CHAPTER

7

Phylogeographic Patterns in Populations of Cichlid Fishes from Rocky Habitats in Lake Tanganyika

CHRISTIAN STURMBAUER
Department of Zoology
University of Innsbruck
A-6020 Innsbruck, Austria

ERIK VERHEYEN
Royal Belgian Institute of Natural Sciences
B-1000 Brussels, Belgium

LUKAS RÜBER
Zoological Museum of the University of Zürich
Switzerland

AXEL MEYER
Department of Ecology and Evolution
State University of New York
Stony Brook, New York 11794

I. Lake Tanganyika and Its Cichlid Species Flock

The cichlid species flocks of the great East African lakes represent the most diverse assemblages of freshwater fishes in the world. Lake Tanganyika is by far the oldest of the three major East African rift lakes with an estimated age of about 9 to 12 million years (Cohen *et al.*, 1993). Its geological history is relatively well known (reviewed in Tiercelin and Mondeguer, 1991). The lake is formed of three basins which have been fused to one large lake about 5 to 6 million years ago. Seismic data show that about 200,000 (Tiercelin and Mondeguer, 1991) to 75,000 (Scholz and Rosendahl, 1988; C. A. Scholz personal communication) years ago the level of Lake Tanganyika dropped 600 m below its present level, possibly even splitting the lake into three sublakes for several tens of thousands of years. This vicariant event must have had severe effects on several habitats and their fish populations. After this period the lake level rose again with additional minor fluctuations in the more recent history. At present times the lake has reached its largest extension due to addition of a large tributary river, the Ruzizi in the northern edge of the lake. It was formed about 10,000 years ago by the formation of the Virunga volcano chain in Rwanda, blocking the formal connection of this area with the Nile system. The influx of the Ruzizi River also ended a long period of isolation from the Zaire River system and caused an overflow of Lake Tanganyika via the Lukuga into the Lualaba, the upper reaches of the Zaire River.

Although the cichlid flock of Lake Victoria is considered to be monophyletic (Meyer *et al.*, 1990), the Malawi and Tanganyika cichlid flocks are probably of polyphyletic origin (Greenwood, 1981; Nishida, 1991; Sturmbauer and Meyer, 1993; Moran *et al.*, 1994; Sturmbauer *et al.*, 1994; Kocher *et al.*, 1993, 1995). Lake Malawi harbours a small sub-flock of five endemic species of Tilapiine cichlids (Eccles and Trewavas, 1989; Axelrod, 1993), in addition to its subflock of "haplochromines" which is considered monophyletic (Moran *et al.*, 1994). The Lake Tanganyika cichlid flock is composed of several lineages, assigned to 12 tribes (Poll,

1986). The ancestors of some tribes are likely to be older than the lake and probably have colonized the proto-lakes of Tanganyika to radiate in parallel into subflocks. The Victoria and Malawi cichlids are all, without exception, maternal mouthbrooders (females brood their eggs by buccal incubation; reviewed by Barlow, 1991; Keenleyside, 1991), and the Tanganyika flock contains several lineages of mouthbrooders as well as substrate breeders (Nishida, 1991; Sturmbauer *et al.*, 1994; Kocher *et al.*, 1995). The Tanganyikan cichlid fauna is morphologically, ecologically and behaviorally the most diverse species flock of the African lakes (Fryer and Iles, 1972; Greenwood, 1984). Due to its old age, the radiation may be in a highly advanced stage and the phylogeographic history of species and populations may reach back far in time compared to the cichlids of Lake Malawi and Lake Victoria. Several species are split into numerous populations which might have complex histories. Some are likely to be old, and therefore may have highly diversified genetically, to an extent that their history can be deduced from gene sequences.

A. Modeling Adaptive Radiation

The Tanganyika cichlid species flock thus provides an excellent model system to elucidate the evolutionary mechanisms which induce and trigger explosive speciation events. An important aspect of understanding adaptive radiations is concerned with the mode of speciation which led to their diversification. Specifically, the relative importance of intrinsic biological characteristics such as ecology, anatomy, and behavior versus abiotic factors such as geological history, geographic structuring of the lake basin, barriers to gene flow, and fluctuations of the lake level is controversial. Although abiotic factors are thought to provide or prevent the opportunities for dispersal, biotic factors may define the dispersal capability of each species once an opportunity for gene flow is provided. Abiotic factors may thus be viewed as shape parameters of habitats in the lake ecosystem defining their location, size, and discontinuity in time and space. Changes in any abiotic parameter might reshape habitats, and existing barriers might be "torn down" at one time whereas others might arise at another. The degree of habitat change extends from small-scale fluctuations to vicariant events affecting almost all habitats and their species communities. Because abiotic factors most likely affect the whole species communities equally in their habitats, differences in the distribution patterns among species may primarily arise due to species-specific biological differences.

Among biotic factors presumed to affect the dispersal and consequently the amount of gene flow among cichlid populations are ecological specialization and niche partitioning, e.g., habitat specifity, site fidelity or territoriality, homing behavior, and social organization (Fryer and Iles, 1972; McKaye and Gray, 1984; McElroy and Kornfield, 1990; Yanagisawa and Nishida, 1991; Hert, 1992; Sturmbauer and Dallinger, 1995). These species-specific characteristics may influence to which extent species will be split into distinct populations, to which extent populations will be isolated from each other, and also to which extent physical changes might affect their population structures.

During periods of physical separation, genetic differences between populations will accumulate. Prezygotic isolation mechanisms might evolve as byproducts of genetic isolation, possibly driven by sexual selection on traits involved in social and/or reproductive behavior. This mechanism was suggested for color patterns of males being the decisive criterion of mate recognition and choice (Mayr, 1984; Dominey, 1984). Given the behavioral diversity in Tanganyikan mouthbrooders, the relative importance of sexual selection may also vary among species or lineages.

II. Speciation and DNA

The comparison of genetic patterns among species assemblages living sympatrically in geographically isolated populations is expected to provide insights into the dynamics of population histories and their evolutionary causes (e.g., reviewed in Avise, 1994). The amount of genetic divergence within and among populations, as well as frequencies and distributions of different genotypes, will provide information about their historical demography. By relating the observed patterns to ecology, habitat specificity and behavior, the decisive characteristics triggering the degree of isolation may be identified for several species on a comparative basis. Identifying the causes of isolation in various species is the goal of such an approach, and ultimately identifying possible patterns for various groups of species of similar biology.

This chapter combines results of mitochondrial (mt)DNA sequence data presently available for three endemic Tanganyika cichlid lineages: *Tropheus* (Sturmbauer and Meyer, 1992), *Simochromis* (Meyer *et al.*, 1996), and the members of the tribe Eretmodini (Rüber, 1994; Verheyen *et al.*, 1996). MtDNA was shown to be a sensitive marker for population differentiation because it evolves 5 to 10 times faster than nuclear DNA (Avise, 1994). It is exclusively maternally transmitted in cichlids, making it more sensitive to population size fluc-

tuations (reviewed in Meyer, 1993; Avise, 1994). This chapter focuses on results based on the mitochondrial control region because it is the most variable region of the entire genome (reviewed in Meyer, 1993, 1994) and thus most suitable in addressing phylogenetic questions at the population level. All species in this chapter inhabit rock and cobble shores along the lake where they often occur in sympatry. They all are epilithic algae feeders and are habitat specific to different degrees (Sturmbauer *et al.*, 1992). For all three taxa, geographically distinct populations have been described, distinguishable only by minor, if any, morphological variation, but sometimes pronounced differences in coloration.

DNA sequence data are available for populations of all three taxa along the central eastern coast of Lake Tanganyika.[1] This shoreline contains the major breakpoints which correspond to the locations of the three main basins of the lake and thus covers habitats in shallow water which are more strongly affected by fluctuations of lake level, as well as habitats situated at very steep shorelines which were probably not affected by periods of low lake level (Fig. 1A). Additional, yet unpublished, data were added to the *Tropheus* data set to increase geographical overlap with the data sets for *Simochromis* and the Eretmodini in the central eastern region of the lake.

Species of the genus *Tropheus* are strictly confined to rock habitats for foraging and mating, and have a limited capacity for dispersal across open water (Brichard, 1989; Sturmbauer and Dallinger, 1995). Six nominal species are described (Poll, 1986), some of which have overlapping distribution (Snoeks *et al.*, 1994), and more than 70 distinctly colored "races" have been reported (P. Schupke, personal communication). Samples of 23 localities are included in Fig. 1A.

The genus *Simochromis* is closely related to *Tropheus* (Nishida, 1991; Sturmbauer and Meyer, 1992; Kocher *et al.*, 1995) and both genera are classified within the same tribe, the Tropheini (Poll, 1986). The two *Simochromis* species studied so far, *S. babaultii* and *S. diagramma*, appear to be similar to *Tropheus* in their ecology, but they are typically less abundant. Their number of described geographical "races" is much smaller than *Tropheus* and some behavioral differences also exist between the taxa of the two genera. Although both sexes of *Tropheus* are highly sedentary, *Simochromis* species have been observed to move about in schools, and only dominant males keep territories (Brichard, 1989; C. Sturmbauer *et al.*, unpublished observations). In contrast to *Tropheus* and to the Eretmodini, *Simochromis* is sexually dichromatic. Samples of 11 and 13 localities were analyzed for *S. babaultii* and *S. diagramma*, respectively.

Species of the tribe Eretmodini are small, stenotopic cichlids and are the only group of cichlids that are adapted to living in shallow coastal areas, exposed to wave action. Three genera and four nominal species, *Eretmodus cyanostictus*, *Spathodus erythrodon*, *Spathodus marlieri*, and *Tanganicodus irsacae*, have been described. Because eretmodines have a reduced swimbladder, they actually "sit" on the substrate, like the Gobiidae and the marine Blenniidae. The species differ in their ecology and dental morphology: three species were classified as epilithic algae feeders and one species (*Tanganicodus*) tends to feed on higher portions of invertebrates (Yamaoka *et al.*, 1986). As in the case of *Tropheus*, numerous geographically isolated populations have been described for all four species, but color differences among populations are less pronounced than in *Tropheus*. Samples of 43 specimens from 32 localities were analyzed for the Eretmodini.

A. Genetic Variation in Tropheus

Comparisons of the amount of sequence variation found within the genus *Tropheus* to that within the haplochromine species flocks of Lake Malawi and Lake Victoria suggested that *Tropheus* may be roughly twice the age of the whole Malawi species flock and six times the age of the Lake Victoria cichlid flock (Sturmbauer and Meyer, 1992).

Tropheus duboisi is the most basal species in the genus, sister group to seven distinct lineages comprising the remaining five presently recognized species (Fig. 1b). Although the average corrected sequence divergence among these seven different lineages amounted to 9.1% (standard deviation 1.6%), the average genetic divergence among populations of the same mitochondrial lineage was only 3.0% (standard deviation 1.1%). On the basis of the observed short branches and thus similar levels of genetic divergence defining the major mitochondrial lineages, and of similar levels among populations within each lineage, two successive radiations are hypothesized: in the primary radiation, *Tropheus* colonized rocky shores along the entire lake and the seven lineages originated. Relatively recently, each of those lineages underwent secondary radiations during which time geographically separated populations diversified to the patterns presently observed. The major fluctuation of the lake level was suggested as the trigger of the secondary radiations.

[1]The nucleotide sequences in this chapter are available from EMBL/GenBank and are as follows: Z12047 to Z12100 and Z75694 to Z75709 for *Tropheus*; X90593 to X90638 for the eretmodines; U40524 to U40532 for *Simochromis babaultii*; and U38808 and U38984 to U38995 for *Simochromis diagramma*.

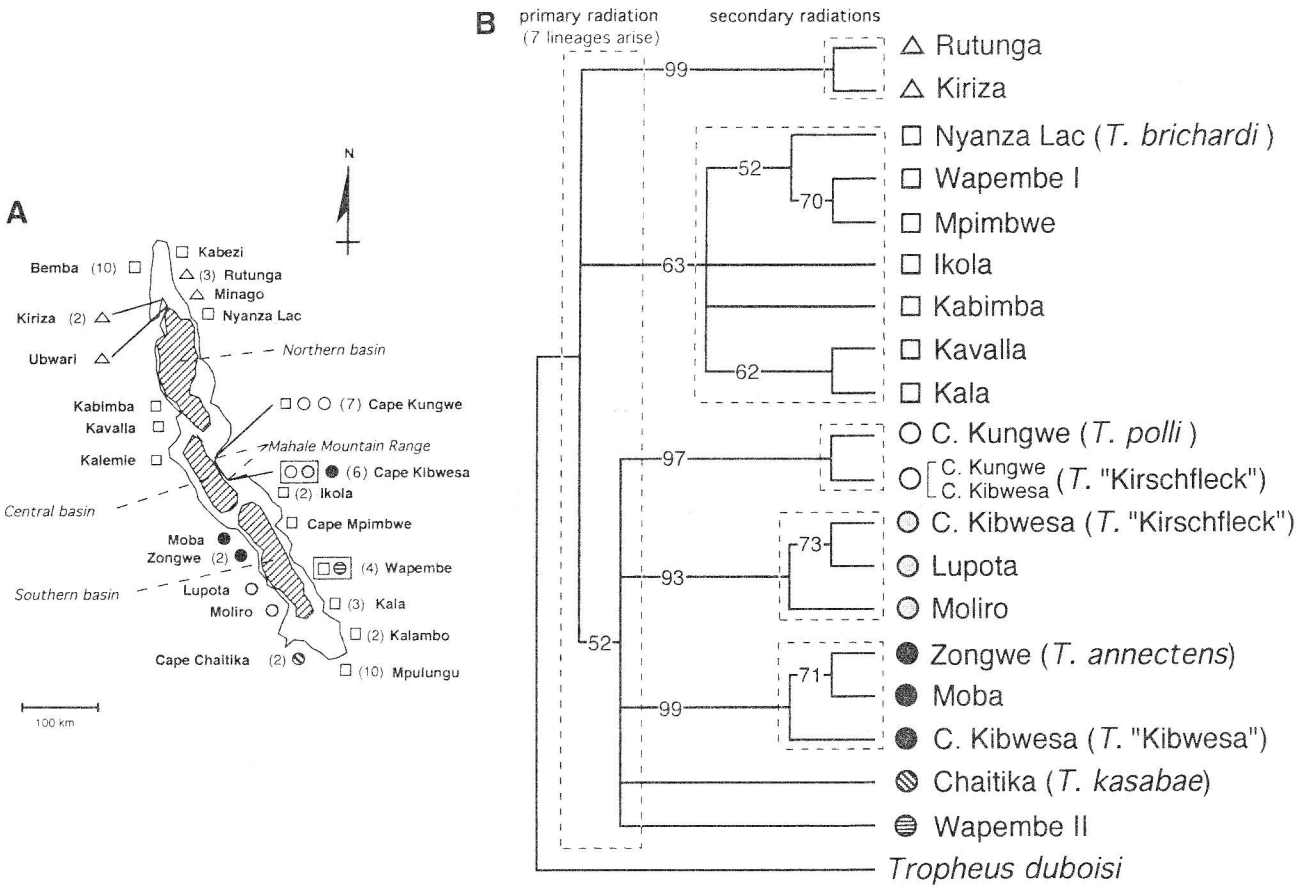

FIGURE 1 Mitochondrial phylogeny and geographic distribution of the Tanganyikan genus *Tropheus*. Seventy-two individuals, assigned to six currently recognized species, were sequenced (Poll, 1986): *Tropheus moorii*, Boulenger, 1898; *T. annectens*, Boulenger, 1898; *T. duboisi*, Marlier, 1959; *T. brichardi*, Nelissen and Thys, 1976; *T. kasabae*, Nelissen, 1977; and *T. polli*, Axelrod, 1978. The populations are identified by the name of the nearest village (Brichard, 1989). (A) Map of Lake Tanganyika with localities of 23 *Tropheus* populations and distribution of the seven recognized major mitochondrial lineages. Stippled areas represent the hypothesized shoreline at a depth of 600 m, illustrating the locations of the three lake basins and the steepness of the shoreline. Numbers of individuals analyzed from each locality are given in parentheses if larger than one. Boxed genotype symbols indicate the presence of two mitochondrial genotypes assigned to different major lineages within a single geographical "race." (B) Molecular phylogeny of the genus *Tropheus*. The species names are given according to the current taxonomic assignments; a name in quotation marks refers to that used in the text. All populations from which species names are omitted are presently classified as *T. moorii*. The symbols indicate genetically distinct lineages based on the phylogenetic analysis of mitochondrial sequence data. The primary radiation and the succeeding more recent secondary radiations are indicated by stippled boxes. This phylogeny was obtained by parsimony analysis (using PAUP 3.1.1; Swofford, 1993). A bootstrap consensus tree (Felsenstein, 1985; 1000 replicates, 50% majority rule) based on 442 bp of the control region is shown. *T. duboisi*, identified as the closest living relative of all other *Tropheus* species (Sturmbauer and Meyer, 1992), was used as the outgroup. Transversions were weighted nine times over transitions based on the average observed frequency among taxa of up to 5% uncorrected sequence divergence. Three insertions were observed; each was weighted like a single transversion. This analysis included 20 representative taxa and resulted in three equally parsimonious trees [heuristic search with random addition of taxa and 20 replications; ACCTRANS option; weighted tree length, 393 steps; unweighted tree length, 172 base substitutions; consistency index excluding uninformative characters (Kluge and Farris, 1969), 0.50]. Bootstrap values are given on the branches.

B. *Phylogeographic Patterns in Tropheus*

The distributions of six of the seven major genetic lineages show a clear phylogeographic pattern. Each lineage is confined to discrete shore sections of a single basin, typically centering along steeply sloping habitats (see Fig. 2). Their genetic distances to each other suggest that these six lineages might have colonized

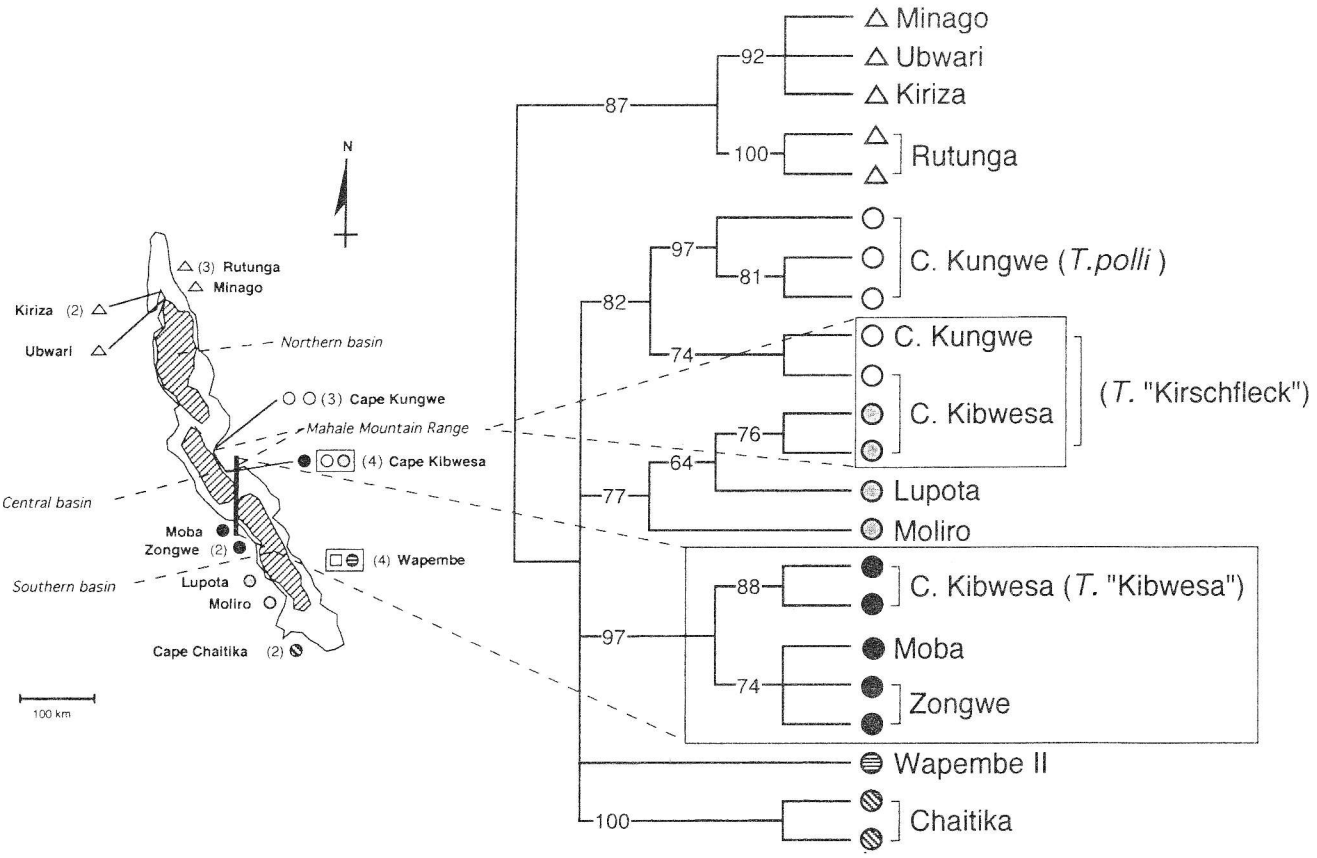

FIGURE 2 Mitochondrial phylogeny obtained by parsimony analysis and locations of *Tropheus* populations with geographically restricted distribution along the three major basins of Lake Tanganyika (indicated by stippled areas on the map). The populations are identified by the name of the nearest village (Brichard, 1989). Numbers of individuals analyzed from each locality are given in parentheses if larger than one. Boxed genotype symbols indicate the presence of two mitochondrial genotypes assigned to different major lineages within a single population. A bootstrap consensus tree (1000 replicates, 50% majority rule) is shown, based on 382 bp of the control region. Five individuals of a lineage from the northern shores (indicated by open triangle symbols) were used as the outgroup based on the analysis presented in Fig. 1B. Transversions were weighted nine times over transitions based on the average observed frequency among taxa of up to 5% uncorrected sequence divergence. Three insertions were observed; each was weighted like a single transversion. This analysis included 22 representative taxa and resulted in 14 equally parsimonious trees (heuristic search with random addition of taxa and 20 replications; ACCTRANS option; weighted tree length, 312 steps; unweighted tree length, 128 base substitutions; consistency index excluding uninformative characters, 0.64). Bootstrap values are given on the branches. The symbols indicate genetically distinct lineages based on the phylogenetic analysis of mitochondrial sequence data. A split of the lake between the central and the southern basin during a period of low lake level, suggested by the distribution of closely related mitochondrial genotypes at opposite shores, is indicated on the map and connected to the relevant part of the phylogeny (lower box). The upper boxed section of the phylogeny indicates the presence of two distinct genotypes assigned to two major mitochondrial lineages in *Tropheus* "Kirschfleck."

their habitats during the primary radiation, well before the reported period of the low lake level. Due to the stability of their habitats, these lineages are likely to have survived the drop of the lake level intact. When the lake rose again, they did not seem to have significantly expanded their ranges during their secondary radiations.

Only a single lineage (square symbols in Fig. 1B and Fig. 3) is widespread throughout the entire lake, ranging from Bemba at the very northwestern end down to Mpulungu at the very south of Lake Tanganyika (Fig. 1B). Despite this, all members of the "square" lineage, even from distant populations, are genetically closely related, as closely as the populations within each primary lineage. Thus, the square lineage may have significantly expanded its range of distribution during its secondary radiation when the lake level rose after its low period.

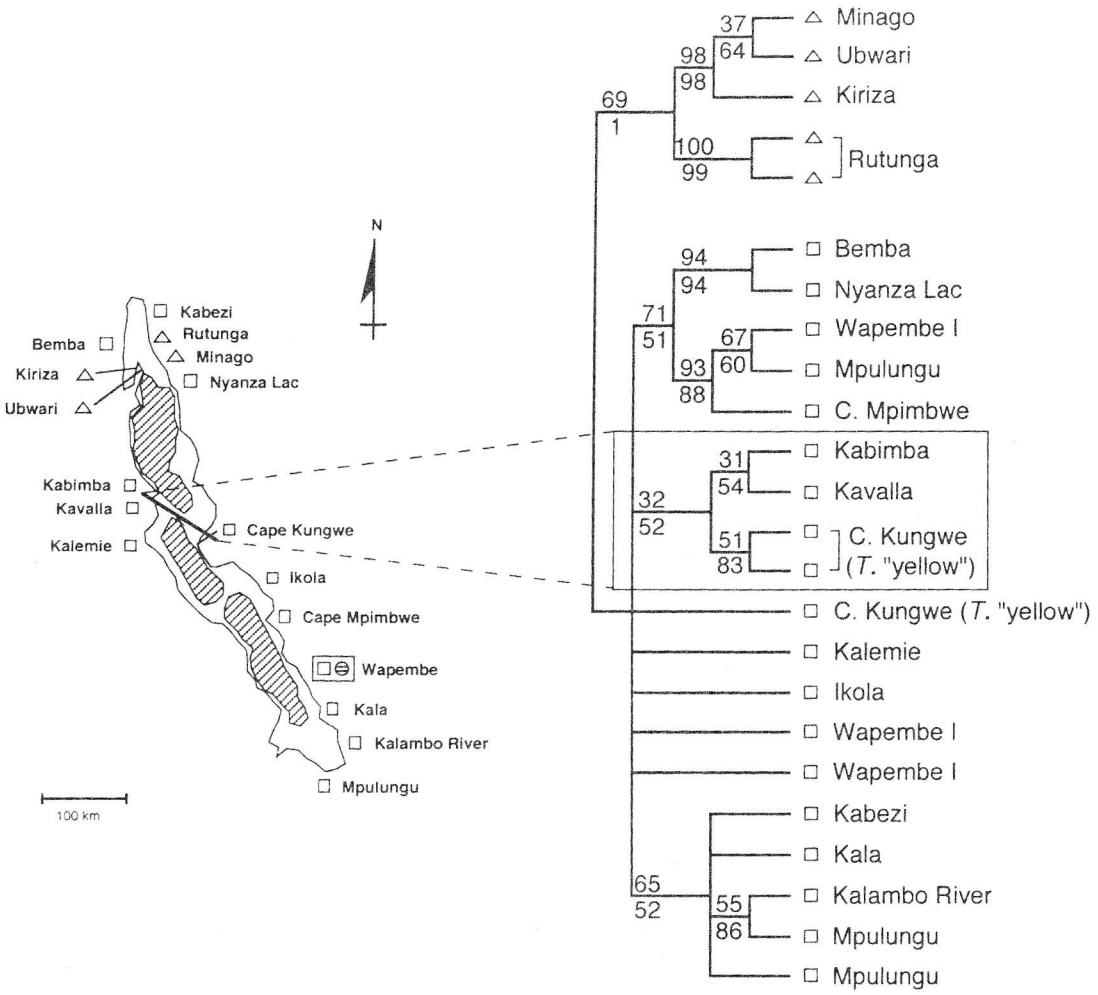

FIGURE 3 Mitochondrial phylogeny based on 382 bp of the control region, obtained by parsimony analysis and neighbor joining (Saitou and Nei, 1987), and locations of *Tropheus* populations with geographically widespread distribution (termed "square lineage" in the text) along the three major basins of Lake Tanganyika are shown (indicated by stippled areas on the map). The populations are identified by the name of the nearest village (Brichard, 1989). The boxed genotype symbols at Wapembe indicate the presence of two mitochondrial genotypes assigned to different major lineages within a single population. A bootstrap consensus tree (1000 replicates in both, parsimony and neighbor joining) is shown in which all branches that were found in less than 50% of the replicates of both methods are collapsed. Five individuals of a lineage from the northern shores (indicated by open triangle symbols) were used as the outgroup based on the analysis presented in Fig. 1B. Transversions and transitions were weighted equally, as all taxa of the ingroup are closely related. Three insertions were observed; each was weighted like a single base substitution. In neighbor joining, Jukes–Cantor distances (Jukes, 1980) were used to correct for multiple substitutions. This analysis included 24 representative individuals and resulted in 50 equally parsimonious trees (heuristic search with random addition of taxa and 20 replications; ACCTRANS option; tree length, 97 base substitutions; consistency index excluding uninformative characters, 0.59). Bootstrap values above the branches are from parsimony, whereas those below the branches are from neighbor joining. A split of the lake between the northern and the central basin during a period of low lake level, suggested by this phylogenetic analysis, is indicated on the map and is connected to the relevant part of the phylogeny (boxed section).

In addition, the distribution of mitochondrial genotypes within and among populations of the "square lineage" does not suggest any obvious phylogeographic structure. Of 10 individuals sequenced from the population at Mpulungu at the southern end of the lake, 8 different mitochondrial genotypes were identified, clustering with mitochondrial genotypes of several other localities of this lineage (three individuals were included in the analysis presented in Fig. 3).

During their period of geographic expansion the

members of the "square lineage" seem to have entered regions that were already colonized by other lineages. Data of a population near Wapembe at the southeastern shore of the lake suggest an introgression event in the history of this population between the indigenous lineage (from the primary radiation of *Tropheus*) and a second lineage of invaders. Among four individuals sequenced, all were different: three mitochondrial genotypes are assigned to the "square lineage" (square symbols in Figs. 1B and 3), whereas one individual represents a separate and heretoforth undescribed major mitochondrial lineage, in addition to the previously recognized lineages (hatched circle symbol in Figs. 1B and 2). This genotype may be a relict of the indigenous lineage from the primary radiation.

The central eastern shore of Lake Tanganyika along the Mahale Mountain range (see Fig. 1A) represents a continuous, steeply sloping rock habitat. Its northern edge at Cape Kungwe is situated close to the border of the northern and the central basin, and its southern edge is at the border between the central and the southern basin. Along this range more than one taxon of *Tropheus* occurs in sympatry (Snoeks *et al.*, 1994), pointing to high frequencies of secondary contact between multiple lineages at the junctions of the intermediate lake basins. At Cape Kungwe, the northern edge of the Mahale Mountain range, four sympatric *Tropheus* species were found: *T. duboisi* (the sister group of all other *Tropheus*), *Tropheus* "yellow," *T. polli*, and *Tropheus* "Kirschfleck." *Tropheus* "yellow" belongs to the "square lineage" with widespread distribution. Two of the three analyzed individuals appear to be most closely related to the individuals sampled at Kavalla and Kabimba on the western shore of the lake, exactly where the northern basin touches the current western shore line (see boxed section in Fig. 3), suggesting that Lake Tanganyika was actually split into sublakes between the northern and the central basin during the low period.

Three species live sympatrically at the southern edge of the Mahale Mountain range at Cape Kibwesa. Two species, *T. polli* and *T.* "Kirschfleck," were also found at Cape Kungwe. The third sympatric *Tropheus*, *Tropheus* "Kibwesa" (black filled circle in Figs. 1 and 2), was only found at a stretch of some hundred meters around Cape Kibwesa. These individuals were resolved in a clade together with fish from two populations of the southwestern shore at Moba and Zongwe (see lower boxed section in Fig. 2). Because *Tropheus* is highly stenotopic and most probably unable to cross several kilometers of open water (Brichard, 1978; Yanagisawa and Nishida 1991; Sturmbauer and Dallinger, 1995), the observed phylogeographic affinities between populations at opposite shores suggest that Lake Tanganyika was also split into sublakes between the central and the southern basin.

T. polli, and *Tropheus* "Kirschfleck" occur in sympatry all along the shore of the Mahale Mountain range. Of the individuals sampled at Cape Kungwe, *T. polli* and *Tropheus* "Kirschfleck" have closely related genotypes which belong to the same mitochondrial lineage (open circles in Fig. 2). The amount of genetic divergence between these two species is in line with other genetic divergences of individuals of the same major genetic lineage which diversified during secondary radiations. However, this close genetic relationship is contrasted by their clearly expressed differences in coloration and in the shape of their caudal fins. The individuals of *Tropheus* "Kirschfleck" sampled at the southern edge of the Mahale Mountain range around Cape Kibwesa were genetically heterogeneous (see upper boxed section in Fig. 2). One genotype is almost identical to that sampled at Cape Kungwe and thus is also closely related to the genotypes found in *T. polli*. The second genotype belongs to a different major mitochondrial lineage and is closely related to populations at Lupota and Moliro at the southwestern shore of the lake (shaded circles in Figs. 1 and 2). Thus, one species, probably *T. polli*, may be indigenous and *Tropheus* "Kirschfleck" might have invaded the area from the opposite shoreline when the lake was split. Introgression or hybridization upon secondary contact between invading *Tropheus* might explain the observed distribution of genotypes.

C. Genetic Variation in Eretmodines

The mitochondrial phylogeny is not fully concordant with the present taxonomy of the eretmodines, pointing to the existence of yet undescribed species and in a few cases, to hybridization among species on secondary contact. This chapter focuses on the phylogeography of mitochondrial genotypes and discusses the taxonomic heterogeneities only briefly, as a study including morphology, mtDNA, and nuclear markers is currently underway to adequately address these problems. Of the four presently recognized species, *Spathodus marlieri*, *Spathodus erythrodon*, *Eretmodus cyanostictus*, and *Tanganicodus irsacae*, two major mitochondrial lineages have been identified (symbolized as A and B in Fig. 4), subdivided into seven clades. Because the average corrected sequence divergences between the two eretmodine lineages are similar to those found among the seven major lineages of the genus *Tropheus* (9.2%), the Eretmodini may have about the same age as the primary radiation of *Tropheus* (see Fig. 1B). Species occur widely sympatrically with *Tropheus* and they also seem to have undergone succes-

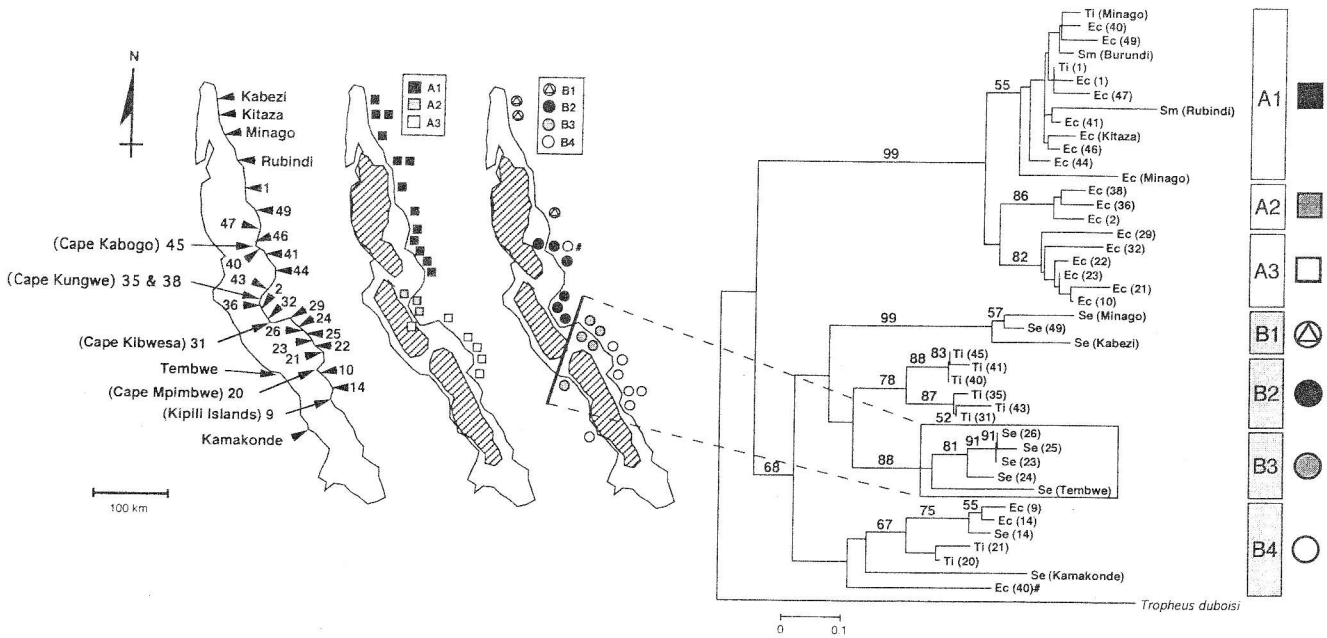

FIGURE 4 Mitochondrial phylogeny of the Eretmodini based on 336 bp of the control region obtained by neighbor joining (Saitou and Nei, 1987); locations of sampled populations are shown on the left map, and geographic distribution of mitochondrial lineages along the three major basins of Lake Tanganyika (indicated by stippled areas on the map) are shown on the center and right map. Populations are identified either by the name of the nearest village or by the number of the sample location during a collection in 1992. Bootstrap values are given on those branches that were obtained in more than 50% of the replications. *Tropheus duboisi* was used as the outgroup based on a phylogenetic analysis of several Tanganyikan mouthbrooders (Sturmbauer and Meyer, 1993). Kimura distances were used to correct for multiple substitutions (Kimura, 1980). This analysis included 43 representative individuals. Two major lineages (A and B) were defined on the basis of the phylogeny and the observed genetic distances among clades. Lineage A was subdivided into three clades (A1, A2, and A3) whereas lineage B was subdivided into four clades (B1 to B4). The lineages and clades were not only defined on the basis of the mitochondrial control region reported here, but also on the basis of cytochrome b (Rüber, 1994; Verheyen *et al.*, 1996). The geographic distribution of lineage A is shown on the map in the center, whereas that of lineage B is shown on the right map. A split of the lake between the central and the southern basin during a period of low lake level, suggested by the distribution of clade B3, is indicated on the right map and the relevant section of the phylogeny is boxed. The species names are given according to the current taxonomic assignments: Ec, *Eretmodus cyanostictus*; Sm, *Spathodus marlieri*; Se, *Spathodus erythrodon*; and Ti, *Tanganicodus irsacae*. Locality names or numbers are given in parentheses. Ec (40)# of clade B4 indicates a morphologically and genetically distinct *Eretmodus* sympatric to another *Eretmodus*, Ec (40) of clade A1.

sive radiations. As in *Tropheus*, several habitats along the lake may have been colonized during a primary radiation establishing the two major mitochondrial lineages. Each lineage has then undergone secondary radiations, but in contrast to *Tropheus*, these secondary radiations did not proceed simultaneously in both lineages and may have been triggered by different causes. The Kimura distances between the clades within lineage A range from 3.1 to 3.4%, similar to the distances found in the secondary radiations of *Tropheus* (3.0%). The considerably higher Kimura distances (4.5 to 7.7%) observed among the four clades within lineage B indicate that their split is considerably older. The phylogram depicted in Fig. 4 also shows that the secondary radiations within lineage A occurred much more recently than the clade formation within lineage B.

D. Phylogeographic Patterns in Eretmodines

Lineage A is subdivided into three clades (A1, A2, and A3; see Fig. 4), each of which has limited distributions, so that the habitats of each subclade can be assigned to shores of one of the three lake basins. As depicted in the central map of Fig. 4, clade A1 ranges from Burundi to the northern edge of the Mahale Mountain range at Cape Kungwe. Surprisingly, individuals of different species clustered within clade A1.

Clade A2 is restricted to the northern half of the Mahale Mountain range and is exclusively composed by *E. cyanostictus*. Clade A3 ranges from the southern part of the Mahale Mountain range to Cape Mpimbwe and is morphologically homogeneous, comprising only *E. cyanostictus*.

The individuals assigned to lineage B are subdivided into four clades. Lineages B1, B2, and B3 have restricted distributions (see the right map on Fig. 4). Lineage B1 is comprised exclusively of *S. erythrodon* and is limited to the northeastern shores of the northern basin (circle–triangle symbols in Fig. 4). Lineage B2, composed of *T. irsacae*, is found in the central basin, extending further to the north, to the southeastern shores of the northern basin. The distribution of lineage B3 seems to be restricted to the southern edge of the central basin and is found both on the eastern and on the western shores of the lake. Clade B4 is also morphologically heterogeneous and, like clade A1, contains individuals classified as *Spathodus*, *Eretmodus*, and *Tanganicodus* (filled circles in Fig. 4). In contrast to clades B1, B2, and B3, clade B4 it was found in more than one basin, at the southernmost Tanzanian localities, the southwestern shore at Kamakonde, and also in a single individual of *E. cyanostictus* on locality 40 in the northern basin [symbolized as Ec (40)# in Fig. 4]; this individual is morphologically and genetically distinct to a sympatric *Eretmodus* [Ec (40) of clade A1]. Individual Ec (40)# may also represent a "mitochondrial relict" genotype of a previously more widespread clade because this genotype was resolved on the basis of clade B4.

Like the "square lineage" of *Tropheus*, lineage A seems to have undergone a secondary radiation during which three clades (A1, A2, and A3 in Fig. 4) were formed and the range of distribution was significantly expanded to shores of all three basins. During their spread, populations of clades A1, A2, and A3 may have reached secondary contact with populations of lineage B which seem to have spread and diversified earlier. At locality 40 (marked by # in the right map in Fig. 4), individuals of three clades (A1, B2, and B4) were found sympatrically.

E. Genetic Variation in Simochromis

The two species analyzed, *S. babaultii* and *S. diagramma*, had an average genetic distance of 3.8% (standard deviation 0.6%). Thus, *S. babaultii* and *S. diagramma* would seem to be much younger species than most *Tropheus* and most eretmodines. They probably originated at about the same time when *Tropheus* and eretmodines underwent their secondary radiations. Among 25 sampled individuals of *S. babaultii* from 11 localities, nine mitochondrial genotypes were identified. The average genetic divergence among these nine genotypes was 0.9% (standard deviation 0.8%). Among 28 sampled individuals of *S. diagramma* from 13 localities, 13 genotypes with an average genetic distance of 1.2% (standard deviation 0.7%) were identified.

F. Phylogeographic Patterns in Simochromis

The branching order among different haplotypes did not seem to reflect the geographic distribution of populations in either *S. babaultii* and *S. diagramma* in any obvious way, although in both species the two most ancestral haplotypes were found in the most southern populations. The geographic distribution of the mitochondrial genotypes observed in *S. babaultii* and *S. diagramma* was variable among haplotypes; however, when more than one individual was sampled from a given locality, both species tended to contain more than one haplotype per locality. Several genotypes were shared among geographically distant localities (Fig. 5) that did not correlate with the partitions of the lake according to the three basins.

III. From Patterns toward an Understanding of Processes

A. Habitat Characteristics, Lake Level, and Phylogeography

The phylogeographic patterns of mtDNA variation show striking parallels in *Tropheus* and the Eretmodini in that both seem to have simultaneously undergone (at least) two consecutive radiations. The majority of these lineages are confined to small sections of a single basin (see Fig. 2 for *Tropheus* and Fig. 4 for the Eretmodini) and thus show a high degree of intralacustrine endemism (Snoeks *et al.*, 1994). They all originated from a primary radiation, which occurred roughly at the same time in *Tropheus* and the Eretmodini. The lineages with restricted distribution are likely to have colonized their habitats before the period of the dramatically low lake level. Their habitats were situated at steeply sloping shore sections and may thus have in common that they were probably not wiped out by the drop of the lake level, but shifted along a continuous slope. After the lake rose again, the populations shifted back along the slope and did not seem to have expanded their ranges of distribution significantly.

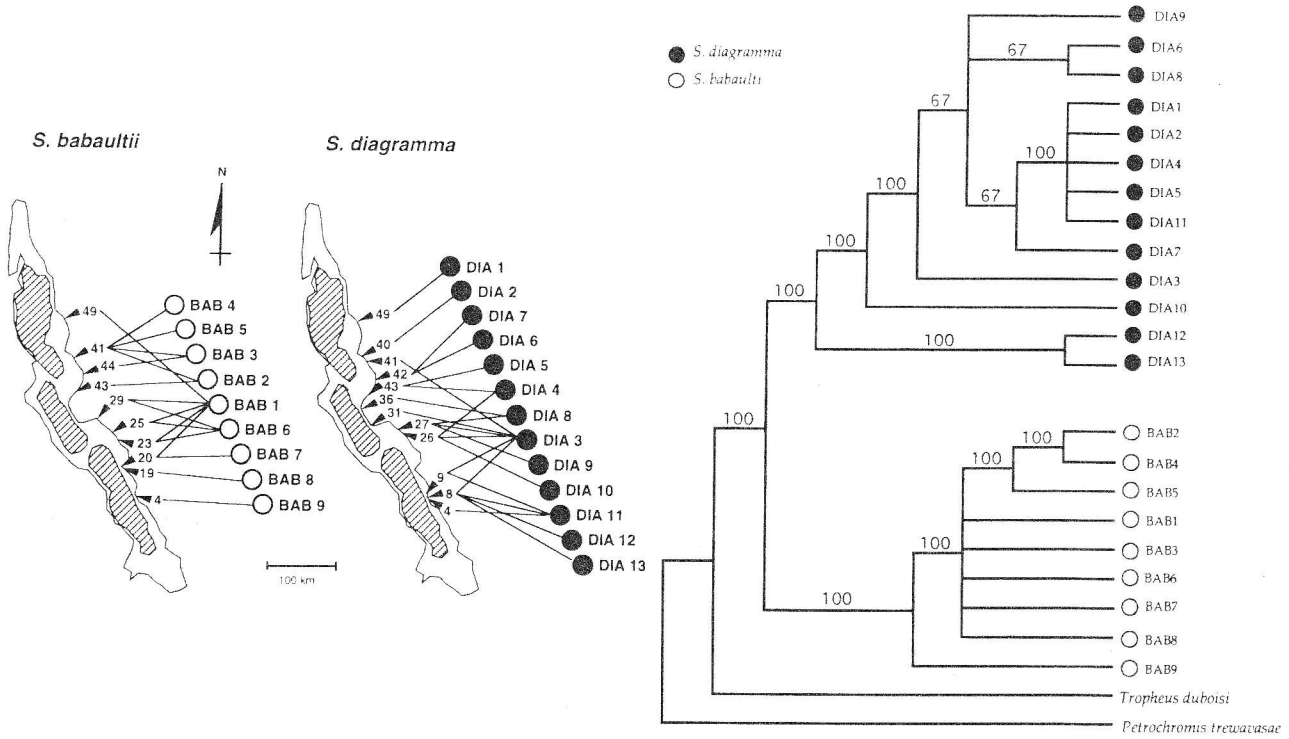

FIGURE 5 Mitochondrial phylogeny of two species of the genus *Simochromis* based on 414 bp of the control region, locations of sampled populations, and the geographic distribution of mitochondrial genotypes along the three major basins of Lake Tanganyika (indicated by stippled areas on the maps). Populations are identified according to the number of the sample location during a collection in 1992. Parsimony analysis used equal weights for transversions and transitions, and *Tropheus duboisi* and *Petrochromis trewawasae*, both classified within the same tribe (Tropheini), were used as outgroups (Poll, 1986). A heuristic search resulted in minimum length trees of 64 substitutions and a consistency index of 0.79. A bootstrap consensus tree (1000 replicates, 50% majority rule) is shown with bootstrap values on the branches. This analysis included 25 individuals (nine mitochondrial haplotypes) for *S. babaultii* and 28 individuals (13 mitochondrial haplotypes) for *S. diagramma*. The geographic distribution of the nine identified haplotypes in *S. babaultii* is shown on the map on the left, whereas that of the 13 identified haplotypes in *S. diagramma* is shown on the right map.

As a second striking parallel, the "square lineage" in *Tropheus* and the A-lineage in the Eretmodini seem to have simultaneously expanded their distributions to all three basins of the lake because the average genetic divergences within the two groups are small and similar (around 3%). Clade B4 of the eretmodines might also have extended its range at about the same time because their members were found at both the eastern and the western shore of the southern basin and also at locality 40 in the northern basin (see the right map in Fig. 4) and because the average Kimura distances within clade B4 also amounted to 3.2%. It seems reasonable that the spread of all three groups was triggered by the same event, a substantial rise in the lake level.

However, there are differences with respect to phylogeographic structuring among populations: no clear phylogeographic pattern emerged in the "square lineage" of *Tropheus*. If the ancestors of the square lineage originally inhabited more shallow coasts, their populations must have been most severely affected when the lake level retreated. Several populations, previously spread over a wide stretch of shallow coastline, could have been fused into one large population for the period of the low lake level, which might explain the present genetic heterogeneity of this lineage. When the lake level rose after the low period the shallow bays were flooded again and quickly recolonized by the square lineage, which now contained several mitochondrial genotypes. The origin of the square lineage may be situated at the southern section of the northern basin and possibly at the western shore of the central basin; these are the only regions where no individuals with genotypes of the other lineages of limited distribution have been found.

In contrast to the square lineage of *Tropheus*, the three clades of the A lineage of the Eretmodini do show strong phylogeographic structuring (Fig. 4, cen-

ter map); all three clades are genetically and also geographically distinct, suggesting that their parental population may not have been as large and genetically heterogeneous as that of *Tropheus* and thus may not have originated from a shallow and unstable habitat.

The heterogeneity in the genetic patterns of six versus a single lineage in *Tropheus* highlights the importance of intrinsic characteristics of species for their ability to disperse. The enormous success of the "square lineage" in colonizing newly available habitats, and even in replacing at least one original population after the vicariant event, strongly indicates significant differences in their ecology and/or behavior in comparison to the remaining lineages. One might assume that their occurrence in more unstable habitats in shallower regions of the lake, which were repeatedly affected, even by smaller lake level fluctuations, might have forced these populations to become more mobile than those populations situated at steeply sloping habitats which remained intact, regardless of the water level. It is also striking that the square lineage did not seem to be sucessful in colonizing the majority of stable habitats and in replacing indigenous lineages. This observation might point to the possibility that communities at equilibrium are resilient to invasion.

S. babaultii and *S. diagramma* are likely to be much younger than *Tropheus* and the eretmodines. Because none of the genotypes were shared between the two species, lineage sorting may be complete, despite their young age. The genetic distances of about 3% to each other suggest that their origin might also be directly connected to the reported substantial drop of the lake level. The same vicariant event might thus have triggered both: speciation in *Simochromis* and secondary radiation in *Tropheus* and the Eretmodini. As suggested by the branching order in both, *S. babaultii* and *S. diagramma*, *Simochromis* might have speciated in the southern basin and then colonized habitats in the central and northern basin after the lake level of Tanganyika rose again.

The second important observation is that both species of *Simochromis* lack any phylogeographic structure. This is in contrast to the findings for the six *Tropheus* lineages with limited distribution and the eretmodines. However, it is strikingly similar to the pattern found for the square lineage of *Tropheus*. Because none of the mitochondrial genotypes were shared among the two species, all identified genotypes are likely to have arisen after the speciation event, despite their lack of phylogeographic structure. This pattern might be explained by the fact that these genotypes were already present in the founder population, before they spread and colonized shores along all three basins. Populations remained genetically heterogeneous due to the young age of these populations and the species. Moreover, the genetic heterogeneity may be caused by high rates of contemporary gene flow among populations all along the lake, even if they are separated from each other by large distances. This explanation is based on the assumption that *Simochromis* species are less sedentary than *Tropheus* and the Eretmodini. Ecological differences, combined with small-scale lake level fluctuations, may result in higher rates of gene flow among populations. In *Simochromis*, speciation may only occur as a consequence of vicariant events, such as an actual split of the lake, allowing for secondary contacts between more strictly isolated populations, such as those from opposite shores.

Similarly, the lack of phylogeographic structure such as observed in the square lineage of *Tropheus* and *Simochromis* has also been reported for Malawi cichlids (Bowers *et al.*, 1994; Moran and Kornfield, 1993). This parallel to Lake Malawi cichlids may be interesting because most Malawi species, particularly those found at the shallow and thus more unstable southern shore regions, are substantially younger, even than *Simochromis*. DNA sequences of such closely related species tend to differ from each other by too few point mutations and thus will not provide enough synapomorphies for phylogenetic analyses. Due to incomplete lineage sorting, mitochondrial genotypes may even still be shared by different species which can only be characterized genetically by different frequency patterns of mitochondrial RFLP haplotypes (Moran and Kornfield, 1995). Thus, more variable genetic markers, particularly microsatellites, may actually be more useful for phylogenetic analyses than DNA sequences (A. Parker and I. Kornfield, personal communication). A study on closely related Malawi cichlids has been undertaken by Kornfield and Parker (1997). As a future perspective, mtDNA sequences and those microsatellites used for extremely young Malawi species could be analyzed in parallel in *Simochromis*, allowing for a better understanding of the accumulation of homoplasy in microsatellite markers with an increasing time of divergence and to sort out maternal (mitochondrial) and nuclear (microsatellite DNA) perspectives on these issues.

B. Split of Lake Tanganyika

In both *Tropheus* and the Eretmodini, closely related genotypes were found at opposite shorelines, despite their restricted dispersal ability through open water. Their low capability of dispersal has been demonstrated at a rock habitat near Rubiza in the northern Lake Tanganyika which emerged in the early seventies after a storm. This habitat was isolated from the next

rock habitat by 15 km of sand shore (Brichard, 1978). *Tropheus*, *Simochromis* and the Eretmodini could not bridge 15 km of sand to colonize these new habitats; they appear to be too philopatric. Thus, it is highly unlikely that any of the three groups can cross several tens of kilometers of open water to colonize shores at opposite sides of the lake. The observation of closely related genotypes on opposite shores corroborates an actual split of Lake Tanganyika during the period of the extremely low lake level which was suggested on the basis of seismic data (Scholz and Rosendahl, 1988; Tiercelin and Mondeguer, 1991). Habitats at the border regions of the basins at either side may have been interconnected by newly forming shorelines through the lake, causing a connection of previously separate populations, so that even philopatric rock cichlids could have established secondary contact across the lake. The reported low stand of the lake level 200,000 to 75,000 years ago is likely to have severely impacted the present distributions of all three groups. However, the observed breakpoints among lineages, or among populations of the same lineage, are different. None of the lineages of the Eretmodini reached a lakewide distribution, indicating that their ability for dispersal is more similar to that of the six lineages of *Tropheus* with restricted distribution than to that of the "square lineage."

The average genetic divergences of the lineages arising during the secondary radiations, especially the finding of closely related populations at both sides of the lake in the border regions of the lake basins, may be suitable to calibrate the rates of sequence divergence in cichlids as soon as more accurate datings of the dramatically low lake level and the split of Lake Tanganyika become available.

C. Secondary Contact, Hybridization, and Speciation

According to the allopatric model of speciation, temporary geographic isolation is required to form reproductive barriers. Whenever two populations gain secondary contact, they may interbreed, depending on whether they still recognize each other as members of the same reproductive unit. Species tend to lose mobility with an increasing level of specialization (Fryer and Iles, 1972). Specialists may be subdivided into populations according to the discontinuous presence of habitats, such as rocky habitats that are interleaved by sandy shores. Data of *Tropheus* from Wapembe, where two genotypes of two major mitochondrial lineages were found in individuals of a single geographical "race," suggest a past hybridization event between an indigenous lineage and a second lineage of invaders (see Fig. 1). The three individuals assigned to the "square lineage" may represent the descendants of the invaders, whereas the fourth individual seems to represent a separate and previously unidentified primary mitochondrial lineage, a relict of the indigenous lineage.

A potential case of hybridization was found between *T. polli* and *Tropheus* "Kirschfleck" at the Mahale Mountain range (see upper boxed section of Fig. 2). One species, *T. polli*, may be indigenous and *Tropheus* "Kirschfleck" might have invaded the area from the opposite shore line when the lake was split. Introgression or hybridization upon secondary contact between invading *Tropheus* might explain the observed distribution of genotypes. Because both species remained separate and because the observed genetic distances correspond to the splits of the secondary radiations, hybridization may have only occured during the initial phase after secondary contact, and this potential hybrid zone is limited to this one locality.

Prezygotic isolation mechanisms may have been perfected due to reinforcement after secondary contact, as was suggested by Butlin (1991). The second alternative, that hybridization may still be going on at very low frequencies so that species boundaries remain intact despite ongoing hybridization, seems less likely because the Kimura distances among heterospecific individuals found so far were always about 3%. As a third alternative, an ancient polymorphism may have persisted, as was shown for cichlid species of Lake Malawi (Moran and Kornfield, 1993). Which of the alternative scenarios is correct may become known when more representative samples become available and when nuclear markers are included in future analyses.

Speciation without morphological change can be viewed as one of the characteristics of geographically separated sister species living in complex species communities of similar composition (Sturmbauer and Meyer, 1992). However, sympatry of four morphologically monomorphic species has only been reported for *Tropheus* so far (Snoeks *et al.*, 1994; C. Sturmbauer *et al.*, personal observations). Whenever sympatric, they are segregated by water depth (Kohda and Yanagisawa, 1992). One species is invariably found in the very upper water layer to a depth of about 2 m, whereas the other species shifts to a greater water depth of 2–6 m. For example, wherever *T. polli* and *Tropheus* "Kirschfleck" live sympatrically, *T. polli* always inhabits the upper zone and *Tropheus* "Kirschfleck" the deeper zone. The sympatric occurrences of *Tropheus* species that are virtually identical morphologically may point to the possibility that long-term coexistence may occur by depth segregation rather than morphological change. In the Eretmodini, which only inhabit the up-

permost shore sections, sympatric taxa always seem to differ morphologically and hence, ecologically, pointing to the possibility that ecological diversification may be more important for the coexistence of two eretmodines than for two *Tropheus*.

Most eretmodines (it has not been reported for *S. marlieri*) have a highly complicated system of mouthbrooding (Kuwamura *et al.*, 1989). They are sexually monochromatic and live in permanent pairs. The female broods the eggs and transfers the newly hatched fry to the male which completes the mouthbrooding. In contrast, *Tropheus* forms temporary pairs in the territory of the male, and the female leaves the male's territory after spawning takes place (Yanagisawa and Nishida, 1991; Sturmbauer and Dallinger, 1995). *Tropheus* seems to live sedentarily in densely packed communities where both sexes are territorial (Yanagisawa and Nishida, 1991; Sturmbauer and Dallinger, 1995). Color patterns may play a much more important role in the social system of *Tropheus* and sexual selection may thus be more intense than in the eretmodines. *Simochromis* species have been observed to move about in schools, and only dominant males keep territories (Brichard, 1989; C. Sturmbauer *et al.*, unpublished observations). In contrast to *Tropheus* and the Eretmodini, they are sexually dichromatic and more similar to the cichlids of Lake Victoria and Lake Malawi. They may be much more mobile than *Tropheus* and the eretmodines, which could also explain their lack of phylogeographic structure.

D. Evolutionary Characteristics of Mitochondrial DNA

One of the major characteristics of mtDNA results from its one-dimensional path of coalescence. mtDNA phylogenies do not necessarily reflect the true species phylogenies. Sampling error among (maternally inherited) genotypes, hybridization upon secondary contact, differential extinction due to random genetic drift, or the retention of ancient polymorphisms can result in para- or polyphyly of mitochondrial genotypes in a single biological species. However, the paraphyletic placement of taxa does not necessarily imply that mitochondrial phylogenies are incorrect; paraphyly is an inevitable consequence of the evolution of a new species: if one out of several populations of a species undergoes speciation and the other populations remain reproductively compatible, the "older" species becomes paraphyletic. The possible paraphyletic placement of *S. erythrodon* in lineages B1 and B3 may thus represent a consequence of the speciation of *Tanganicodus* (in clade B2) within an originally conspecific assemblage (see Fig. 4). In conclusion, populations may have strikingly different histories. Although hybridization upon secondary contact may better explain the observed patterns in some populations (such as those along the break points of the lake basins), different alternatives may apply to other situations.

Large-scale patterns of the most frequent genotypes are likely to be detected by small sample sizes. A more finely structured understanding of the history of populations, and also about underlying processes, will emerge with increasing sample sizes. The parallel analysis of mitochondrial and nuclear markers will add a second, nonlinear dimension to population studies, resulting from the genetics of nuclear DNA. Taking advantage of the information record in both genomes will be an important task for future studies.

IV. Conclusions

The authors' comparative results emphasize the following hierarchy of factors influencing speciation:

1. Most lineages of *Tropheus* and of the eretmodines show a high degree of intralacustrine endemism. This observation has important implications for taxonomists and future taxonomic work should account for that. Genetic data should be incorporated into species descriptions.
2. A split of Lake Tanganyika during the period of a low lake level, suggested on the basis of seismic data, is independently corroborated by DNA sequences of *Tropheus* and the eretmodines. This major vicariant event affected the phylogeograpy of all investigated groups of rock-dwelling cichlids.
3. This general trend was overlayed by patterns resulting from specific characteristics of habitats which determine how severely abiotic factors act on each population and thus might explain some of the differences observed.
4. The degree of philopatry may be the dominating biological characteristic of a species, differentially affecting the phylogeographic structures among populations.
5. Finally, each population may adapt to living in specific habitats by evolving new ecological and behavioral characteristics which may also affect their dispersal ability.

References

Avise, J. C. 1994. "Molecular Markers, Natural History and Evolution." Chapman and Hall, New York.

Axelrod, H. R. 1993. "The Most Complete Colored Lexicon of Cichlids." T.F.H., Neptune City, NJ.

Barlow, G. W., 1991. Mating systems among cichlid fishes. *In* "Cich-

lid Fishes: Behaviour, Ecology and Evolution" (M. H. A. Keenleyside, ed.), pp. 173–190. Chapman and Hall, London.

Bowers, N., Stauffer, J. R. and Kocher, T. D. 1994. Intra- and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malawi. *Mol. Phyl. Evol.* **3**:75–82.

Brichard, P. 1978. Un cas d'isolement de substrats rocheux au milieu de fonds de sable dans le nord du lac Tanganyka. *Rev. Zool. Afr.* **92**:518–524.

Brichard, P. 1989. "Cichlids of Lake Tanganyika." T.F.H. Publications, Neptune City, NJ.

Butlin, R. 1991. Reinforcement of premating isolation. *In* Speciation and Its Consequences" (D. Otte and J. A. Endler, eds.), pp. 158–197. Sinauer, Sunderland, MA.

Cohen, A. S., Soreghan, M. J. and Scholz, C. A. 1993. Estimating the age of formation of lakes: An example from Lake Tanganyika, East-African rift system. *Geology* **21**:511–514.

Dominey, W. J. 1984. Effect of sexual selection and life history on speciation: Species flocks in African cichlids and Hawaiian *Drosophila*. *In* "Evolution of Fish Species Flocks (A. A. Echelle and I. Kornfield, eds.), pp. 231–249. University of Maine at Orono Press, Orono.

Eccles, D. H., and Trewavas, E. 1989. "Malawian Cichlid Fishes: The Classification of Some Haplochromine Genera." Lake Fish Movies, Herten.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783–791.

Fryer, G., and Iles, T. D. 1972. "The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution." Oliver and Boyd, Edinburgh.

Greenwood, P. H. 1981. "The Haplochromine Fishes of the East African Lakes." Kraus International Publications, München.

Greenwood, P. H. 1984. African cichlids and evolutionary theories. *In* "Evolution of Fish Species Flocks" (A. A. Echelle and I. Kornfield, eds.), pp. 141–154. University of Maine at Orono Press, Orono.

Hert, E. 1992. Homing and home-site fidelity in rock-dwelling cichlids (Pisces: Teleostei) of Lake Malawi, Africa. *Environ. Biol. Fish.* **33**:229–237.

Jukes, T. H. 1980. Silent nucleotide substitutions and the molecular evolutionary clock. Science **210**:973–978.

Keenleyside, M. H. A. 1991. Parental care. *In* "Cichlid Fishes: Behaviour, Ecology and Evolution" (M. H. A. Keenleyside, ed.), pp. 191–208. Chapman and Hall, London.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**:111–120.

Kluge, A. G. and Farris, J. S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**:1–32.

Kocher, T. D., Conroy, J. A. McKaye, K. R. and Stauffer, J. R. 1993. Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. *Mol. Phyl. Evol.* **2**:158–165.

Kocher, T. D., Conroy, J. A., McKaye, K. R., Stauffer, J. R., and Lockwood, S. F. 1995. Evolution of NADH dehydrogenase subunit 2 in East African cichlid fish. *Mol. Phyl. Evol.* **4**:420–432.

Kohda, M., and Yanagisawa, Y. 1992. Vertical distribution of two herbivorouos cichlid fishes of the genus *Tropheus* in Lake Tanganyika, Africa. *Ecol. Freshwater Fish.* **1**:99–103.

Kornfield, I., and Parker, A. 1997. Molecular systematics of a rapidly evolving species flock: The Mbuna of Lake Malawi and the search for phylogenetic signal. *In* "Molecular Systematics of Fishes" (T. D. Kocher and C. A. Stepien, eds.). Academic Press, San Diego.

Kuwamura, T., Nagoshi, M. and Sato, T. 1989. Female-to-male shift of mouthbrooding in a cichlid fish, *Tanganicodus irsacae*, with notes on breeding habits of two related species in Lake Tanganyika. *Environ. Biol. Fish.* **24**:187–198.

Mayr, E. 1984. Evolution of fish species flocks: A commentary. *In* "Evolution of Fish Species Flocks" (A. A. Echelle and I. Kornfield, eds.), pp. 3–11. University of Maine at Orono Press, Orono.

McElroy, D. M., and Kornfield, I. 1990. Sexual selection, reproductive behavior, and speciation in the Mbuna species flock of Lake Malawi (Pisces: Cichlidae). *Environ. Biol. Fish.* **28**:273–284.

McKaye, K. R., and Gray, W. N. 1984. Extrinsic barriers to gene flow in rock-dwelling cichlids of Lake Malawi: Macrohabitat heterogeneity and reef colonization. *In* "Evolution of Fish Species Flocks" (A. A. Echelle and I. Kornfield, eds.), pp. 169–183. University of Maine at Orono Press, Orono.

Meyer, A. 1993. Evolution of mitochondrial DNA in fishes. *In* "Molecular Biology Frontiers, Biochemestry and Molecular Biology of Fishes" (P. W. Hochacka and T. P. Mommsen, eds.), Vol. 2, pp. 1–38. Elsevier, Amsterdam.

Meyer, A. 1994. DNA technology and phylogeny of fish: Molecular phylogenetic studies of fish. *In* "Genetics and Evolution of Aquatic Organisms" (A. R. Beaumont, ed.), pp. 219–249. Chapman and Hall, London.

Meyer, A., Knowles, L., and Verheyen, E. 1996. Widespread geographic distribution of mitochondrial haplotypes in Lake Tanganyika rock-dwelling fishes. *Mol. Ecol.* **5**:341–350.

Meyer, A., Kocher, T. D., Basasibwaki, P. and Wilson, A. C. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**:550–553.

Moran, P., and Kornfield, I. 1993. Retention of an ancestral polymorphism in the Mbuna species flock (Teleostei: Cichlidae) of Lake Malawi. *Mol. Biol. Evol.* **10**:1015–1029.

Moran, P., and Kornfield, I. 1995. Were population bottlenecks associated with the radiation of the Mbuna species flock (Teleostei: Cichlidae) of Lake Malawi? *Mol. Biol. Evol.* **12**:1085–1093.

Moran, P., Kornfield, I., and Reinthal, P. N. 1994. Molecular systematics and radiation of the haplochromine cichlids (Teleostei: Perciformes) from Lake Malawi. *Copeia* **1994**:274–288.

Nishida, M. 1991. Lake Tanganyika as an evolutionary reservoir of old lineages of East African cichlid fishes: Inferences from allozyme data. *Experientia* **47**:974–979.

Poll, M. 1986. Classification des cichlidae du lac Tanganika. Tribus, genres et aspeces. Acad. Roy. de Belg. Memories de la classe des sciences, Collection in −8° −2° serie, T. XLV—Fascicule **2**:1–163.

Rüber, L. 1994. "Phylogenetic and Phylogeographic Patterns in the Endemic Tanganyikan Cichlid Tribe Eretmodini, Inferred from mtDNA Sequences." Masters thesis, Zoological Museum of the University of Zürich, Switzerland.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

Scholz, C. A., and Rosendahl, B. R. 1988. Low lake stands in Lakes Malawi and Tanganyika, delineated with multifold seismic data. *Science* **240**:1645–1648.

Snoeks, J., Rüber, L., and Verheyen, E. 1994. The Tanganyika problem: Taxonomy and distribution of its ichthyofauna. *In* "Speciation in Ancient Lakes" (K. Martens, B. Goddeeris and G. Coulter, eds.), *Adv. in Limnol.* **44**:357–374.

Sturmbauer, C., and Dallinger, R. 1995. Diurnal variation of spacing and foraging behaviour in *Tropheus moorii* (Cichlidae) in Lake Tanganyika, eastern Africa. *Neth. J. Zool.* **45**:386–401.

Sturmbauer, C., Mark, W., and Dallinger, R. 1992. Ecophysiology of Aufwuchs-eating cichlids in Lake Tanganyika: Niche separation by trophic specialization. *Environ. Biol. Fish.* **35**:283–290.

Sturmbauer, C., and Meyer, A. 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature* **359**:578–581.

Sturmbauer, C., and Meyer, A. 1993. Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes of Lake Tanganyika, East Africa. *Mol. Biol. Evol.* **10**:751–768.

Sturmbauer, C, Verheyen, E., and Meyer, A. 1994. Mitochondrial phylogeny of the Lamprologini, the major substrate spawning lineage of cichlid fishes from Lake Tanganyika in eastern Africa. *Mol. Biol. Evol.* **11**:691–703.

Swofford, D. L. 1993. "Phylogenetic Analysis Using Parsimony (PAUP) Version 3.1.1." Smithsonian Institution, Washington, D.C.

Tiercelin, J. J., and Mondeguer, A. 1991. The geology of the Tanganyika Trough. *In* "Lake Tanganyika and Its Life" (G. W. Coulter, ed.), pp. 7–48. Oxford University Press, London Oxford and New York.

Verheyen, E., Rüber, L., Snoeks, J., and Meyer, A. 1996. Mitochondrial phylogeography of rock dwelling cichlid fishes reveals evolutionary influence of historic lake level fluctuations in Lake Tanganyika, Africa. *Phil. Trans. Roy. Soc. Ser. B.* **351**:797–805.

Yamaoka, K., Hori, M., and Kuratani, S. 1986. Ecomorphology of feeding in "goby-like" cichlid fishes in Lake Tanganyika. *Physiol. Ecol. Jap.* **23**:17–29.

Yanagisawa, Y., and Nishida, M. 1991. The social and mating system of the maternal mouthbrooder *Tropheus moorii* (Cichlidae) in Lake Tanganyika. *Jap. J. Ichthyol.* **38**:43–50.