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Similar Morphologies of Cichlid Fish in Lakes Tanganyika and Malawi Are Due to Convergence

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The species flocks of cichlid fishes in the lakes of East Africa are the most spectacular example of adaptive radiation among living vertebrates. Similar highly derived morphologies are found among species in different lakes. These similarities have been variously interpreted either as evidence for migration of ancestral species between the lakes, or of striking convergence of morphology. To distinguish among these competing hypotheses we sequenced a portion of the mitochondrial DNA control region from six pairs of morphologically similar taxa from Lakes Malawi and Tanganyika. Our results clearly indicate a separate origin of these morphologies in the two lakes. They also suggest that the Tanganyikan radiation is relatively old, and that the species flocks in lakes Malawi and Victoria are derived from one of the ancient lineages found in Lake Tanganyika. These findings have important implications for understanding the pattern of morphological and behavioral evolution in these fish. © 1993 Academic Press, Inc.

INTRODUCTION

The explosive speciation of cichlid fish in the lakes of East Africa has long been a focus for controversy among evolutionary theorists (Mayr, 1963; Fryer and Iles, 1972). Lake Malawi, with over 400 species of endemic cichlid, has more species of fish than any other lake in the world. Lakes Victoria (>200 species) and Tanganyika (>170 species) run a close second and third. These species probably arose in a relatively short period of time. Recent geological studies suggest that Lake Tanganyika is no more than 12 MY old (Cohen *et al.*, 1993), while Lake Malawi is thought to be considerably more recent, probably less than 2 MY old (Banister and Clarke, 1980). Several authors have suggested an extremely recent (<200,000 years) common ancestor for the Lake Victoria cichlid flock (Sage *et al.*, 1984; Meyer *et al.*, 1990). The mechanisms by which so many species have arisen in such a short time, within closed lake basins, are a fascinating subject for research.

A separate, monophyletic origin for the species flock in each lake has often been assumed, partly because of the high levels of endemicity found in the cichlid fauna, and partly because of the ancient and isolated history of each lake (Fryer and Iles, 1972). The lake basins have arisen separately by a gradual lengthening and deepening of the East African rift (Malawi and Tanganyika) or by tilting of the Tanzanian shield (Victoria). Each lake now drains through a separate river, which effectively isolates extant lacustrine forms. Suggestions persist, however, that some lakes may have been connected at various times through intermediate lakes (e.g., the connection of lakes Malawi and Tanganyika through Lake Rukwa; Banister and Clarke, 1980), or by stream capture (Greenwood, 1974). All of this leads to the conclusion that geologic evidence is not sufficient to determine whether the flocks evolved separately in each lake.

Morphological taxonomists have failed to reach a consensus concerning relationships of fish in different lakes. Regan (1920) created a basal dichotomy between "haplochromine" and "tilapiine" lineages based on the shape of the neurocranial apophysis. Implied in this distinction were numerous instances of morphological convergence between certain tilapiine species from Lake Tanganyika with haplochromine species from lakes Malawi and Victoria. Myers (1936), while generally accepting the idea of a separate origin of the fishes in each flock, suggested that the Tanganyikan genus *Petrochromis* and Lake Malawi *Pseudotropheus* were so similar that the genera should be merged. Trewavas (1949), while studying the Malawi flock, reaffirmed Regan's hypothesis of a separate origin of the fish in different lakes and this view has been promulgated by most workers since (Fryer and Iles, 1972).

A series of papers by Greenwood (1978, 1979, 1980)

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and colleagues (Stiassny, 1981) exposed inconsistencies in the characters used by others to demonstrate the close relationship of fish within each lake. Neither the structure of the apophysis, squamation and shape of the caudal fin, nor the structure of anal fin spots is consistent among the members of a single flock. Eventually, Greenwood (1983, p. 209) concluded "that the species flocks of lakes Victoria, Malawi and Tanganyika are each composed of several distinct lineages, and that members of at least some lineages occur in more than one lake." The search for morphological characters useful for cladistic analyses has continued. Despite the morphological diversity of these fishes, however, few synapomorphic characters exist for resolving phylogeny (Stiassny, 1991). Repatterning of cichlids has mostly occurred by changes in relative growth rate among structures (Strauss, 1984). In any case, construction of phylogenies from morphological characters creates a tautology for studies of the evolution of these traits. An independent set of characters is required.

Kornfield (1991), on the basis of allozyme characters, suggested that *Cyphotilapia frontosa* of Lake Tanganyika was more closely related to members of the Malawi flock than to other Tanganyikan species tested. To date, this has been the only test of the relationship between Malawi and Tanganyikan cichlids using molecular characters.

We have used the sequence of nucleotides in mitochondrial DNA to test the independent origin hypothesis. These characters are expected to evolve independently from the genes which control the morphology of these fishes. The recency of the East African cichlid radiations (Sage *et al.*, 1984) led us to sequence a rapidly evolving portion of the mitochondrial genome, the control or D-loop region.

MATERIALS AND METHODS

Sources of DNA

We chose six pairs of morphologically similar fishes to illustrate the range of morphological and behavioral similarities of species in lakes Malawi and Tanganyika (Fig. 1). *Petrochromis* (Tanganyika) and *Petrotilapia* (Malawi) share a highly developed rasping jaw morphology and were considered by Fryer and Iles (1972) to represent one of the most remarkable similarities in the two fauna. The morphology and ecology of the elongate predator *Bathybates* (T) closely mimics that of *Rhamphochromis* (M). The fleshy lips of *Lobochilotes* (T) closely resemble the fat lips of *Placidochromis milomo* (M). *Tropheus* (T) and *Pseudotropheus* (M) are morphologically and behaviorally similar taxa which live over rock substrates. *Cyphotilapia frontosa* (T) has an enlarged nuchal hump which is also found in *Cyrtocara moorii* (M). Some species of the rock-dwelling *Julidochromis* (T) display a strong horizontal striping reminiscent of *Melanochromis auratus* (M).

Most specimens were collected by the authors directly from nature: *Bathybates* sp. Boulenger; *Cyrtocara moorii* Boulenger; *Lobochilotes labiatus* Boulenger; *Melanochromis auratus* (Boulenger); *Placidochromis milomo* (Oliver); *Petrotilapia* sp.; *Rhamphochromis* sp. Several Tanganyikan specimens were provided by Mireille Shreyen (Fishes of Burundi, Bujumbura): *Cyphotilapia frontosa* (Boulenger); *Petrochromis orthognathus* Matthes; *Tropheus moorii* var. Moba Boulenger. Two species common in the aquarium trade were purchased from dealers in the United States: *Julidochromis regani* Poll and *Pseudotropheus zebra* (Boulenger). All vouchers will be deposited in the Pennsylvania State University Museum.

DNA Methods

DNA was extracted from tissues of frozen or ethanol-preserved specimens using standard proteinase K digestion and phenol extraction protocols (Kocher *et al.*, 1989). Amplifications were performed in 50-μl volumes of 0.67 M Tris (pH 8.8), 2 mM $MgCl_2$, 1 mM of each dNTP, 5 μM of each primer, 1–1000 ng of template DNA and 1.5 units *Taq* polymerase (Perkin Elmer-Cetus). The primers used were THR (5′-AGCTCAGCGCCAGAGCGCCGGTCTTGTAAA-3 and TDKD (5′-CCTGAAGTAGGAACCAGATG-3′). The temperature profile for 30–40 cycles of double-stranded amplification was 93°C for 30 s, 50°C for 60 s and 72°C for 120 s. Amplified products were separated on a 1% LMP agarose gel. Desired fragments were purified from the gel by hot phenol extraction and precipitation with isopropanol. The DNA (approximately 0.5 μg) was resuspended in 20–40 μl of dH_2O and 10–12 μl sequenced using the TAQ DyeDeoxy Terminator cycle sequencing protocol (Applied Biosystems). Centri-Sep columns (Princeton Separations) were used to remove unincorporated nucleotides prior to loading on an automated sequencer (373A, Applied Biosystems).

Phylogenetic Analyses

Sequence alignment was performed using the sequential algorithm of the program PILEUP (Devereux *et al.*, 1984). A gap penalty of 1 and a gap length penalty of 0.3 were used, but the alignment was not particularly sensitive to variations in these parameters. No manual adjustments of the alignment were performed.

Minimum length networks relating the taxa were found using the branch and bound algorithm of PAUP (Swofford, 1992). Statistical significance of particular groupings was estimated by branch and bound searches of 2000 bootstrap samples from the original data set. For the tree relating 16 taxa, computational limitations prevented the examination of a large num-

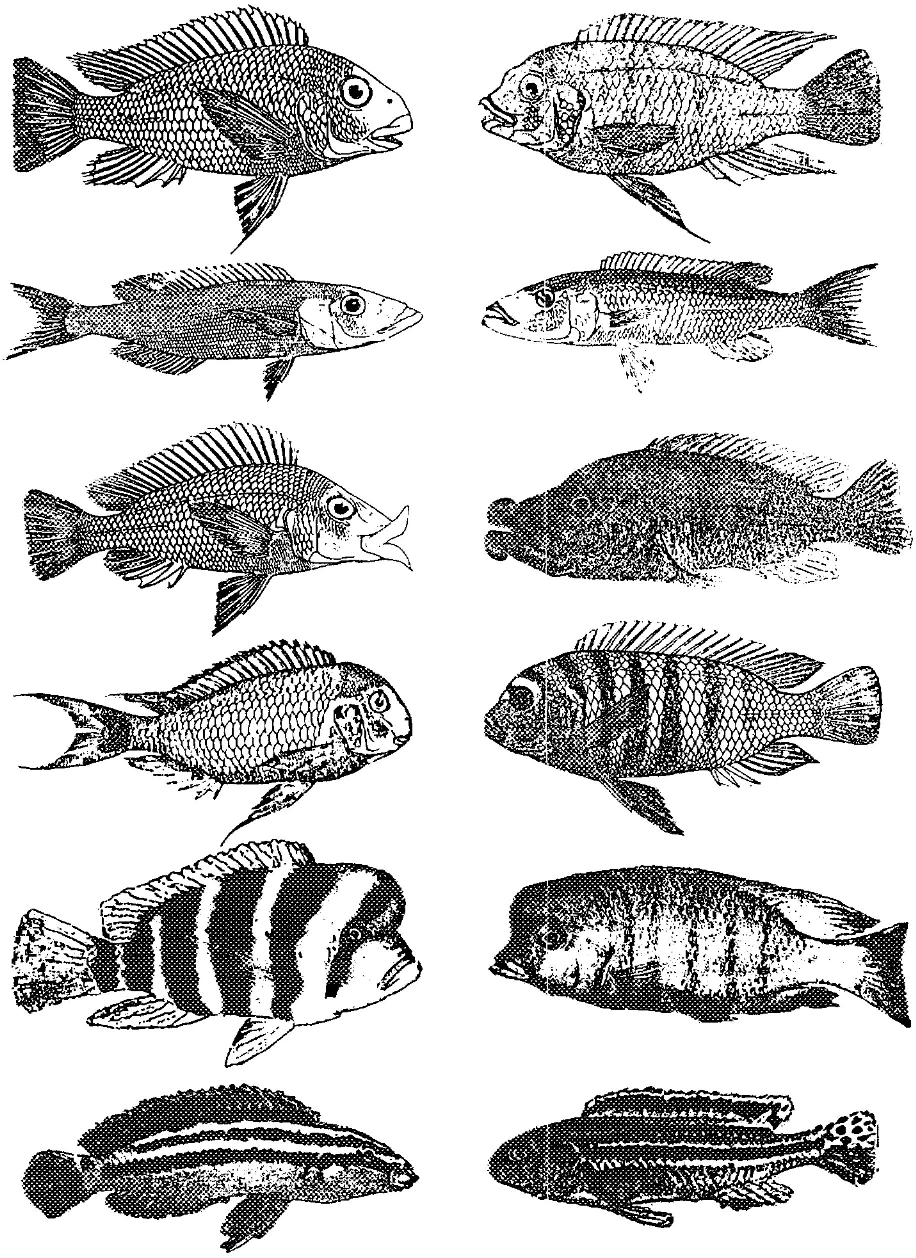

FIG. 1. Six pairs of morphologically similar cichlids from Lakes Tanganyika and Malawi. Tanganyikan fish are in the left column, Malawi fish in the right. The specific features shared are: rasping jaw morphology, fusiform body, fleshy lips, mbuna habit, nuchal hump, and horizontal striping.

ber of bootstrap samples. Instead, the majority-rule consensus of trees no more than five steps longer than the shortest tree (954 trees of length ≤281) was constructed.

RESULTS

The sequences of a portion of the control region, beginning immediately after the proline tRNA, for each of the 12 species is shown in Fig. 2. Only 15, mostly single base pair, insertion/deletion events were invoked to align approximately 350 bp from all species. In the complete alignment of 357 positions, 126 sites are variable among these 12 species.

The matrix of observed differences among the sequences is shown in Table 1. The difference among species ranges from 7 to 74 (2.0–20.7%). No close affinities (<9% sequence difference) exist between the species in lakes Malawi and Tanganyika. *Julidochromis* is most distant from the other species, showing an av-

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Petrotilapia    CCGGGCTCTGCCTTCCATGTAAACGCAATGCATATATGTATTAACACCATTATTTTATATTAAACATATCCTATATATTAATACATACCATTTATAAAA-ACATAGACAAATATACCACA 119
Pseudotropheus  CCGGGCTCTGCCTTCCATGTAAACGCAATGCATATATGTATTAACACCATTGTTTTATATCAAACATATCCTATATATTAATACATACCATTTATAAAA-ACATAGACAAATATACCACA 119
Melanochromis   CCGGGCTCTGCCTTCCATGTAAACGCAATGCATATATGTATTAACACCATTATTTTATATCAAACATATCCTATATATTAATACATATCATTTACAAAA-ACATAGACAAATATACCACA 119
Cyrtocara       CCGGGCTCTGCCTTTCATGTAAACGCAATGCATATATGTATTATCACCATTATTTTATATTAAACATATCCTATATATTAAGACATATCATTTAC-AAA-ACATAGACAAATATTCCACA 118
Placidochromis  CCGGGCTCTGCCTTTCATGTAAACGCAATGCATATATGTATTATCACCATTATTTTATGTTAAACATATCCTATATATTAATACATATCATTTACAAAA-ACATAGATAAATATACCACA 119
Rhamphochromis  CCGGGCTCTGCCTTACATGTAAACGCAATGCATATATGTATTATCACCATTATTTTATATCAAACATATCCTATATATTAATACATACCAT-TATAAAAGATATAGACAGATATAACACA 119
Petrochromis    CCGGGCTCTGCCTTGGATGTAAACGCAATGCATATATGTATTAACACCATTATTTTATATCAAACATATCCTATATATAAATACATAATATTTAC-AAAGACATAGATTAATTCACCACA 119
Lobochilotes    CCGGGCTCTGCCTTGGATATAAACGCAGTACATATATGTATTAACACCATTATTTTATATTAAACATATCCTATATATAAATACATATTATTTAC-AAAGACATAGACTAATTTACCACA 119
Tropheus        CCGGGCTCTGCCTTGGATGAAAACGCAATGCATATATGTATTATCACCATTATTTTATCTCAAACATATCCTATATATAAATACATATTATTTAC-AAAAACATAGATTAATACCCCACA 119
Bathybates      CCGGGCTCTGCCCTGTATGTAAACACAATGCATATATGTATTATCACCATATTTTTATATCAAACATATCCTATATATAAATACATATATTTTAC-AAAGACATAGACTGTTCCCCCACA 119
Cyphotilapia    CCGGGCTCTGCCTCCAATGTATATGCAATACATATATGTATTATCACCATTATTTTATATCGAACATATCCTATATATAAATACATAACATTCAAAAGA-ACATAGATTGACCCCCCACA 119
Julidochromis   CCGGGCTCTGCCT-CGATGTAAACGTCGTGCATATATGTATTATCACCATTATCTTATATCAAACATAACCTATATATAAATACATAAAATTTAC-AAAGACATAGACCTGTCCCCAACA 118

Petrotilapia    TCCTTGTTTAAACCATTT-TAACTAAAAGGTACATAAACCAT-AACTGAATATTCTCCAATA-AAT-ATCTACTAATTACTAAACGATAGTTTAAGACCGATCACAACTCTCAC-TAGTT 234
Pseudotropheus  TACTTGTTTAAACCATTC-TAACTAAAGGGTACATAAACCAT-AACTGAATATTCTCCAATA-AAT-ATCTATTAATTACTAAACGATAGTTTAAGACCGATCACAACTCTCAC-TAGTT 234
Melanochromis   TATTTGTTTAAACCATTT-TAACTAAAGGGTACATAAACCAT-AACTGAATATTCTCCAATA-AAT-ACCTATTAATTACTAAACGATAGTTTAAGACCGATCACAACTCTCAC-TAGTT 234
Cyrtocara       TATTTGTTTAAACCATTT-TAACTAAGGGGTACATAAACCAT-AACTGGATATACTCCAATA-AAC-CTTAATCAACCACTGAACGATAGTTTAAGACCGATCACAACTCTCAC-TAGTT 233
Placidochromis  TATTTGTTTAAACCATTT-TAACTAAGGGGTACATAAACCAT-AACTGAATATACTCCAATA-AAC-CTT-ATTAACCACTGAACGATAGTTTAAGACCGACCACAACTCTCAC-CGGTT 233
Rhamphochromis  TCTTTGTTTACACCATTC-TAACTAAGGGGTACATAAACCAT-AACTGAATACTATCCAATA-AAT-ATTCATTGTCCACTGAACGATAGTTTAAGACCGATCACAACTCTCAC-TAGTT 234
Petrochromis    TATTTGTCATCAACATTT-TAACTAAGAAGTACATAAACCAT-AACTGAATATTTTCCAATA-ATT-ATTTATAAATTGCGGAACGATAGTTTAAGACCGATCACACCTCTCACATAGTT 235
Lobochilotes    TATTTGTCAACAACATTT-TAACTAAGAAGAACATAAACCAT-AACTGAATATTCTCCAATG-ATT-ATTTATAAATTGCGGAACGATAGTTTAAGACCGATCACACCTCTCACATAGTT 235
Tropheus        TATTTGCTAACACCATTT-CAACTAAGAAATACATAAACCAT-GATTGGAAATCTTCCAACA-ATTAATTTATAAATTGCTGAACGATAGTTTAAGACCGATCACACCTCTCACATAGTT 236
Bathybates      TATTTGCCATCAACAAT--ATACGAAGAA-CACATAAACCAATAACTGAA-ATATTCCAACAAA---ATCTATAAACCACTGAACGATAGTTTAAGACCGACCACAACTCTCACATAGTC 232
Cyphotilapia    TATTTGCCAACAACATTTACAACTGAAAA-AACATAAACCATGAACTGAA-ACTTTCCAAAATAAGTATAAATAAATTACTGAACGATAGTTTAAGACCGATCACACCTCTCACATAGTC 237
Julidochromis   TATTTGCTATGGACATGTACGATTCAAGGGT-CATAAACCAT-AACTG-ATATA-TCCAACATA-TATTTAATTAATTACTGGACGATGATTTAAGACCGATCACACCTCTCATACAGTT 233

Petrotilapia    AAGTTATACCAAGTACCCACCATCCTATTCATTACCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTTCTTAATGTCAACGGTTCTTGAAGGTCAAGGACAAGTATTCGTG   351
Pseudotropheus  AAGTTATACCAAGTACCCACCATCCTATTCATTACCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTTAATGTCAACGGTTCTTGAAGGTCAAGGACAAGTATTCGTG   351
Melanochromis   AAGTTATACCAAGTACCCACCATTCTATTCATTACCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTTAATGTCAACGGTTCTTGAAGGTCAAGGACAAGTATTCGTG   351
Cyrtocara       AAGTTATACCAAGTACCCACCATCCTATTCATTACCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTAAATGTTAACGGTTCTTGAAGGTCAAGGACAAGTATTCGTG   350
Placidochromis  AAGTTATACCAAGTACCCACCATCCTATTTATTACCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTAAATGTTAACGGTTCTTGAAGGTCAAGGACAAGTATTCGTG   350
Rhamphochromis  AAGTTATACCAAGTAGCCACCATCCTATTTGCTACCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTTAATGTTAACGGTTCTTGAAGGTCAAGGACAAATATTCGTG   351
Petrochromis    AAGATATACCAAGTACCCACCATCCTATACATTTCCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTTCTTAATGCTAACGGTTCTTGAAGGTCAAGGACAACTATTCGTG   352
Lobochilotes    AAGATATACCAAGTACCCACCATCCTATTCATTTCCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTTAATGTCAACGGTTCTTGAAGGTCAAGGACAGTTATTCGTG   352
Tropheus        AAGATATACCAAGTACCCACCATCCTATTCATTTCCCATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTTAATGCTAACGGTTCTTGAAGGTCAAGGACAGATATTCGTG   353
Bathybates      TAGATATACCAAGTATCCACCATCCTATAGTTTAAAATTATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTAAATGTTAACGGTTCTTGAAGGTCAAGGACAGTTATTCGTG   349
Cyphotilapia    AAGATATACCAAGTACCCACCATTCTATTTATTTACAATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTTCTCAATGTCCACGGTTCTTGAAGGTCAAGGACAATTACTTGTG   354
Julidochromis   AAGATATACCAAGTACCCACCATCCTATTCGTTTACAATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTCAATGTTAACGGTTATTGAAGGTGAGGGACAATTACTTGTG   350
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FIG. 2. Aligned control region sequences of 12 species of cichlid fish. Gaps which have been introduced to maximize sequence similarity are indicated by dashes. These sequences have been submitted to the GenBank data library under Accession Nos. U01105–U01116.

erage of 66.3 differences (18.6%). Among the most similar sequences, the ratio of observed transitions and transversions is about 10:1. The observed ratio declines with sequence difference. Comparisons involving *Julidochromis* show the lowest transition to transversion ratios (1:1), suggesting that it is the most distantly related of the species studied.

TABLE 1

Matrix of Differences Observed among the Mitochondrial DNA Control Region Sequences of Cichlids from Lakes Malawi and Tanganyika

	1	2	3	4	5	6	7	8	9	10	11
1 Petrotilapia	—										
2 Pseudotropheus	7	—									
3 Melanochromis	10	7	—								
4 Cyrtocara moorii	25	24	21	—							
5 Placidochromis	26	25	22	12	—						
6 Rhamphochromis	30	27	30	31	30	—					
7 Petrochromis	38	39	37	42	44	47	—				
8 Lobochilotes	37	38	35	40	44	49	17	—			
9 Tropheus moori	48	47	44	43	47	53	25	31	—		
10 Bathybates	70	67	66	61	63	67	52	56	62	—	
11 Cyphotilapia	60	61	58	66	66	68	51	54	55	63	—
12 Julidochromis	71	68	66	66	69	74	60	64	55	73	64

Phylogenetic Trees

From these sequences we found the shortest networks relating the six pairs of species using the branch and bound algorithm of PAUP. The grouping of the six Malawi taxa, to the exclusion of the six Tanganyikan taxa, was found in 99% of 2000 bootstrap samples (Fig. 3). This tree clearly demonstrates that the six species from Lake Malawi share a unique common ancestor. All species from Lake Malawi, including the morphologically and genetically distinct *Rhamphochromis*, are more closely related to each other than to their morphological cognates in Lake Tanganyika.

To examine hypotheses about the characteristics of the ancestors of these flocks, we also determined the most parsimonious reconstruction of the phylogeny which includes the sequences of *Astatotilapia burtoni* (Gunther) from Tanganyika, *Astatotilapia calliptera* (Gunther) from Malawi (Meyer *et al.*, 1991) and *Astatotilapia piceatus* Greenwood and *Astatoreochromis alluaudi* Pellegrin from Lake Victoria (Meyer *et al.*, 1990). Two shortest trees of length 276 exist, but at least one internodal segment is ambiguous. In the consensus of the 954 shortest trees (Fig. 4), *Astatoreochromis* falls outside the clade containing *Astatotilapia* and the Malawi flock. There is a strong suggestion that

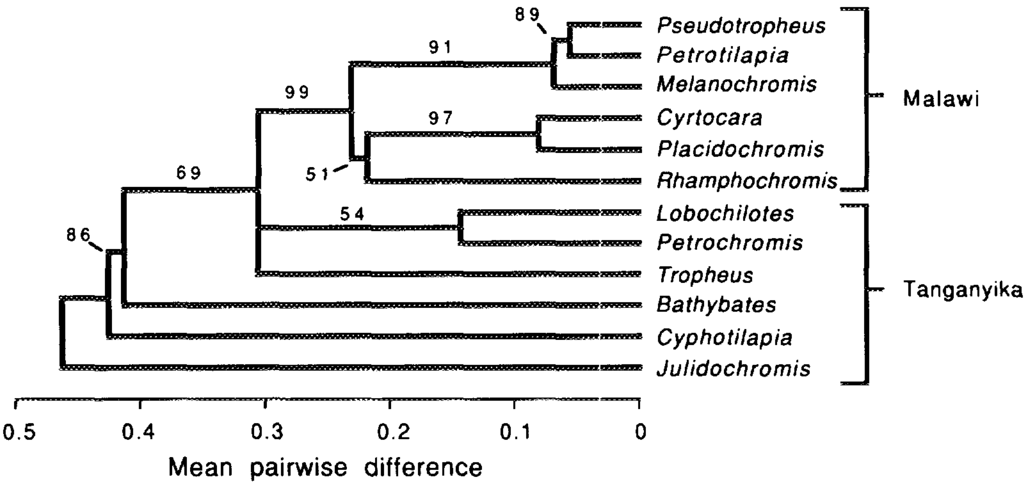

FIG. 3. Bootstrapped parsimony tree with branch lengths drawn proportional to the uncorrected difference between sequences. Analysis of 2000 bootstrap samples was performed using the branch and bound algorithm of PAUP (Swofford, 1992).

Astatotilapia is polyphyletic, since *A. calliptera* clusters as one of four main lineages in the Malawi flock, but this clustering is not statistically significant. The shortest tree constructed under the constraint that Malawi taxa other than *A. calliptera* be monophyletic is only two steps longer (length = 278). Constraining the topology so that *Astatotilapia* is monophyletic results in a tree two steps longer still (length = 280).

DISCUSSION

The sequences reported here clearly refute postulates of a close relationship among morphologically similar fish from lakes Malawi and Tanganyika. The taxa from Lake Malawi have highly similar mtDNA sequences, demonstrating a relatively recent common ancestor. In contrast, the species from Lake Tanganyika are more distantly related, which suggests that this flock began to diversify much longer ago. Together, these observations establish separate evolutionary origins of the morphologically similar fishes in the two lakes.

Monophyly of the Malawi Flock

The high levels of sequence similarity among the Malawi taxa suggest a recent, monophyletic origin for most of the cichlids in that lake. Although ours is a limited sampling of the taxa in Lake Malawi, a more complete sampling using restriction fragment length polymorphisms did not discover any taxa more divergent than those studied here (Moran *et al.*, 1993). We doubt that any of the endemic haplochromine cichlids (i.e., excluding *Tilapia, Oreochromis, Serranochromis*) of Lake Malawi will show greater divergence from *Rhamphochromis*, but this prediction should be tested by sequencing additional species.

So far, four main lineages can be identified within

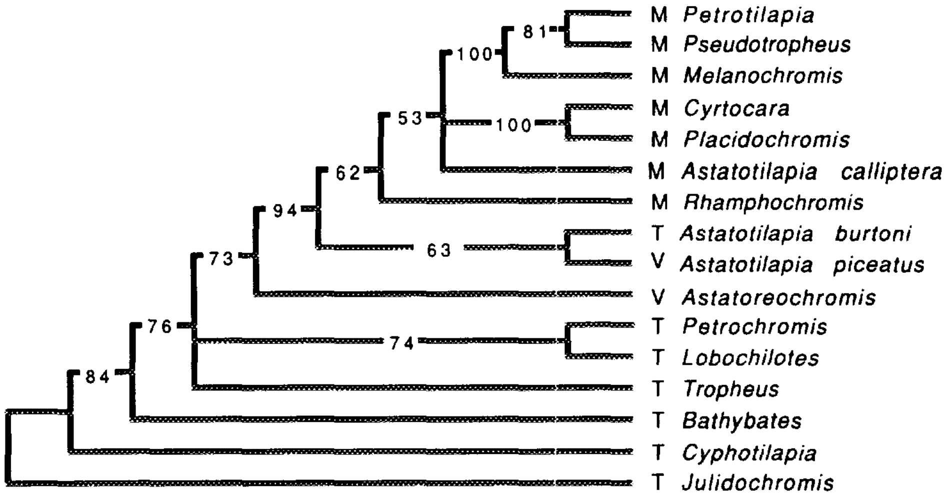

FIG. 4. Majority-rule consensus tree for 16 taxa, including 3 suspected ancestral forms. The branch and bound algorithm of PAUP was used to discover 954 trees of length 281 or shorter. Branch lengths are arbitrary.

the Malawi flock: selected rock-dwelling cichlids (mbuna), the sand-dwelling haplochromines, *A. calliptera,* and *Rhamphochromis.* We suspect that mtDNA sequences will have some utility for studying generic-level relationships within Lake Malawi, but our enthusiasm must be qualified by the suggestion of Moran and Kornfield (1993) that ancestral mtDNA polymorphisms have been maintained during the radiation of the Malawi flock.

Tanganyikan Flock Is Not Monophyletic

The Tanganyikan flock cannot be considered monophyletic (*sensu* Hennig, 1966) since both the Malawi and Victoria flocks are nested within the clade containing the Tanganyikan species. This is consistent with the finding that some clades in Lake Tanganyika contain more variation than the entire Malawi flock (Sturmbauer and Meyer, 1992). As suggested by Nishida (1991), Lake Tanganyika appears to be a reservoir of ancient lineages of cichlids. It is not yet clear whether the radiation of taxa in Lake Tanganyika began before the formation of the current lake. It is possible that the Tanganyikan cichlids are oligophyletic, having arisen from a number of distinct riverine taxa. These questions could be answered by sampling of additional taxa from outside the lake, and by comparing molecular clock estimates of the divergence of lacustrine taxa with geologic estimates of the age of the lake. Of particular interest is whether the genus *Astatotilapia* arose from lacustrine ancestors in the margins of Lake Tanganyika.

Ancestors of the Malawi and Victoria Flock

The characteristics of the ancestors of the flocks in lakes Malawi and Victoria is a topic of continuing discussion (Crapon de Caprona and Fritzsch, 1991). Trewavas (1949) suggested that *A. calliptera* was representative of the morphology and behavior of the ancestor of the Malawi flock. Since that time, it has generally been assumed that the ancestors of these flocks were morphologically generalized riverine cichlids which may have migrated extensively through the river systems of East Africa (Fryer and Iles, 1972).

Greenwood (1979, 1980) proposed that the Malawi flock had a polyphyletic origin, and that the closest relatives of some lineages might be found in the Lake Victoria flock. Indeed, he later suggested (Greenwood, 1983) that *Macropleurodus bicolor* (V) and *Chilotilapia rhoadesi* (M) "are members of a monophyletic lineage within the haplochromine cichlids, and that representatives of this lineage occur in lakes Malawi, Victoria and Tanganyika."

Oliver (1984) elaborated on this idea, suggesting that the mbuna-like taxa of Lake Tanganyika are closely related to the Lake Malawi mbuna. He proposed that characteristics of the anal-fin spots identified two classes of fish in Lake Malawi—those similar to *A. calliptera* (mostly mbuna) and those similar to *Serranochromis* (sand-dwelling haplochromines). While mitochondrial data support the idea of separate mbuna and sand-haplochromine clades, there is no indication that *A. calliptera* is more closely related to either one. And, as Eccles and Trewavas (1989) point out, it is unlikely that highly specialized species, such as mbuna, could have successfully traveled between lakes through fluvial connections.

Because of uncertainty in the branching order of *Rhamphochromis* and *A. calliptera* in our phylogeny, the characteristics of the immediate ancestor of the Malawi flock remain unclear. The hypothesis of an *Astatotilapia*-like progenitor remains the most likely scenario. Our study confirms Greenwood's (1979) claim that *Astatotilapia* is not a monophyletic assemblage. A more complete study of East African astatotilapiines is of central importance to understanding the colonization of lakes in this region and should be the focus of additional research. The suggestion that *Serranochromis* or *Chetia* contributed to the flock (Greenwood, 1979) should also be investigated with mtDNA sequencing.

Convergence or Parallelism?

Instances of morphological similarity have traditionally been classified as either convergence or parallelism. Authors differ on the definition and usage of these terms, and contradictory usage sometimes occurs on the same page! Futuyma (1986) defines convergence as the "evolution of similar features independently in unrelated taxa, usually from different antecedent features or by different developmental pathways." The distinction from parallelism rests on three points. If taxa are closely related, the term parallelism is preferred, but for organisms of intermediate or undetermined relatedness the definition becomes arbitrary. Parallelism is also indicated if the ancestral character states for the taxa are the same, but this depends on the definition of the character. Finally, if similar developmental pathways are involved, it is considered parallelism. However, developmental pathways can be described at a variety of levels, and currently very little is known about the molecular basis for processes which have been described at larger scales.

The conclusion of the present paper is that the pairs of similar Malawi and Tanganyika fish are distant relatives, not sister taxa. The immediate common ancestors of each flock were not contemporaneous (probably separated by about 10 MY; T. D. Kocher *et al.*, in preparation). The Malawi flock appears to be monophyletic and was derived from a riverine, *Astatotilapia*-like ancestor. While it is possible that similar developmental pathways are followed in each species pair, it seems unlikely that identical substitutions are involved; the similarities probably arise from mutations in different, if overlapping, sets of genes. We prefer the term convergence until the morphological characteristics of the ancestors of these fish are discovered, or until the developmental genetic basis for the similarities is described.

Age of the Species Flocks

There is considerable uncertainty about the geologic age of each of the lakes. The mitochondrial DNA data suggest a recent origin for the Malawi taxa, consistent with a recent origin for that lake (Fryer and Iles, 1972). The data are also compatible with a middle Miocene date for the origin of the Tanganyikan flock (Cohen *et al.*, 1993). Nishida (1991) has suggested that the Tanganyikan flock contains remnants of at least seven lineages of cichlids older than 5 MY, and suggested that these lineages were present before the formation of the present lake, estimated at 2 MY ago by Banister and Clarke (1980). Our work has confirmed the presence of multiple old lineages in Lake Tanganyika, but it would be premature to speculate on the timing of branching events based on the sequences presented in this paper. The occurrence of multiple substitutions at the same nucleotide position is clearly evident in comparisons among distantly related taxa. Because the pattern of accumulation of sequence change is still not well understood for the control region (Kocher and Wilson, 1991), it is difficult to make the appropriate corrections to the difference data which would account for multiple substitutions at the same site. Therefore, this sequence is not suitable for determining the absolute time scale over which these radiations have occurred. Analysis of a sequence more suited to molecular clock analysis (mitochondrially encoded protein-coding gene) is now in progress for a number of these taxa (T. D. Kocher *et al.*, in preparation).

Rapid Evolution of the Species Flocks

The evolutionary history of the major cichlid species flocks in East Africa is gradually coming into focus. The sequences we have obtained clearly demonstrate that extreme morphologies have arisen separately in each lake. An independent origin of these flocks has long been suspected (Regan, 1922), but this is the first time that genetic data have been brought together for species in all three lakes.

The phenomenon of rapid and separate evolution of the complex traits which distinguish these species is confirmed. It is particularly interesting to note that within the basin of Lake Malawi, a full range of morphological diversity (comparable to that in Lake Tanganyika) has arisen within the last 2 million years. The challenge which remains is to understand the genetic and ecological mechanisms by which species in these lakes came to be so similar. Phylogenies developed from mtDNA sequences hold great promise for structuring further studies of this unique phenomenon.

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