Lake Level Fluctuations Synchronize Genetic Divergences of Cichlid Fishes in African Lakes

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Water level fluctuations are important modulators of speciation processes in tropical lakes, in that they temporarily form or break down barriers to gene flow among adjacent populations and/or incipient species. Time estimates of the most recent major lowstands of the three African Great Lakes are thus crucial to infer the relative timescales of explosive speciation events in cichlid species flocks. Our approach combines geological evidence with genetic divergence data of cichlid fishes from the three Great East African Lakes derived from the fastest-evolving mtDNA segment. Thereby, we show for each of the three lakes that individuals sampled from several populations which are currently isolated by long geographic distances and/or deep water form clusters of equally closely related haplotypes. The distribution of identical or equally closely related haplotypes in a lake basin allows delineation of the extent of lake level fluctuations. Our data suggest that the same climatic phenomenon synchronized the onset of genetic divergence of lineages in all three species flocks, such that their most recent evolutionary history seems to be linked to the same external modulators of adaptive radiation. A calibration of the molecular clock of the control region was elaborated by gauging the age of the Lake Malawi species flock through the divergence among the *utaka*-cichlid and the *mbuna*-cichlid lineages to minimally 570,000 years and maximally 1 Myr. This suggests that the low-lake-level period which established the observed patterns of genetic relatedness dates back less than 57,000 years, probably even to 17,000–12,400 years ago, when Lake Victoria dried up and Lakes Malawi and Tanganyika were also low. A rapid rise of all three lakes about 11,000 years ago established the large-scale population subdivisions observed today. Over that period of time, a multitude of species originated in Lakes Malawi and Victoria with an impressive degree of morphological and ecological differentiation, whereas the Tanganyikan taxa that were exposed to the same habitat changes hardly diverged ecologically and morphologically. Our findings also show that patterns of genetic divergences of stenotopic organisms provide valuable feedback on geological and sedimentological time estimates for lake level changes.

Introduction

The Great East African Lakes have been established as major model systems for the study of adaptive radiation. Each lake harbors flocks of cichlid fishes including hundreds of endemic species. It is now clear that the flocks of cichlid fishes in Lakes Victoria, Malawi, and Tanganyika arose independently via intralacustrine speciation (Kocher et al. 1993; Meyer 1993; Nagl et al. 2000). Despite several stunning similarities, the three species flocks differ from each other in age, species number, complexity, and overall degree of morphological diversity (Fryer and Iles 1972; Greenwood 1980, 1984; Poll 1986; Eccles and Trewavas 1989). All three lakes have complex geological histories, characterized by dynamic basin morphology, as well as by lake level fluctuations, caused by variations in amount of rainfall, temperature, evaporation, and, for some lakes, tectonic activity (Scholz and Rosendahl 1988; Gasse et al. 1989; Tiercelin and Mondeguer 1992; Cohen, Soreghan, and Scholz 1993; Delvaux 1995; Johnson et al. 1996; Lezzar et al. 1996; Cohen et al. 1997). Lake Tanganyika is the oldest of the three major East African lakes. Its central basin was formed 9–12 MYA. The structure of the lake basin suggests that a meandric river gave rise to at least three shallow, swampy proto-lakes (9–12 MYA), which progressively deepened to fuse finally into a single deep lake (5–6 MYA; Tiercelin and Mondeguer 1991; Cohen, Soreghan, and Scholz 1993). The Lake Malawi rift basin started to develop about 8.6 MYA, but deepwater conditions were acquired only 4.5 MYA. With an estimated age of about 400,000 years, Lake Victoria is substantially younger than the other two lakes, is much shallower, and has a different geological origin (Johnson et al. 1996).

The age of a species flock may not correspond to the geological age of a lake, since climatic or geological events may have caused a temporary dry-up, such that preexisting species flocks may have gone extinct. Moreover, it was also shown by recent molecular studies that the dynamics of diversification events in African cichlid fishes are likely to be connected to fluctuations in the lake level (Sturmbauer and Meyer 1992; Johnson et al. 1996; Sturmbauer et al. 1997; Rüber et al. 1998; Nagl et al. 2000). The evolutionary consequences of such severe environmental changes are well known for European terrestrial faunas (Hewitt 1996) but are much less understood for tropical ecosystems. Lakes Malawi and Tanganyika were severely affected by the change to a drier climate in the late Pliocene/early Pleistocene, resulting in a drop of the lake level by 650–700 m about 1.1 MYA in Lake Tanganyika (Lezzar et al. 1996; Cohen et al. 1997) and an almost complete dry-up of Lake

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Malawi from 1.6 MYA until 1.0–0.57 MYA (Delvaux 1995). After that minimum, both lakes rose until about 400,000 years ago. New regressions started about 390,000 years ago in Lake Tanganyika (Cohen et al. 1997) and 420,000 years ago in Lake Malawi (Delvaux 1995), followed by a period of fluctuating lake level in both lakes. In Lake Tanganyika, the minima were dated at 390,000–360,000 years ago, 290,000–260,000 years ago, and 190,000–170,000 years ago; those of Lake Malawi were not precisely dated by Delvaux (1995). Lake Tanganyika rose to its present level 170,000–40,000 years ago, and the rise of Lake Malawi to its present level was estimated at about 250,000–120,000 years ago. Concerning their most recent history, several studies agree that the lake levels of all three lakes were substantially lower during the late Pleistocene ice ages, when the climate in much of north and equatorial Africa became progressively more arid. Two recent studies carried out in the north part of Lake Tanganyika demonstrated three periods of low lake level for Lake Tanganyika in its recent past, the first 40,000–35,000 years ago, the second 23,000 years ago, and the third 18,000 years ago (Lezzar et al. 1996; Cohen et al. 1997). The lowstands at 23,000 and 18,000 years ago were also found in a sediment study in the very south part of the lake by Gasse et al. (1989). A minimum water level of about 400 m below the present level was reached 18,000 years ago, and the lake was lower until 13,000 years ago abruptly rising in two steps 13,000 and 10,600 years ago (Gasse et al. 1989). An earlier study tentatively dated the latest major lowstand for Lakes Malawi (250–500 m) and Tanganyika (600 m) at about 25,000 years ago (Scholz and Rosendahl 1988; C. A. Scholz, personal communication). This age estimate has been obtained by extrapolating sedimentation rates and may correspond to the minimum at 18,000 years ago found in the more recent studies (Gasse et al. 1989; Lezzar et al. 1996; Cohen et al. 1997). As suggested for Lake Tanganyika, Lake Malawi was also at least 400 m lower 18,000–10,700 years ago (Brooks and Robertshaw 1990; Owen et al. 1990; Finney and Johnson 1991), and seismic reflection profile and piston core analyses from Lake Victoria suggest that Lake Victoria was (almost) dry from 17,300 years ago until 12,400 years ago (Johnson et al. 1996; see also and Stager, Reinthal, and Livingstone 1986; Talbot and Livingstone 1989). In summary, the lake levels of all three Great East African Lakes seem to have been influenced in a similar way by the same global climatic changes. They were generally low 18,000–12,000 years ago, quickly rising to present levels with few and less severe fluctuations, with a maximum of 150 m in the Holocene and in historic times (Owen et al. 1990).

In order to compare the evolutionary consequences of the most recent minima of the lake levels on the cichlid faunas of Lakes Tanganyika, Malawi, and Victoria, we investigated the geographic distribution of closely related genotypes. Therefore, we selected species and populations of littoral cichlid fishes in each of the three lakes that have been shown to be weak dispersers and thus likely to be greatly affected by lake level changes (Meyer et al. 1990; Sturmbauer and Meyer 1992; Moran and Kornfield 1993; Verheyen et al. 1996; Sturmbauer et al. 1997; van Oppen et al. 1997; Albertson et al. 1999; Arnegard et al. 1999; Markert et al. 1999). The samples included in our study were chosen according to the basin structure of the lakes. Our approach was based on the observation that lake level fluctuations temporarily form or break down barriers among habitats and thus either promote or prevent gene flow among adjacent populations and/or incipient species (Sturmbauer 1998). The degree of habitat change enforced by water level fluctuations may range from small-scale effects to major vicariant events that affect species communities in most habitats. Any drop in the lake level will establish secondary contact and admixis among previously isolated populations in shallow regions of a lake, leading to an increase in genetic diversity in admixed populations. A rise in the lake level may promote population subdivision due to the colonization of new habitats. Newly formed ecological barriers interrupt gene flow, such that genetic differences can accumulate independently and lineage sorting can proceed. Populations of cichlid fishes specialized to particular habitats such as rocks in the littoral zone are likely to become isolated to a higher degree than less stenotopic and thus more mobile species. Only populations that have become isolated in the very recent past should share identical or closely related haplotypes, even if they are now separated by long distances or are situated on opposite shores. When the time of divergence between two populations is short, not many new mutations arise and lineage sorting is likely to be incomplete. In this case, any individual sampled from one population is expected to be as similar to individuals from different populations that were formed by the same founder event as it is to some individuals sampled from its own population. Any set of genetically heterogeneous populations is expected to contain several clusters of equally closely related genotypes, since lineage sorting is likely to be incomplete, as long as the number of generations after the split is equal to or less than twice the population size (Tajima 1983).

It is important to note that our approach is not affected by ancient DNA sequence polymorphisms due to recent speciation events, because only gene trees referring to the geographic distribution of genotypes are used to derive relative time estimates for allopatric divergence. While most rock-dwelling cichlid species of Lake Tanganyika are easily distinguishable by means of mtDNA sequences, and incipient speciation mostly proceeds in absence of ecomorphological innovation, species of Lakes Victoria and Malawi are much younger and still tend to share mitochondrial haplotypes, even if they sometimes differ dramatically in terms of their trophic morphologies and are placed in different genera (Greenwood 1980, 1984; Eccles and Trewavas 1989; Meyer et al. 1990; Moran and Kornfield 1993; Kornfield and Parker 1997; Parker and Kornfield 1997; Nagl et al. 2000). Thus, mtDNA-based phylogenies still represent gene trees and not species trees for Lake Victoria and Lake Malawi cichlids. Recent studies using more vari-

able genetic markers or behavioral data, however, showed that these young species are genetically distinct and have evolved complete prezygotic isolation (Seehausen, van Alphen, and Witte 1997; Seehausen and van Alphen 1998; van Oppen et al. 1998, 2000; Knight et al. 1999).

Materials and Methods

Using DNA sequences of the most variable section of the control region (358–360 bp following the tRNA Pro; Lee et al. 1995), we searched for genetic traces of lake level fluctuations in populations of cichlid fishes which were caused by a temporary fusion of several previously isolated populations and/or incipient species and their subsequent split. To assess the consequences of the most recent minimum lake level over 400–600 m in Lake Tanganyika and 250–500 m in Lake Malawi, we analyzed populations at steeply sloping shores close to the lake basin(s) that are likely to have remained unaffected by lake level fluctuations, as well as populations from shallow regions of the lakes which would have been most severely affected by lake level fluctuations. To investigate the consequences of the dry-up in Lake Victoria, we used all available sequence data of individuals from distant localities in the lake and in surrounding water bodies representing potential refuge areas for colonizers after the dry-up (Nagl et al. 2000). For each lake, we plotted all presently available mitochondrial haplotypes on their location of origin and related them to the inferred paleoshorelines during the last major lowstand or to potential refuge areas in the case of Lake Victoria, to compare the pattern with the predictions. The species assignments were disregarded, and the phylogenies were taken as mitochondrial gene trees bearing phylogeographic information. For Lake Tanganyika, we selected taxa of the genera Tropheus, Eretmodus, and Ophthalmotilapia that exhibited strong phylogeographic structuring pointing to a limited ability for dispersal across sand or mud coasts and open water (Brichard 1978; Sturmbauer and Meyer 1992; Sturmbauer and Dallinger 1995; Verheyen et al. 1996; Sturmbauer et al. 1997). Of 243 DNA sequences available for the genus Tropheus, 29 haplotypes (60 individuals) from 15 localities relevant to assessment the consequences of the latest dramatic lake level fluctuation were selected, some of which were published earlier (Sturmbauer and Meyer 1992; Sturmbauer et al. 1997; see map in fig. 1*A*). The Tropheus samples represent four mitochondrial lineages from three regions of the lake and are presently assigned to three nominal species (*Tropheus moorii, Tropheus kasabae,* and *Tropheus brichardi*). Of 50 published DNA sequences of *Eretmodus* cf. *cyanostictus* (lineage A as defined in Rüber, Verheyen, and Meyer 1999), three mitochondrial haplotypes from four localities formed a monophyletic cluster that was used for our analyses of divergence levels. Of 39 DNA sequences (50 individuals; Hanssens et al., unpublished data) available for the genus Ophthalmotilapia, five sequences of *Ophthalmotilapia nasuta* from six localities formed a phylogeographically informative cluster that was used in the analysis. The EMBL accession numbers of the DNA sequences of *Eretmodus* cf. *cyanostictus* relevant to this paper are Z97412 (Bemba), Z97411 (Luhanga), X90612 (Ngombe 92/1), and X90632 (Cape Kabogo 92/40). Those of *Ophthalmotilapia boops* are Z95983—Z95995, those of *Ophthalmotilapia heterodonta* are Z95996—Z96001, those of *Ophthalmotilapia nasuta* are Z96002—Z96015, and those of *Ophthalmotilapia ventralis* are Z96016—Z96020. The accession numbers for Tropheus are AJ295902–AJ295924, in addition to those already published.

The analyzed DNA sequences of Lake Malawi rock-dwelling cichlids, termed *mbuna,* were published previously (Bowers, Stauffer, and Kocher 1994; Parker and Kornfield 1997) and comprise 26 haplotypes (50 individuals of 24 nominal species of 13 locations) from both the central steeply sloping core region of the lake and the southernmost shallow sections of the lake (see map in fig. 2*A*). Due to incomplete lineage sorting among *mbuna* species, identical genotypes were frequently found among individuals of different species, even if hybridization did not occur among sympatric species (Albertson et al. 1999). The DNA sequences available for Lake Victoria haplochromines (Meyer et al. 1990; Nagl et al. 2000) include 56 specimens of 24 described plus 9 undescribed species. All analyzed taxa belong to a monophyletic assemblage (called lineage VC in Nagl et al. 2000) in which the majority of individuals are Lake Victoria endemics, and a few others stem from surrounding water bodies. Of the localities analyzed from Lake Victoria, three were situated at the northeastern shore, four at the southern edge of the lake basin near Mwanza, and four additional localities situated in surrounding rivers and lakes (see map in fig. 3*A*).

All three data sets used in this analysis were tested for their relative rates of base substitutions using the computer program PHYLTEST (Kumar 1996). Therefore, we defined three monophyletic lineages, the first comprising Tropheus (29 taxa), the second comprising Lake Malawi *mbuna* (45 taxa), and the third comprising Lake Victoria cichlids (15 taxa). Three consecutive analyses were performed so that each of the three lineages was once used as the reference group. For each data set, a mitochondrial phylogeny was constructed using the parsimony and neighbor-joining algorithms of PAUP*, version 4.64d (Swofford 1998). We then selected one of the most-parsimonious trees that in all three analyses was most similar to the neighbor-joining tree to plot genotypes and their locations of origin in the form of minimum spanning trees, since they best illustrate all genotypes descending from the same ancestral lineage at the time of their most recent admixis (see figs. 1–3). Genotype clusters relevant to assess the extent of lake level fluctuations had to contain identical or very closely related genotypes found both in habitats with steep slopes that are most likely also available during periods of low lake level and in habitats that are only colonizable during high-water-level periods. In some cases, habitats were situated at opposite shores of a lake, separated from each other by deep water. For each haplotype cluster, the geographic extension of the latest sec-

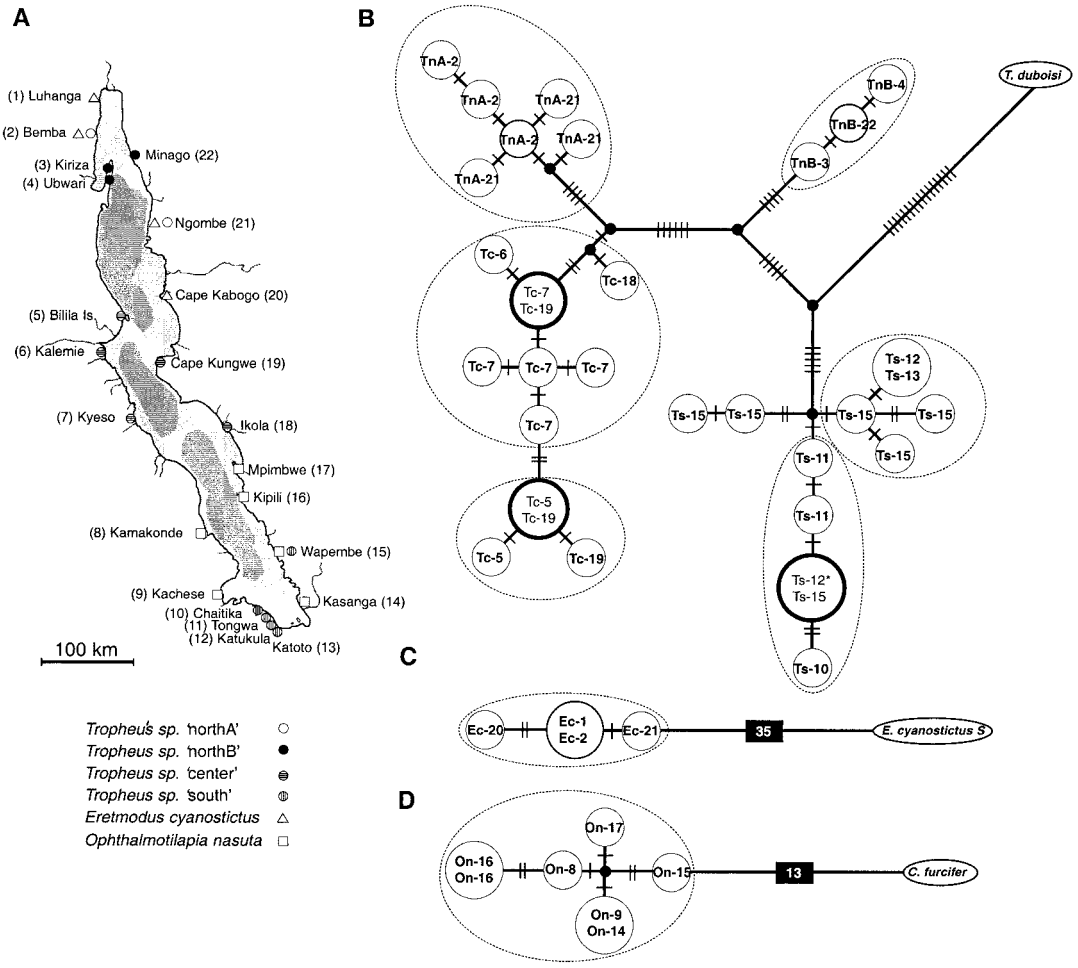

FIG. 1.—Distribution of genetically closely related individuals of three rock-dwelling cichlid species of Lake Tanganyika. *A*, Map of Lake Tanganyika indicating the sampling locations. Triangles indicate sampling localities of Eretmodus, squares indicate those of Ophthalmotilapia, and circles indicate those of Tropheus. Numbers in parentheses correspond to locality numbers in the minimum spanning trees. Shaded areas represent the inferred shorelines of the paleolake that would result from a water level drop of 600 m in Lake Tanganyika (Scholz and Rosendahl 1988). *B*, Minimum spanning tree of 60 individuals of Tropheus, comprising 29 haplotypes from 15 localities. The minimum spanning tree depicts mitochondrial haplotypes for Tropheus, with individuals labeled by species abbreviation and locality number. It is derived from one out of 400 most-parsimonious trees found by parsimony analysis (74 evolutionary steps; consistency index excluding uninformative characters = 0.65) that was identical to the neighbor-joining tree. *C*, Minimum spanning tree of selected individuals of Eretmodus representing a phylogeographically informative genotype cluster. The tree is derived from one out of four most-parsimonious trees found by parsimony analysis (38 evolutionary steps; consistency index excluding uninformative characters = 0.67). *D*, Minimum spanning tree of selected individuals of Ophthalmotilapia representing a phylogeographically informative genotype cluster, derived from one out of eight most-parsimonious trees found by parsimony analysis (20 evolutionary steps; consistency index excluding uninformative characters = 0.67). Cross bars on the branches give the minimum number of base substitutions among genotypes; circled genotypes represent phylogeographically informative genotype clusters relevant for divergence calculations. The taxonomic assignment of the haplotypes, which is irrelevant for our phylogeographic analysis, is listed using the following abbreviations, also giving the number of the sampling location on the map: TnA = *Tropheus* sp. 'north-A'; TnB = *Tropheus* sp. 'north-B'; Tc = *Tropheus* sp. 'center'; Ts = *Tropheus* sp. 'south.'

ondary admixis and dispersal event was derived from the haplotype distribution. To compare the relative ages of haplotype clusters within and among the lakes, average mutation differences among haplotypes belonging to a phylogeographically informative cluster were calculated. This was done by defining an ancestral haplotype for each cluster and subsequently calculating arithmetic means and standard deviations of all mutation counts between the ancestral and the derived haplotypes. Identical haplotypes were counted according to their frequencies.

An absolute age estimate was attempted by calibrating the molecular divergences by a new age estimate for Lake Malawi using average genetic divergences of two basal lineages of the Lake Malawi cichlid species flock. The geological history of the Lake Malawi basin is complex, so the age of the lacustrine habitat can only be dated in the form of a minimum-maximum estimate. While the geological age is assumed to be 4–5 Myr, the lake was most likely almost or completely dry for several thousands of years in the late Pleistocene from 1.6 MYA until maximally 1 MYA and minimally 570,000 years ago. We thus estimated the age of its species flock to be between 570,000 years and 1 Myr (Delvaux 1995), assuming that the emergence of the ecological diversity of Lake Malawi cichlids did not predate this period, be-

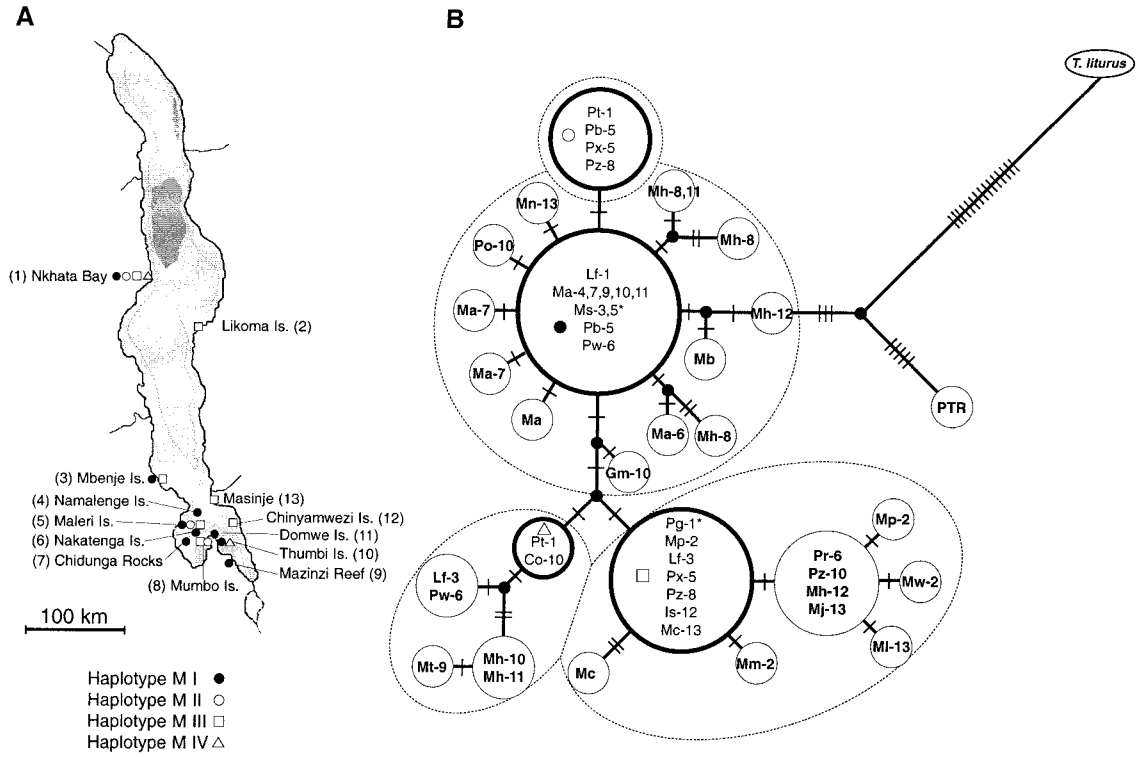

FIG. 2.—Distribution of 26 closely related genotypes (50 individuals of 24 nominal species of 13 locations) of Lake Malawi rock-dwelling cichlids (Bowers, Stauffer, and Kocher 1994; Parker and Kornfield 1997). *A,* Map of Lake Malawi indicating the sampling locations of *mbuna* cichlids. Numbers in parentheses correspond to locality numbers in the minimum spanning trees. Circles, squares, and triangles identify four distinct haplotypes found at several localities. Shaded areas represent the inferred shorelines of the paleolake that would result from water level drops of 200 and 500 m (Scholz and Rosendahl 1988). *B,* Minimum spanning tree of *mbuna* genotypes. The tree was derived from one out of four most-parsimonious trees found by parsimony analysis that was identical to the neighbor-joining tree (59 evolutionary steps; consistency index excluding uninformative characters = 0.55). The taxonomic assignment of the haplotypes, which is irrelevant for our phylogeographic analysis, is listed using the following abbreviations, also giving the number of the sampling location on the map: Co = *Cyathochromis obliquidens*; Gm = *Genyochromis mento*; Is = *Iodotropheus sprengerae*; Lf = *Labeotropheus fuelleborni*; Ma = *Melanochromis auratus*; Mb = *Melanochromis* sp. 'blue'; Mc = *Melanochromis* sp. 'blotch'; Mh = *Melanochromis heterochromis*; Mj = *Melanochromis johanni*; Ml = *Melanochromis* sp. 'lepidophage'; Mm = *Melanochromis* sp. 'maingano'; Mn = *Melanochromis simulans*; Mp = *Melanochromis parallelus*; Ms = *Melanochromis* sp. 'slab'; Mt = *Melanochromis melanopterus*; Mw = *Melanochromis* sp. 'black and white johanni'; Pb = *Pseudotropheus barlowi*; Pg = *Pseudotropheus zebra* 'gold'; Po = *Pseudotropheus tropheops* 'orange chest'; Pr = *Pseudotropheus zebra* 'red dorsal'; Pt = *Pseudotropheus tropheops* 'black'; Pw = *Pseudotropheus williamsi*; Px = *Pseudotropheus xanstomachus*; Pz = *P. zebra* 'BB'; PTR = *Petrotilapia* sp.; *Tramitichromis liturus* was the outgroup. GenBank accession numbers: U01113, U01926–U01930, U01932–U01945, U01951–U01953, U90759–U90780, U90782.

cause most suitable lacustrine habitats were lacking in a shallow and swampy lake.

To calibrate the mutation rate of the 359-bp segment of the control region, we calculated the average genetic distance among two ancient Lake Malawi cichlid lineages (Meyer et al. 1990). We compared published DNA sequences of 26 *mbuna* (Kocher et al. 1993; Bowers, Stauffer, and Kocher 1994; Parker and Kornfield 1997), 13 *utaka* (Meyer et al. 1990; Lee et al. 1995; Parker and Kornfield 1997), and five additional species sequenced by us for this study: *Aulonocara jacobfreibergi*, *Fossorchromis rostratus*, *Melanochromis caeruleus* 'yellow', *Nimbochromis livingstoni*, and *Nimbochromis venustus* (table 1). After performing a relative-rate test (Takezaki, Rzhetsky, and Nei 1995), average pairwise Kimura distances were calculated among all taxa of each lineage. Two *mbuna* sequences and three *utaka* sequences were excluded because of different substitution rates.

Results and Discussion

The relative-rate tests, in which three monophyletic lineages were defined to represent the taxa of a lake, revealed constant evolutionary rates among fishes from the three different lakes ($P < 0.05$). The branch length for the Tropheus clade was 0.072, that for the Malawi *mbuna* was 0.046, and that for the Lake Victoria cichlids was 0.062. Using Tropheus as the reference group, a Z value of 0.906 was obtained; using Lake Malawi *mbuna*, the Z value was 0.509; and using Lake Victoria cichlids, the Z value was 1.376; in all cases rate constancy was not rejected. The minimum spanning tree for the Tanganyikan genus Tropheus identified six phylogeographically informative clusters of individuals having extremely small genetic differences from each other (average genetic distance = 1.33 base substitutions; SD = 0.63; 36 pairwise comparisons; see fig. 1*A* and *B*). The clusters were found in three regions of the lake, and taxa

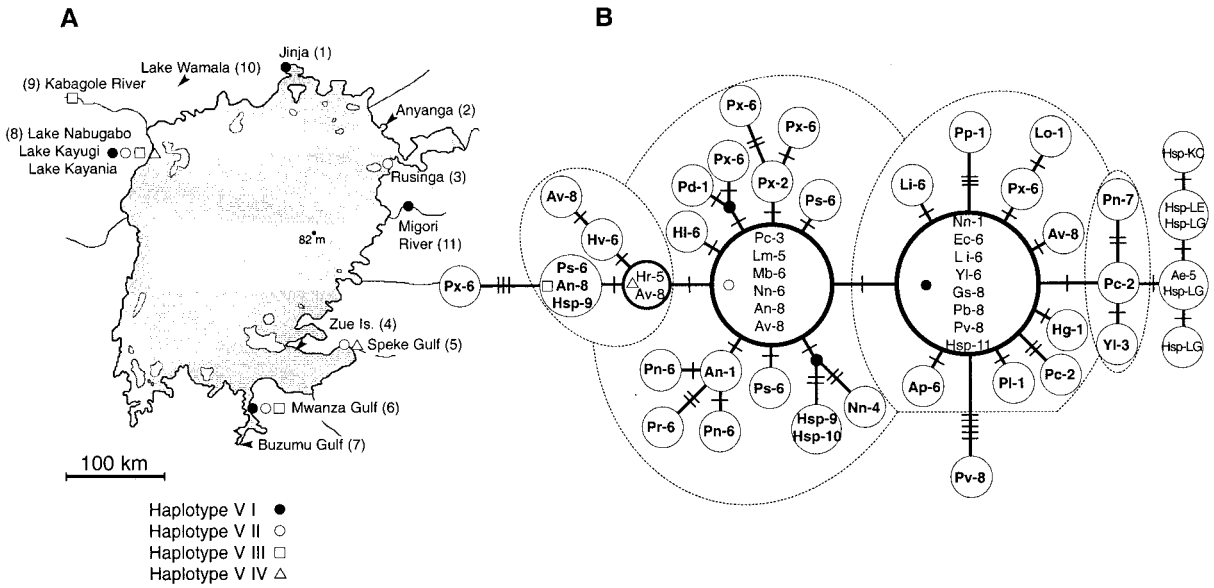

FIG. 3.—Distribution of closely related genotypes in Lake Victoria cichlids (Meyer et al. 1990; Nagl et al. 2000). *A*, Map of Lake Victoria indicating the sampling locations. Numbers in parentheses correspond to locality numbers in the minimum spanning trees. Circles, squares, and triangles identify four distinct genetic lineages found at several localities. The deepest location is indicated (Nagl et al. 2000). *B*, The minimum spanning tree of 56 specimens shows that four identical haplotypes were found in more than one lake region. The tree was derived from one out of 500 trees found by parsimony analysis (53 evolutionary steps; consistency index excluding uninformative characters = 0.60) that was most similar to the neighbor-joining tree. The taxonomic assignment of the haplotypes, which is irrelevant for our phylogeographic analysis, is indicated by the following abbreviations, also giving the number of the sampling location on the map: Ae = *Astatotilapia elegans*; An = *Astatotilapia nubilis*; Ap = *Astatotilapia piceatus*; Av = *Astatotilapia velifer*; Ec = *Enterochromis cinctus*; Gs = *Gaurochromis simpsoni*; Hg = *Harpagonochromis guiarti*; Hl = *Haplochromis lividus*; Hr = *Haplochromis* sp. 'rock kribensis'; Hsp = *Haplochromis* sp.; Hv = *Haplochromis* sp. 'velvet black'; Li = *Labrochromis ishmaeli*; Lm = *Lipochromis melanopterus*; Lo = *Lipochromis obescus*; Mb = *Macropleurodus bicolor*; Nn = *Neochromis nigricans*; Pb = *Paralabidochromis beadlei*; Pc = *Paralabidochromis chilotes*; Pd = *Prognathochromis dentex*; Pl = *Prognathochromis longirostris*; Pn = *Paralabidochromis plagiodon*; Pp = *Prognathochromis paraguiarti*; Pr = *Psammoochromis riponianus*; Ps = *Ptyochromis sauvagei*; Pv = *Prognathochromis ventator*; Px = *Ptyochromis xenognathus*; Yl = *Yssichromis laparogramma.* KC = Kazinga Channel; LE = Lake Edward; LG = Lake George. GenBank accession numbers: AF213518–AF213540, AF213542, AF213543, AF213545–AF213547, AF213549–AF213553, AF213557, AF213558, AF213560, AF213566, AF213581–AF213584, AF213588.

often were collected at opposite shores. The most striking similarities among populations were found in the central and southern regions of the lake, where individuals of one side of the lake had mitochondrial genotypes identical to those found at the opposite shoreline (fig. 1*A* and *B* and table 2). For *Eretmodus cyanostictus*, closely related individuals were also found in four localities in the northern basin of Lake Tanganyika (one base substitution between Bemba and Ngombe, two base substitutions between Luhanga and Cape Kabogo as well as Luhanga and Ngombe; represented by triangles in fig. 1*A*; see also fig. 1*C*). Closely related individuals of *O. nasutus* were found in four localities of the southern basin of Lake Tanganyika (one to three base substitutions; represented by squares in fig. 1*A*, see also fig. 1*D*). Average genetic distances were not calculated for the latter two species due to the small sample size.

The analyzed DNA sequences of rock-dwelling cichlids from Lake Malawi of 13 populations from both the central steeply sloping core region of the lake and the southernmost shallow sections of the lake contained four distinct haplotypes which were found in more than one species and in populations at the steeply sloping central region, to where the lake would retreat at a lake stand 500 m below its present level, as well as at localities in shallow water at the southern end of the lake (fig. 2 and table 2). These haplotypes also correspond to four phylogeographically informative clusters (average genetic distance = 1.27 base substitutions; SD = 0.96; 171 pairwise comparisons; see fig. 2).

The DNA sequences of Lake Victoria haplochromines from seven localities in the lake and four localities from surrounding rivers and lakes contained 4 out of 38 haplotypes that were shared by up to eight species originating from opposite sides of the lake, again defining geographically informative clusters of genotypes (average genetic distance = 1.33 base substitutions; SD = 0.97; 212 pairwise comparisons; see fig. 3 and table 2).

The presence of individuals having identical or very closely related mitochondrial haplotypes at distant localities in all three lakes, together with the remarkable degree of genetic heterogeneity found in many populations, consistently indicates an event of secondary admixis of previously isolated populations in the very recent past, followed by a range expansion. In each of the three lakes, this event was triggered by a major fluctuation of the lake level. Even though base substitutions occur stochastically, they can be taken as measures of divergence time when three criteria are met. First, all taxa have similar rates of base substitution in the analyzed gene segment. Second, the stochastic variation of mutation rates among individuals is homogenized by averaging multiple pairwise comparisons of taxa. Third, incomplete lineage sorting must be taken into account

Table 1
Sequences of the First Part of the Mitochondrial Control Region from Two Groups of Lake Malawi Cichlids that Were Used for the Calibration of the Molecular Clock

Taxon	Accession No.	Reference
mbuna		
Aulonocara jacobfreibergi	AJ295929	Present study
Cyathochromis obliquidens	U90759	Parker and Kornfield (1997)
Genyochromis mento	U90779	Parker and Kornfield (1997)
Iodotropheus sprengerae	U90767	Parker and Kornfield (1997)
Melanochromis auratus clone 2	U01927	Bower, Stauffer, and Kocher (1994)
M. auratus clone 3	U01928	Bowers, Stauffer, and Kocher (1994)
M. auratus clone 4	U01929	Bower, Stauffer, and Kocher (1994)
M. auratus clone 5	U01930	Bower, Stauffer, and Kocher (1994)
Melanochromis heterochromis	U90780	Parker and Kornfield (1997)
M. heterochromis clone 3	U01938	Bower, Stauffer, and Kocher (1994)
M. heterochromis clone 4	U01939	Bower, Stauffer, and Kocher (1994)
M. heterochromis clone 5	U01940	Bowers, Stauffer, and Kocher (1994)
M. heterochromis clone 6[a]	U01941	Bowers, Stauffer, and Kocher (1994)
Melanochromis melanopterus	U01952	Bowers, Stauffer, and Kocher (1994)
Melanochromis parallelus	U90769	Parker and Kornfield (1997)
Melanochromis sp. 'black + white johanni'	U01935	Bowers, Stauffer, and Kocher (1994)
Melanochromis sp. 'block'	U01933	Bowers, Stauffer, and Kocher (1994)
Melanochromis sp. 'blue'	U01934	Bowers, Stauffer, and Kocher (1994)
Melanochromis sp. 'lepidophage'	U01943	Bowers, Stauffer, and Kocher (1994)
Melanochromis sp. 'maingano'	U01951	Bowers, Stauffer, and Kocher (1994)
Melanochromis sp. 'slab'	U01954	Bowers, Stauffer, and Kocher (1994)
Petrotilapia sp.[a]	U01113	Kocher et al. (1993)
Pseudotropheus sp. 'red dorsal'	U90760	Parker and Kornfield (1997)
Pseudotropheus sp. 'orange chest'	U90778	Parker and Kornfield (1997)
Pseudotropheus williamsi	U90772	Parker and Kornfield (1997)
P. williamsi	U90775	Parker and Kornfield (1997)
Pseudotropheus xanstomachus	U90762	Parker and Kornfield (1997)
utaka		
Buccochromis atritaeniatus[b]	AJ291409	Meyer et al. (1990)
Champsochromis spilorhynchus	U12553	Lee et al. (1995)
C. spilorhynchus	AJ291413	Meyer et al. (1990)
Cyrtocara moorii	U12554	Lee et al. (1995)
Dimidiochromis compressiceps	AJ291408	Meyer et al. (1990)
Fossorochromis rostratus	AJ295925	Present study
''Haplochromis'' diaboli[a]	AJ291411	Meyer et al. (1990)
Labidochromis caeruleus	AJ291406	A. Meyer (unpublished data)
L. caeruleus 'yellow'	AJ295926	Present study
Lethrinops auritus	U12551	Lee et al. (1995)
Lethrinops gossei	U90781	Parker and Kornfield (1997)
Maravichromis labidodon	AJ291412	Meyer et al. (1990)
Nimbochromis livingstonii[a]	AJ295928	Present study
Nimbochromis polystigma[b]	AJ291407	Meyer et al. (1990)
Nimbochromis venustus	AJ295927	Present study
Protomelas annectens	AJ291414	Meyer et al. (1990)
Sciaenochromis gracilis[a]	AJ291410	Meyer et al. (1990)
Tramitichromis lituris	AJ291405	A. Meyer (unpublished data)
Outgroup		
Astatotilapia calliptera	X58152	Meyer, Kocher and Wilson (1991)

[a] Excluded from calibration of the molecular clock on the basis of the results of the branch length test.
[b] Identical DNA sequences.

by comparing those individuals from a given population only to their counterparts from another population that are direct descendants of the same ancestral mitochondrial haplotype. All haplotype clusters that have diverged after the most recent admixis thus exhibit similar levels of genetic variation.

The strikingly similar average numbers of base substitutions in all phylogeographically informative haplotype clusters suggest that the latest periods of low lake level in Lakes Tanganyika, Malawi, and Victoria happened, or at least ended, roughly at the same time. The finding of identical genotypes of Tropheus at opposite shores in the central region of Lake Tanganyika (fig. 1*A* and *B*) suggests a retreat of the lake level by a minimum of 550 m, which would be sufficient to shift a continuous band of rock bottom into the depth limit of Tropheus (about 50 m). The distribution of identical or very closely related genotypes in Lake Malawi, and particularly their occurrence at the edge of the deep basin at locality 1 (fig. 2), suggests a retreat of about 500 m, but certainly more than 400 m, below its present level.

Since all data sets were found to have similar evolutionary rates in the analyzed segment of the control region, the inference of relative age estimates seems justified. The published rate estimates for the control region on various organisms differ widely (Vigilant et al. 1991;

Table 2
Identical Haplotypes Found at Distant Localities at Lakes Tanganyika, Malawi, and Victoria

	Taxon	Locality (no. of haplotypes)
Lake Tanganyika		
Haplotype T1	*Tropheus moorii*	Bilila Island (5)
	T. moorii	Cape Kungwe (19)
Haplotype T2	*T. moorii*	Kyeso (7)
	T. moorii	Cape Kungwe (19)
Haplotype T3	*T. moorii*	Katukula (12)[a]
	T. moorii	Wapembe (15)
Lake Malawi (Bowers, Stauffer, and Kocher 1994; Parker and Kornfield 1997)		
Haplotype M1	*Labeotropheus fuelleborni*	Nkhata Bay (1)
	Melanochromis auratus	Namalenge Island (4)
	M. auratus	Chidunga Rocks (7)
	M. auratus	Mazinzi Reef (9)
	M. auratus	Thumbi Island (10)
	M. auratus	Domwe Island (11)
	Melanochromis sp. 'slab'	Mbenje Island (3)[a]
	Melanochromis sp. 'slab'	Maleri Island (5)[a]
	Pseudotropheus barlowi	Maleri Island (5)
	Pseudotropheus williamsi	Nakatenga Island (6)
Haplotype M2	*Pseudotropheus tropheops* 'black'	Nkhata Bay (1)
	P. barlowi	Maleri Island (5)
	Pseudotropheus xanstomachus	Maleri Island (5)
	Pseudotropheus zebra BB	Mumbo Island (8)
Haplotype M3	*P. zebra* 'gold'	Nkhata Bay (1)[a]
	Melanochromis parallelus	Likoma Island (2)
	L. fuelleborni	Mbenje Island (3)
	P. xanstomachus	Maleri Island (5)
	P. zebra 'BB'	Mumbo Island (8)
	Iodotropheus sprengerae	Chinyamwezi Island (12)
	Melanochromis sp. 'blotch'	Masinje (13)
Haplotype M4	*P. tropheops* 'black'	Nkhata Bay (1)
	Cyathochromis obliquidens	Thumbi Island (10)
Lake Victoria (Meyer et al. 1990; Nagl et al. 2000)		
Haplotype VI	*Neochromis nigricans*	Jinja (1)
	Enterochromis cinctus	Mwanza Gulf (6)
	Labrochromis ishmaeli	Mwanza Gulf (6)
	Yssichromis laparogramma	Mwanza Gulf (6)
	Gaurochromis simpsoni	Lake Nabugabo (8)
	Paralabidochromis beadlei	Lake Nabugabo (8)
	Prognathochromis ventator	Lake Nabugabo (8)
	Haplochromis sp.	Migori River (11)
Haplotype V2	*Paralabidochromis chilotes*	Rusinga (3)
	Lipochromis melanopterus	Speke Gulf (5)
	Macropleurodus bicolor	Mwanza Gulf (6)
	N. nigricans	Mwanza Gulf (6)
	Astatotilapia nubilis	Lake Nabugabo (8)
	Astatotilapia velifer	Lake Nabugabo (8)
Haplotype V3	*Ptyochromis sauvagei*	Mwanza Gulf (6)
	A. nubilis	Lake Nabugabo (8)
	Haplochromis sp.	Kabagole River (9)
Haplotype V4	*Haplochromis* sp. 'rock kribensis'	Speke Gulf (5)
	A. velifer	Lake Nabugabo (8)

[a] One additional base insertion/deletion is not included.

Quinn 1992; Brown, Beckenbach, and Smith 1993; Stewart and Baker 1994). Since no calibration of the evolutionary rate was available for cichlid fishes, we used new evidence for the history of Lake Malawi (Delvaux 1995) and the average genetic distance among two ancestral lineages of Lake Malawi cichlids to derive a rate estimate. Among 25 *mbuna* and 14 *utaka* haplotypes (350 pairwise comparisons), an average Kimura distance of 6.54% (SD = 0.98%) was found. Our rate estimate for the most variable section of the control region thus amounts to 6.5%–8.8% per Myr, depending on which age is assumed for the Lake Malawi lacustrine ecosystem (fig. 4). The highly similar average genetic distances within clusters of closely related genotypes in cichlids of all three lakes (Lake Tanganyika, 1.33 mutations, 0.37%; Lake Malawi, 1.27 mutations, 0.35%; Lake Victoria, 1.33 mutations, 0.37%) would translate into an age range of between 57,000 and 40,000 years. This estimate would fit to a period of moderately low lake level (−160 m) in Lake Tanganyika 40,000–35,000 years ago (Lezzar et al. 1996; Cohen et al. 1997) but is older than the dating of the most recent period of very

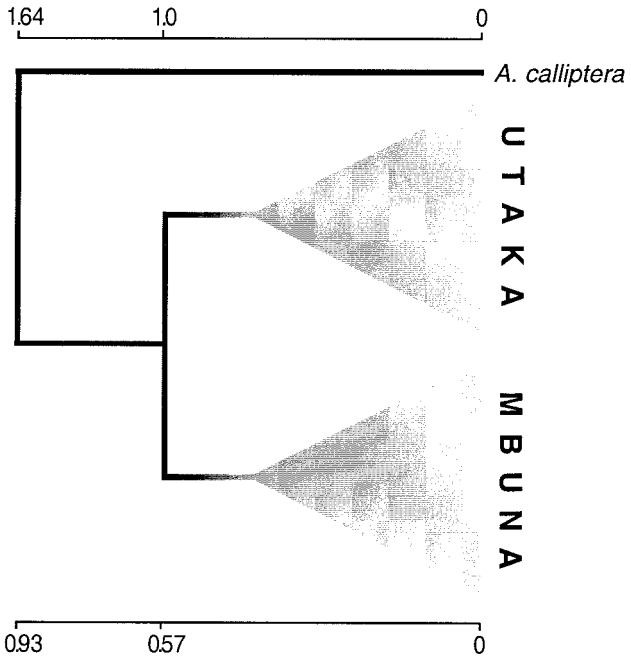

FIG. 4.—Calibration of the molecular clock of a 359-bp segment of the control region using the observed average genetic divergence among two basal lineages of the Lake Malawi cichlid species flock. Linearized Kimura distances among 25 *mbuna* and 14 *utaka* were calculated using the computer program LINTRE (Takezaki, Rzhetsky, and Nei 1995) after performing a relative-rate test (Takezaki, Rzhetsky, and Nei 1995; see table 1). The observed average genetic divergence of 6.54% (SD = 0.98%) was calibrated by a new age estimate for Lake Malawi, ranging from maximally 1 Myr and minimally 570,000 years (Delvaux 1995). Our rate estimate for the most variable section of the control region is thus 6.5%–8.8% per Myr. The scale on top of the phylogram corresponds to the maximum age estimate, and that on the bottom corresponds to the minimum age estimate (in Myr). *Astatotilapia calliptera* was used as the outgroup.

low lake level in all three lakes (18,000–12,000 years ago). This discrepancy may be due to the still relatively imprecise age estimate for Lake Malawi. Alternatively, it was repeatedly shown that mtDNA mutation rates seem faster when studied over relatively few generations, due to rapid accumulation of mutations at hypervariable sites. These mutation hot spots rapidly saturate after short periods of divergence, or genotypes may be quickly removed from populations due to selection against slightly deleterious mutations (Parsons et al. 1997; Gibbons 1998). We thus feel that the time estimate for the latest major dispersal event between 40,000 and 57,000 years ago is likely to be too old. To us, the most likely time for an almost synchronous spread in all three lakes would be their rise dated about 11,000 years ago by geological and sedimentological evidence, because the drop in the lake level 40,000–35,000 years ago was not sufficiently large to allow for crossing of the lake at the central basin by individuals of Tropheus (Gasse et al. 1989; Owen et al. 1990; Finney and Johnson 1991; Johnson et al. 1996; Lezzar et al. 1996; Cohen et al. 1997). The fluctuation of Lake Malawi over, at most, 150 m between A.D. 1500 and A.D. 1840 (Owen et al. 1990; but see Nicholson 1998) is not likely to have resulted in the observed pattern of genotype distribution, because this would have not fused all studied populations.

The observed phylogeographic patterns of genetic divergences not only corroborate the suggested synchrony of the latest most severe dessication events in the three major East African lakes (Broecker et al. 1998), but also demonstrate equally severe consequences for their cichlid faunas. The same climatic phenomenon caused synchronization of genetic divergences of lineages within and among distinct species flocks and thus links the most recent evolutionary history and the stability of the fish communities of all three lakes to the same external modulators of adaptive radiation. Our findings show that patterns of genetic divergences of stenotopic organisms provide valuable feedback on geology-based time estimates for events affecting lacustrine ecosystems. They have important implications for future works, since they open the possibility to compare simultaneous processes of species diversification among lakes that differ widely in the absolute ages of their radiating species communities (Sturmbauer 1998). Over the same period, a multitude of species originated in Lake Victoria and Lake Malawi with an impressive degree of ecological differentiation, whereas the Tanganyikan taxa that were exposed to the same habitat changes have hardly diverged ecologically and morphologically at all in the recent past. Future studies may allow identification of the characteristics and the driving modulators dominating an early stage of adaptive radiation as prevailing in Lake Victoria, in comparison with the intermediate stage in Lake Malawi and the highly advanced stage in Lake Tanganyika.

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