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Evolutionary history of the cichlid fish species flocks of the East African great lakes inferred from molecular phylogenetic data

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With 6 figures in the text

Abstract

The species flocks of cichlid fishes in the largest East African Lakes, Victoria, Malawi and Tanganyika, are well-known examples of adaptive radiations and "explosive speciation". These species assemblages are the most species-rich and the most diverse, morphologically, ecologically and behaviorally among vertebrates. Phenotypic and genotypic data sets are expected to provide concordant phylogenetic information about these species assemblages, since both share identical evolutionary histories. Molecular data however have some advantages for phylogeny reconstruction over morphological data. Our understanding of the phylogenetic relationships 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 molecular data. Phylogenetic analyses of recent molecular data in the context of the geological history of the East African lakes helped to elucidate some aspects of the evolutionary history and evolutionary processes that might have led to the origin of these extraordinary fish faunas. The molecular studies on the whole confirm many previous morphology-based hypotheses, however, they also offer alternative phylogenetic hypotheses that differ from previous phylogenies.

Introduction

The cichlid fish faunas of the three East African Great Lakes, Victoria, Tanganyika, and Malawi, are enormously diverse and a testimony to the evolutionary victory of cichlid fish. In each of these lakes (Fig. 1) a radiation of several hundred species (FRYER & ILES 1972) almost all of which are endemic to their particular lake are found. These species flocks make the Cichlidae one of the most species-rich family of vertebrates. The special history of cichlids is highlighted by the coexistence of other families of fish in each of these three lakes, that have not undergone this kind of spectacular radiation. The evolutionary origin and ecological maintenance of the enormous cichlid species diversity (Fig. 2) has been much researched and debated (e.g. MAYR 1942, 1984, FRYER & 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 phylogenetically independent of each other (STIASSNY 1981, GREENWOOD 1983).

The species flocks (defined as monophyletic assemblages of species inhabiting one lake, GREENWOOD 1984) of all three of the lakes contain a sweeping array of morphologically and behaviorally highly specialized cichlids (FRYER & ILES 1972). Part of the reason for the evolutionary success and diversification of cichlids might be a morphological novelty only

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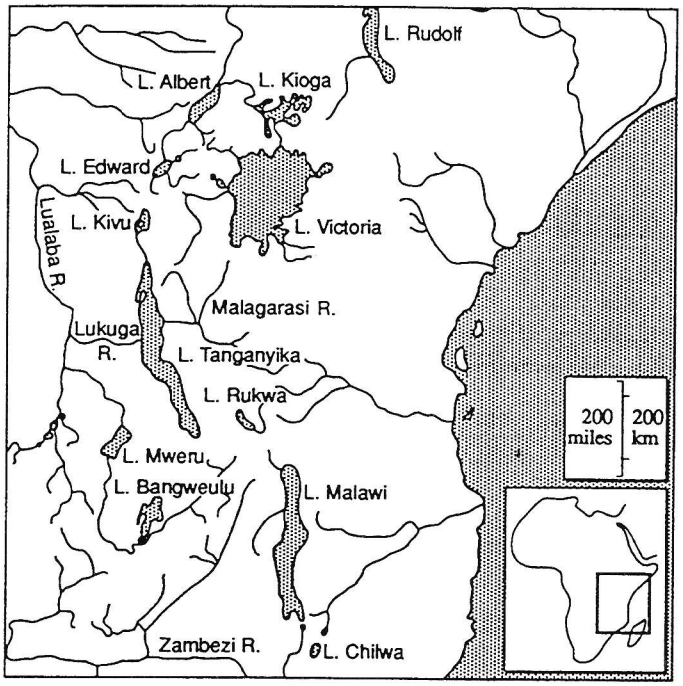

Fig. 1. Geography of East Africa showing the locations of the three great lakes and the major river systems. Figure redrawn after FRYER & ILES (1972).

they possess (LIEM 1973, OSSE & LIEM 1975). Cichlids have a second set of jaws in back of the buccal cavity, the modified pharyngeal 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 stenotopy and fine 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 & LIEM 1975). Similar morphological solutions to the same ecological problems have been found in more than one cichlid species flock (GREENWOOD 1983). Some highly derived specializations are similar between species endemic to different lakes. In East African cichlids, both the Victoria endemic *Macropleurodus bicolor* and the Malawi endemic *Chilotilapia rhoadesi* exhibit a highly derived oral jaw dentition, jaw structure and feeding behavior – they prey on gastropods by crushing their shells with their oral jaws (FRYER & ILES 1972, GREENWOOD 1983). Likewise, SCHLUTER & MCPHAIL (1993) described that temperate fishes repeatedly diversified in a predictable way when new habitats became available.

Despite much effort in elucidating the evolutionary relationships among the species assemblages, the question of whether each of the assemblages is a monophyletic flock that can be traced back to a single ancestral species and consequently whether the morphological similarities between the members of different flocks evolved as convergences, remained unanswered. Alternatively, specializations could have arisen only once and indicate polyphyletic origins 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 & ILES 1972, GREENWOOD 1983) and that these radiations cannot be traced back to single ancestral species.

The tempo and mode of evolution and the origin of morphological solutions to ecological problems can be studied based on an understanding of the relationships among the species flocks. It is desireable to have knowledge of the phylogenetic relationships within and between the three cichlid radiations. Furthermore, estimates on rates of speciation in these flocks will hinge on basic knowledge (like the monophyly versus paraphyly or polyphyly) and the age of the species assemblages. Based on these phylogenies one might gain knowledge about the

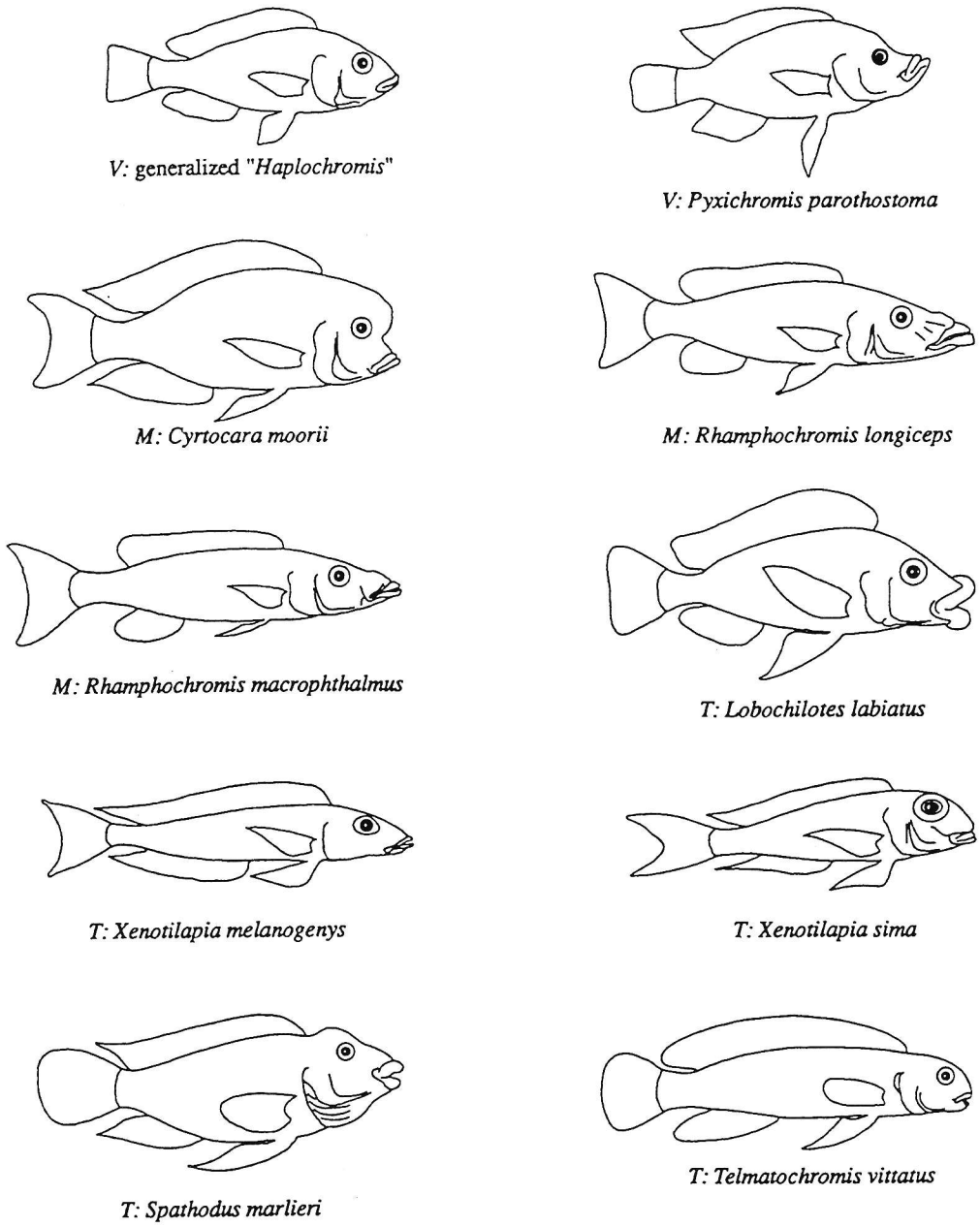

Fig. 2. Variation in body from in East African cichlid fish from Lake Victoria (V), Lake Tanganyika (T) and Lake Malawi (M). Figure redrawn after FRYER & ILES (1972) and GREENWOOD (1984).

potential ancestors of these species flocks and what might have made them successful colonizers of these lakes and founders of species flocks.

The use of morphology and molecules in tracing evolutionary histories

Each organism's phenotype and genotype share the same evolutionary history (except in probably rare cases of horizontal gene transfer), hence, both general types of data sets should provide the same estimates of phylogenetic relationships (HILLIS 1987, PATTERSON 1987). Therefore, data derived from the phenotypes of organisms, which traditionally consist of

morphological characters, biochemical data reflecting the genotype are expected to provide equally reliable phylogenetic markers. But, 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 cladistic analysis which aims to reconstruct the phylogeny of the species under consideration. Also, the number of molecular characters that can be extracted from species is limitless since each species' genome is made up of billions of DNA base pairs each of which can be used as a phylogenetic character and potentially contains phylogenetic information. 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 cladistic analysis. Molecular data can have the added advantage over morphological data sets that they can be collected in "universal" 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 a universal metric is e.g. small nuclear ribosomal RNA gene 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 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) and provide useful phylogenetic information, and the signal-to-noise-ratio is often similar in both kinds of data sets (HILLIS 1987, SANDERSON & DONOGHUE 1989; and also behavioral characters: DEQUEIROZ & WIMBERGER 1993). Also, morphology-based phylogenetic hypotheses are often the basis for later molecular phylogenetic studies that aim to test predictions made based on morphological characters. Molecular studies have the advantage that since they use characters that are independent from morphology, they are able to trace the evolution of morphological traits based on a molecular phylogenetic hypothesis, avoiding circularity and allowing for immediate identification of potential homoplasies and morphological parallelisms.

Various types 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 (MEYER 1994). Since the advent of the polymerase chain reaction (PCR) in 1985–1986 (SAIKI et al. 1985, 1988, WRISHNIK et al. 1987), our knowledge about DNA and the phylogeny of vertebrates has increased dramatically (e.g. reviewed in MEYER 1993a,b, 1994).

Since congruence in the phylogenetic estimates is expected from both kinds of data sets, it has been argued that the combination of 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 & MADDISON 1992).

The polymerase chain reaction

PCR is an enzymatic cloning technique that allows the amplification of portions of genes (within size limits of currently 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 (bp) in length and define the 5' and 3' ends 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.

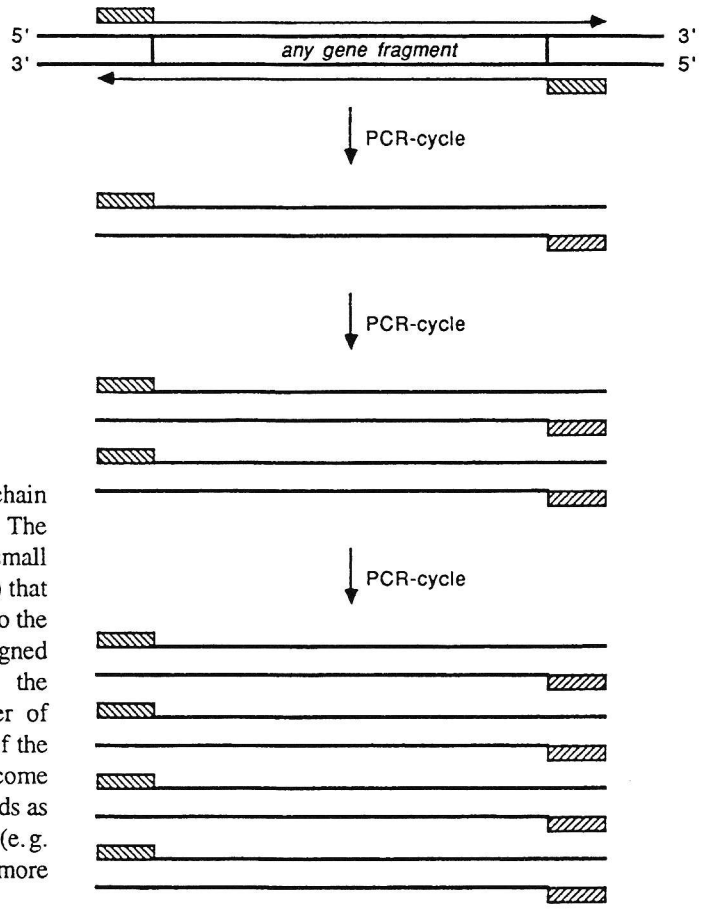

Fig. 3. The principle of the polymerase chain reaction. After WRISCHNIK et al. 1987. The hatched boxes represent "primers" small oligonucleotides (about 20 bp in length) that have a DNA sequence complementary to the stretch of DNA for which they are designed to attach. During each cycle of the polymerase chain reaction the number of copies that are defined by the 5' ends of the two primers get doubled. Primers become incorporated into the copied DNA strands as indicated. See text and cited references (e.g. ERLICH 1989, WITTE et al. 1989) for more details.

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 thermal cycler in about three hours. PCR is much faster and cheaper than convential 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 & ERLICH 1988) or digested with an exonuclease to product single-stranded DNA for direct sequencing of single-stranded DNA. Sequencing 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 time-consuming, costly and technically involved. 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 can also be stored in data banks (e.g. EMBL, GenBank) and are universally usable, powerful data. The increased costs of DNA sequences compared to 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 new studies. Again, these obvious advantages of molecular data do not negate or diminish the value or necessity of morphological

inquiries, but rather should be viewed as a complementary approach that builds on previous comparative morphological work. 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 that are sometimes in conflict with previous morphological phylogenetic hypotheses (KORNFIELD 1991, MEYER et al. 1990, 1991, STURMBAUER & MEYER 1992, MORAN et al. 1994, STURMBAUER et al. 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 & BALL 1990, MEYER 1993b). This raises the important issue of 'gene-trees' versus 'species trees', however, this will not be discussed here.

Monophyly of the Lake Victoria super-flock

Lake Victoria is the youngest of the three large East African Lakes. It started to form about 250,000 to 750,000 years ago (FRYER & ILES 1972), yet it contained a species flock of more than 300 endemic haplochromine cichlids (the entire flock is threatened by extinction: e.g. WITTE et al. 1992). The lake had its origin 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 & ILES 1972). The geological history of the formation of Lake Victoria indicates that during the Pleistocene a connection existed between this lake and several smaller ones to the west of it: Lakes Edward, George, and Kivu (Fig. 1). Therefore, the Lake Victoria flock should be considered a super-species-flock that goes beyond the current shores of Lake Victoria. GREENWOOD was the first to point this out, coined 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).

Almost all of the endemic cichlids of Lake Victoria were originally assigned to a single genus "*Haplochromis*"; now they are divided into more than 20 genera (GREENWOOD 1980). Until recently, it was not known whether more than one riverine ancestral species provided the initial inoculum to the present diversity in Lake Victoria. Among cichlid taxonomists, the tendency was to believe that the Lake Victoria haplochromine cichlid assemblage had more than one ancestor (FRYER & ILES 1972); GREENWOOD argued that neither the Victoria super-flock nor the Lake Malawi cichlid assemblage should be considered a single species flock (GREENWOOD 1983). However, electrophoretic data showed that the members of this cichlid flock are extremely closely related (the mean genetic distance being only 0.006 substitutions per locus) which suggested that they might have recently arisen from only a single ancestral species (SAGE et al. 1984).

Mitochondrial DNA (mtDNA) sequences, in general, evolve faster than nuclear DNA (BROWN et al. 1979; reviewed in MEYER 1993a). Phylogenies based on mtDNA (particularly of the fast evolving control-region), therefore, have the ability to resolve evolutionary relationships among young species (e.g. MEYER et al. 1990). 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 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 of the mitochondrial genome (MEYER et al. 1990). Most of the mutations are transition substitutions rather than transversions indicating a very recent divergence (MEYER 1993a). More variation has been documented in the homologous portion of mtDNA in *Homo sapiens* than was found among the studied 14 species of nine representative endemic genera of Lake Victoria haplochromine cichlids (VIGILANT et al. 1989).

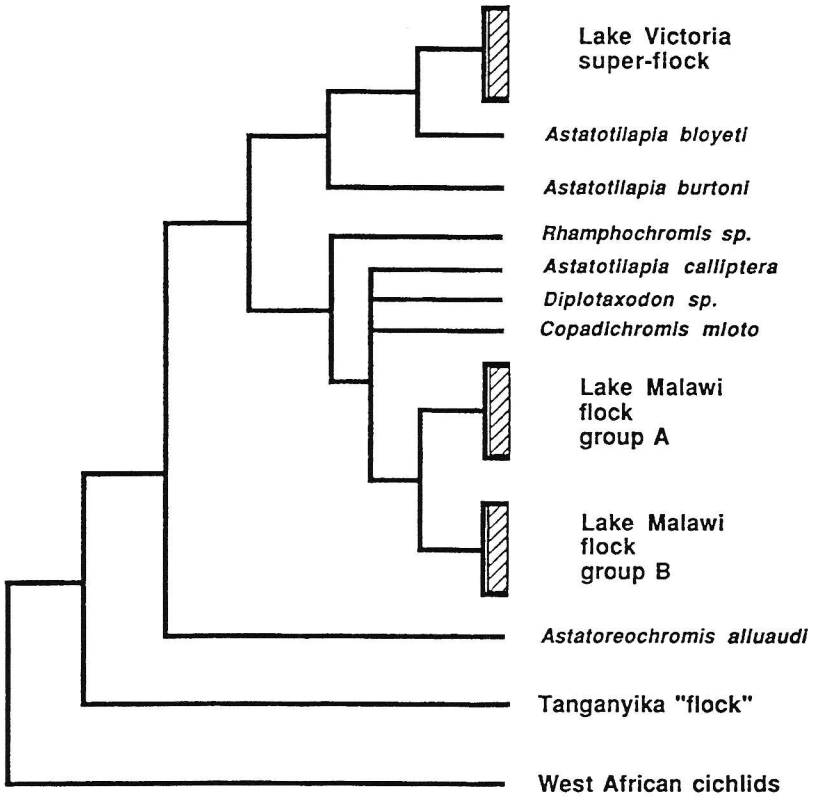

Fig. 4. Phylogenetic tree relating the three endemic species flocks of Lake Victoria, Lake Malawi and some riverine species of haplochromine cichlids from East Africa to part of the Lake Tanganyika flock (see Fig. 5) based on MEYER et al. (1990, 1991) and MORAN et al. (1994). Presumed monophyletic assemblages are indicated with shaded boxes. Branches are not drawn to indicate time since divergence. *Astatoreochromis alluaudi* is a widespread East African species that also lives in Lake Victoria. *Astatotilapia bloyeti* is a generalized haplochromine (Fig. 2) that is found in several rivers of East Africa and probably resembles the ancestral species of the Lake Victoria flock.

This high degree of mtDNA similarity and the earlier allozyme data suggested a very young age for this flock, probably less than 200,000 years (SAGE et al. 1984, MEYER et al. 1990) (Fig. 4). The molecular-based age estimate for the species flock is younger than the lake, and supports the notion of intra-lacustrine speciation; i.e. the adaptive radiation of this species flock is likely to have occurred in Lake Victoria itself rather than being due to several independent immigrations of 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 (and also the Malawi flock) originated from single ancestral species (MEYER et al. 1990, 1991) (Fig. 4). The phylogeny of the Lake Victoria super-flock and the Lake Malawi flock) in relation to riverine East African cichlids is currently being investigated further (MEYER & MONTERO, unpubl. data).

Despite this extremely low level of mitochondrial DNA 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 & BALL 1990). These data might also argue that the estimated number of species in Lake Victoria is supported by genetic differences and that they might indeed be reproductively isolated biological species. However, the interpretation of mitochondrial DNA to address the question of the validity of biological species has to be interpreted with caution (AVISE & BALL 1990). The mtDNA data tentatively indicate that founding populations of these species might have been small and the mtDNA variability low in the progenitor population (MEYER et al. 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 (MEYER et al., in prep.).

Recent nuclear DNA data from the MHC locus (KLEIN et al. 1993, ONO et al. 1993) for Lake Malawi cichlids suggests that the amount of variation contained in the members of the Malawi flock is large, arguing against small population sizes for the founders or at least against the existence of a prolonged bottleneck, which would tend to decrease genetic variation. New mitochondrial DNA studies on intraspecific variation in Lake Malawi mbuna detected quite limited amounts of variation (BOWERS et al. 1994).

The Lake Malawi flock and its relationships to the Victoria super-flock

Electrophoretic data determined that the endemic cichlids of Lake Malawi are exceedingly closely related (KORNFIELD 1978) and suggested that the Lake Malawi flock is not polyphyletic (KORNFIELD et al. 1985). In agreement with the electrophoretic evidence, data from mtDNA sequences also suggest that the Lake Malawi species flock appears to be monophyletic (MEYER et al. 1990). Species from the Victoria and Malawi flocks differ from each other by at least 54 DNA-substitutions (in the 803 base pairs compared from two mitochondrial genes); hence any morphological or behavioral similarity that appears to link particular species from these lakes should be interpreted as parallelism or homoplasy rather than as an indicator of common descent (MEYER et al. 1990). This is also true for the Lake Malawi-Tanganyika comparison (KORNFIELD et al. 1985, MEYER et al. 1990, MEYER 1993a, KOCHER et al. 1993). The Malawi and the Victoria flocks, despite being genetically distinct, are still very closely related, relative to other comparisons. 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).

Within Lake Malawi two genetically monophyletic groups, each composed of about 200 species (ECCLES & TREWAVAS 1989), were characterized based on mtDNA sequences; they differ from each other by at least 24 substitutions (MEYER et al. 1990, see also MORAN et al. 1994) (Fig. 4). Based on mtDNA sequence divergence, the age of this flock has been estimated at around 700,000 years, suggesting that this radiation took place in the Lake Malawi basin which is 1–2 million years old (FRYER & 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 largely assigned to the genus *Haplochromis* (ECCLES & TREWAVAS 1989). Mitochondrial data suggest that both groups can be traced back to a common ancestral species for probably the whole Lake Malawi flock with the exception of the *Astatotilapia calliptera* lineage (MEYER et al. 1990, MORAN et al. 1994). *Astatotilapia calliptera*, which is not strictly endemic to Lake Malawi, is, based mitochondrial DNA data, distinct from the two major lineages and might be representative of the ancestral stock (MEYER et al. 1991, MORAN et al. 1994) (Fig. 4, 5); which had been previously suggested by morphological data (TREWAVAS 1949). Since no phylogenetic hypothesis based on cladistically

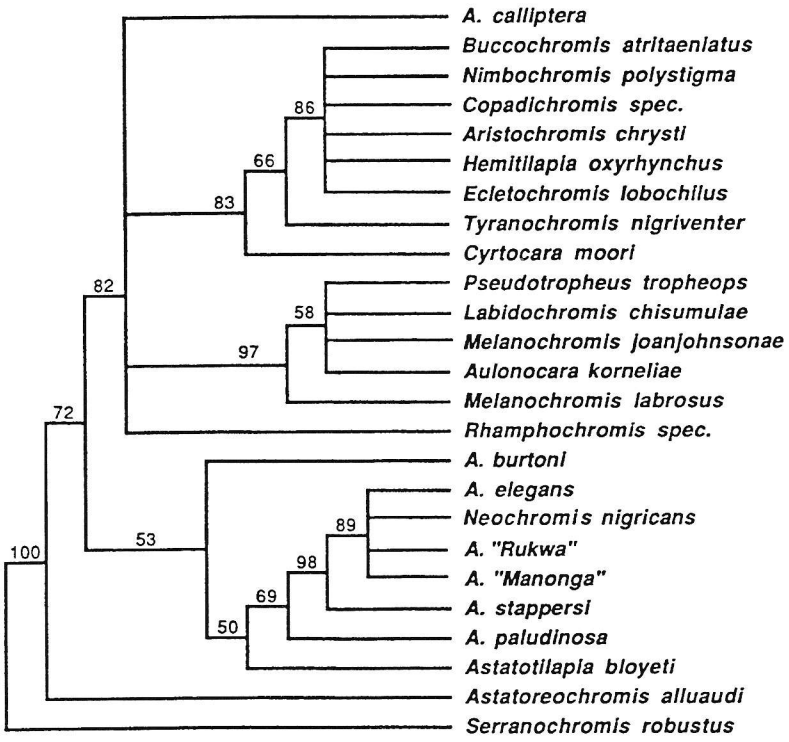

Fig. 5. 50% majority rule bootstrap concensus tree analyzed with PAUP (200 replications) of unpublished mitochondrial control region sequences of Lake Malawi, Lake Victoria and East African non-endemic haplochromine cichlids (MEYER & MONTERO, unpubl. data). "A" stands for *Astatotilapia* indicating tentative assignment of these riverine haplochromine species to this mitochondrial not-monophyletic genus.

analyzed morphological characters it is available comparisons to the molecular phylogenetic hypothesis are currently not possible.

More recent interesting work by 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 member lineage; this species was not 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 mloto* may represent other discrete endemic lineages for a total of six (not considering *Serranochromis robustus*) (Fig. 4). Further, these data tentatively indicate that the *Rhamphochromis* lineage may be more basal than *Astatotilapia calliptera* and that the mbuna group is paraphyletic (MORAN et al. 1994) (Fig. 4). Fig. 4 is a composite of MORAN et al.'s (1994) and our (MEYER et al. 1990, 1991, STURMBAUER & MEYER 1992, 1993) work. MORAN et al.'s (1994) data also suggest that the mbuna group is paraphyletic and that the genus *Copadichromis* is polyphyletic and in need of taxonomic revision. MORAN et al. (1994) further suggest the need for several other taxonomic revisions of Lake Malawi cichlids and discuss hybridization as an explanation for some unexpected placements of species.

Recently, we (MEYER, MONTERO & SPREINAT, unpubl. data) 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 (MEYER et al. 1990, 1991). The mtDNA sequences generally confirm MORAN et al.'s (1994) recent findings (Fig. 5). Since *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 Malawian species flocks plus some of the East African non-endemic haplochromines. We can confirm MORAN et al.'s (1994) findings that *Rhamphochromis* represents another independent lineage of the Lake Malawi species flock, increasing to four the lineages represented in the lake (not counting *Serranochromis*) (Fig. 5). Further sequencing work on *Copadichromis* will be required to test whether or not it represents an independent lineage, whether the genus is not monophyletic as suggested by MORAN et al. (1994) or whether some members of the genus belong to the non-mbuna group. Also *Diplotaxodon* should be sequenced to test whether it is another independent lineage. Whether *Rhamphochromis* is the most basal Malawian clade, even more basal then *Astatotilapia calliptera* (Fig. 4) as has been suggested by MORAN et al. (1994), was not clear from our sequence data (see also KOCHER et al. 1993). Our majority rule bootstrap tree based on a parsimony analysis of the control region sequences (Fig. 5, MEYER, MONTERO & SPREINAT, unpubl. data) does not resolve the branching order among the four Malawian lineages, but our most parsimonious trees agree with MORAN et al. (1994) in placing *Rampkochromis* most basal within the Lake Malawi cichlids. In contrast to MORAN et al. (1994) we find that within the non-mbuna group *Cyrtocara* and *Tyranochromis* represent the most basal groups; within the mbuna group *Melanochromis labrosus* appears to be the most basal member (Fig. 5). However, these findings must remain suggestive since (1) the bootstrap support was not unambiguously high and (2) until a more complete representation of Malawian taxa is achieved.

MtDNA sequences tentatively 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 & MONTERO, unpubl. data) (Fig. 4, 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 affinities to other riverine Tanzanian *Astatotilapia* species of uncertain species status (L. SEEGERS and L. DE VOS collected and kindly provided samples). Some members of the widespread mainly riverine genus *Astatotilapia* are likely to represent the body plan and lifestyle of the ancestors of the Victoria and possibly the Malawi species flocks (MEYER et al. 1991). *Astatotilapia* is, mitochondrially, an unnatural group; GREENWOOD (1979) previously recognized that this genus was defined on shared primitive characters and is in need of taxonomic revision.

Lake Tanganyika, an evolutionary fountain for species diversity

Lake Tanganyika is estimated be 9–12 million years old (COHEN et al. 1993), making it by far the oldest of the large 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 a relatively small number) in 49 endemic genera that are assigned to twelve tribes (FRYER & ILES 1972, POLL 1986, COULTER 1991). 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

(1986) classification and assignments of genera into tribes (STURMBAUER & MEYER 1993, STURMBAUER et al. 1994). Comparisons of electrophoretic and mtDNA data demonstrated that several Tanganyikan lineages are much older than the lineages of Lake Victoria and Malawi (NISHIDA 1991, STURMBAUER & MEYER 1992, 1993, STURMBAUER et al. 1994, KOCHER et al. 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 & MEYER 1993, STURMBAUER et al. 1994). However, the age estimates for the lakes themselves and the cichlid lineages in them remain uncertain until a more reliable calibration of the cichlid molecular clock is achieved (see also KOCHER et al. 1993).

The existence of several old lineages 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 re-evaluation of the geological age of Lake Tanganyika indicated that the age of the lake is probably greater than that 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 et al. 1994).

Electrophoretic and mtDNA sequences suggest that the Victoria and Malawi flocks are closely related to particular Tanganyikan tribes; the Tropheini and Haplochromini (NISHIDA 1991, STURMBAUER & MEYER 1993) (Fig. 6). This may not be surprising since previously considerable similarities between *Tropheus* and *Pseudotropheus* from Lake Malawi had been interpreted to argue for a polyphyletic original Lake Malawi cichlid (FRYER & 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 (MEYER et al. 1990, KOCHER et al. 1993) (Figs. 5, 6).

The Tanganyika flock can be viewed as a reservoir of old phylogenetic lineages that gave rise to the ancestors (which in both cases were likely to have been riverine generalists similar to *Astatotilapia*) of the Victoria and Malawi flocks (NISHIDA 1991, MEYER et al. 1990, 1991). Lake Tanganyika does not harbor all the members and descendants of some of its endemic lineages (e.g. Lamprologini, STURMBAUER et al. 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.

Intra-lacustrine speciation: potential causes and mechanisms

The current Lake Victoria, with an area of 68,000 km^2 roughly 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 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

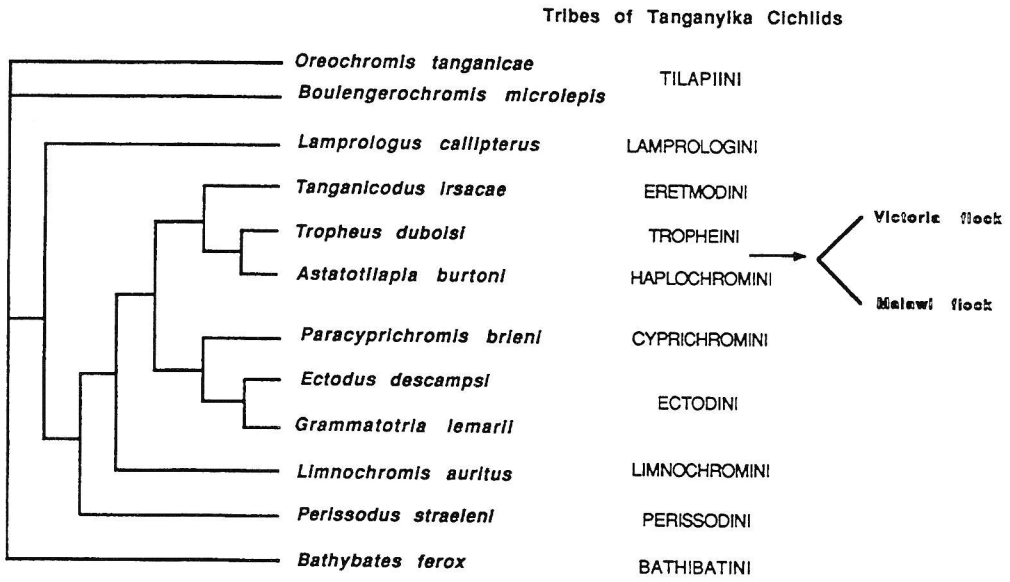

Fig. 6. Molecular phylogenetic relationships of some representative species of nine of eleven tribes (names of tribes in capitals following POLL (1986)) endemic to Lake Tanganyika, figure modified from STURMBAUER & MEYER (1993) and STURMBAUER et al. (1994). In this phylogenetic analysis both the tilapia and *Bathybates* were used as outgroups. 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 two species flocks of Lake Malawi and Lake Victoria is indicated with the arrow on the right and based on NISHIDA (1991) and STURMBAUER & MEYER (1993). Some of the relationships of Tanganyikan tribes remain somewhat tentative and are being tested further (STURMBAUER & MEYER 1993).

smaller bodies of water after the lake level rose again is likely to have occurred (MAYR 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. 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 Malawi (STAGER et al. 1986, SCHOLZ & ROSENDAHL 1988, GASSE et al. 1989, ROCHE 1991, TIERCELIN & MONDEGUER 1991). The lake topography consists 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 & 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 & ROSENDAHL 1988, GASSE et al. 1989).

Intralacustrine speciation, probably by micro-allopatric speciation, through isolation by distance or appropriate habitat type, would appear to the most important mode of speciation for all three species flocks. *Tropheus* (they 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 & 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 & 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 seem that physical separation of water masses is not a necessary precondition for the establishment of genetic discontinuities and speciation.

Intra-lacustrine speciation should not be automatically equated with sympatric speciation and should not be interpreted as refutation of allopatric speciation models (KONDRASHOV & 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 & ILES 1972). Only very few species occur throughout a whole lake (FRYER & ILES 1972) and species often are restricted to single localities in which population size can be as small as few hundred individuals (RIBBINK et al. 1983). Most cichlids are poor dispersers, they are philopatric, they show homing, and males tend to defend breeding territories for several years (HERT 1992, but 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 & JOHNSTON 1987).

Recently, however, a case for sympatric speciation as an actual mechanism for intralacustrine speciation was made for two small radiations of Cameroon tilapiine cichlids (SCHLIEWEN et al. 1994). This case of small flocks originating within extremely small crater lakes would seem to be the strongest so far for sympatric speciation to be responsible for diversification in cichlids. However, the picture is not complete and more data on species status, ecology, and behavior of the Cameroon tilapias are eagerly awaited. Nonetheless, molecular data strongly argue for monophyly of these species assemblages. The Cameroon flocks might provide the best workable small replicate of the East African radiations that might allow the experimental manipulation and testing of dispersal and other important ecological factors that have been implicated to explain alternative speciation mechanisms.

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 appears unlikely (but not unthinkable) 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 (MEYER & MONTERO, unpubl. data) (Fig. 4, 5).

That rates of speciation in cichlids can be astonishingly fast has been known since the discovery of five endemics in Lake Nabugabo (GREENWOOD 1965), a small lake that is less than 4,000 years old and separated from Lake Victoria only by 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).

Even faster rates of speciation were suggested by the finding that the southern end of Lake Malawi was arid only two centuries ago and is now inhabited by numerous endemic species and 'color morphs' that are found there and are believed to have originated during the

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