The Limnology, Climatology and Paleoclimatology of the East African Lakes

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Molecular Phylogenetic Inferences About the Evolutionary History of East African Cichlid Fish Radiations

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Abstract — The species flocks of cichlid fishes from the Great East African Lakes, Victoria, Malawi and Tanganyika, are well-known among evolutionary biologists as extreme examples for adaptive radiation and explosive speciation. Of all radiations involving vertebrates, these species assemblages are the most species-rich and the most diverse, morphologically, ecologically and behaviorally. Traditionally, all knowledge about the evolution and phylogenetic relationships within and between these species flocks was derived from morphological analyses but recently molecular DNA-based data sets have provided new insights into the phylogenetic and biogeographic history of East African cichlid fishes. Phenotypic (e.g., morphological) and genotypic (e.g., DNA sequences of genes) data sets are expected to provide concordant phylogenetic information about these species assemblages, since both share identical evolutionary histories. Molecular data however have several advantages for phylogeny reconstruction over morphological data, e.g., DNA sequences tend to diverge with some regularity over time, which may or may not be true for morphological data. This "molecular clock" allows one to make time estimates of speciation events in the absence fossils. Our understanding of the phylogenetic relationships, history and evolutionary processes among East African cichlid fish species flocks has increased rapidly since the recent invention of the polymerase chain reaction (PCR) which dramatically facilitated the collection of DNA sequence data. Phylogenetic analyses of recent molecular data (mostly mitochondrial DNA sequences) in the context of the geological history of the Great East African Lakes helped to elucidate some aspects of the evolutionary historical patterns and evolutionary processes that might have led to the origin of these extraordinary fish faunas. Here, we summarize recent findings on the molecular phylogenetic relationships of endemic species of Lakes Malawi and Victoria and non-endemic, riverine species of haplochromine cichlids that phylogenetically connect these two species flocks. The DNA-sequence phylogeny confirms that the endemic species flock of Lake Tanganyika is by far the oldest and provided an evolutionary reservoire for the species diversity of East Africa. The Tropheini, among the tribes endemic to Lake Tanganyika, are found to be the closest living relatives to the haplochromine cichlids from Lake Tanganyika and outside of it. New mitochondrial control region DNA sequences, collected for this study, confirm that the vast majority of Lake Malawi cichlids can be assigned to two genetic and ecological groups, one that lives over sandy bottoms, and another one that lives over rocky substrate. Three other lineages are identified for Lake Malawi: *Rhamphochromis*, *Astatotilapia calliptera*, and possibly *Diplotaxodon*. *Copadichromis* which had been suggested to be another separate lineage based on mitochondrial restriction data (Moran, Reinthal and Kornfield, 1994) could, based on mitochondrial DNA sequences not be confirmed to be distinct from the "sand" group (called group "A" according to Meyer et al., 1990). Both Lake Victoria and Malawi species flocks are equally distantly related to the Tropheini. Non-endemic East African haplochromine cichlids (e.g., *Serranochromis*, *Astatoreochromis*, *Astatotilapia*, *Orthochromis*, and *Schwetzochromis*) are more closely related to these endemic flocks (and provide biogeographic links) than any Tanganyikan endemic cichlid species.

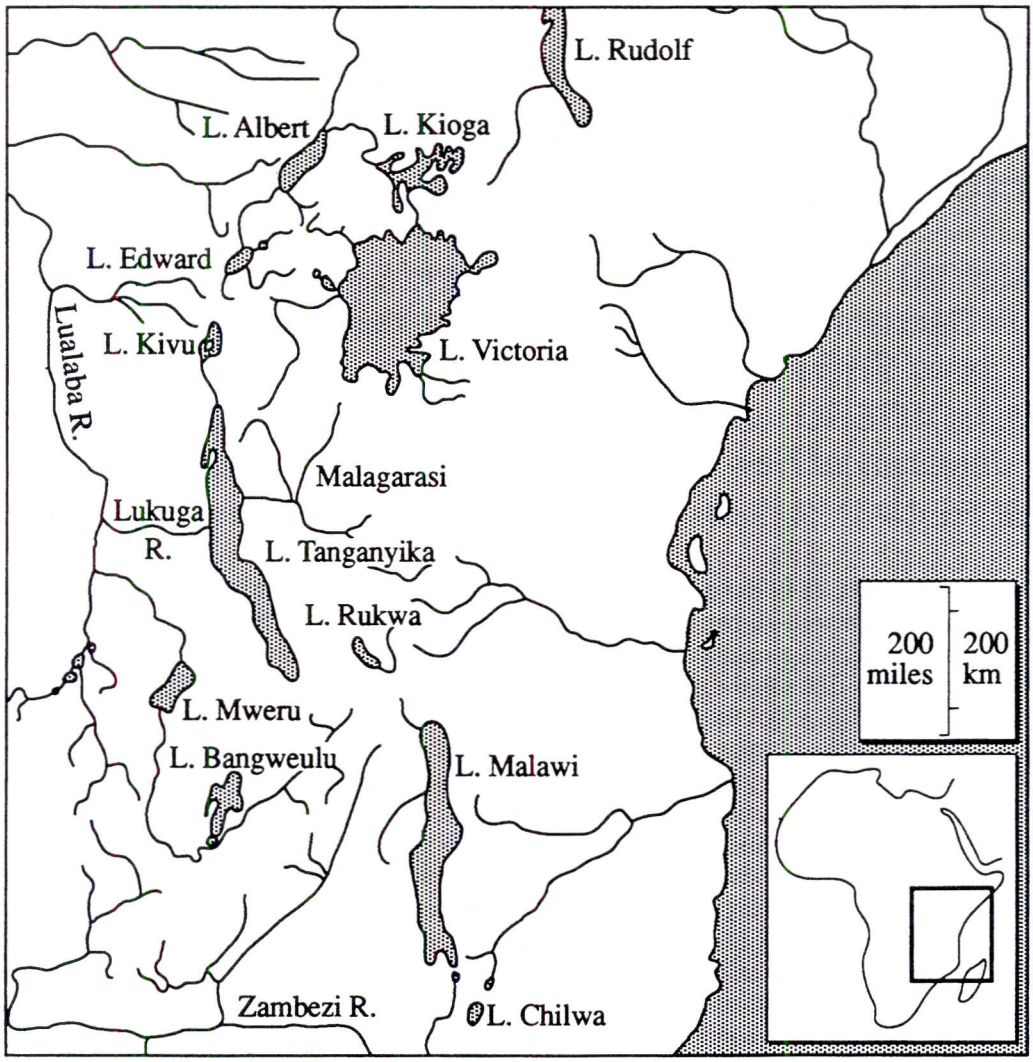

Figure 1. Map of East Africa. Figure redrawn after Fryer and Iles (1972).

INTRODUCTION

The cichlid fish faunas of the three Great East African Lakes, Victoria, Tanganyika, and Malawi, are enormously diverse and a testimony to the evolutionary success of cichlid fishes. Each of these lakes (Fig. 1) harbors a radiation of several hundred species (Fryer and Iles, 1972) almost all of which are endemic to their particular lake. These species flocks make the Cichlidae one of the most species-rich family of vertebrates (Fig. 2). The special history of cichlids is highlighted by the coexistence of other families of fishes in each of these three lakes, that have not undergone this kind of spectacular evolution. The evolutionary origin and ecological maintenance of the enormous cichlid species diversity has been much researched and debated (e.g., Mayr, 1942, 1984; Fryer and Iles, 1972; Coulter, 1991; Keenleyside 1991). Despite this long, still ongoing debate, the phylogenetic relationships among the endemic cichlid faunas have remained largely unresolved since no morphological feature could be found to be characteristic of all members of a particular radiation that might have unambiguously indicated that each of these radiations are, to some degree, independent of each other (e.g., Stiassny, 1981; Greenwood, 1983).

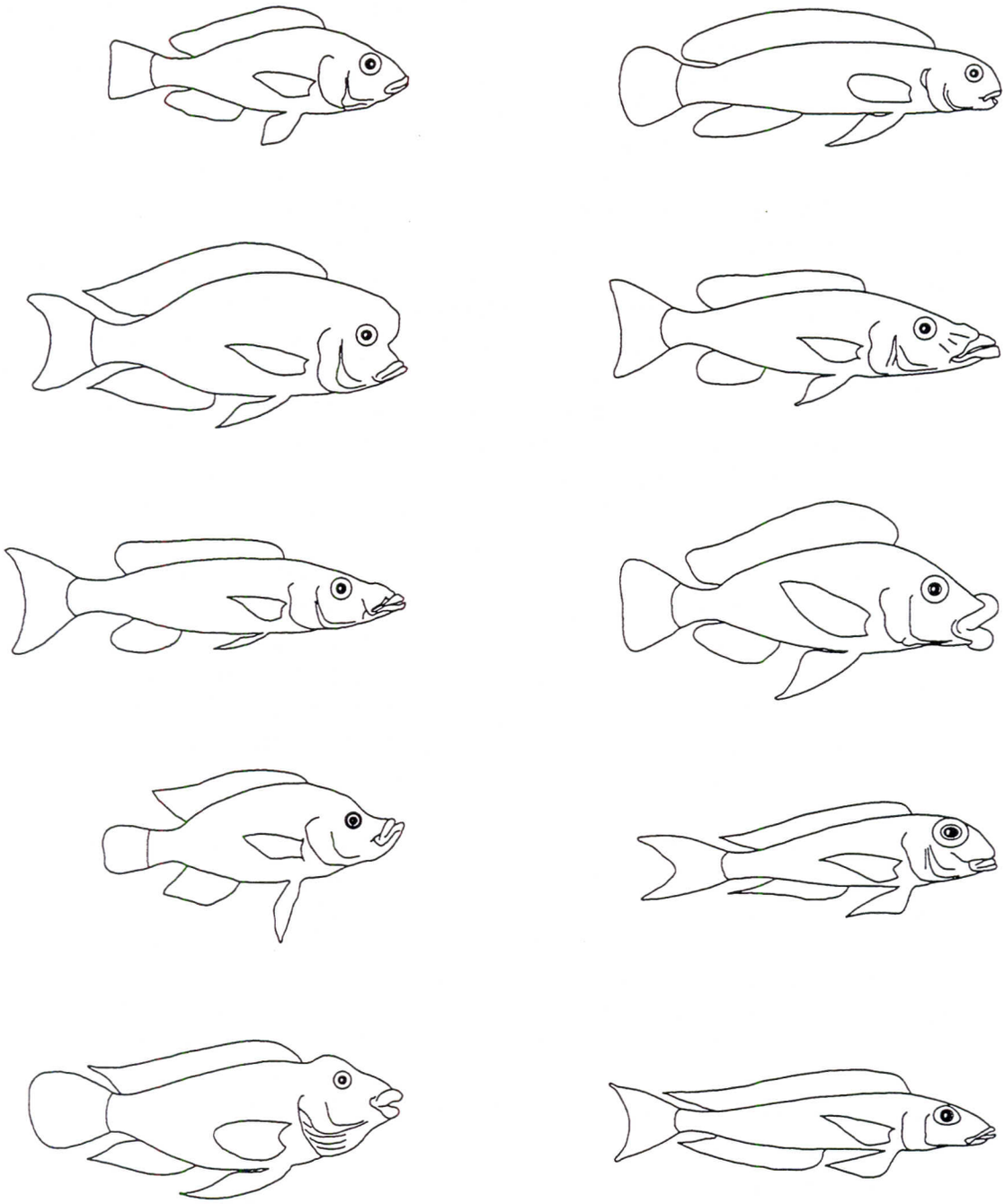

Figure 2. Body form variation found among East African cichlid fish from Lake Victoria (V), Lake Tanganyika (T) and Lake Malawi (M). Figure redrawn after Fryer and Iles (1972) and Greenwood (1984). The fish are (row by row) from left top to lower right: (V) generalized "*Haplochromis*," (T) *Telmatochromis vittatus*, (M) *Cyrtocara moori*, (M) *Rhamphochromis longiceps*, (M) *Rhamphochromis macrophthalmus*, (T) *Lobochilotes labiatus*, (V) *Pyxichromis parothostoma*, (T) *Xenotilapia sima*, (T) *Spathodus malieri*, (T) *Xenotilapia melanogenys*.

The species flocks (defined as monophyletic, i.e., containing a single ancestral species and all of its descendent species which inhabit one lake, Greenwood, 1984) of all three of the lakes contain a sweeping array of morphologically and behaviorally highly specialized

cichlids (Fryer and Iles, 1972). An often mentioned reason for the evolutionary success and diversification of cichlids is a morphological novelty only they possess (Liem, 1973; Osse and Liem, 1975). Cichlids have a second set of jaws in back of their buccal cavity, the modified pharyngeal jaws, that are functionally de-coupled from their oral jaws. This key innovation is believed to have allowed cichlids to become highly specialized on particular types of prey and to have facilitated the evolution of fine ecological niche-partitioning. This second set of jaws might allow cichlids to out-compete other fish inhabiting the Great East African Lakes that do not possess them (Liem, 1973; Osse and Liem, 1975).

Some highly derived morphological and ecological specializations are similar between species endemic to different lakes, i.e., similar morphological solutions to the same ecological problems have been found in more than one cichlid species flock (Greenwood, 1983). The Lake Victoria endemic *Macropleurodus bicolor* and the Lake Malawi endemic *Chilotilapia rhoadesi* both have highly derived dentition, jaw structures and feeding behaviors — they prey on gastropods by crushing their shells with their oral jaws (Fryer and Iles, 1972; Greenwood, 1983). Unfortunately, the evolutionary relationships among the species assemblages remained unknown, the question of whether each of the assemblages is monophyletic, i.e., can be traced back to a single ancestral species, and consequently whether the above mentioned morphological similarities between members of different flocks evolved more than once independently, as parallelisms, remained unanswered. Alternatively, specializations could have arisen only once and would indicate polyphyletic origins (several ancestral species per lake) for the species flocks with each of several lineages having a geographic distribution that extends beyond the boundaries of a single lake (Stiassny, 1981; Greenwood, 1983). This interpretation would indicate that relationships of recent common ancestry exist among many of the members of the three species flocks (Fryer and Iles, 1972; Greenwood, 1983).

Much insight about the tempo and mode of evolution and about the origin of morphological solutions to ecological problems can be gained from an understanding of the evolutionary relationships among and between members of these species flocks. Estimates on rates of speciation in these flocks will hinge on basic knowledge (like monophyly versus polyphyly) and the age of the species assemblages. The identification of sister group relationships will help to pinpoint which characteristics in ancestors of these species flocks might have made them successful colonizers of these lakes and founders of species flocks.

TRACING EVOLUTIONARY HISTORIES WITH MORPHOLOGY AND MOLECULES

Each organism's phenotype and its underlying genotype have experienced the same evolutionary history, except for presumably rare cases of horizontal gene transfer. Hence, both general types of data sets should provide the same reliable estimates of phylogenetic relationships among species (Hillis, 1987; Patterson, 1987). Data derived from the phenotypes of organisms, which traditionally consist of morphological characters and various kinds of biochemical data reflecting the genotype are expected to share identical evolutionary histories. Molecular data sets are usually easier to obtain than morphological data sets. This is because often only experts of a particular group of organisms are able to identify meaningful morphological characters for a phylogenetic analysis which aims to reconstruct the phylogeny of the species under consideration. The number of molecular characters that can be identified in species is essentially without limits since each species' genome is made up of billions of DNA base pairs each of which potentially contains phylogenetic informa-

tion. The number of characters that can be identified in the phenotype of organisms is limited by the morphologist's abilities working on the group to identify characters for a phylogenetic analysis and will tend to be orders of magnitude fewer.

Molecular data can have the added advantage over morphological data sets that they can be collected in objective metrics, e.g., DNA sequences of particular genes from several laboratories can be combined and applied to phylogenetic questions that were not intended in the original study. Such universal metrics are, e.g., small nuclear ribosomal RNA gene (18S) sequences that have been collected for a wide variety of organisms. This potential of some (but not all types of) molecular data sets to be "universal metrics" for the purpose of phylogeny reconstruction is not present in morphological data since each of these data sets must be newly established for every phenotype-based phylogenetic study and such data sets are only rarely transferable between studies. Still, one type of data set is not inherently better than another, both exhibit "phylogenetic noise" (e.g., homoplasy). Both provide useful phylogenetic information, while the signal-to-noise ratio is often similar in both kinds of data sets (Hillis, 1987; Sanderson and Donoghue, 1989; and also behavioral characters: DeQueiroz and Wimberger, 1993).

Since congruence in phylogenetic estimates is expected from both kinds of data sets, it has been argued that the combination of both morphological and molecular data sets should provide "total evidence" (Kluge, 1989). There are, however, numerous problems when both data sets are combined, and when different phylogenetic answers are obtained if these data sets are analyzed separately (reviewed in Swofford, 1991; Maddison and Maddison, 1992).

Several kinds of biochemical data are typically used to infer phylogenetic relationships among species. Allozyme, immunological and DNA–DNA hybridization data have been widely used but are now increasingly replaced by several types of DNA-based data (reviewed in Meyer, 1993b). Since the advent of the polymerase chain reaction (PCR) in 1985–86 (Saiki et al., 1985, 1988; Wrishnik et al., 1987), our knowledge about DNA and phylogeny of vertebrates has increased dramatically (reviewed in Kocher et al., 1989; Meyer, 1993a,b).

THE POLYMERASE CHAIN REACTION AND DIRECT SEQUENCING

The polymerase chain reaction (PCR) is an enzymatic cloning technique that allows the amplification of portions of genes (within size limits of maximally several thousand base pairs) that are defined by synthetic oligonucleotide "primers" (Saiki et al., 1985, 1988; reviews in, e.g., White et al., 1989; Arnheim et al. 1990; refs. in Erlich 1989; Innis et al., 1990) (Fig. 3). The primers are usually around 20 base pairs in length and define the beginning and the end of the double-stranded piece of DNA that is going to be amplified. The specificity of the amplification is accomplished through the need for an almost-perfect fit of the primers to the template DNA (Kwok et al., 1990). During each cycle of PCR, the number of copies of the DNA-fragment delineated by the primers at either end is doubled (Fig. 3). Usually 25–40 cycles are completed in a computer controlled heating block (thermal cycler) in about three hours creating millions and millions of identical copies of a piece of a DNA. PCR is much faster and cheaper than conventional cloning techniques. First, a double-stranded PCR product is produced that is then either sequenced (double stranded sequencing, or alternatively "cycle-sequenced"), or subcloned and then sequenced, or cut with restriction enzymes (RFLP data) or used as template DNA for a subsequent asymmetric amplification (Gyllensten and Erlich, 1988) or digested with an exonuclease to produce single-stranded DNA for direct sequencing of single-stranded DNA. Sequencing

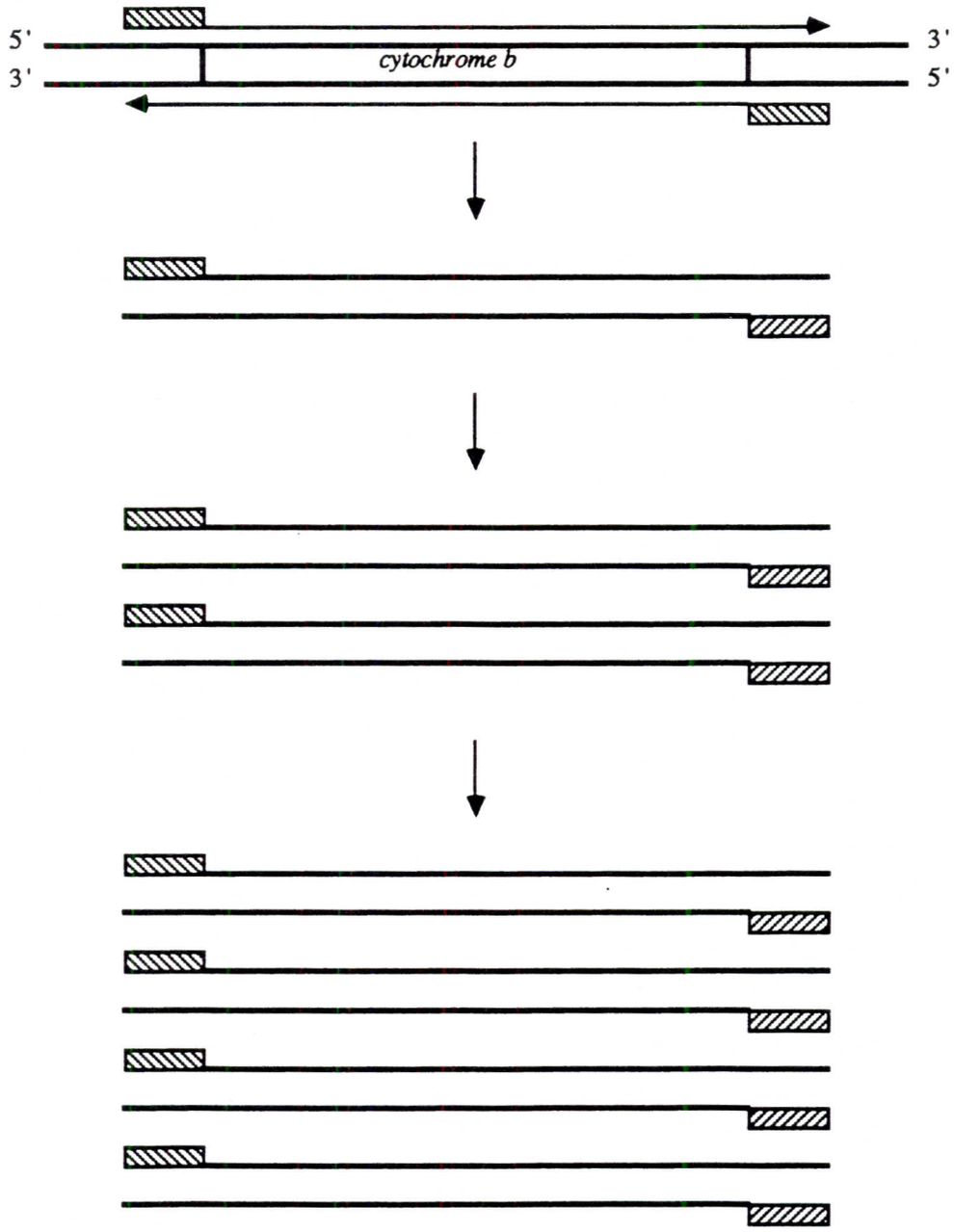

Figure 3. The principle of the polymerase chain reaction. Modified after Wrishnik et al., 1987. The hatched boxes represent "primers" small oligonucleotides (about 20 base pairs in length) whose DNA sequence is complementary to the stretch of DNA for which they are designed to attach, in this example the regions flanking the mitochondrial cytochrome *b* gene. During each cycle of the polymerase chain reaction the number of copies that are defined by the 5′ ends of the two primers is doubled leading to an exponential increase in the number of identical pieces of DNA with each PCR-cycle. Primers are incorporated into the copied DNA strands as indicated. See text and cited references (e.g., Erlich, 1989; White et al., 1989) for more details.

gels of single-stranded DNA often allow one to read more base pairs than sequencing gels of double-stranded DNA. Single-stranded PCR amplified DNA can be as clean as sub-cloned DNA and routinely more than 300–400 bp can be unambiguously determined from a single sequencing reaction.

The determination of DNA sequences tends to be more time-consuming, costly and technically involved than several other molecular data sets that can be used for phylogenetic analysis, however DNA sequences of homologous mitochondrial and nuclear genes will allow direct comparisons and study of DNA from different species that have been determined in different laboratories — DNA sequences of the same genes are "universal metrics" that can be transferred between different studies and laboratories. DNA sequences are stored in data banks (e.g., EMBL, GenBank) and are universally usable, powerful data. The increased costs of DNA sequences compared to, e.g., RFLP data are far outweighed by their advantage as a universally retrievable, and applicable type of data, since homologous data from independent laboratories can be used in direct comparisons for several studies.

MOLECULAR DATA AND THE EVOLUTIONARY HISTORY OF EAST AFRICAN CICHLID RADIATIONS

Until lately, evolutionary studies on cichlids relied exclusively on morphological characters and were subject to the danger of circularity by interpreting the evolution of the same morphological specializations that were used to construct the phylogenetic relationships. Molecular approaches, specifically the study of the mitochondrial genome through restriction enzyme analysis and more recently through DNA sequences are providing many new insights and some surprising results (Kornfield, 1991; Meyer et al., 1990, 1991; Sturmbauer and Meyer, 1992, 1993; Kocher et al., 1993; Sturmbauer, Verheyen and Meyer, 1994). In these molecular studies, as in most other similar studies, evolutionary relationships among mitochondrial DNA haplotypes are used as proxy for the phylogenetic relationships among species (Avise and Ball, 1990; Meyer 1993b). Both morphological and molecular data are analyzed by identical phylogenetic methods (reviewed, e.g., in Swofford 1991, for fishes see Meyer, 1993b). All of these methods have weaknesses, strengths, and underlying assumptions; space does not allow the review of these methods here but excellent reviews are available (Felsenstein, 1988, Swofford and Olsen, 1990; Swofford, 1991). The paucity of cichlid fossils in Africa (VanCouvering, 1982) makes the molecular approach particularly valuable. DNA sequences of two sister species diverge with relative regularity ("molecular clock") over time from their ancestral species. If the approximate "ticking rate" of the molecular clock for a particular gene in a particular lineage is know, one can back-calculate how long ago a common ancestor of two species might have lived, based on the amount of DNA-sequence divergence observed. The regularity of the molecular clock is disputed and several simplifying assumptions enter into these calculations, which is why caution must be exercised in the interpretation of these data.

A SINGLE ANCESTOR FOR THE LAKE VICTORIA SUPER-FLOCK

Of the three large East Afrikan lakes, Lake Victoria is the youngest. Its origin is dated back to about about 250,000 to 750,000 years ago (Fryer and Iles, 1972), nonetheless it harbors a species flock of more than 300 endemic haplochromine cichlids, much of which is now threatened by extinction through the introduction of the Nile Perch: e.g., Witte et al., 1992). Lake Victoria originated from two westward flowing rivers, the proto-Kagera and the proto-Katonga, that were back-ponded in the Pleistocene by the uplifted western margin of the Victoria basin (Fryer and Iles, 1972). Geological data on the formation of Lake Victoria indicate that during the Pleistocene a connection existed between it and several smaller lakes to the west of it: Lakes Edward, George, and Kivu (Fig. 1). Hence, Lake Victoria species flock should be considered a super-species flock that goes beyond the current shores of Lake

Victoria (Greenwood, 1984). Greenwood christened the term super-species flock and laid out criteria for the use of "species flock" for species assemblages: (1) high levels of endemicity, (2) monophyly, (3) geographic circumscription (Greenwood, 1984).

Nearly all endemic cichlids of Lake Victoria had been assigned to the single genus "*Haplochromis*"; Greenwood later divided them into more than 20 different genera (Greenwood, 1980). It was not known for long whether more than one riverine ancestral species provided the initial "seed" to the present diversity in Lake Victoria. Among cichlid taxonomists, most believed that the Lake Victoria haplochromine cichlid assemblage had more than one ancestor (Fryer and Iles, 1972); Greenwood argued that neither the Victoria super-flock nor the Lake Malawi cichlid assemblage should be considered as single species monophyletic flocks (Greenwood, 1983). But, electrophoretic data demonstrated that the species in the Victoria cichlid super-species flock are extremely closely related (the mean genetic distance being only 0.006 substitutions per locus); this suggested that these species might have recently arisen from only a single ancestral species (Sage et al., 1984).

Mitochondrial DNA (mtDNA) sequences evolve faster than nuclear DNA (Brown et al., 1979; reviewed in Meyer, 1993a). Phylogenies based on mtDNA (particularly of the fast evolving part of the mitochondrial genome, the control region), therefore, can resolve evolutionary relationships among young, very closely related species (e.g., Meyer et al., 1990, 1991; Sturmbauer and Meyer, 1992). The amount of mtDNA variation among fishes of the Victoria flock was investigated and found to be extremely small (Meyer et al., 1990). In fact, no variation was detected in 363 base pairs (bp) of the cytochrome *b* gene, and only about 2–3 substitutions differentiate mitochondrial haplotypes and presumably species of Lake Victoria cichlids in 440 bp of the control region (Meyer et al., 1990). More variation had been found in the homologous portion of mtDNA genome in the single species, *Homo sapiens* than was found among the all 14 species of nine representative endemic genera of Lake Victoria haplochromine cichlids which had been studied (Vigilant et al., 1989). This high degree of mtDNA similarity and the earlier allozyme data suggested a very young age for this flock, it was estimated to be probably less than 200,000 years of age (Sage et al., 1984; Meyer et al., 1990) (Fig. 4). This age estimate for the Lake Victoria super species flock is younger than the lake itself, and supports the notion of intra-lacustrine speciation; i.e., the adaptive radiation of this species flock is likely to have occurred in the lake itself rather than being due to several founding species from different ancestral lineages. Phylogenetic relationships within the Victoria super-flock could not be established with certainty since too little phylogenetic information was contained even in the fastest evolving portion of the mitochondrial genome (Meyer et al., 1990). Comparisons of mtDNA sequences from Lake Victoria endemics with those from Lake Malawi, Lake Tanganyika, non-endemics and riverine cichlids of East and West Africa indicate that the Lake Victoria super-flock (which includes endemics from satellite lakes like Lake Edward) make it likely that the Victoria super-flock originated from a single ancestral species (Meyer et al., 1990, 1991) (Fig. 4). The mitochondrial-based suggestion of monophyly of the Lake Victoria super-flock still holds with the inclusion of more riverine East African cichlid species (see below, and Meyer and Montero, unpublished data).

For Lake Victoria, despite this extremely low level of mtDNA variation among morphologically very different species of cichlids (Fig. 2), there was only one case in which identical mtDNA haplotypes were detected among two morpho-types interpreted to be good biological species (Meyer et al., 1990). This might argue that lineage sorting of mtDNA haplotypes was fast and almost complete even among these very young species (Avise and

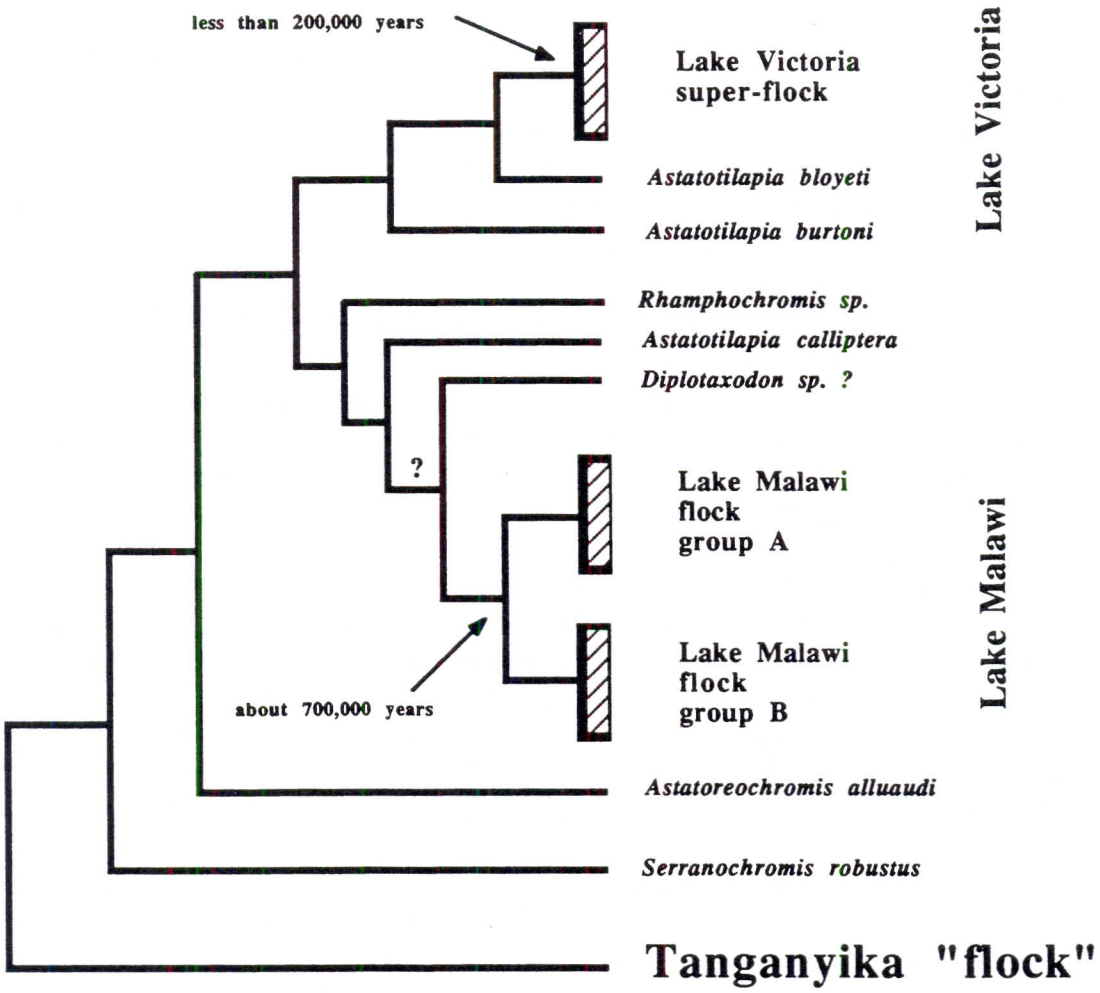

Figure 4. Phylogenetic tree relating the three endemic species flocks of Lake Victoria, Lake Malawi and some riverine species of haplochromine cichlids from East Africa to part of the Lake Tanganyika flock (the tribe Tropheini is the sister group to the haplochromine cichlids that are found in East African rivers and whose ancestors colonized Lakes Victoria and Malawi to form their species flocks, see Fig. 6) based on (Meyer et al., 1990, 1991; Moran, Reinthal and Kornfield, 1994; Sturmbauer and Meyer, 1993; Sturmbauer, Verheyen and Meyer, 1994). Presumed monophyletic assemblages are indicated with shaded boxes. Branches are not drawn to scale with time since divergence. *Astatoreochromis alluaudi* and *Serranochromis robustus* are widespread East African species. *A. alluaudi* also lives in Lake Victoria. The "?" is meant to indicate that we have not tested the finding that *Diplotaxodon* is a separate lineage from the other four found in Lake Malawi as suggested by a RFLP mitochondrial DNA study (Moran et al., 1994). *Astatotilapia bloyeti* is a generalized haplochromine (Fig. 2) that is found throughout much or East Africa and probably resembles the ancestral species of the Lake Victoria super-flock. *Astatotilapia burtoni* is found in Lake Tanganyika and surrounding areas. *Astatotilapia calliptera* is not strictly endemic to Lake Malawi.

Ball, 1990). These data might also argue that the estimated number of species in Lake Victoria which were solely based on, at times, slight morphological differences, is supported by genetic differences. The data might cautiously be interpreted to suggest that the different mitochondrial DNA haplotypes might indeed represent reproductively isolated biological species (Avise and Ball, 1990). A more detailed characterization, with larger intraspecific

sample sizes and the inclusion of nuclear DNA markers will provide more insights into the question of the validity of the species ranks and the dynamics of speciation in Lake Victoria haplochromine cichlids. Preliminary data with larger sample sizes than the original study (Meyer et al., 1990), confirm that intraspecific levels of variation in endemic Lake Victoria cichlids are extremely low (Meyer et al., in prep.). However, the level of variation in the nuclear major histocompatibility complex was found to be extensive among Lake Malawi cichlids (Klein et al., 1993; Ono et al., 1993).

PHYLOGENY OF THE LAKE MALAWI FLOCK AND ITS RELATIONSHIP TO THE VICTORIA SUPER-FLOCK

Preliminary electrophoretic data determined that the endemic cichlids of Lake Malawi are extremely closely related (Kornfield, 1978) but suggested that the Lake Malawi and Tanganyika flocks are not monophyletic but share at least one lineage (Kornfield et al., 1985). In contrast, data from mtDNA sequences, suggested that the Lake Malawi species flock appears to be monophyletic (Meyer et al., 1990; Kocher et al., 1993). MtDNA of the highly derived *Macropleurodus-Chilotilapia* species pair from Lake Victoria and Malawi respectively (which had been used to argue for a polyphyletic origin of both flocks [Greenwood, 1983]) were compared, and demonstrated that these two species are not sister taxa but rather are more closely related to the rest of their monophyletic Victoria and Malawi assemblages (Meyer et al., 1990). Species from the Lake Victoria and Lake Malawi species flocks differ by at least 54 substitutions (in the 803 base pairs compared from two mitochondrial genes) from each other; therefore any morphological or behavioral similarity that appears to link particular species from these lakes must now be interpreted as parallelism or homoplasy rather than as an indicator of common descent (Meyer et al., 1990). Kocher et al. (1993) compared other species pairs from these species flocks that show striking morphological similarities and also concluded that in all cases these morphological similarities are merely parallelism and do not represent evidence for common descent. The Malawi and the Victoria flocks, despite being genetically distinct, are still very closely related: in the mitochondrial cytochrome *b* gene, they differ by only 5% sequence divergence whereas congeneric cichlid species of the Neotropical genus *Cichlasoma* differ by up to 11% (Meyer et al., 1990; Kocher et al., 1993).

Among the members of the Lake Malawi species flock two genetically distinct groups can be identified — each is composed of about 200 species (Eccles and Trewavas, 1989). Based on mtDNA sequences, these groups differ from each other by at least 24 substitutions (Meyer et al., 1990; Meyer and Montero, unpublished data) (Fig. 4). Based on mtDNA sequence divergence, the age of the this flock has been preliminarily estimated at around 700,000 years, suggesting that this radiation took place in the 1–2 million years old Lake Malawi basin (Fryer and Iles, 1972). One group of species is largely confined to rocky habitats (the mbuna), and the second lives over sandy habitats, and is composed of species that were until recently (Eccles and Trewavas, 1989) largely assigned to the genus *Haplochromis*. We suggested that both groups can be traced back to a common ancestral species for probably almost the whole Lake Malawi flock with the exception of the *Astatotilapia calliptera* lineage (Meyer et al., 1990, 1991). *Astatotilapia calliptera*, which is not strictly endemic to Lake Malawi, is, based on mitochondrial DNA sequences (Meyer et al., 1991), distinct from the two major lineages and might be representative of the ancestral stock which colonized the early Lake Malawi from rivers in East Africa (Meyer et al., 1991) (Fig. 4); this had been previously suggested by morphological data (Trewavas, 1949).

The origin of the Lake Malawi flock is probably due to a very small number of ancestral lineages (Meyer et al., 1990, 1991; Moran and Kornfield 1993; Moran, Reinthal, and Kornfield 1994; reviewed in Meyer, 1993b; but see Klein et al., 1993; and Ono et al., 1993 on *MHC* variation in Lake Malawi cichlids). Moran et al. (1994) based on mitochondrial RFLP data, suggest that there are six independent lineages in Lake Malawi: *Serranochromis robustus* is clearly a basal lineage; this species had not been included in our original studies (Meyer et al. 1990, 1991). Moran et al.'s (1994) recent restriction analyses of mtDNA further suggests that aside from the two major groups, the mbuna and non-mbuna (Fig. 4) also *Rhamphochromis*, *Diplotaxodon*, *Astatotilapia calliptera* and *Copadichromis* may represent other discrete endemic lineages for a total of six (not considering *Serranochromis robustus*) (Fig. 4). Further, these data tentatively indicated that the *Rhamphochromis* lineage may be more basal than *Astatotilapia calliptera* (Moran et al., 1994) (Fig. 4). Figure 4 is a composite of Moran et al.'s (1994) and our (Meyer et al., 1990, 1991; Sturmbauer and Meyer 1992, 1993) work.

We extended our mtDNA sequence analysis of East African cichlid species to include several other Malawian and riverine haplochromine taxa that had not been studied previously. The mtDNA sequences confirm some of Moran et al.'s (1994) findings, but differ somewhat in others (Fig. 5). *Serranochromis robustus* is a distant relative of both the Malawi and the Victoria haplochromine cichlids, we used it as an outgroup in our analysis of the relationships among the Victoria and Malawi species flocks plus some of the East African non-endemic haplochromines. We can confirm Moran et al.'s (1994) finding that *Rhamphochromis* represents another independent lineage of the Lake Malawi species flock, bringing to four the lineages represented in the lake (not counting *Serranochromis*) (Figs. 4 and 5). We disagree with Moran et al. (1994) in that we find that *Copadichromis* does not represent an independent lineage but appears to be a member of the non-mbuna group. We have not sequenced *Diplotaxodon*, and cannot comment as to whether it is another independent lineage (indicated with a "?" in Fig. 4). Whether *Rhamphochromis* is the most basal lineage in Lake Malawi, even more basal than *Astatotilapia calliptera* (Figs. 4 and 5) as has been suggested by Moran et al. (1994), was not clear from our data. Our 50% majority rule bootstrap tree based on a parsimony analysis of the control region sequences (Fig. 5) does not resolve the branching order among the four Malawian lineages, but our most parsimonious trees agree with Moran et al. (1994) in placing *Rhamphochromis* most basal. Within the non-mbuna group we find that *Cyrtocara* and *Tyranochromis* represent the most basal groups; within the mbuna group *Melanochromis labrosus* appears to be the most basal member (Fig. 5), these findings must remain tentative until a more complete representation of Lake Malawi species is accomplished.

MtDNA sequences identified the non-endemic *Astatotilapia burtoni*, a generalist species found in Lake Tanganyika and surrounding waters, to be the closest living relative of the Lake Victoria flock (Meyer et al., 1991). However, the bootstrap values supporting this branching are rather low (Fig. 5) making this finding tentative. More recently, several other non-endemic East African riverine cichlids from the Malagarasi river, the Ruahu river, Lake Rukwa and Lake Kitangiri (e.g., *Astatotilapia bloyeti*) have been characterized mitochondrially and are found to be even more closely related to the Victoria flock than *Astatotilapia burtoni* (Meyer and Montero, unpublished data) (Figs. 4 and 5). The Victoria super-flock, mitochondrially speaking, appears to include the endemics of Lake Victoria and its satellite lakes plus some riverine haplochromine cichlids of East Africa, e.g., a species of *Astatotilapia* from the Manago river from Tanzania (collected by L. Seegers) and has very close

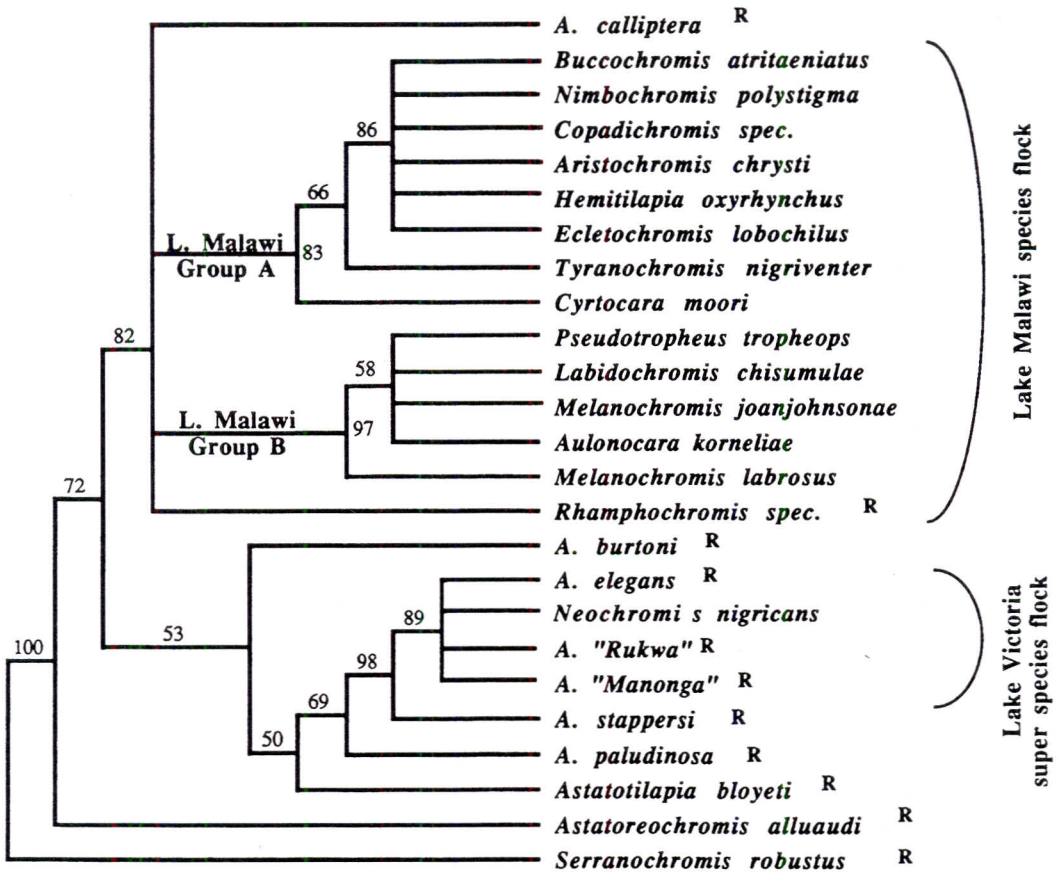

Figure 5. 50% majority rule bootstrap consensus tree analyzed with PAUP (Swofford 1991) (200 replications) of previously unpublished mitochondrial control region sequences of Lake Malawi, Lake Victoria and East African non-endemic haplochromine cichlids (Meyer and Montero, unpublished data). "A" stands for *Astatotilapia* indicating tentative assignment of these riverine, non-endemic haplochromine species (and "R" stands for riverine species) to this mitochondrial not-monophyletic genus.

affinities to other riverine Tanzanian *Astatotilapia* species of uncertain species status (collected and provided by Lothar Seegers and Tuur Vos). Some members of the widespread mainly riverine genus *Astatotilapia* (mitochondrially, an unnatural group, Figs. 4 and 5; see Meyer et al., 1991) is likely to represent the body plan and lifestyle of the ancestors of the Victoria and possibly the Malawi species flocks (Meyer et al., 1991). We are currently expanding our phylogenetic mitochondrial DNA analysis to include more East African riverine haplochromines (Meyer, Voos, Seegers, Montero unpublished data). Greenwood previously recognized that *Astatotilapia* is not monophyletic, when he assigned species into this genus, that this genus is a "depository"-genus for generalized non-endemic haplochromine cichlids.

LAKE TANGANYIKA, THE EVOLUTIONARY RESERVOIR FOR EAST AFRICAN CICHLIDS

Lake Tanganyika is estimated to have an age of 9–12 million years (Cohen et al., 1993, or even 26 mya Johnson, this volume), making it by far the oldest of the Great East African

lakes. Being the oldest, it may not be surprising that it harbors the morphologically and behaviorally most diverse cichlid fauna, consisting of about 171 species (however, by comparison with the Victoria and Malawi flocks this is a relatively small number) in 49 endemic genera that are assigned to twelve tribes (Fryer and Iles, 1972; Poll, 1986; Coulter, 1991). These tribes are assemblages of genera that seem to represent ecologically, morphologically, as well as behaviorally well defined phylogenetic lineages (Poll 1986). Morphological and electrophoretic data suggest that the lineages of cichlids from Lake Tanganyika are old and can be traced back to at least seven distinct ancestral lineages (Poll, 1986; Nishida, 1991). Phenotypic differences between the tribes are pronounced, and mtDNA data turned out to be generally in good agreement with Poll's classification (Poll, 1986) and assignments of genera into tribes (Sturmbauer and Meyer, 1993; Sturmbauer, Verheyen and Meyer, 1994). Comparisons of electrophoretic and mtDNA data demonstrated that several Tanganyikan lineages are much older than the lineages of Lakes Victoria and Malawi (Nishida, 1991; Sturmbauer and Meyer, 1992, 1993; Kocher et al., 1993; Sturmbauer, Verheyen and Meyer, 1994).

The age of some endemic Tanganyikan cichlid lineages was recently estimated for two tribes of cichlids, the Tropheini and the Ectodini (Sturmbauer and Meyer 1992, 1993). The genus *Tropheus* was estimated to be about twice as old as the entire cichlid species flock from Lake Malawi and six times older than the entire flock of endemic haplochromines from Lake Victoria (Sturmbauer and Meyer, 1992). The Ectodini, represented by twelve endemic genera, seem to be approximately twice as old as *Tropheus* and five times older than the Malawi flock (Sturmbauer and Meyer, 1993). Estimates based on mtDNA sequences suggest that the Ectodini, a large variable tribe of endemics, are probably about 3.5 to 4 million years old, and some other lineages (e.g., Bathibatini and Lamprologini) might be even older than 5 million years (Nishida, 1991; Sturmbauer and Meyer, 1993; Kocher et al., 1993; Sturmbauer, Verheyen and Meyer, 1994.). The "short branches" at the base of the phylogenetic tree relating the major lineags of Lake Tanganyika cichlids suggests that the formation of lineages proceeded rapidly and that the tempo and mode of speciation and morphological diversification at the early stage of the Tanganyika radiation was dramatic (Sturmbauer, Verheyen and Meyer, 1994.).

The existence of several old lineages of cichlids might be evidence for the polyphyletic origin of the Lake Tanganyika species flock if it could be shown that more than one of these lineages is older than the lake itself or if basal members of more than one of those lineages are found outside the lake. Based on an earlier lower age estimate for Lake Tanganyika (2–4 million years) it had been assumed that an age of 5 million years for the old lineages implied a polyphyletic origin for this species flock (Nishida, 1991). A reevaluation of the geological age of Lake Tanganyika indicated that the age of the lake is likely to be greater than those of the tribes (Cohen et al., 1993) which might argue that virtually the whole Tanganyika flock could have evolved within the lake basin from a single ancestral lineage. This remains to be tested. Lake Tanganyika cichlids, probably unlike those of the other two species flocks, apparently have been able to leave the confines of the lake — several species of the *Lamprologus* group occur in the Zaire river. They appear be closely related to derived endemic lamprologine cichlids and are not basal lamprologine lineages (Sturmbauer, Verheyen and Meyer, 1994).

Both electrophoretic and mtDNA sequences suggest that the Victoria and Malawi flocks are closely related to a particular Tanganyikan tribe, the Tropheini (Nishida, 1991; Sturmbauer and Meyer, 1993; Sturmbauer, Verheyen and Meyer, 1994) (Fig. 6). This may

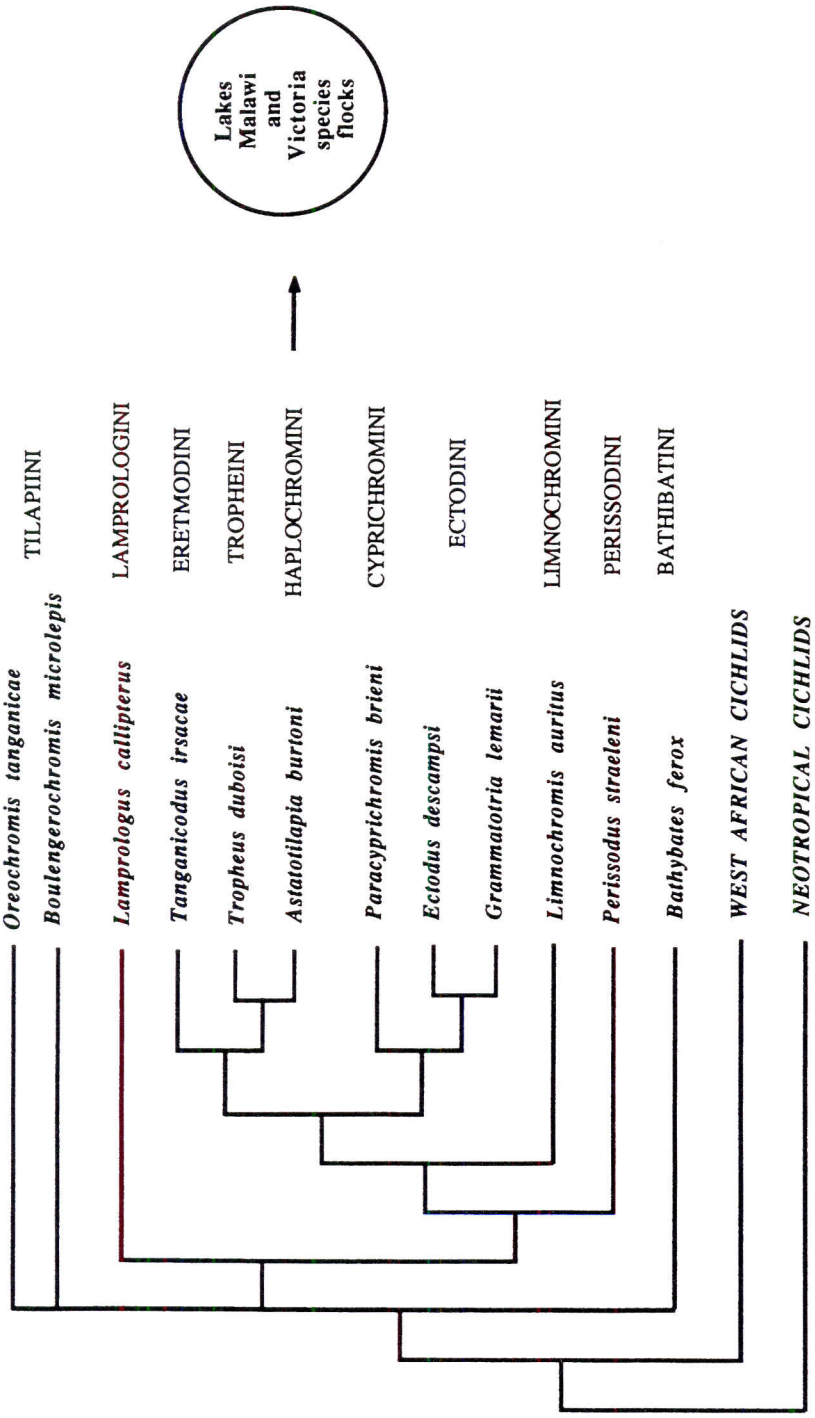

Figure 6. Molecular phylogenetic relationships of some representative species of ten of the eleven tribes (names of tribes in capitals following Poll (1986) endemic to Lake Tanganyika, figure based on (Sturmbauer and Meyer, 1993; Sturmbauer, Verheyen and Meyer, 1994). Some of the members of these tribes are shown in Fig. 2. e.g., *Lobochilotes* belongs to the same tribe as *Tropheus*, the Tropheini; *Spathodus* belongs to the Eretmodini; *Xenotilapia* to the Ectodini. The sister group relationship of the endemic Lake Tanganyika tribe Tropheini to the Lake Tanganyika haplochromine cichlids and the haplochromines of the two species flocks of Lakes Malawi and Victoria is indicated with the arrow and the bubble on the right and based on Nishida (1991) and Sturmbauer and Meyer (1993). Some of the relationships of Tanganyikan tribes remain somewhat tentative, they are indicated here as polytomies (and are being tested further, Sturmbauer and Meyer, in preparation).

not be surprising since considerable similarities between *Tropheus* and *Pseudotropheus* from Lakes Tanganyika and Malawi respectively, had been interpreted to argue for a polyphyletic origin of Lake Malawi cichlids (Fryer and Iles, 1972). However, the molecular phylogeny strongly suggests that these similarities are merely homoplasies due to convergent evolution since *Pseudotropheus* is genetically more closely related to all other species of Lake Malawi even including Malawian morphological generalists that have no resemblance to *Tropheus* from Lake Tanganyika (Kocher et al., 1993; Figs. 4–6).

Unlike the monophyletic species-flocks of the lakes Victoria and Malawi (Meyer et al., 1990, 1991), the cichlid fauna of Lake Tanganyika is believed to be of polyphyletic origin with affinities to the cichlid faunas of other African regions (Fryer and Iles, 1972; Poll, 1986; Nishida, 1991). The species of the tribe Lamprologini comprise species from Lake Tanganyika, as well as from the Zaïre river. Some "haplochromine" cichlids from Lake Tanganyika (i.e., *Tropheus* and *Astatotilapia burtoni*) appear to be the sister groups of the endemic species flocks of Lakes Malawi and Victoria (Meyer et al. 1990, 1991; Sturmbauer and Meyer 1993; Meyer 1993). The single endemic Tanganyikan species of the Tylochromini even has its closest relatives in central and western Africa (Stiassny, 1990). The Tanganyikan cichlid fauna hence can be viewed as an evolutionary reservoir of ancient African cichlid fishes (Nishida, 1991), the understanding of which might allow to eludicate the origin of the modern African cichlid fauna, as well as the interrelationships between the endemic flocks of the large Eastern African lakes. The Tanganyika flock has been viewed as a reservoir of old phylogenetic lineages that gave rise to the ancestors of the Victoria and Malawi flocks (Nishida, 1991). Lake Tanganyika does not harbor all the members and descendants of some of its endemic lineages since some lamprologine cichlids, which are endemic to the Zaire river were recently found not to be basal in the tribe Lamprologini and must hence be interpreted to have left the confines of the Lake Tanganyika (Sturmbauer, Verheyen and Meyer, 1994). More accurate calibrations of the age estimates for the lineages, and more work on riverine cichlids, particularly from West Africa, will be required to test the presumed polyphyly of the Lake Tanganyika species flock.

SPECIATION WITHIN THE LAKE BASINS (INTRA-LACUSTRINE SPECIATION)

The current Lake Victoria, with a an area of 68,000 km^2 about the size of Ireland, appears to have experienced a period of almost complete desiccation as recently as 14,000 years b.p. (Stager et al., 1986; Roche, 1991). Numerous ponds and rivers around the margins of the lake shore might have persisted and to have provided refugia for fish. Over evolutionary time spans there was probably ample opportunity for spatial isolation within the larger lake basin, providing the necessary preconditions for geographic speciation. Amalgamation of separate faunas of several smaller bodies of water after the lake level rose again is likely to have occurred (Worthington, 1937; Brooks, 1950; Fryer and Iles, 1972; Mayr, 1984; and more reference in Echelle and Kornfield, 1984). Geographic isolation of formerly interbreeding populations brought about by lake level fluctuations which split up larger bodies of water into smaller ones, followed by the acquisition of reproductive isolation before the geographically separated populations reunited, is a likely scenario for speciation in Lake Victoria. Hence, allopatric speciation (inter-lacustrine between separated bodies of water) might have been an important mechanism of speciation (Worthington, 1937; Brooks, 1950; Fryer and Iles, 1972). This possibility should not diminish the likely importance of micro-allopatric speciation for Lake Victoria cichlids.

Periods of aridity that led to drastic lake-level fluctuations (drops in water level of up to 600 m), and splits of the single lake are also documented for Lakes Tanganyika and a lesser extent Lake Malawi (Stager et al., 1986; Scholz and Rosendahl, 1988; Gasse et al., 1989; Roche, 1991; Tiercelin and Mondeguer, 1991). The lake topography consist of two (Lake Malawi) or three (Lake Tanganyika) extremely deep basins (up to 704 and 1470 m, respectively). These lake level changes will have separated populations that once exchanged genes and will have brought into contact populations that previously did not. In species of the *Tropheus* species complex from Lake Tanganyika, mtDNA sequences suggest that these large lake level fluctuations might have influenced the distribution of genetic variation and probably speciation (Sturmbauer and Meyer, 1992). Genetic distances and geographic patterning of genetic variation mirror the topology of the presumed paleo-lake shores during periods of low water levels (Scholz and Rosendahl, 1988; Gasse et al., 1989).

Intra-lacustrine speciation, probably by micro-allopatric speciation, through isolation by distance or appropriate habitat type, would appear to be the most important mode of speciation for all three species flocks. *Tropheus*, which only live over rocky habitats, from different sites in Lake Tanganyika appear to be effectively prevented from exchanging genes by discontinuities in the available habitat (Sturmbauer and Meyer, 1992). For example, long beaches or estuaries are evidently effective barriers to gene flow since large genetic differences were found between populations separated by only a few kilometers of shoreline (Sturmbauer and Meyer, 1992). Particularly for Lakes Tanganyika and Malawi, but probably also for Lake Victoria, it seems that speciation clearly can take place in single bodies of water. It would therefore appear that physical separation of water masses is not a necessary precondition for the establishment of genetic discontinuities and speciation.

Intra-lacustrine speciation should however, not be equated with sympatric speciation and should not be interpreted as refutation of allopatric speciation models (Kondrashov and Mina, 1986). It is often not appreciated that these lakes are vast and have extremely long varied coast lines, and that almost all endemic species have much restricted geographic distributions (Fryer and Iles, 1972). Only very few species, that live along the shores, occur throughout a whole lake (Fryer and Iles, 1972) and species often are restricted to single localities in which population size can be as small as a few hundred individuals (Ribbink et al., 1983). Most cichlids are poor dispersers, they are philopatric, they show homing, and males tend to defend feeding and breeding territories for several years (Hert, 1992; but see Turner, 1994). All of this points toward restricted gene flow. In addition, brood sizes are small and both factors are ingredients for fast diversification by micro-allopatric speciation (Cohen and Johnston, 1987). It seems important to point out, however, that many species of cichlids live in open water, roam freely and seem to be distributed widely or even occur in the whole lake (Coulter, 1991). In these species the models of restricted gene flow etc. do not seem to hold and the processes responsible for speciation might differ from the ones responsible for species restricted to small areas along the shore (George Coulter, pers. comm.). If the open water species return to restricted spawning areas on the shore for reproduction, some of the conditions potentially responsible for reproductive isolation among shore species might be met as well in these open water species.

RATES OF SPECIATION AND MORPHOLOGICAL DIVERSIFICATION, AND THE ROLE OF SEXUAL SELECTION

It is not clear how many species of the current flock of 300+ species of Lake Victoria survived the episode of drying 14,000 years ago. They may have survived in smaller

marginal lakes, springs, or headwaters of rivers and recolonized the lake again after it filled up again. It may or may not appear likely (but not unthinkable, see below) that most of the 300+ species of Lake Victoria arose in less than 14,000 years. It seems possible also, that the Victoria flock is derived from East African riverine haplochromines that recolonized Lake Victoria after this period of aridity (Figs. 4 and 5).

Rates of speciation in cichlids can be astonishingly fast; this has been known since the discovery of five endemic species of cichlids in Lake Nabugabo (Greenwood, 1965), a small lake that is less than 4,000 years old and separated from Lake Victoria only by a sand bar. These five species are believed to have close relatives in Lake Victoria that chiefly differ in the male's breeding coloration, pointing to the potential importance of sexual selection for the fast rates of speciation in cichlids (Dominey, 1984).

Still faster rates of speciation were suggested by the finding that the southern end of Lake Malawi was dry only two centuries ago and is now inhabited by numerous endemic species and "color morphs" that are only found there and are believed to have originated during the last 200 hundred years! (Owen et al., 1990). This in situ speciation hypothesis seems supported by the fact that almost all endemic cichlids in all lakes have restricted geographic distributions (see also Turner, 1994). Ancestral genetic polymorphisms are retained across some species boundaries among some (but not all, Reinthal and Meyer, unpublished data) closely related species of mbuna consistent with the extreme rates of speciation observed in Lake Malawi cichlids (Moran and Kornfield, 1993; Moran, Reinthal and Kornfield, 1994).

Coloration appears to evolve quickly since there are several cases in which genetically closely related species of *Tropheus* and mbuna have dramatically different colorations (Ribbink et al., 1983; Sturmbauer and Meyer, 1992; Moran and Kornfield, 1993). Interestingly, despite pronounced variation in coloration among populations of *Tropheus*, this group of species has remained otherwise virtually unchanged for probably more than one million years (Sturmbauer and Meyer, 1992). Concurrently, the explosive speciation and morphological radiation of the Lake Victoria and Malawi flocks occurred, underscoring that morphological evolution can experience periods of rapid change and long periods of stasis (Avise, 1977).

The potential importance of coloration and sexual selection in the speciation of cichlid fish has been debated for some time (Dominey, 1984; Mayr, 1984; Turner, 1994). Sexually and socially selected traits might undergo more rapid diversification than traits under survival selection and might facilitate or fuel the explosive pace of speciation in cichlids. Coloration is of importance in intraspecific interactions and is a trait that might be more strongly shaped by sexual rather than by survival selection; coloration might act as a reproductive barrier without concordant morphological diversification. In *Tropheus*, coloration can vary tremendously among genetically closely related populations, alternatively it can also be very similar among genetically distant populations (or species) (Sturmbauer and Meyer, 1992). The importance of sexual selection in the formation of the species flocks is hotly debated — the verdict is still out.

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