity levels suggest that they have maintained as a large population with no severe bottlenecking to reduce their total genetic variation which is unusual in speciation event. The large level of genetic variation shown in the chambo appears to be equally shared by all species, and has not been partitioned as has happened in the haplochromines (McKaye *et al.*, 1982, 1984; Sage *et al.*, 1984).

3) The haplochromines show variation in colour (McKaye *et al.*, 1984) and feeding specialization (Reinthal *et al.*, 1989), and consequently they are thought to have evolved in small isolated areas within the lake. These ecological specializations (which usually are the main factors of the rapid evolution, Greenwood, 1981; Witte, 1984) should not have happened in the chambo as they are relatively large pelagic species that can move around the lake, so the spatial isolation is not possible. Differences between the chambo are in depth of

spawning area and spawning colouration (Lowe, 1953) which probably occurred because of isolation caused by changes in lake level (Owen *et al.*, 1990). However, this obviously was not a major bottleneck as much of the underlying variation is still found in all species.

The evidence from allozyme data shows that they are reproductively isolated (large random mating populations with significant differences in allele frequencies at the same locus, Appendix 3), at least in the south of lake, but any isolation will have been relatively recent. They share many uniform but unique morphological features and at least 10 different polymorphic loci, the very low fixation index F_{ST}, the very small genetic distance, and the short branch lengths on the tree suggest that they have undergone recent intralacustrine sympatric evolution from a common ancestor. The geological age of the lake has been estimated at between 1-2 million years (Greenwood, 1984). The age of the haplochromine flock based on gene sequence data was estimated to be about 700,000 years (Meyer *et al.*, 1990). The age of the chambo divergence based on the allozyme data in this study is estimated to be only 100,000 - 250,000 years (despite using the largest proportionality constant $k = 18 \times 10^6$ of Maxson & Wilson, 1974, and the Nei distances of the three chambo from Table 10 Chapter 3 in the equation of time since divergence $t = kD$ of Nei, 1987). This means that the founding event of the chambo lineage is very recent.

Further molecular studies of the chambo using a range of techniques including allozymes, RFLP (restriction fragment length polymorphism) of mtDNA and possibly sequencing would give us a clearer idea of the evolution of this group. More information would also be benificial for our understanding

of the population structure of this important fishery resource, and the link, if any, between northern and southern populations. Morphologically variations in jaw dentition, pharyngeal bone proportions and body shape of *O. (Ny.) karongae* along the lake (Turner, pers. comm.) suggest that isolated populations of this species at least are probably present in Lake Malawi.

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APPENDIX 1

ELECTROPHORETIC BUFFERS & BIOCHEMICAL STAINING RECIPES

1. Buffer solutions used in electrophoresis

AMINE-CITRATE (MORPHOLINE) pH 6.5 (AC)

Stock solution :

(0.04 *M*) citric acid monohydrate	8.4 g/l

Adjust to the desired pH (6.5) by adding *N*-(3-aminopropyl)-morpholine before making up volume.

Electrode : Undiluted stock solution
Gel : 1:20 dilution of stock solution

TRIS-CITRATE/LITHIUM-BORATE pH 8.5 (TCB)

Stock solution A :

(0.3 *M*) boric acid	18.55 g/l
(0.1 *M*) lithium hydroxide	8.4 g/l

Stock solution B :

(0.076 *M*) Tris	9.21 g/l
(0.005 *M*) citric acid	1.05 g/l
(0.015 *M*) boric acid	0.93 g/l
(0.005 *M*) lithium hydroxide	0.21 g/l

Adjust to the desired pH (8.5) before making up volume

Electrode : Undiluted stock solution A
Gel : Undiluted stock solution B

TRIS-CITRATE pH 8 (TC)

Stock solution :	
(0.250 *M*) Tris	30.29 g/l
(0.075 *M*) citric acid	15.76 g/l

Adjust to the desired pH (8) before making up volume

Electrode : Undiluted stock solution
Gel : 1:25 dilution of stock solution

TRIS-BORATE-EDTA pH 8.5 (TBE)

Stock solution :	
(0.500 *M*) Tris	60.57 g/l
(0.240 *M*) boric acid	15.00 g/l
(0.016 *M*) EDTA	5.99 g/l

Adjust to the desired pH (8.5) before making up volume

Electrode : Undiluted stock solution
Gel : 1:10 dilution of stock solution

NOTE: Except the Amine-citrate, all electrode buffers were adjusted to desired pH with HCL or NaOH.

2. The staining recipes for various enzymes

AAT (Aspartate aminotransferase, EC 2.6.1.1) (Dimer)

Tris	**300 mg**
L-Aspartic acid	**65 mg**
α-Ketoglutaric acid	**20 mg**
Pyridoxal-5-phosphate (P5P)	**10 mg**
Polyvinylpyrrlidone (PVP)	**10 mg**
Fast blue RR salt	**25 mg**

Dissolve in 25 ml distilled water, and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 20-30 mins.

ACP (Acid phospatase, EC 3.1.3.2) (Dimer)

4-Methylumbelliferyl phosphate	20 mg

Dissolve in 25 ml 0.2 *M* citrate-NaoH buffer pH 5.5, and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 30-40 mins. and view under UV light (long wavelength).

ADA (Adenosine deaminase, EC 3.5.4.4) (Monomer)

Adenosine	15 mg
MTT (Methyl thiazolyl blue)	5 mg
PMS (Phenazine methosulphate)	1 mg
Xanthine oxidase	0.025 units
Nucleoside phosphorylase	0.625 units

Dissolve in 25 ml 0.05 *M* phosphate buffer pH 7.8, and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 15-20 mins.

ADH (Alcohol dehydrogenase, EC 1.1.1.1) (Dimer)

Absolute ethanol *or* propanol	300 µl
NAD (Nicotinamide adenine dinucleotide)	20 mg
MTT	5 mg
PMS	1 mg
Pyruvic acid	50 µl

Dissolve in 25 ml 0.2 *M* Tris-HCL buffer pH 9, and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 15-20 mins.

AH (Aconitate hydratase, EC 4.2.1.3) (Monomer)

Cis-aconotic acid	75 mg
1 *M* $MgCl_2$	4 ml
NADP (Nicotinamide adenine dinucleotide phosphate)	5 mg
Isocitric dehydrogenase	2 units
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.4 *M* Tris-HCL pH 8, and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 30-35 mins.

AK (Adenylate kinase, EC 2.7.4.3) (Monomer)

AMP (Adenosine 5′-monophosphate)	25 mg
ATP (Adenosine 5′-triphosphate)	25 mg
PEP	25 mg
NADH (Nicotinamide adenine dinucleotide - reduced form)	10 mg
KCl	150 mg
1 *M* $MgCl_2$	400 µl
Pyruvate kinase	40 units
L-Lactic dehydrogenase	275 units

Dissolve in 25 ml 0.2 *M* Tris-HCL pH 8, and add 25 ml 2% agar. Incubate the gel slice at 37 °C for 3-5 mins. and view under UV light.

ALAT (Alanine aminotransferase, EC 2.6.1.2) (Dimer)

L-Alanine	500 mg
α-Ketoglutaric acid	40 mg
NADH	10 mg
L-Lactic dehydrogenase	200 units

Dissolve in 25 ml 'Analar' Tris-HCl pH 7.5, and add 25 ml 2% agar. Incubate the gel slice at 37 °C for 10-20 mins. and view under UV light.

CK (Creatine kinase, EC 2.7.3.2) (Dimer)

Creatine	90 mg
ATP	60 mg
Magnesium acetate	120 mg
Potassium acetate	120 mg
Phospho(*enol*)pyruvate (PEP)	45 mg
NADH	30 mg
Pyruvate kinase	6 units
L-Lactic dehydrogenase	410 units

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8, and add 20 ml 2% agar. Incubate the gel slice at 37 °C for 10-20 mins. and view under UV light.

DDH (Dihydrolipoamide dehydrogenase, EC 1.8.1.4) (Monomer ?)

2,6-Dichlorophenol-indophenol ('DCPIP') solution*	2.5 ml
NADH	7.5 mg
MTT	5 mg

*Dissolve 4 mg of 'DCPIP' in 2.5 ml distilled water, and then filter through filter paper before adding to NADH and MTT. Add the mixture solution with 25 ml 0.025 *M* Tris-HCl pH 8.5 and 20 ml 2% agar. Incubate the gel slice at 37 °C for 1/2-1 hr.

EST (Esterase, EC 3.1.1.-) (Monomer)

α-Naphthyl acetate solution*	1 ml
Fast blue RR salt	40 mg

Soak a gel slice in 200 ml of either 0.05 *M* phosphate buffer pH 6.5 or 0.1 *M* Tris-maleate pH 5.3 and leave at 4 °C for 15 mins. before pouring off buffer.

*Dissolve 10 mg α-naphthyl acetate in 1 ml acetone and then add to fast blue RR salt. Add the mixture solution with 25 ml of the same buffer used for soaking gel and then add 20 ml 2% agar. Incubate the gel slice at 37 °C for 10-15 mins.

ESTD (Esterase-D, EC 3.1.-.-) (Dimer)

4-Methylumbelliferyl acetate*	10 mg

Soak a gel slice in 200 ml of either 0.05 *M* acetate buffer pH 6.4 or 0.1 *M* phosphate buffer pH 6.5 and leave at 4 °C for 15 mins. before pouring off the buffer.

*Dissolve 4-methylumbelliferyl acetate in 1 ml acetone and then add to 25 ml of the same buffer used for soaking gel. Add 20 ml 2% agar. Incubate the gel slice at 37 °C for 10-15 mins. and view under UV light.

FBALD (Fructose-biphosphate aldolase, EC 4.1.2.13) (Dimer)

Fructose-1,6-diphosphate	250 mg
NAD	5 mg
Glycerol-3-phosphate dehydrogenase	40 units
MTT	10 mg
PMS	5 mg

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 20 ml 2% agar. Incubate the gel slice at 37 °C for 1/2-1 hr.

FH (Fumarate hydratase, EC 4.2.1.2) (Tetramer)

Sodium fumarate (Fumaric acid)	60 mg
NAD	20 mg
Sodium pyruvate (Pyruvic acid)	(20 µl) 20 mg
Malic dehydrogenase	60 units
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.5 *M* Tris-HCl pH 8 and then add 20 ml 2% agar. Incubate the gel slice at 37 °C for 15-20 mins.

G3PDH (Glycerol-3-phosphate dehydrogenase, EC 1.1.1.8) (Dimer)

DL-α-glycerophosphate	200 mg
Sodium pyruvate (Pyruvic acid)	(200 µl) 200 mg
EDTA	60 mg
NAD	15 mg
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 20 ml 2% agar. Incubate the gel slice at 37 °C for 10-15 mins.

G6PDH (Glucose-6-phosphate dehydrogenase, EC 1.1.1.49) (Dimer)

D-Glucose-6-phosphate	10 mg
NADP	5 mg
1 *M* $MgCl_2$	1 ml
MTT & PMS	5 mg & 1 mg

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 20-30 mins.

GPI (Glucose-6-phosphate isomerase, EC 5.3.1.9) (Dimer)

D-Fructose-6-phosphate	20 mg
NADP	5 mg
1 *M* $MgCl_2$	100 µl
MTT	5 mg
PMS	1 mg
Glucose-6-phosphate dehydrogenase	1.4 units

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 7-15 mins.

GDA (Guanine deaminase, EC 3.5.4.3) (Dimer)

Guanine solution*	3 ml
Xanthine oxidase	0.1 units
MTT	7 mg
PMS	2 mg

*Dissolve 50 mg guanine in 10 ml warm 0.1 *N* NaOH, and then make up volume to 50 ml.

Add the mixture with 25 ml 0.2 *M* Tris-HCL pH 7.6 and 20 ml 2% agar. Incubate the gel slice at 37 °C for 20-30 mins.

IDHP (Isocitrate dehydrogenase $NADP^+$, EC 1.1.1.42) (Dimer)

DL-Isocitric acid (Na-salt)	50 mg
NADP	6 mg
MTT	7 mg
PMS	1 mg
1 *M* $MgCl_2$	1 ml

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 15-20 mins.

IDDH (L-Iditol dehydrogenase, EC 1.1.1.14) (Tetramer)

D-Sorbitol	125 mg
NAD	20 mg
MTT	5 mg
PMS	1 mg
Pyruvate	(50 µl) 50 mg
Pyrazole	50 mg

Dissolve in 25 ml 0.1 *M* Tris-HCl pH 7.4 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 20-30 mins.

LDH (L-Lactate dehydrogenase, EC 1.1.1.27) (Tetramer)

Sodium lactate (solution)	200 µl
NAD	10 mg
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 10-15 mins.

MDH (Malate dehydrogenase, EC 1.1.1.37) (Dimer)

DL-Malic acid	60 mg
NAD	10 mg
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.2 Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 10-15 mins.

MEP (Malic enzyme $NADP^+$, EC 1.1.1.40) (Tetramer)

L-Malic acid	100 mg
1 *M* $MgCl_2$	500 µl
NADP	5 mg
MTT	5 mg
PMS	1 mg

Dissolve in buffer solution of 0.6 g Tris in 25 ml distilled water, and then add 25 ml 2 % agar. Incubate the gel slice at 37 °C for 15-20 mins.

MPI (Mannose-6-phosphate isomerase, EC 5.3.1.8) (Monomer)

D-Mannose-6-phosphate	20 mg
NADP	5 mg
MTT	5 mg
PMS	1 mg
1 *M* $MgCl_2$	50 µl
Glucose-6-phosphate dehydrogenase	10 units
Glucose-6-phosphate isomerase	25 units

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 5-10 mins.

PEPC (Peptidase-C, EC 3.4.-.-) (Monomer)

Glycyl-L-leucine	20 mg
Peroxidase	10 mg
L-Amino acid oxidase	5 mg
1 *M* $MnCl_2$	100 µl
3-Amino-9-ethyl carbazole	12.5 mg

Dissolve 3-amino-9-ethyl carbazole with 3 ml DMSO, and then add 25 ml 0.2 *M* Tris-HCl pH 8 and 25 ml 2% agar. Incubate the gel slice at 37 °C for 20-30 mins.

PGDH (Phosphogluconate dehydrogenase, EC 1.1.1.44) (Dimer)

6-Phosphogluconate (Na_3) (6-phosphogluconic acid)	10 mg
1 *M* $MgCl_2$	100 µl
NADP	5 mg
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 10-15 mins.

PGM (Phosphoglucomutase, EC 5.4.2.2) (Monomer)

α-D-Glucose-1-phosphate (sodium)	50 mg
1 *M* $MgCl_2$	350 µl
NADP	5 mg
MTT	5 mg
PMS	1 mg
Glucose-6-phosphate dehydrogenase	1.4 units

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice at 37 °C for 5-10 mins.

SOD (Superoxide dismutase, EC 1.15.1.1) (Dimer)

NAD	10 mg
MTT	5 mg
PMS	1 mg

Dissolve in 25 ml 0.2 *M* Tris-HCl pH 8 and then add 25 ml 2% agar. Incubate the gel slice exposed to light at ambient temperature or at 37 °C.

APPENDIX 2

CALCULATING FORMULAS EMPLOYED

1. Chi-square test for goodness-of-fit (Sokal & Rohlf, 1969)

$$\chi^2 = \Sigma (\text{Obs} - \text{Exp})^2(\text{Exp})^{-1}$$

'Obs' = observed genotype frequencies
'Exp' = expected genotype frequencies

The expected genotype frequencies were calculated using Levene's (1949) formula for small samples.

$$E(x_{ii}) = y_i(y_i - 1)(4n - 2)^{-1}$$

$$E(x_{ij}) = y_i y_j(2n - 1)^{-1}$$

where x_{ii} = the number of a_i/a_i homozygotes in the sample, x_{ij} = the number of a_i/a_j heterozygotes in the sample, y_i = the number of a_i alleles in the sample, y_j = the number of a_j alleles in the sample, and n = the sample size.

Example 1. In a sample of *O. (O.) shiranus* population 14 individuals were observed at *AH-2** as 1 A/A homozygotes, 3 A/C heterozygotes, 1 A/D heterozygotes, 5 C/C homozygotes, 3 C/D heterozygotes, and 1 D/D homozygotes

$$y_A = (1\text{x}2) + 3 + 1 = 6$$
$$y_C = (5\text{x}2) + 3 + 3 = 16$$
$$y_D = (1\text{x}2) + 1 + 3 = 6$$

$$E(x_{AA}) = 6(6 - 1)(4\text{x}14 - 2)^{-1} = 0.556$$

$$E(x_{AC}) = (6)(16)(2\text{x}14 - 1)^{-1} = 3.556$$

$$E(x_{AD}) = (6)(6)(2\text{x}14 - 1)^{-1} = 1.333$$

$$E(x_{CC}) = 16(16 - 1)(4x14 - 2)^{-1} = 4.444$$

$$E(x_{CD}) = (16)(6)(2x14 - 1)^{-1} = 3.556$$

$$E(x_{DD}) = 6(6 - 1)(4x14 - 2)^{-1} = 0.556$$

$$\chi^2 = (1 - 0.556)^2(0.556)^{-1} + (3 - 3.556)^2(3.556)^{-1} + (1 - 1.333)^2 (1.333)^{-1} + (5 - 4.444)^2(4.444)^{-1} + (3 - 3.556)^2(3.556)^{-1} + (1 - 0.556)^2(0.556)^{-1} = 1.036$$

The number of **degrees of freedom** (*df*) was calculated as t-(a-1)-1, where t is the total number of classes of data and a is the number of different alleles. In this case $t = 6$ and $a = 3$, therefore the *df* is 6-(3-1)-1 = 3.

The P value at $\chi^2 = 1.036$, $df = 3$ is 0.79; the observed genotypes were not deviated from the Hardy-Weinberg equilibrium.

2. Expected heterozygosity (unbiased estimate of Nei, 1978)

For a single locus, an unbiased estimate of heterozygosity is given by

$$h = 2n(1 - \Sigma p_i^2)/(2n - 1) ,$$

whereas the corresponding unbiased estimate of H averaged over all loci is

$$\hat{H} = \Sigma h_k/r ,$$

where p_i is the frequency of the ith allele at a locus in a sample from the population, h_k the value of h for the kth locus, and r the total number of loci investigated. The sample size n may be vary from locus to locus.

$$S.E. = \sqrt{Var}$$

$$Var = \Sigma (h_k - \hat{H})^2/r(r - 1)$$

Example 2. From the Example 1, $p_A = 6/2x14 = 0.2143$
$p_C = 16/2x14 = 0.5714$
$p_D = 6/2x14 = 0.2143$

$$h = (2x14)[1 - (0.2143^2+0.5714^2+0.2143^2)] / (2x14 - 1)$$
$$= 0.603$$

Similarly, the h values at the other loci were obtained.

$$\therefore \quad \hat{H} = (0.476 + 0.485 + 0.603 + 0.385 + 0.349 + 0.516 + 0.423 + 0.519 + 0 + 0 + ... + 0) / 43$$
$$= 0.087$$

$$Var = [(0.476 - 0.087)^2 + (0.487 - 0.087)^2 + ... + (0.519 - 0.087)^2 + (0 - 0.087)^2 + (0 - 0.087)^2 + ... + (0 - 0.087)^2] / 43(43-1)$$
$$= 0.00082$$

$$S.E. = \sqrt{0.00082} = 0.029$$

3. F-statistics, F_{ST} (the variance component estimation of Wright, 1978)

F_{ST} = (Actual variance)(Limiting variance)$^{-1}$,

Actual variance = Total variance - Sampling variance ,

Total variance = $r^{-1} \Sigma(p - \bar{p})^2$,

Sampling variance = $r^{-1} \Sigma(2n)^{-1}p(1 - p)$,

Limiting variance = $\bar{p}(1 - \bar{p})$,

where p is the frequency of an allele at a locus in a population, $\bar{p}$ the averaged allelic frequency over all populations at that allele and that locus, n the number of individuals sampled for that locus in that population, and r the number of populations.

Example 3. Calculation of F_{ST} for the three populations (species) of the chambo (data as in Table 4, Chapter 2).

At *AAT-2**,

$$\bar{p}_A = (0.919 + 0.758 + 0.750) / 3 = 0.809$$
$$\bar{p}_B = (0.081 + 0.242 + 0.250) / 3 = 0.191$$

Total variance (p_A) = $[(0.919 - 0.809)^2 + (0.758 - 0.809)^2 + (0.750 - 0.809)^2] / 3$
= 0.0061

Total variance (p_B) = $[(0.081 - 0.191)^2 + (0.242 - 0.191)^2 + (0.250 - 0.191)^2] / 3$
= 0.0061

Sampling variance (p_A) = $[(2x37)^{-1}(0.919)(1 - 0.919) + (2x33)^{-1}(0.758)(1 - 0.758) + (2x14)^{-1}(0.750)(1 - 0.750)] / 3$
= 0.0035

Sampling variance (p_B) = $[(2x37)^{-1}(0.081)(1 - 0.081) + (2x33)^{-1}(0.242)(1 - 0.242) + (2x14)^{-1}(0.250)(1 - 0.250)] / 3$
= 0.0035

$\therefore$ Actual variance (p_A) = 0.0061 - 0.0035 = 0.0026
Actual variance (p_B) = 0.0061 - 0.0035 = 0.0026
Actual variance (total) = 0.0026 + 0.0026 = 0.0052

Limiting variance (p_A) = 0.809(1 - 0.809) = 0.1545
Limiting variance (p_B) = 0.191(1 - 0.191) = 0.1545
Limiting variance (total) = 0.1545 + 0.1545 = 0.309

$\therefore$ F_{ST} (*A*) = 0.0026 / 0.1545 = 0.017
F_{ST} (*B*) = 0.0026 / 0.1545 = 0.017
F_{ST} (total) = 0.0052 / 0.309 = 0.017

At *ADA**,

$$\bar{p}_A = (0.125 + 0.074 + 0.250) / 3 = 0.1497$$
$$\bar{p}_B = (0 + 0.537 + 0.200) / 3 = 0.2457$$
$$\bar{p}_C = (0.359 + 0 + 0.450) / 3 = 0.2697$$
$$\bar{p}_D = (0.516 + 0.389 + 0.100) / 3 = 0.335$$

Total variance (p_A) = $[(0.125 - 0.1497)^2 + (0.074 - 0.1497)^2 + (0.250 - 0.1497)^2] / 3$
= 0.0055

Total variance (p_B) = $[(0 - 0.2457)^2 + (0.537 - 0.2457)^2 + (0.200 - 0.2457)^2] / 3$
= 0.0491

Total variance (p_C) = $[(0.359 - 0.2697)^2 + (0 - 0.2697)^2 + (0.450 - 0.2697)^2] / 3$
= 0.0377

Total variance (p_D) = $[(0.516 - 0.335)^2 + (0.389 - 0.335)^2 + (0.100 - 0.335)^2] / 3$
= 0.0303

Sampling variance (p_A) = $[(2x32)^{-1}(0.125)(1 - 0.125) + (2x27)^{-1}(0.074)(1 - 0.074) + (2x10)^{-1}(0.250)(1 - 0.250)] / 3$
= 0.0041

Sampling variance (p_B) = $[(2x32)^{-1}(0)(1 - 0) + (2x27)^{-1}(0.537)(1 - 0.537) + (2x10)^{-1}(0.2)(1 - 0.2)] / 3$
= 0.0042

Sampling variance (p_C) = $[(2x32)^{-1}(0.359)(1 - 0.359) + (2x27)^{-1}(0)(1 - 0) + (2x10)^{-1}(0.45)(1 - 0.45)] / 3$
= 0.0053

Sampling variance (p_D) = $[(2x32)^{-1}(0.516)(1 - 0.516) + (2x27)^{-1}(0.389)(1 - 0.389) + (2x10)^{-1}(0.1)(1 - 0.1)] / 3$
= 0.0043

∴ **Actual variance (p_A) = 0.0055 - 0.0041 = 0.0014**
Actual variance (p_B) = 0.0491 - 0.0042 = 0.0449
Actual variance (p_C) = 0.0377 - 0.0053 = 0.0324
Actual variance (p_D) = 0.0303 - 0.0043 = 0.026

Actual variance (total) = 0.0014 + 0.0449 + 0.0324 + 0.026
= 0.1047

Limiting variance (p_A)	=	0.1497(1 - 0.1497)	=	0.1273
Limiting variance (p_B)	=	0.2457(1 - 0.2457)	=	0.1853
Limiting variance (p_C)	=	0.2697(1 - 0.2697)	=	0.1970
Limiting variance (p_D)	=	0.3350(1 - 0.3350)	=	0.2227
Limiting variance (total)	=	0.1273 + 0.1853 + 0.1970 + 0.2227		
	=	0.7323		
$\therefore$ F_{ST} (A)	=	0.0014 / 0.1273	=	0.011
F_{ST} (B)	=	0.0449 / 0.1853	=	0.242
F_{ST} (C)	=	0.0324 / 0.1970	=	0.164
F_{ST} (D)	=	0.0260 / 0.2227	=	0.117
F_{ST} (total)	=	0.1047 / 0.7323	=	0.143

Similarly:	at *ADH**,	total actual variance	=	0.0043
		total limiting variance	=	0.1817
	at *AH-2**,	total actual variance	=	0.0202
		total limiting variance	=	0.6917
	at *EST-2**,	total actual variance	=	0.0089
		total limiting variance	=	0.1222
	at *GDA**,	total actual variance	=	0.0150
		total limiting variance	=	0.5278
	at *G6PDH-1**,	total actual variance	=	0.0036
		total limiting variance	=	0.5209
	at *G6PDH-2**,	total actual variance	=	0.0518
		total limiting variance	=	0.2130
	at *GPI-1**,	total actual variance	=	0.0011
		total limiting variance	=	0.0389
	at *IDDH-1**,	total actual variance	=	0.0107
		total limiting variance	=	0.1120
	at *IDHP-1**,	total actual variance	=	0.0093
		total limiting variance	=	0.1049

at *LDH-1**,	total actual variance	=	negative value
		≈	0
	total limiting variance	=	0.4980
at *MEP-2**,	total actual variance	=	0.0064
	total limiting variance	=	0.5850
at *PGM**,	total actual variance	=	negative value
		≈	0
	total limiting variance	=	0.0753
at *PGDH**,	total actual variance	=	0.0177
	total limiting variance	=	0.1327
and *SOD**,	total actual variance	=	0.0045
	total limiting variance	=	0.3625

∴ Total actual variance averaged over all loci = (0.0052 + 0.1047 + 0.0043 + 0.0202 + 0.0089 + 0.0150 + 0.0036 + 0.0518 + 0.0011 + 0.0107 + 0.0093 + 0 + 0.0064 + 0 + 0.0177 + 0.0045) / 16
= 0.0165

Total limiting variance averaged over all loci = (0.309 + 0.7323 + 0.1817 + 0.6917 + 0.1222 + 0.5278 + 0.5209 + 0.2130 + 0.0389 + 0.1120 + 0.1049 + 0.4980 + 0.5850 + 0.0753 + 0.1327 + 0.3625) / 16
= 0.3255

∴ F_{ST} over all loci = 0.0165 / 0.3255
= 0.051

NOTE: Negative variance components are sometimes obtained using Wright's (1978) procedure. Resulting from the assumption for computational purposes, the estimated total variance is partitioned orthogonally into estimated variance components. Thus if the sum of a part of the estimated variance components exceeds the estimated total variance, the remaining estimated variance components take on negative values. The program BIOSYS-1 counted these values as zero for the next step of calculation.

4. Genetic distances

4.1 Unbiased distances of Nei (1978)

When x_i and y_i are the frequencies of the ith allele at a particular locus in taxa X and Y respectively, Nei (1978)'s unbiased distance can be defined as

$$D_N = - \ln (J_{XY} / \sqrt{J_X J_Y})$$

where J_{XY}, J_X and J_Y are the arithmetic means across loci of $\Sigma x_i y_i$, $(2n_X \Sigma x_i^2 - 1)/(2n_X - 1)$ and $(2n_Y \Sigma y_i^2 - 1)/(2n_Y - 1)$, respectively.

Example 4.1 The calculation of Nei's unbiased distance between *O. (O.) placidus* and *O. (O.) shiranus* (allele frequency data from Table 4, Chapter 2).

$$J_{XY} = [(1) + (1x0 + 0x0.357 + 0x0.643) + ... + (0x0.5 + 1x0.5) + (1) + (1) + ... + (1)] / 36$$
$$= 0.85$$

$$J_X = \{(2x5x1 - 1)/(2x5 - 1) + (1) + (1) + ... + [2x5(0.75^2 + 0.25^2) - 1]/(2x5 - 1) + (1) + (1)\} / 36$$
$$= 0.988$$

$$J_Y = \{(2x20x1 - 1)/(2x20 - 1) + [2x20(0.375^2 + 0.643^2) - 1]/(2x20 - 1) + ... + [2x20(0.5^2 + 0.5^2) - 1]/(2x20 - 1) + (1) + (1) + ... + (1)\} / 36$$
$$= 0.914$$

$$\therefore \quad D_N = - \ln (0.85/\sqrt{0.988 \times 0.914})$$
$$= 0.115$$

4.2 Arc distance of Cavalli-Sforza & Edwards (1967)

$$D_{arc} = \sqrt{(1/L) \sum_{L} (2\theta/\pi)^2}$$

$$\theta = \cos^{-1} \Sigma \sqrt{x_i y_i}$$

L = number of loci investigated

Example 4.2 With the same data as in Example 4.1, the Cavalli-Sforza & Edwards (1967) arc distance was calculated as the following :

$$\sum_{L} (2\theta/\pi)^2 = [2(\cos^{-1}\sqrt{1})(7/22)]^2 + [2(\cos^{-1}\sqrt{0})(7/22)]^2 + \ldots\ldots\ldots\ldots + [2\{\cos^{-1}(\sqrt{0} + \sqrt{1x0.5})\}(7/22)]^2 + 0 + 0 + \ldots\ldots\ldots\ldots + 0$$

$$= 3.8$$

$$\therefore \quad D_{arc} = \sqrt{(1/36)(3.8)}$$

$$= 0.325$$

APPENDIX 3

CHI-SQUARE TEST FOR HARDY-WEINBERG EQUILIBRIUM, F-STATISTICS VALUES & CONTINGENCY CHI-SQUARE ANALYSES FOR HETEROGENEITY

1. Chi-square test for deviation from Hardy-Weinberg equilibrium

Levene (1949) correction for small sample size employed in chi-square analyses

Population: *O.(O.) SHIRANUS* (OOSH)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
AAT-3						
	A-A	3	1.667			
	A-B	4	6.667			
	B-B	7	5.667			
				2.447	1	.118
ADH						
	B-B	3	4.333			
	B-C	8	5.333			
	C-C	0	1.333			
				3.077	1	.079
AH-2						
	A-A	1	.556			
	A-C	3	3.556			
	A-D	1	1.333			
	C-C	5	4.444			
	C-D	3	3.556			
	D-D	1	.556			
				1.037	3	.792
DDH-1						
	A-A	10	11.154			
	A-B	10	7.692			
	B-B	0	1.154			
				1.966	1	.161
EST-1						
	A-A	1	.556			
	A-B	4	4.889			
	B-B	9	8.556			
				.540	1	.462
LDH-2						
	A-A	4	2.889			
	A-B	5	7.222			
	B-B	5	3.889			
				1.429	1	.232

MEP-2						
	B-B	8	7.037			
	B-C	4	5.926			
	C-C	2	1.037			
				1.652	1	.199
SOD						
	A-A	3	3.370			
	A-B	8	7.259			
	B-B	3	3.370			
				.157	1	.692

Chi-square test with pooling

****

Population: *O.(O.) SHIRANUS* (OOSH)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
AH-2	Homozygotes for most common allele	5	4.444			
	Common/rare heterozygotes	6	7.111			
	Rare homozygotes and other heterozygotes	3	2.444	.369	1	.543

Significance test using exact probabilities

****

Population: *O.(O.) SHIRANUS* (OOSH)

Locus	R1	R2	R3	P
AAT-3	7	4	3	.238
ADH	3	8	0	.191
AH-2	5	6	3	.621
DDH-1	10	10	0	.277
EST-1	9	4	1	.490
LDH-2	5	5	4	.316
MEP-2	8	4	2	.258
SOD	3	8	3	1.000

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: *O.(NY.) KARONGAE* (ONYK)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
AAT-2						
	A-A	32	31.205			
	A-B	4	5.589			
	B-B	1	.205			
				3.544	1	.060
ADA						
	A-A	1	.444			
	A-C	3	2.921			
	A-D	3	4.190			
	C-C	3	4.016			
	C-D	14	12.048			
	D-D	8	8.381			
				1.625	3	.654
ADH						
	A-A	0	.012			
	A-B	2	1.976			
	B-B	40	40.012			
				.012	1	.912
AH-2						
	A-A	2	.554			
	A-B	3	4.985			
	A-C	1	.692			
	A-D	1	2.215			
	B-B	11	9.692			
	B-C	2	2.769			
	B-D	9	8.862			
	C-C	1	.154			
	C-D	0	1.231			
	D-D	3	1.846			
				12.368	6	.054
EST-2						
	A-A	31	29.096			
	A-B	8	11.807			
	B-B	3	1.096			
				4.657	1	.031
GDA						
	A-A	8	7.901			
	A-B	18	18.197			
	B-B	10	9.901			
				.004	1	.947
G6PDH-1						
	A-A	4	6.000			
	A-B	20	16.000			
	B-B	8	10.000			
				2.067	1	.151

GPI-1						
	A-A	0	.120			
	A-B	5	4.759			
	B-B	37	37.120			
				.133	1	.715
IDDH						
	B-B	9	9.370			
	B-C	5	4.259			
	C-C	0	.370			
				.514	1	.473
LDH-1						
	A-A	13	13.024			
	A-B	21	20.952			
	B-B	8	8.024			
				.000	1	.988
MEP-2						
	A-A	1	.280			
	A-B	3	4.573			
	A-C	2	1.867			
	B-B	17	15.680			
	B-C	12	13.067			
	C-C	3	2.533			
				2.686	3	.443
PGM						
	A-A	39	39.036			
	A-B	3	2.928			
	B-B	0	.036			
				.038	1	.846
PGDH						
	A-A	1	.878			
	A-B	7	7.244			
	B-B	13	12.878			
				.026	1	.871
SOD						
	A-A	1	.467			
	A-B	5	6.067			
	B-B	17	16.467			
				.814	1	.367

Chi-square test with pooling
****

Population: *O.(NY.) KARONGAE* (ONYK)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
ADA	Homozygotes for most common allele	8	8.381			
	Common/rare heterozygotes	17	16.238			
	Rare homozygotes and other heterozygotes	7	7.381	.073	1	.787
AH-2	Homozygotes for most common allele	11	9.692			
	Common/rare heterozygotes	14	16.615			
	Rare homozygotes and other heterozygotes	8	6.692	.844	1	.358
MEP-2	Homozygotes for most common allele	17	15.680			
	Common/rare heterozygotes	15	17.640			
	Rare homozygotes and other heterozygotes	6	4.680	.879	1	.349

Significance test using exact probabilities
****

Population: *O.(NY.) KARONGAE* (ONYK)

Locus	R1	R2	R3	P
AAT-2	32	4	1	.197
ADA	8	17	7	1.000
ADH	40	2	0	1.000
AH-2	11	14	8	.484
EST-2	31	8	3	.063
GDA	10	18	8	1.000
G6PDH-1	8	20	4	.277
GPI-1	37	5	0	1.000
IDDH	9	5	0	1.000
LDH-1	13	21	8	1.000
MEP-2	17	15	6	.478
PGM	39	3	0	1.000
PGDH	13	7	1	1.000
SOD	17	5	1	.414

Chi-square test for deviation from Hardy-Weinberg equilibrium
****

Population: ***O.(NY.) LIDOLE*** **(ONYL)**

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
AAT-2						
	A-A	**21**	**18.846**			
	A-B	**8**	**12.308**			
	B-B	**4**	**1.846**			
				4.267	**1**	**.039**
ADA						
	A-A	**0**	**.113**			
	A-B	**2**	**2.189**			
	A-D	**2**	**1.585**			
	B-B	**8**	**7.660**			
	B-D	**11**	**11.491**			
	D-D	**4**	**3.962**			
				.275	**3**	**.965**
ADH						
	A-A	**1**	**.046**			
	A-B	**1**	**2.723**			
	A-C	**0**	**.185**			
	B-B	**27**	**26.323**			
	B-C	**4**	**3.631**			
	C-C	**0**	**.092**			
				21.135	**3**	**.020**
AH-2						
	A-A	**2**	**1.418**			
	A-B	**4**	**4.491**			
	A-C	**3**	**4.018**			
	A-D	**2**	**1.655**			
	B-B	**3**	**3.109**			
	B-C	**6**	**5.873**			
	B-D	**3**	**2.418**			
	C-C	**3**	**2.473**			
	C-D	**2**	**2.164**			
	D-D	**0**	**.382**			
				1.276	**6**	**.973**
GDA						
	A-A	**9**	**9.154**			
	A-B	**17**	**16.692**			
	B-B	**7**	**7.154**			
				.012	**1**	**.914**
G6PDH-1						
	A-A	**5**	**3.220**			
	A-B	**10**	**13.559**			
	B-B	**15**	**13.220**			
				2.157	**1**	**.142**
IDHP-1						
	A-A	**10**	**10.345**			
	A-B	**5**	**4.310**			
	B-B	**0**	**.345**			
				.467	**1**	**.495**

LDH-1						
	A-A	5	5.455			
	A-B	15	14.091			
	B-B	8	8.455			
				.121	1	.728
MEP-2						
	A-A	3	3.554			
	A-B	13	11.508			
	A-C	3	3.385			
	B-B	8	8.631			
	B-C	5	5.231			
	C-C	1	.692			
				.517	3	.915
PGM						
	A-A	32	32.000			
	A-B	1	1.000			
	B-B	0	.000			
				.000	1	1.000
SOD						
	A-A	3	2.229			
	A-B	7	8.543			
	B-B	8	7.229			
				.628	1	.428

Chi-square test with pooling

Population: *O.(NY.) LIDOLE* (ONYL)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
ADA	Homozygotes for most common allele	8	7.660			
	Common/rare heterozygotes	13	13.679			
	Rare homozygotes and other heterozygotes	6	5.660	.069	1	.793
ADH	Homozygotes for most common allele	27	26.323			
	Common/rare heterozygotes	5	6.354			
	Rare homozygotes and other heterozygotes	1	.323	1.724	1	.189
AH-2	Homozygotes for most common allele	3	3.109			
	Common/rare heterozygotes	13	12.782			
	Rare homozygotes and other heterozygotes	12	12.109	.009	1	.926
MEP-2	Homozygotes for most common allele	8	8.631			
	Common/rare heterozygotes	18	16.738			
	Rare homozygotes and other heterozygotes	7	7.631	.193	1	.660

Significance test using exact probabilities
****

Population: *O.(NY.) LIDOLE* (ONYL)

Locus	R1	R2	R3	P
AAT-2	21	8	4	.060
ADA	8	13	6	1.000
ADH	27	5	1	.298
AH-2	3	13	12	1.001
GDA	9	17	7	1.000
G6PDH-1	15	10	5	.216
IDHP-1	10	5	0	1.000
LDH-1	8	15	5	1.000
MEP-2	8	18	7	.735
PGM	32	1	0	1.000
SOD	8	7	3	.612

Chi-square test for deviation from Hardy-Weinberg equilibrium
****

Population: *O.(NY.) SQUAMIPINNIS* (ONYS)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
AAT-2						
	A-A	8	7.778			
	A-B	5	5.444			
	B-B	1	.778			
				.106	1	.745
ADA						
	A-A	1	.526			
	A-B	1	1.053			
	A-C	2	2.368			
	A-D	0	.526			
	B-B	0	.316			
	B-C	2	1.895			
	B-D	1	.421			
	C-C	2	1.895			
	C-D	1	.947			
	D-D	0	.053			
				2.192	6	.901
ADH						
	B-B	10	10.345			
	B-C	5	4.310			
	C-C	0	.345			
				.467	1	.495

AH-2						
	A-A	1	.286			
	A-B	2	1.905			
	A-D	0	1.524			
	B-B	2	2.143			
	B-D	4	3.810			
	D-D	2	1.333			
				3.667	3	.300
EST-2						
	A-A	16	16.000			
	A-B	1	1.000			
	B-B	0	.000			
				.000	1	1.000
GDA						
	A-A	1	.848			
	A-B	5	5.333			
	A-C	1	.970			
	B-B	7	7.000			
	B-C	3	2.667			
	C-C	0	.182			
				.272	3	.965
G6PDH-1						
	A-A	2	1.667			
	A-B	6	6.000			
	A-C	1	1.667			
	B-B	5	4.636			
	B-C	2	2.727			
	C-C	1	.303			
				2.159	3	.540
G6PDH-2						
	A-A	2	1.333			
	A-B	4	5.333			
	B-B	5	4.333			
				.769	1	.380
LDH-1						
	A-A	6	5.758			
	A-B	8	8.485			
	B-B	3	2.758			
				.059	1	.808
MEP-2						
	A-A	1	.840			
	A-B	5	3.920			
	A-C	0	1.400			
	B-B	2	3.640			
	B-C	5	2.800			
	C-C	0	.400			
				4.595	3	.204
PGM						
	A-A	13	13.034			
	A-B	2	1.931			
	B-B	0	.034			
				.037	1	.847
SOD						
	A-A	1	.316			
	A-B	2	3.368			
	B-B	7	6.316			
				2.112	1	.146

Chi-square test with pooling

Population: *O.(NY.) SQUAMIPINNIS* (ONYS)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
ADA	Homozygotes for most common allele	2	1.895			
	Common/rare heterozygotes	5	5.211			
	Rare homozygotes and other heterozygotes	3	2.895	.018	1	.893
AH-2	Homozygotes for most common allele	2	2.143			
	Common/rare heterozygotes	6	5.714			
	Rare homozygotes and other heterozygotes	3	3.143	.030	1	.862
GDA	Homozygotes for most common allele	7	7.000			
	Common/rare heterozygotes	8	8.000			
	Rare homozygotes and other heterozygotes	2	2.000	.000	1	1.000
G6PDH-1	Homozygotes for most common allele	5	4.636			
	Common/rare heterozygotes	8	8.727			
	Rare homozygotes and other heterozygotes	4	3.636	.125	1	.723
MEP-2	Homozygotes for most common allele	2	3.640			
	Common/rare heterozygotes	10	6.720			
	Rare homozygotes and other heterozygotes	1	2.640	3.359	1	.067

Significance test using exact probabilities

Population: *O.(NY.) SQUAMIPINNIS* (ONYS)

Locus	R1	R2	R3	P
AAT-2	8	5	1	1.000
ADA	2	5	3	1.001
ADH	10	5	0	1.000
AH-2	2	6	3	1.001
EST-2	16	1	0	1.000
GDA	7	8	2	1.000
G6PDH-1	5	8	4	1.000
G6PDH-2	5	4	2	.538
LDH-1	6	8	3	1.000
MEP-2	2	10	1	.112
PGM	13	2	0	1.000
SOD	7	2	1	.297

2. F-statistics observed within different levels of taxa of tilapias

GENUS *TILAPIA*

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2						
	C	.18580	.00066	.13743	.15128	.908
	D	.55560	.00451	.15226	.24691	.617
	F	.05640	.00255	.00000	.05322	.000
	G	.20220	.00478	.06100	.16132	.378
	Total	---	.01249	.35069	.61272	.572
ACP						
	A	.60000	.00000	.24000	.24000	1.000
	B	.40000	.00000	.24000	.24000	1.000
	Total	---	.00000	.48000	.48000	1.000
ADA						
	F	.18860	.00451	.05827	.15303	.381
	H	.41140	.00451	.14739	.24215	.609
	I	.03340	.00464	.00000	.03228	.000
	J	.36660	.00464	.19975	.23220	.860
	Total	---	.01830	.40540	.65967	.615
ADH						
	B	.04000	.00180	.00060	.03840	.016
	C	.40000	.00000	.24000	.24000	1.000
	D	.54180	.00263	.19308	.24825	.778
	E	.01820	.00083	.00050	.01787	.028
	Total	---	.00525	.43418	.54452	.797
AK-2						
	B	.05560	.00201	.01036	.05251	.197
	C	.35700	.00260	.17500	.22955	.762
	D	.03640	.00298	.00232	.03508	.066
	E	.16360	.00298	.10408	.13684	.761
	F	.38740	.00059	.22492	.23732	.948
	Total	---	.01115	.51669	.69129	.747
ALAT						
	A	.60000	.00000	.24000	.24000	1.000
	B	.40000	.00000	.24000	.24000	1.000
	Total	---	.00000	.48000	.48000	1.000
CK						
	A	.80000	.00000	.16000	.16000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.32000	.32000	1.000
EST-1						
	B	.60000	.00000	.24000	.24000	1.000
	C	.40000	.00000	.24000	.24000	1.000
	Total	---	.00000	.48000	.48000	1.000
EST-2						
	C	.20000	.00000	.16000	.16000	1.000
	E	.80000	.00000	.16000	.16000	1.000
	Total	---	.00000	.32000	.32000	1.000

ESTD					
	B	.20000	.00000	.16000	.16000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	D	.60000	.00000	.24000	.24000	1.000
	Total	---	.00000	.56000	.56000	1.000
GDA					
	A	.04440	.00173	.00616	.04243	.145
	B	.75560	.00173	.14840	.18467	.804
	D	.20000	.00000	.16000	.16000	1.000
	Total	---	.00345	.31456	.38710	.813
GPI-1					
	C	.40000	.00000	.24000	.24000	1.000
	E	.60000	.00000	.24000	.24000	1.000
	Total	---	.00000	.48000	.48000	1.000
GPI-2					
	A	.02000	.00090	.00070	.01960	.036
	C	.78000	.00090	.15270	.17160	.890
	D	.20000	.00000	.16000	.16000	1.000
	Total	---	.00180	.31340	.35120	.892
G6PDH-1					
	A	.60000	.00000	.24000	.24000	1.000
	B	.40000	.00000	.24000	.24000	1.000
	Total	---	.00000	.48000	.48000	1.000
G6PDH-2					
	A	.60000	.00000	.24000	.24000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	E	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.56000	.56000	1.000
IDDH					
	C	.40000	.00000	.24000	.24000	1.000
	E	.40000	.00000	.24000	.24000	1.000
	F	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.64000	.64000	1.000
IDHP-1					
	B	.80000	.00000	.16000	.16000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.32000	.32000	1.000
LDH-1					
	B	.80000	.00000	.16000	.16000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.32000	.32000	1.000
LDH-3					
	B	.80000	.00000	.16000	.16000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.32000	.32000	1.000
MEP-2					
	D	.60000	.00000	.24000	.24000	1.000
	E	.40000	.00000	.24000	.24000	1.000
	Total	---	.00000	.48000	.48000	1.000
PEPC					
	B	.60000	.00000	.24000	.24000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	D	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.56000	.56000	1.000

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
PGDH						
	B	.25000	.00375	.14625	.18750	.780
	C	.75000	.00375	.14625	.18750	.780
	Total	---	.00750	.29250	.37500	.780
SOD						
	B	.80000	.00000	.16000	.16000	1.000
	C	.20000	.00000	.16000	.16000	1.000
	Total	---	.00000	.32000	.32000	1.000
Average		----	.00261	.42380	.46702	.907

SUBGENUS *T. (COPTODON)*

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2						
	C	.30967	.00110	.19069	.21377	.892
	D	.25933	.00751	.03433	.19208	.179
	F	.09400	.00424	.00000	.08516	.000
	G	.33700	.00796	.05624	.22343	.252
	Total	---	.02082	.28126	.71445	.394
ADA						
	F	.31433	.00752	.05759	.21553	.267
	H	.68567	.00752	.05759	.21553	.267
	Total	---	.01504	.11518	.43106	.267
ADH						
	B	.06667	.00300	.00000	.06222	.000
	D	.90300	.00438	.00000	.08759	.000
	E	.03033	.00138	.00046	.02941	.016
	Total	---	.00876	.00046	.17923	.003
AH-2						
	B	.09267	.00335	.01383	.08408	.164
	C	.26167	.00433	.10229	.19320	.529
	F	.64567	.00098	.20812	.22878	.910
	Total	---	.00866	.32424	.50606	.641
ALAT						
	A	.33333	.00000	.22222	.22222	1.000
	B	.66667	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
CK						
	A	.66667	.00000	.22222	.22222	1.000
	C	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
GDA						
	A	.07400	.00288	.00807	.06852	.118
	B	.92600	.00288	.00807	.06852	.118
	Total	---	.00576	.01615	.13705	.118

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
GPI-2						
	A	.03333	.00150	.00072	.03222	.022
	C	.96667	.00150	.00072	.03222	.022
	Total	---	.00300	.00144	.06444	.022
G6PDH-1						
	A	.66667	.00000	.22222	.22222	1.000
	B	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
IDDH						
	E	.66667	.00000	.22222	.22222	1.000
	F	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
IDHP-1						
	B	.66667	.00000	.22222	.22222	1.000
	C	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
LDH-1						
	B	.66667	.00000	.22222	.22222	1.000
	C	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
PEPC						
	B	.66667	.00000	.22222	.22222	1.000
	C	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
Average		----	.00477	.29614	.39565	.749

GENUS *SAROTHERODON*

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2						
	B	.66667	.00000	.22222	.22222	1.000
	D	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
ADA						
	F	.33333	.00000	.22222	.22222	1.000
	I	.66667	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
ADH						
	C	.05567	.00232	.00388	.05257	.074
	D	.94433	.00232	.00388	.05257	.074
	Total	---	.00464	.00776	.10514	.074
AH-2						
	C	.39167	.00906	.06858	.23826	.288
	D	.25000	.00313	.12188	.18750	.650
	E	.35833	.01219	.00000	.22993	.000
	Total	---	.02437	.19045	.65569	.290

ALAT					
	A	.33333	.00000	.22222	.22222	1.000
	B	.66667	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
EST-1					
	A	.70000	.00150	.17850	.21000	.850
	B	.30000	.00150	.17850	.21000	.850
	Total	---	.00300	.35700	.42000	.850
ESTD					
	D	.66667	.00000	.22222	.22222	1.000
	E	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
FH-2					
	B	.33333	.00000	.22222	.22222	1.000
	C	.66667	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
GDA					
	A	.46667	.01103	.03286	.24889	.132
	B	.53333	.01103	.03286	.24889	.132
	Total	---	.02206	.06572	.49778	.132
GPI-1					
	B	.04667	.00201	.00235	.04449	.053
	C	.95333	.00201	.00235	.04449	.053
	Total	---	.00401	.00470	.08898	.053
GPI-2					
	B	.02000	.00094	.00000	.01960	.000
	C	.98000	.00094	.00000	.01960	.000
	Total	---	.00188	.00000	.03920	.000
G3PDH-1					
	A	.12500	.00488	.02637	.10938	.241
	B	.87500	.00488	.02637	.10938	.241
	Total	---	.00977	.05273	.21875	.241
G6PDH-1					
	A	.66667	.00000	.22222	.22222	1.000
	B	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
G6PDH-2					
	A	.66667	.00000	.22222	.22222	1.000
	B	.33333	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
IDHP-1					
	A	.68000	.00064	.20416	.21760	.938
	B	.32000	.00064	.20416	.21760	.938
	Total	---	.00128	.40832	.43520	.938
MDH-2	A	.33333	.00000	.22222	.22222	1.000
	B	.66667	.00000	.22222	.22222	1.000
	Total	---	.00000	.44444	.44444	1.000
MDH-3	A	.16667	.00417	.05139	.13889	.370
	B	.83333	.00417	.05139	.13889	.370
	Total	---	.00833	.10278	.27778	.370
MPI	A	.97633	.00110	.00002	.02311	.001
	B	.02367	.00110	.00002	.02311	.001
	Total	---	.00220	.00004	.04621	.001

PEPC					
	A	.58333	.00391	.17665	.24306	.727
	B	.41667	.00391	.17665	.24306	.727
	Total	---	.00781	.35330	.48611	.727
PGM					
	B	.91667	.00313	.01076	.07639	.141
	D	.08333	.00313	.01076	.07639	.141
	Total	---	.00625	.02153	.15278	.141
Average		----	.00478	.25599	.34896	.734

GENUS *OREOCHROMIS*

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2						
	C	.06285	.00158	.00912	.05890	.155
	D	.66731	.00214	.12758	.22201	.575
	E	.09215	.00147	.02990	.08366	.357
	F	.17769	.00032	.12567	.14612	.860
	Total	---	.00551	.29226	.51068	.572
AAT-3						
	B	.22592	.00145	.13088	.17488	.748
	C	.69715	.00145	.16713	.21113	.792
	D	.07692	.00000	.07101	.07101	1.000
	Total	---	.00290	.36902	.45702	.807
ADA						
	A	.06254	.00039	.04654	.05863	.794
	B	.00038	.00000	.00000	.00038	.000
	C	.01438	.00039	.00209	.01418	.148
	D	.04254	.00016	.02156	.04073	.529
	E	.00523	.00004	.00029	.00520	.055
	G	.02877	.00015	.00978	.02794	.350
	H	.02308	.00054	.00585	.02254	.260
	I	.11146	.00047	.07046	.09904	.711
	J	.29000	.00158	.14001	.20590	.680
	K	.06223	.00059	.02103	.05836	.360
	L	.07731	.00064	.02627	.07133	.368
	M	.03077	.00062	.01075	.02982	.360
	N	.00385	.00018	.00000	.00383	.000
	O	.09362	.00033	.07145	.08485	.842
	P	.03846	.00064	.01711	.03698	.463
	R	.07692	.00000	.07101	.07101	1.000
	S	.03846	.00064	.01711	.03698	.463
	Total	---	.00737	.53130	.86771	.612
ADH						
	B	.04554	.00073	.00982	.04346	.226
	C	.08654	.00042	.07022	.07905	.888
	D	.78569	.00121	.12115	.16838	.720
	E	.00531	.00007	.00010	.00528	.019
	F	.07692	.00000	.07101	.07101	1.000
	Total	---	.00243	.27230	.36718	.742

AH-2					
	B	.03846	.00064	.01711	.03698	.463
	C	.17931	.00173	.06451	.14716	.438
	D	.22992	.00270	.06344	.17706	.358
	E	.25785	.00314	.06325	.19136	.331
	F	.05769	.00136	.01935	.05436	.356
	G	.15985	.00164	.05253	.13430	.391
	H	.07692	.00000	.07101	.07101	1.000
	Total	---	.01121	.35119	.81222	.432
AK					
	A	.92308	.00000	.07101	.07101	1.000
	B	.07692	.00000	.07101	.07101	1.000
	Total	---	.00000	.14201	.14201	1.000
ALAT					
	A	.52562	.00036	.23829	.24934	.956
	B	.47438	.00036	.23829	.24934	.956
	Total	---	.00071	.47657	.49869	.956
CK					
	A	.53846	.00000	.24852	.24852	1.000
	B	.07692	.00000	.07101	.07101	1.000
	C	.38462	.00000	.23669	.23669	1.000
	Total	---	.00000	.55621	.55621	1.000
DDH-1					
	A	.05769	.00036	.03958	.05436	.728
	B	.17308	.00036	.12834	.14312	.897
	C	.76923	.00000	.17751	.17751	1.000
	Total	---	.00072	.34543	.37500	.921
EST-1					
	A	.17031	.00032	.12804	.14130	.906
	B	.82969	.00032	.12804	.14130	.906
	Total	---	.00065	.25608	.28261	.906
EST-2					
	B	.03546	.00080	.00933	.03420	.273
	C	.00915	.00007	.00094	.00907	.103
	D	.85862	.00115	.07076	.12140	.583
	E	.09677	.00028	.06978	.08740	.798
	Total	---	.00230	.15081	.25207	.598
FBALD-2					
	B	.22308	.00023	.16616	.17331	.959
	C	.70000	.00023	.20285	.21000	.966
	D	.07692	.00000	.07101	.07101	1.000
	Total	---	.00046	.44001	.45432	.969
FH-1					
	A	.07692	.00000	.07101	.07101	1.000
	B	.84615	.00000	.13018	.13018	1.000
	C	.07692	.00000	.07101	.07101	1.000
	Total	---	.00000	.27219	.27219	1.000
FH-2					
	A	.03592	.00016	.01533	.03463	.443
	B	.96408	.00016	.01533	.03463	.443
	Total	---	.00032	.03065	.06927	.443
GDA					
	B	.62208	.00121	.16038	.23510	.682
	C	.28231	.00157	.11538	.20261	.569
	D	.09562	.00044	.06961	.08647	.805
	Total	---	.00322	.34537	.52418	.659

GPI-1					
	B	.00462	.00005	.00020	.00459	.044
	C	.99154	.00008	.00032	.00839	.038
	D	.00385	.00003	.00015	.00383	.038
	Total	---	.00016	.00067	.01682	.040
GPI-2					
	A	.19862	.00148	.12331	.15917	.775
	C	.80138	.00148	.12331	.15917	.775
	Total	---	.00297	.24661	.31833	.775
G3PDH-2					
	A	.00123	.00004	.00000	.00123	.000
	B	.99654	.00011	.00000	.00345	.000
	C	.00223	.00007	.00000	.00223	.000
	Total	---	.00023	.00000	.00690	.000
G6PDH-1					
	A	.16115	.00082	.08149	.13518	.603
	B	.82754	.00087	.08666	.14272	.607
	C	.01131	.00019	.00134	.01118	.120
	Total	---	.00188	.16950	.28908	.586
G6PDH-2					
	A	.33569	.00036	.20484	.22300	.919
	B	.66431	.00036	.20484	.22300	.919
	Total	---	.00071	.40968	.44601	.919
IDDH					
	A	.40000	.00041	.22728	.24000	.947
	B	.01446	.00039	.00212	.01425	.149
	C	.57177	.00094	.20856	.24485	.852
	D	.01377	.00013	.00214	.01358	.158
	Total	---	.00187	.44010	.51268	.858
IDHP-1					
	B	.86177	.00113	.08662	.11912	.727
	D	.13823	.00113	.08662	.11912	.727
	Total	---	.00225	.17324	.23825	.727
LDH-1					
	C	.89185	.00089	.03897	.09646	.404
	D	.10815	.00089	.03897	.09646	.404
	Total	---	.00177	.07795	.19291	.404
LDH-2					
	A	.03569	.00048	.01481	.03442	.430
	B	.96431	.00048	.01481	.03442	.430
	Total	---	.00096	.02962	.06884	.430
MDH-2					
	B	.92308	.00000	.07101	.07101	1.000
	C	.07692	.00000	.07101	.07101	1.000
	Total	---	.00000	.14201	.14201	1.000
MDH-3					
	B	.84615	.00000	.13018	.13018	1.000
	C	.07692	.00000	.07101	.07101	1.000
	D	.07692	.00000	.07101	.07101	1.000
	Total	---	.00000	.27219	.27219	1.000
MEP-1					
	A	.95723	.00063	.02132	.04094	.521
	B	.04277	.00063	.02132	.04094	.521
	Total	---	.00127	.04263	.08188	.521

MEP-2						
	A	.02200	.00013	.00568	.02152	.264
	B	.08931	.00041	.05680	.08133	.698
	C	.53615	.00137	.20137	.24869	.810
	D	.05338	.00064	.01126	.05053	.223
	E	.23038	.00221	.08474	.17731	.478
	F	.06877	.00096	.01057	.06404	.165
	Total	---	.00572	.37042	.64342	.576
PGDH						
	D	.09338	.00015	.07157	.08466	.845
	E	.90662	.00015	.07157	.08466	.845
	Total	---	.00031	.14314	.16933	.845
PGM						
	B	.96823	.00074	.00368	.03076	.120
	C	.01923	.00048	.00396	.01886	.210
	D	.01254	.00026	.00021	.01238	.017
	Total	---	.00147	.00784	.06200	.126
SOD						
	B	.55000	.00311	.13860	.24750	.560
	C	.44808	.00315	.13648	.24730	.552
	D	.00192	.00005	.00000	.00192	.000
	Total	---	.00631	.27507	.49672	.554
Average		----	.00212	.24620	.33544	.734

SUBGENUS *O. (OREOCROMIS)*

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2	C	.06300	.00124	.00900	.05903	.152
	D	.68033	.00166	.14416	.21748	.663
	F	.25667	.00047	.16125	.19079	.845
	Total	---	.00337	.31440	.46730	.673
AAT-3	B	.27078	.00071	.16307	.19746	.826
	C	.61811	.00071	.20166	.23605	.854
	D	.11111	.00000	.09877	.09877	1.000
	Total	---	.00141	.46349	.53227	.871
ADA	A	.09033	.00056	.06472	.08217	.788
	B	.00056	.00000	.00000	.00056	.000
	C	.02078	.00056	.00289	.02035	.142
	D	.06144	.00023	.02997	.05767	.520
	E	.00756	.00006	.00040	.00750	.053
	G	.04156	.00022	.01360	.03983	.341
	H	.03333	.00078	.00811	.03222	.252
	J	.33700	.00151	.17443	.22343	.781
	M	.04444	.00089	.01491	.04247	.351
	N	.00556	.00026	.00000	.00552	.000
	O	.13522	.00047	.09759	.11694	.835
	P	.05556	.00093	.02377	.05247	.453
	R	.11111	.00000	.09877	.09877	1.000
	S	.05556	.00093	.02377	.05247	.453
	Total	---	.00740	.55291	.83236	.664

ADH					
B	.04044	.00064	.01244	.03881	.321
C	.11111	.00000	.09877	.09877	1.000
D	.73733	.00064	.16731	.19367	.864
F	.11111	.00000	.09877	.09877	1.000
Total	---	.00129	.37728	.43001	.877
AH-2					
B	.05556	.00093	.02377	.05247	.453
C	.19789	.00171	.08953	.15873	.564
D	.18333	.00264	.06684	.14972	.446
E	.31167	.00331	.07747	.21453	.361
F	.08333	.00197	.02581	.07639	.338
G	.05711	.00066	.01117	.05385	.207
H	.11111	.00000	.09877	.09877	1.000
Total	---	.01122	.39336	.80445	.489
AK					
A	.88889	.00000	.09877	.09877	1.000
B	.11111	.00000	.09877	.09877	1.000
Total	---	.00000	.19753	.19753	1.000
ALAT					
A	.75922	.00052	.16683	.18280	.913
B	.24078	.00052	.16683	.18280	.913
Total	---	.00103	.33366	.36561	.913
CK					
A	.66667	.00000	.22222	.22222	1.000
B	.11111	.00000	.09877	.09877	1.000
C	.22222	.00000	.17284	.17284	1.000
Total	---	.00000	.49383	.49383	1.000
DDH-1					
A	.08333	.00052	.05503	.07639	.720
B	.25000	.00052	.16615	.18750	.886
C	.66667	.00000	.22222	.22222	1.000
Total	---	.00104	.44340	.48611	.912
EST-1					
A	.24600	.00047	.16633	.18548	.897
B	.75400	.00047	.16633	.18548	.897
Total	---	.00093	.33265	.37097	.897
EST-2					
B	.05122	.00116	.01266	.04860	.261
C	.01322	.00010	.00130	.01305	.100
D	.81756	.00142	.09485	.14916	.636
E	.11800	.00016	.09745	.10408	.936
Total	---	.00284	.20627	.31488	.655
FBALD-2					
B	.32222	.00033	.20806	.21840	.953
C	.67778	.00033	.20806	.21840	.953
Total	---	.00067	.41612	.43679	.953
FH-1					
A	.11111	.00000	.09877	.09877	1.000
B	.77778	.00000	.17284	.17284	1.000
C	.11111	.00000	.09877	.09877	1.000
Total	---	.00000	.37037	.37037	1.000
FH-2					
A	.05189	.00023	.02131	.04920	.433
B	.94811	.00023	.02131	.04920	.433
Total	---	.00046	.04262	.09839	.433

GDA					
	B	.65000	.00060	.19606	.22750	.862
	C	.22500	.00101	.13038	.17437	.748
	D	.12500	.00041	.09682	.10938	.885
	Total	---	.00202	.42326	.51125	.828
GPI-1					
	C	.99444	.00004	.00020	.00552	.037
	D	.00556	.00004	.00020	.00552	.037
	Total	---	.00009	.00041	.01105	.037
GPI-2					
	A	.28689	.00214	.15278	.20458	.747
	C	.71311	.00214	.15278	.20458	.747
	Total	---	.00429	.30557	.40917	.747
G3PDH-2					
	A	.00178	.00006	.00000	.00177	.000
	B	.99500	.00016	.00000	.00497	.000
	C	.00322	.00010	.00000	.00321	.000
	Total	---	.00033	.00000	.00996	.000
G6PDH-1					
	A	.11111	.00000	.09877	.09877	1.000
	B	.88889	.00000	.09877	.09877	1.000
	Total	---	.00000	.19753	.19753	1.000
G6PDH-2					
	A	.44444	.00000	.24691	.24691	1.000
	B	.55556	.00000	.24691	.24691	1.000
	Total	---	.00000	.49383	.49383	1.000
IDDH					
	A	.46667	.00059	.23052	.24889	.926
	B	.02089	.00057	.00293	.02045	.143
	C	.51244	.00116	.21395	.24985	.856
	Total	---	.00232	.44739	.51919	.862
IDHP-1					
	B	.81889	.00139	.11705	.14831	.789
	D	.18111	.00139	.11705	.14831	.789
	Total	---	.00279	.23410	.29662	.789
LDH-2					
	A	.05156	.00069	.02057	.04890	.421
	B	.94844	.00069	.02057	.04890	.421
	Total	---	.00138	.04115	.09780	.421
MDH-2					
	B	.88889	.00000	.09877	.09877	1.000
	C	.11111	.00000	.09877	.09877	1.000
	Total	---	.00000	.19753	.19753	1.000
MDH-3					
	B	.77778	.00000	.17284	.17284	1.000
	C	.11111	.00000	.09877	.09877	1.000
	D	.11111	.00000	.09877	.09877	1.000
	Total	---	.00000	.37037	.37037	1.000
MEP-1					
	A	.93822	.00091	.02962	.05796	.511
	B	.06178	.00091	.02962	.05796	.511
	Total	---	.00183	.05924	.11592	.511

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
MEP-2						
	A	.03178	.00019	.00789	.03077	.256
	B	.12900	.00059	.07692	.11236	.685
	C	.66333	.00198	.15497	.22332	.694
	E	.14411	.00192	.07172	.12334	.581
	F	.03178	.00057	.00751	.03077	.244
	Total	---	.00525	.31902	.52056	.613
PGDH						
	D	.11111	.00000	.09877	.09877	1.000
	E	.88889	.00000	.09877	.09877	1.000
	Total	---	.00000	.19753	.19753	1.000
PGM						
	B	.96722	.00085	.00524	.03170	.165
	C	.02778	.00069	.00548	.02701	.203
	D	.00500	.00016	.00004	.00498	.008
	Total	---	.00171	.01076	.06368	.169
SOD						
	B	.61800	.00297	.15018	.23608	.636
	C	.37922	.00303	.14674	.23541	.623
	D	.00278	.00007	.00000	.00277	.000
	Total	---	.00607	.29691	.47426	.626
Average		----	.00199	.28442	.35624	.798

SUBGENUS *O. (NYASALAPIA)*

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2						
	C	.06250	.00234	.00938	.05859	.160
	D	.63800	.00322	.08905	.23096	.386
	E	.29950	.00478	.03508	.20980	.167
	Total	---	.01035	.13350	.49935	.267
AAT-3						
	B	.12500	.00313	.04375	.10938	.400
	C	.87500	.00313	.04375	.10938	.400
	Total	---	.00625	.08750	.21875	.400
ADA						
	I	.36225	.00152	.13815	.23102	.598
	J	.18425	.00174	.04640	.15030	.309
	K	.20225	.00192	.04002	.16134	.248
	L	.25125	.00209	.04167	.18812	.222
	Total	---	.00728	.26625	.73080	.364
ADH						
	B	.05700	.00091	.00374	.05375	.070
	C	.03125	.00137	.00156	.03027	.052
	D	.89450	.00249	.00021	.09437	.002
	E	.01725	.00023	.00012	.01695	.007
	Total	---	.00500	.00563	.19535	.029

AH-2					
	C	.13750	.00177	.00569	.11859	.048
	D	.33475	.00283	.03990	.22269	.179
	E	.13675	.00275	.01007	.11805	.085
	G	.39100	.00386	.06841	.23812	.287
	Total	---	.01120	.12407	.69745	.178
CK					
	A	.25000	.00000	.18750	.18750	1.000
	C	.75000	.00000	.18750	.18750	1.000
	Total	---	.00000	.37500	.37500	1.000
EST-2					
	D	.95100	.00055	.00423	.04660	.091
	E	.04900	.00055	.00423	.04660	.091
	Total	---	.00111	.00845	.09320	.091
FBALD-2					
	C	.75000	.00000	.18750	.18750	1.000
	D	.25000	.00000	.18750	.18750	1.000
	Total	---	.00000	.37500	.37500	1.000
GDA					
	B	.55925	.00258	.07438	.24649	.302
	C	.41125	.00283	.05762	.24212	.238
	D	.02950	.00052	.00209	.02863	.073
	Total	---	.00593	.13409	.51724	.259
GPI-1					
	B	.01500	.00017	.00051	.01477	.034
	C	.98500	.00017	.00051	.01477	.034
	Total	---	.00034	.00101	.02955	.034
G6PDH-1					
	A	.27375	.00267	.02432	.19881	.122
	B	.68950	.00282	.03191	.21409	.149
	C	.03675	.00063	.00342	.03540	.097
	Total	---	.00612	.05966	.44830	.133
G6PDH-2					
	A	.09100	.00116	.02369	.08272	.286
	B	.90900	.00116	.02369	.08272	.286
	Total	---	.00232	.04737	.16544	.286
IDDH					
	A	.25000	.00000	.18750	.18750	1.000
	C	.70525	.00044	.17070	.20787	.821
	D	.04475	.00044	.00557	.04275	.130
	Total	---	.00087	.36377	.43812	.830
IDHP-1					
	B	.95825	.00053	.00470	.04001	.118
	D	.04175	.00053	.00470	.04001	.118
	Total	---	.00105	.00940	.08001	.118
LDH-1					
	C	.64850	.00288	.04113	.22795	.180
	D	.35150	.00288	.04113	.22795	.180
	Total	---	.00576	.08226	.45590	.180
MEP-2					
	C	.25000	.00000	.18750	.18750	1.000
	D	.17350	.00207	.01575	.14340	.110
	E	.42450	.00287	.05960	.24430	.244
	F	.15200	.00184	.00743	.12890	.058
	Total	---	.00679	.27029	.70409	.384

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
PGDH						
	D	.05350	.00050	.00809	.05064	.160
	E	.94650	.00050	.00809	.05064	.160
	Total	---	.00100	.01617	.10128	.160
PGM						
	B	.97050	.00047	.00016	.02863	.006
	D	.02950	.00047	.00016	.02863	.006
	Total	---	.00094	.00032	.05726	.006
SOD						
	B	.39700	.00342	.07873	.23939	.329
	C	.60300	.00342	.07873	.23939	.329
	Total	---	.00685	.15746	.47878	.329
Average		----	.00417	.13248	.35057	.378

THE CHAMBO

LOCUS	Allele	Mean frequency	Sampling variance	Actual variance	Limiting variance	F ST
AAT-2						
	A	.80883	.00350	.00257	.15462	.017
	B	.19117	.00350	.00257	.15462	.017
	Total	---	.00699	.00515	.30925	.017
ADA						
	A	.14969	.00412	.00135	.12728	.011
	B	.24568	.00420	.04491	.18532	.242
	C	.26979	.00532	.03244	.19700	.165
	D	.33484	.00427	.02598	.22272	.117
	Total	---	.01791	.10468	.73233	.143
ADH						
	A	.02309	.00031	.00003	.02255	.001
	B	.90115	.00211	.00131	.08908	.015
	C	.07576	.00183	.00291	.07002	.042
	Total	---	.00426	.00426	.18165	.023
AH-2						
	A	.18344	.00391	.00000	.14979	.000
	B	.44643	.00634	.00077	.24713	.003
	C	.12644	.00161	.01503	.11046	.136
	D	.24369	.00508	.00441	.18430	.024
	Total	---	.01695	.02021	.69168	.029
EST-2						
	A	.93464	.00083	.00444	.06109	.073
	B	.06536	.00083	.00444	.06109	.073
	Total	---	.00166	.00889	.12218	.073
GDA						
	A	.41261	.00418	.01211	.24236	.050
	B	.54818	.00465	.00080	.24768	.003
	C	.03922	.00102	.00206	.03768	.055
	Total	---	.00984	.01496	.52772	.028

G6PDH-1					
	A	.36479	.00466	.23172	.000
	B	.58619	.00496	.24257	.000
	C	.04902	.00123	.04662	.077
	Total	---	.01085	.52091	.007
G6PDH-2					
	A	.12121	.00351	.10652	.243
	B	.87879	.00351	.10652	.243
	Total	---	.00701	.21304	.243
GPI-1					
	A	.01984	.00022	.01945	.029
	B	.98016	.00022	.01945	.029
	Total	---	.00044	.03890	.029
IDDH					
	B	.94048	.00175	.05598	.095
	C	.05952	.00175	.05598	.095
	Total	---	.00349	.11196	.095
IDHP-1					
	A	.94444	.00154	.05247	.088
	B	.05556	.00154	.05247	.088
	Total	---	.00309	.10494	.088
LDH-1					
	A	.53140	.00482	.24901	.000
	B	.46860	.00482	.24901	.000
	Total	---	.00965	.49803	.000
MEP-2					
	A	.23156	.00401	.17794	.036
	B	.56612	.00545	.24563	.000
	C	.20233	.00349	.16139	.000
	Total	---	.01295	.58496	.011
PGM					
	A	.96082	.00090	.03764	.000
	B	.03918	.00090	.03764	.000
	Total	---	.00181	.07529	.000
PGDH					
	A	.07143	.00134	.06633	.134
	B	.92857	.00134	.06633	.134
	Total	---	.00267	.13265	.134
SOD					
	A	.23776	.00574	.18123	.012
	B	.76224	.00574	.18123	.012
	Total	---	.01148	.36246	.012
Average		----	.00757	.32550	.051

3. Contingency chi-square analyses for heterogeneity

AMONG ALL THREE CHAMBO SPECIES

Locus	No. of alleles	Chi-square	D.F.	P
AAT-2	2	7.767	2	.02058
ADA	4	66.804	6	.00000
ADH	3	14.718	4	.00532
AH-2	4	23.247	6	.00072
EST-2	2	15.224	2	.00049
GDA	3	22.099	4	.00019
G6PDH-1	3	20.301	4	.00044
G6PDH-2	2	57.206	2	.00000
GPI-1	2	7.082	2	.02899
IDDH	2	21.459	2	.00002
IDHP-1	2	23.036	2	.00001
LDH-1	2	2.325	2	.31268
MEP-2	3	13.285	4	.00996
PGM	2	1.726	2	.42181
PGDH	2	26.359	2	.00000
SOD	2	5.072	2	.07918
(Totals)		327.710	48	.00000

BETWEEN EACH PAIR OF SPECIES :

1) *O. (NY.) KARONGAE & O. (NY.) LIDOLE*

Locus	No. of alleles	Chi-square	D.F.	P
AAT-2	2	6.856	1	.00883
ADA	4	55.552	3	.00000
ADH	3	5.876	2	.05296
AH-2	4	15.332	3	.00155
EST-2	2	12.132	1	.00050
GDA	2	.465	1	.49546
G6PDH-1	2	1.416	1	.23401
GPI-1	2	4.064	1	.04381
IDDH	2	12.448	1	.00042
IDHP-1	2	14.642	1	.00013
LDH-1	2	1.720	1	.18963
MEP-2	3	13.164	2	.00139
PGM	2	.602	1	.43778
PGDH	2	15.429	1	.00009
SOD	2	4.781	1	.02878
(Totals)		164.480	21	.00000

2) *O. (NY.) KARONGAE & O. (NY.) SQUAMIPINNIS*

Locus	No. of alleles	Chi-square	D.F.	P
AAT-2	2	5.212	1	.02243
ADA	4	20.984	3	.00011
ADH	3	15.195	2	.00050
AH-2	4	3.047	3	.38441
EST-2	2	4.109	1	.04265
GDA	3	12.323	2	.00211
G6PDH-1	3	10.181	2	.00616
G6PDH-2	2	33.039	1	.00000
GPI-1	2	3.092	1	.07870
IDDH	2	9.540	1	.00201
LDH-1	2	.081	1	.77553
MEP-2	3	5.179	2	.07505
PGM	2	.505	1	.47731
PGDH	2	11.876	1	.00057
SOD	2	.230	1	.63185
(Totals)		134.593	23	.00000

3) *O. (NY.) LIDOLE & O. (NY.) SQUAMIPINNIS*

Locus	No. of alleles	Chi-square	D.F.	P
AAT-2	2	.006	1	.93772
ADA	4	35.650	3	.00000
ADH	3	3.925	2	.14051
AH-2	4	12.104	3	.00704
EST-2	2	1.961	1	.16143
GDA	3	13.638	2	.00109
G6PDH-1	3	9.492	2	.00868
G6PDH-2	2	26.400	1	.00000
IDHP-1	2	8.889	1	.00287
LDH-1	2	1.702	1	.19207
MEP-2	3	.453	2	.79734
PGM	2	1.808	1	.17875
SOD	2	1.579	1	.20897
(Totals)		117.607	21	.00000

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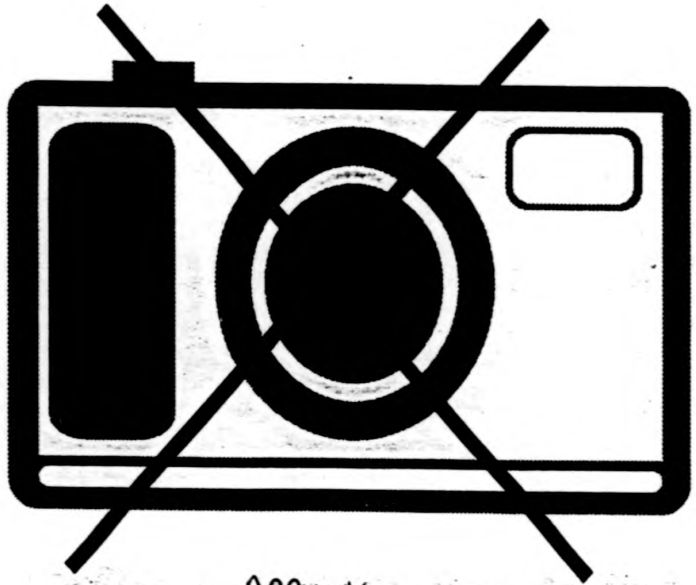

APPx 4