



Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism

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Received 20 March 2003; in revised form 11 April 2003; accepted 11 April 2003

Key words: mtDNA sequences, adaptive radiation, Lake Tanganyika cichlid fishes, Tropheini, trophic specialization

Abstract

One of the most surprising outcomes of recent molecular studies on cichlid fishes of the three Great East African Lakes Victoria, Malawi and Tanganyika, was the stunning rapidity of speciation and cladogenesis at early stages of adaptive radiation. Despite their rapid pace, speciation events were so far intuitively assumed to proceed in a bifurcating and tree-like fashion, even if they could not be resolved by gene phylogenies due to a lack of resolution. On the basis of phylogenetic analyses of the Tropheini, a lineage of endemic rock-dwelling cichlid fishes from Lake Tanganyika, we suggest a pathway of explosive speciation that accounts for a non-bifurcating manner of cladogenesis. This pattern is likely to be the result of the contemporaneous origin of a multitude of founder populations in geographically isolated rock habitats among which gene flow was interrupted simultaneously by a major change of the lake habitat in the form of a rapid rise of the lake level. As a consequence, all new species arising from that vicariance event must exhibit almost equal genetic distances to each other, within the scope of genetic diversity of the founder population(s), even if the actual processes of subsequent speciation and eco-morphological diversification followed independent routes. Our phylogeny also suggests a high frequency of parallel evolution of equivalent trophic specialization in the Tropheini. This phenomenon seems to be an inherent feature of this pathway of speciation, due to the action of similar selective forces on the same set of species colonizing isolated habitats of the same type. Explosive speciation via synchronization of genetic divergence triggered by rapid environmental changes seems to be particularly likely to occur at advanced stages of adaptive radiation, when species are already adapted to particular habitats and have a reduced ability for dispersal.

Introduction

The cichlid fishes of the Great Lakes in the East African rift valley represent a prime model system for the study of adaptive radiation (Fryer & Iles, 1972; Mayr, 1984; Greenwood, 1984; Coulter et al., 1986; Rossiter, 1995; Sturmbauer, 1998; Kornfield & Smith, 2000). Since the first reports on African cich-

lid species flocks appeared (Boulenger, 1898), many scientists have entered this area of research and substantial knowledge has accumulated in the fields of geology, climatology, limnology, as well as ecology, comparative morphology, behavioral sciences and taxonomy. Important insights concerning key innovations (Fryer & Iles, 1972; Liem, 1973), alternative modes of speciation (Rensch, 1933; Kosswig, 1947; Brooks,

1950; Schlieven et al., 1994; Turner, 1994; Danley & Kocher, 2001; Salzburger et al., 2002a), the role of sexual selection in speciation (Seehausen et al., 1997; Galis & Metz, 1998), and the occurrence of convergent evolution have been gained from these model systems (Kocher et al., 1993; Rüber et al., 1999).

Adaptive radiation is thought to be driven by two types of modulators: intrinsic factors in anatomy, ecology or behavior supplying the potential, and external events providing the opportunity, for radiation. An outstanding feature of the African Great Lakes – viewed as an important external mainspring to promote cichlid radiations (Coulter, 1994; Sturmbauer, 1998; Kornfield & Smith, 2000; Sturmbauer et al., 2001) – are water level changes, caused by variations in rainfall, temperature and evaporation, and for some lakes tectonic activity (Scholz & Rosendahl, 1988; Tiercelin & Modéguer, 1991; Cohen et al., 1993; Johnson et al., 1996; Lezzar et al., 1996; Cohen et al., 1997). For example, the 1997 El Niño event resulted in a marked increase of rainfall in East Africa causing Lake Tanganyika to rise by 2 m within 6 months. Any rise of the lake level may promote population subdivision and the colonization of new habitats by shifting the shoreline according to the basin structure of the lake. The degree of habitat change enforced by such water level fluctuations ranges from small-scale effects to major events that affect species communities throughout a lake simultaneously. In this way, lake level fluctuations may induce cycles of concerted allopatric speciation. Wherever the distance among suitable habitats exceeds the dispersal ability of a species, gene flow is interrupted, genetic differences will accumulate independently and lineage sorting will proceed to ultimately lead to the formation of new species.

The cichlid faunas of African lakes are subdivided into communities specialized to particular habitat types (Fryer & Iles, 1972; Coulter, 1994). Coexistence of a great number of species is possible by sometimes small differences in trophic specialization promoting effective resource partitioning (Hori, 1991; Sturmbauer et al., 1992; Genner et al., 1999), at least during critical periods of time when resources are scarce (McKaye & Marsh, 1983). Furthermore, eco-morphological and behavioral specialization to particular types of habitats such as rocky shores and sand bays affects the dispersal ability. More specialized species become isolated to a higher degree than less stenotopic and thus more mobile species. With 24 currently described species the endemic Tanganyikan

tribe Tropheini represents such a highly diverse assemblage of coastal fishes (Poll, 1986). The tribe's actual species number is probably much higher since almost all circumlacustrine taxa represent arrays of sister species (Sturmbauer & Meyer, 1992; Snoeks et al., 1994; Rüber et al., 1999; Baric et al., 2003). Most species of the Tropheini are epilithic algae feeders, specialized to feed on filamentous or unicellular algae, as well as on detritus. They have evolved spectacular ways to deal with food uptake, and marked differences in the pharyngeal anatomy and dentition served as basis for their subdivision at the generic level (Boulenger, 1898; Yamaoka, 1983; Poll, 1986). For example, all species assigned to the genus *Petrochromis* have elongated tricuspid teeth as adaptation to comb unicellular algae and detritus from the rock surface. Likewise, all members of the genus *Tropheus* possess an outer row of bicuspid teeth with a continuous cutting edge and curved conical teeth on the sides of the premaxillary bone (Poll, 1986). Due to their strict specialization to particular niches on rocky habitats most species have a limited ability for dispersal over ecological barriers (Brichard, 1978; Sturmbauer & Dallinger, 1995). This is reflected in the presence of numerous distinctly colored populations and/or sister species. Sister group of the Tropheini are haplochromine cichlids of the genus *Astatotilapia* (Sturmbauer & Meyer, 1993; Salzburger et al., 2002b), which live in swampy zones of Lake Tanganyika, as well as in surrounding rivers and lakes (Poll, 1986). Comparisons of average genetic distances to other lineages of cichlid fishes in Lake Tanganyika suggest that the Tropheini have evolved at a later stage of the radiation than many other tribes (Sturmbauer & Meyer, 1992, 1993; Sturmbauer et al., 1994, 1997). Their age, however, exceeds that of all *mbuna*- and *utaka*-species of Lake Malawi (Sturmbauer & Meyer, 1992; Sturmbauer et al., 2001), and the occurrence of incomplete lineage sorting at the species level has not been observed in all the Lake Tanganyika cichlid species analyzed so far. However, the phenomenon of ancient incomplete lineage sorting, as recently described by Takahashi et al. (2001), might have taken place at the onset of the radiation of the Tropheini.

We used a molecular approach to study the pathway of diversification of this lineage of cichlid fishes within a complex species flock at an advanced stage of radiation. We tested for the strength of the phylogenetic signal in all ancestral branchings of the Tropheini radiation and analyzed the possibility of contemporaneous cladogenesis. We also addressed the phe-

nomenon of parallel evolution of species having equivalent trophic specialization (see e.g. Kocher et al., 1993; Verheyen et al., 1996; Rüber et al., 1998, 1999; Baric & Sturmbauer, 1999; Hanel & Sturmbauer, 2000).

Materials and methods

We analyzed DNA sequences of two mitochondrial gene segments (402 bp of cytochrome *b*; and 385 bp of the control region) of a total of 38 specimens of nine genera of the Tropheini. *Astatotilapia burtoni*, which was identified as the sister group of the Tropheini (Sturmbauer & Meyer, 1993; Salzburger et al., 2002b), and *Haplochromis obesus* were used as outgroup (Table 1). We also included two specimens of the haplochromine cichlid *Ctenochromis horei* in our analyses, since it was tentatively placed within the Tropheini in earlier studies (see also Nishida, 1997), and further two specimens of the limnochrome cichlid *Gnathochromis pfefferi*, which was already shown to branch within the Tropheini (Kocher et al., 1995; Salzburger et al., 2002b). Voucher specimens are deposited at the Royal Museum for Central Africa in Tervuren, Belgium, under the numbers listed in Table 1, or are available from the authors. DNA-extraction, PCR-amplification, and automatic DNA-sequencing were performed according to standard methods (see Salzburger et al., 2002b) using published primers for both gene segments (Kocher et al., 1989; Salzburger et al., 2002b). DNA sequences were aligned by Clustal W (Thompson et al., 1994) and alignments were further increased by eye in the case of the control region. Phylogenetic analyses were performed in three steps. First, the strength of the phylogenetic signal was evaluated for the whole data set. Then phylogenetic trees were constructed using three alternative algorithms, and finally the resulting topologies were statistically evaluated. The different topologies were compared by the nonparametric two-tailed Wilcoxon signed rank test implemented in PAUP* 4.062a (Swofford, 2000), maximum-likelihood scores of the topologies were evaluated by the non-parametric Shimodaira-Hasegawa test (Shimodaira & Hasegawa, 1999) as implemented in PAUP* (Swofford, 2000). Finally, to test whether or not our data support monophyly of the genera in the Tropheini, we performed the topology-dependent permutation tail probability (T-PTP) test available in PAUP* (Swofford, 2000), using a con-

strained topology in which we forced monophyly of the genera, and 100 replications.

In the first step of the analysis the presence of a phylogenetic signal in the entire data set was tested by means of a likelihood mapping analysis using the computer program Puzzle 4.0 (Strimmer & von Haeseler, 1996). Likelihood mapping is a permutation method evaluating the likelihood support for the three alternative topologies of multiple sets of four taxa (quartets) to assess the overall phylogenetic signal in the data set. The likelihoods of the three alternative topologies of all evaluated taxon quartets are mapped on a triangle representing a barycentric coordinate system. The triangle is partitioned into seven distinct regions: the central region representing completely unresolved topologies, the three corners representing fully resolved topologies, and three intermediate regions between the corners representing partially resolved topologies. This analysis was carried out twice, with and without the outgroup taxa. Next, the relative rates of base substitutions were evaluated for all 42 ingroup taxa. We also carried out this test with a more inclusive data set representing all twelve Tanganyikan lineages. These tests were necessary to justify the use of genetic distances for estimating relative ages of the lineages of the Tropheini, as well as for the determination of relative ages with respect to other Tanganyikan lineages. To this end, we performed the branch length test, which is implemented in the computer program LINTRE (Takezaki et al., 1995).

For phylogenetic reconstruction we performed maximum parsimony, neighbor-joining and maximum likelihood analyses in parallel using the computer-program PAUP* 4.0b2a (Swofford, 2000). All base substitutions and indels were weighted equally in maximum parsimony, Kimura-2-Parameter (Kimura, 1980) and Jukes-Cantor distances (Jukes & Cantor, 1969) were used for neighbor-joining. The appropriate model of molecular evolution for the maximum likelihood analysis was evaluated by a likelihood ratio test implemented in the computer program Modeltest 3.0 (Posada & Crandall, 1998). This test justified the use of the HKY model of molecular evolution (Hasegawa et al., 1985) including invariable sites and a gamma correction. We applied the proportion of invariable sites (0.497), the gamma shape parameter (0.607), the base frequencies (A: 0.295; C: 0.259; G: 0.130; T: 0.316), and the transition/transversion ratio (4.407) estimated during the search. We assessed the robustness of the resulting topologies by applying standard measures of confidence (bootstrap and quartet-puzzling

Table 1. Characterization of the studied species

Tribe ¹	Taxon	GenBank Accession Number		Voucher	Habitat	Diet
		Cytochrome <i>b</i>	Control region			
Haplochromini	<i>Astatotilapia burtoni</i>	Z21773 ³	Z215751 ³		swamps	Invertebrates
	<i>Haplochromis obesus</i>	Meyer et al., 1990 ³	U12552 ³		L. Victoria	
	<i>Ctenochromis horei</i>	AY301926	AY301952	1145-T1992	ubiquitous	Invertebrates
Limnochromini	<i>Gnathochromis pfefferi</i>	AY301928	AY301954	893-T1992	rock/cobble	Invertebrates
	<i>Gnathochromis pfefferi</i>	AY301929	AY301955	1089-T1992	rock/cobble	Invertebrates
Tropheini	<i>Limnotilapia dardennii</i>	AY301930	AY301956	244-T1992	rock/cobble	Invertebrates
	<i>Limnotilapia dardennii</i>	AY301931	AY301957	1158-T1992	rock/cobble	Invertebrates
	<i>Lobochilotes labiatus</i>	AY301932	AY301958	1376-T1992	rock	Invertebrates
	<i>Lobochilotes labiatus</i>	AF428170 ³	AF400733 ³		rock	Invertebrates
	<i>Petrochromis ephippium</i>	AY301933	AY301959	1084-T1992	rock	Aufwuchs
	<i>Petrochromis famula</i>	AY301934	AY301960	450-T1992	rock	Aufwuchs
	<i>Petrochromis famula</i>	AY301935	AY301961	579-T1992	rock	Aufwuchs
	<i>Petrochromis famula</i>	AY301936	AY301962	995-T1992	rock	Aufwuchs
	<i>Petrochromis famula</i>	AY301937	AY301963	1019-T1992	rock	Aufwuchs
	<i>Petrochromis orthognathus</i>	AY301938	AY301964		rock	Aufwuchs
	<i>Petrochromis orthognathus</i>	AY301939	AY301965		rock	Aufwuchs
	<i>Petrochromis orthognathus</i>	AY301940	AY301966	1242-T1992	rock	Aufwuchs
	<i>Petrochromis polyodon</i> "elongate"	AY301941	AY301967	391-T1992	rock	Aufwuchs
	<i>Petrochromis polyodon</i> "elongate"	AY301942	AY301968	603-T1992	rock	Aufwuchs
	<i>Petrochromis polyodon</i> "blue-fin"	AY301943	AY301969	692-T1992	rock	Aufwuchs
	<i>Petrochromis polyodon</i> "blue-fin"	AY301944	AY301970	693-T1992	rock	Aufwuchs
	<i>Petrochromis sp.</i> UBWARI	AY301945	AY301971		rock	Aufwuchs
	<i>Petrochromis trewavasae</i>	AY301946	AY301972	331-T1992	rock	Aufwuchs
	<i>Pseudosimochromis curvifrons</i>	AY301947	AY301973	1345-T1992	rock/sand	Aufwuchs
	<i>Pseudosimochromis curvifrons</i>	AY301948	AY301974	1346-T1992	rock/sand	Aufwuchs
	<i>Simochromis babaulti</i>	Z12045 ³	AF400736 ³		rock	Aufwuchs
	<i>Simochromis babaulti</i>	AY301949	AY301975	724-T1992	rock	Aufwuchs
	<i>Simochromis babaulti</i>	AY301950	AY301976	726-T1992	rock	Aufwuchs
	<i>Simochromis diagramma</i>	AY301951	AY301977	793-T1992	rock	Aufwuchs
	<i>Tropheus brichardi</i> NYANZA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus duboisi</i>	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus duboisi</i>	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus moorii</i> BULU	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus moorii</i> CHAITIKA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus moorii</i> IKOLA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus moorii</i> KALA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus moorii</i> KAVALLA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
	<i>Tropheus moorii</i> KIRIZA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs
<i>Tropheus moorii</i> LUPOTA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs	
<i>Tropheus moorii</i> RUTUNGA	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs	
<i>Tropheus moorii</i> WAPEMBE	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs	
<i>Tropheus moorii</i> ZONGWE	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs	
<i>Tropheus polli</i> BULU	Sturmbauer & Meyer, 1992 ³	Sturmbauer & Meyer, 1992 ³		rock	Aufwuchs	

¹According to Poll (1986)²Deposited at the Royal Museum for Central Africa, Tervuren, Belgium³Sequences published elsewhere

frequencies) with 1000 pseudo-replicates (bootstrap) and the evaluation of 10 000 random quartets (quartet-puzzling), and in the case of maximum likelihood under the fast stepwise addition option in PAUP* 4.0b2a (Swofford, 2000).

To evaluate the supports for distinct internal branches critical for the interpretation of the evolu-

tionary pathways, a series of four-cluster likelihood analyses (Strimmer & von Haeseler, 1997) were performed. In this method four clusters of taxa are pre-defined to calculate the relative frequencies of each of the three possible topologies interrelating the members of the four taxon clusters. Likelihoods of all evaluated quartet trees for each subset of four taxa are mapped

on a triangle. The triangle is now partitioned in three regions, each defining the likelihood frequencies of one of the three alternative topologies. This method also allows to delineating the onset of major diversification events, which are indicated by clusters of very short internal branches. For this analysis we used a reduced data-set of 17 taxa (plus one outgroup taxon) with a similar rate of base substitution according to the branch length test implemented in LINTRE (Takezaki et al., 1995). In the second data-set with a reduced number of taxa, each species or closely related assemblage, that was consistently identified by the first phylogenetic analyses, was represented by a single sequence only. From each cluster we selected the sequence with a root-to-tip distance that was closest to the average root-to-tip distance of 0.067 identified in the branch length test in LINTRE. For the second data-set, we again performed parsimony, neighbor-joining (Saitou & Nei, 1987) and maximum likelihood analyses and declaring *Astatotilapia burtoni* as outgroup. Modeltest 3.0 (Posada & Crandall, 1998) identified the HKY model (Hasegawa et al., 1985) with invariable sites and a gamma correction as optimal model of molecular evolution. We used the proportion of invariable sites (0.529), the gamma shape parameter (0.598), the base frequencies (A: 0.283; C: 0.264; G: 0.144; T: 0.309), and the transition/transversion ratio (4.566) estimated during the search.

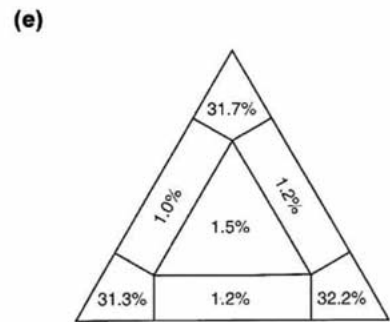
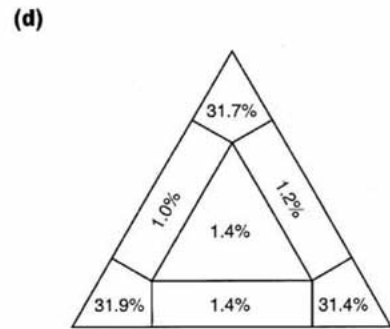
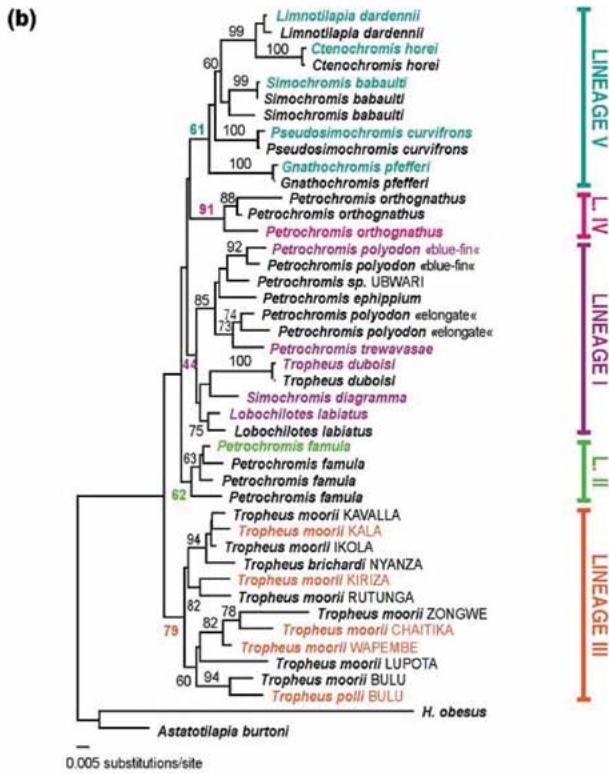
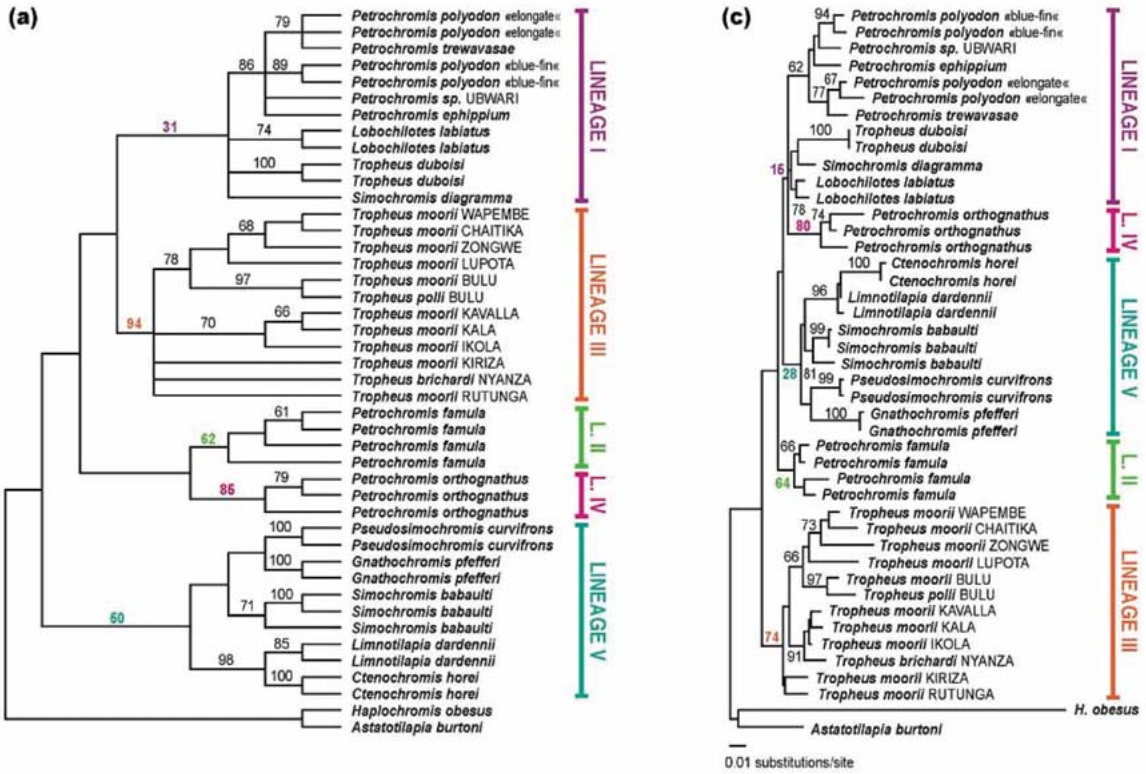
In the first three four-cluster analyses of the second data set the outgroup was defined as the first cluster and the five lineages identified were grouped into the remaining three clusters according to the different topologies found by the three tree-building methods. Then the outgroup was omitted and four new clusters were defined, depending on which discrete internal branch was under evaluation. The five lineages (defined according to the phylogenetic analyses) were clustered into the ten possible sets of quartets, and all 30 possible quartet topologies were tested for their relative supports.

As a complementary approach to estimate the relative age of the Tropheini and the time frame of lineage formation, we calculated the average genetic distances among all five lineages of the Tropheini by including all possible pairwise groupings of taxa. We then compared minimum, maximum and average Kimura distances of the Tropheini with those published for the Lamprologini and the genus *Tropheus* (Sturmbauer & Meyer, 1993; Sturmbauer et al., 1994; see also Baric et al., 2003). This comparison was based on sequences of the control region only.

Results

Phylogenetic analyses suggested the formation of five distinct lineages at the very base of the radiation of the Tropheini (named lineage I – V in Figs 1 and 2), which were consistently recovered in all analyses. Lineage I consisted of *Lobochilotes labiatus*, *Simochromis diagramma*, *Tropheus duboisi*, and a clade including *Petrochromis trewavasae*, *P. sp. UBWARI*, *P. polyodon* ‘elongate’, *P. polyodon* ‘blue-fin’, and *P. ehippium*. *Petrochromis polyodon* ‘elongate’ consistently clustered with *P. trewavasae*, while *P. polyodon* ‘blue-fin’ was resolved in a second clade in neighbor-joining and maximum-likelihood, together with *P. sp. UBWARI* and *P. ehippium*. Lineage II contained the four representatives of *Petrochromis famula*. Lineage III combined the different local forms of *Tropheus moori*, as well as *T. polli* and *T. brichardi*. Lineage IV comprised three specimens of *Petrochromis orthognathus*. Finally, lineage V consisted of *Gnathochromis pfefferi*, *Pseudosimochromis curvifrons*, *Simochromis babaulti*, and a clade formed by *Limnotilapia dardennii* and *Ctenochromis horei*.

The monophyly of four lineages (II, III, IV and V in Fig. 1) was supported by high numbers of synapomorphs, as well as by adequate bootstrap- and likelihood values in all analyses. The fifth lineage (lineage I) was consistently found by all three phylogenetic methods, but was defined by relatively few synapomorphs. The most striking observation was that – despite the excellent overall phylogenetic signal in the data set (Fig. 1d,e) – there was substantial conflict in the branching order that defined the emergence of the major lineages at the base of the radiation. Maximum parsimony yielded 32 most parsimonious trees of a tree length of 570 evolutionary steps (consistency index excluding uninformative sites, 0.41; retention index, 0.67; the strict consensus topology is depicted in Fig. 1a). Lineage V occupied the most ancestral branch in all 32 most parsimonious trees, sister group to a clade comprised by lineages II and IV, and a clade formed by lineages I and III. In neighbor-joining, lineage III occupied the most ancestral position, sister group to a clade containing lineage II, lineage V, lineage IV, and lineage I (Fig. 1b). In the maximum likelihood tree lineage III formed the most ancestral branch, followed by a clade comprising lineages II, I, IV and V (Fig. 1c). Neither in the Wilcoxon signed rank test ($P < 0.05$) nor in the Shimodaira-Hasegawa test ($P < 0.05$) a particular topology was favored.



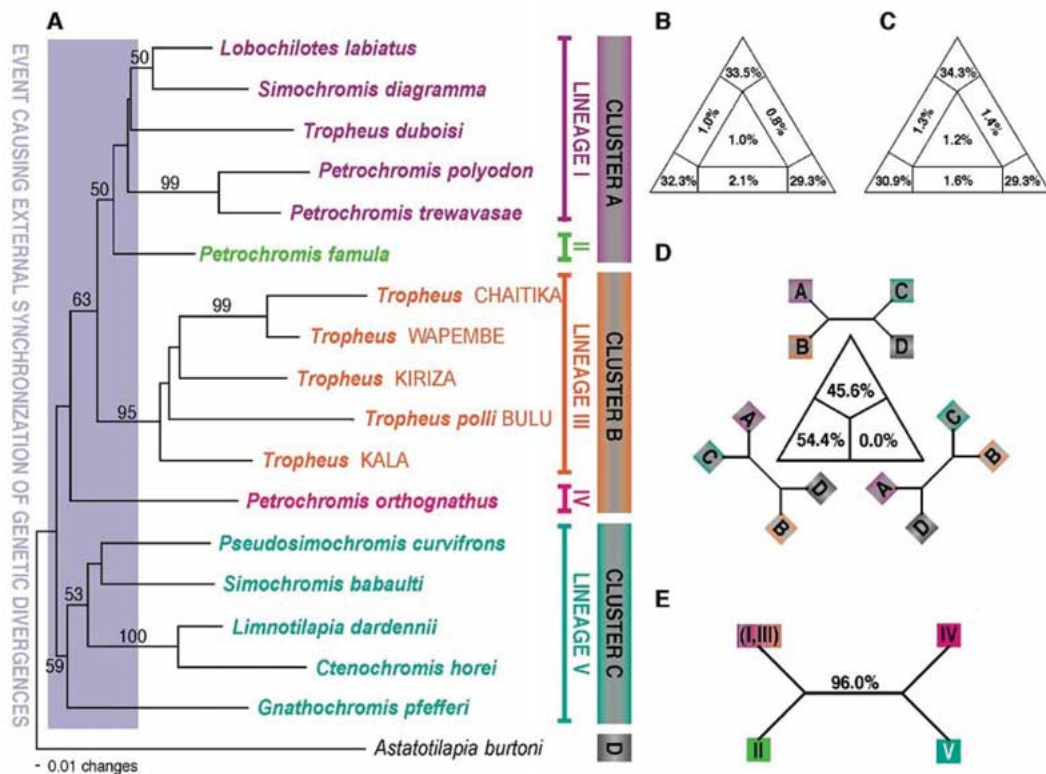


Figure 2. One example for a four-cluster likelihood analysis (Strimmer & Von Haeseler, 1997) evaluating the support for distinct internal branches. For this analysis a reduced data-set including 17 taxa (plus the outgroup *Astatotilapia burtoni*) was used. (a) Neighbor-joining tree based upon Kimura-2-parameter distances of a 402 bp segment of the cytochrome *b* and a 385 bp segment of the control region. (b), (c) Likelihood mapping analysis demonstrating the presence of a strong overall phylogenetic signal in the data set including (b) and excluding (c) the outgroup. (d) Four-cluster likelihood mapping analysis testing the relative likelihoods of the three alternative topologies of the four taxon-clusters (A, B, C and D in (a)) derived from the neighbor joining phylogeny. None of the three possible topologies is clearly favored pointing to a lack of phylogenetic signal in these basal branches. This suggests a contemporaneous origin of the lineages. Using this method we tested all alternative topologies that were obtained in our phylogenetic analyses using alternative tree building algorithms. (e) Example of a quartet topology excluding the outgroup with a different grouping of lineages in comparison to (d). See text and Figure 3 for further details.

The topology dependent T-PTP test, however, rejected monophyly of the genera ($P = 0.01$).

The maximum parsimony analysis of the second data set with a reduced number of taxa yielded three most parsimonious trees of a length of 305 steps (consistency index excluding uninformative sites, 0.48; retention index, 0.54, tree not shown). Lineage II was

resolved as sister group to the remaining four lineages in all three most parsimonious trees. In the neighbor-joining tree, lineage V was resolved as sister group to the remaining four lineages, sister group to a clade containing lineage IV, lineage III, lineage II, and lineage I (Fig. 2a). In the maximum likelihood tree, lineage III formed the most ancestral branch, sister

Figure 1. Phylogenetic analyses of the endemic Tanganyikan tribe Tropheini based upon 402 bp of the mitochondrial cytochrome *b* gene and 385 bp of the mitochondrial control region. *Astatotilapia burtoni* and *Haplochromis obesus* were used as outgroups. Bootstrap values larger than 60% are depicted above the corresponding branches, except for the branches defining the five major lineages (I, II, III, IV, V), for which the values were also depicted when they were smaller than 60%. The generally short internal branches at the basis highlight the rapidity of lineage formation in this group of cichlid fishes. (a) Strict consensus tree obtained from the 32 most parsimonious trees (tree length, 570; consistency index excluding uninformative sites, 0.41). (b) Neighbor-joining topology based upon Kimura-2-parameter distances. (c) Maximum likelihood tree applying the HKY+I+ Γ model for molecular evolution. Further details are given in the material and method section. (d), (e) Likelihood mapping analysis (Strimmer & von Haeseler, 1997) demonstrating the presence of a strong overall phylogenetic signal in the data set including (d) and excluding (e) the outgroup.

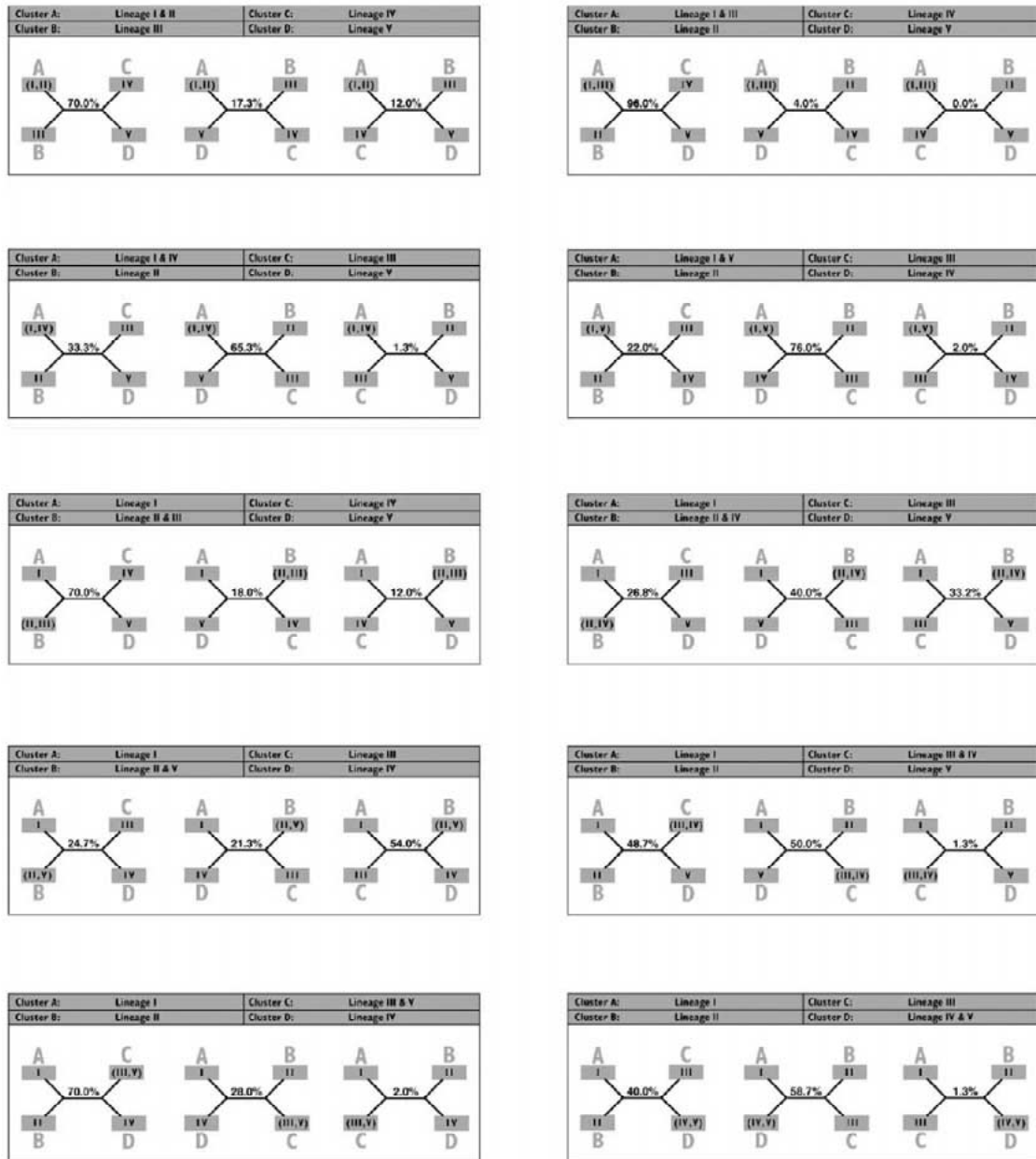


Figure 3. Four-cluster likelihood mapping analyses of 30 possible alternative topologies of the five lineages identified in the Tropheini (I–V). The lineages are defined in Figures 1 and 2. Each box contains the results of one four-cluster likelihood mapping analysis of 10 possible four cluster-groupings of the five lineages. The relative percentage fractions were inconsistent with respect to the tested topologies and highlight the lack of a clear phylogenetic signal.

group to lineage IV and a clade comprising lineages IV, V and I (not shown).

In the four-cluster likelihood mapping analyses, in which all alternative topologies from the three tree building algorithms of the reduced data-set were systematically evaluated, the absence of phylogenetic

signal at the base of the radiation became further evident. None of the four-cluster likelihood mapping analyses clearly favored one particular topology. This is depicted for the neighbor-joining tree in Figure 2d, in which two out of the three possible cluster topologies were found to be equally favored (45.6%; 54.4%;

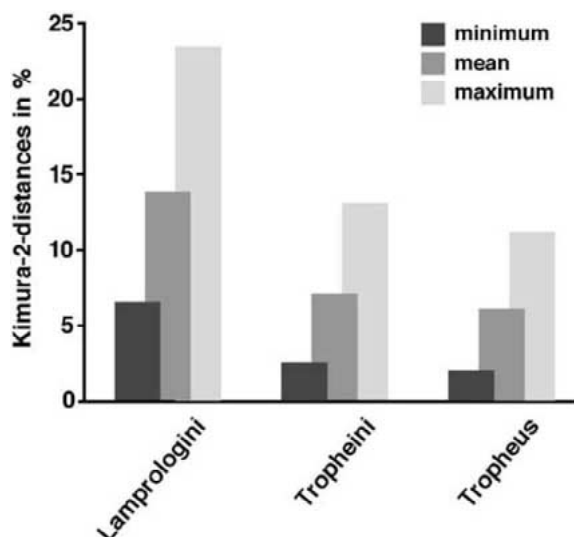


Figure 4. Minimum, average and maximum Kimura distances among lineages of the Tanganyikan tribes Lamprologini (seven lineages, Sturmbauer et al., 1994) and Tropheini (five lineages), compared to those found within the genus *Tropheus* alone (Sturmbauer & Meyer, 1992; Sturmbauer et al., 1997; Baric et al., 2003), based upon 383 bp of the control region. The comparison highlights the younger evolutionary age of the Tropheini as compared to the Lamprologini. The Lamprologini must have undergone radiation during an earlier phase in the lake history. The almost identical ranges of genetic distances observed in the Tropheini and the genus *Tropheus* alone indicates an immediate subdivision of the *Tropheus*-lineage (Lineage III in Fig. 1a) after its formation.

0.0%). The same was found in the four-cluster likelihood analyses of the maximum likelihood tree (46.7%; 50.0%; 3.3%), and of the three most parsimonious trees (59.0%; 34.7%; 6.3%). The lack of support for one particular branching order of the five lineages was further signaled by the fact that the most highly supported topologies differed in all three algorithms. The four-cluster likelihood mapping analyses of all 30 possible groupings of the five lineages also gave conflicting results and did not favor one particular branching order (Fig. 3).

The comparison of genetic distances corroborates the almost contemporaneous origin of the five lineages. The average genetic distances among the five lineages of the Tropheini amounted to 7.01% (standard deviation = 0.018; minimum = 2.57%; maximum = 13.28%; number of pairwise comparisons = 665). Moreover, the average genetic distance within the genus *Tropheus* alone (lineage III) was only slightly smaller than that observed among the five lineages of the Tropheini (average = 6.14%; minimum = 4.6; maximum 7.9%; see Baric et al., 2003). This ob-

servations suggests that *Tropheus* diversified further immediately after its emergence (Fig. 4). The smaller average genetic distances among the five lineages of the Tropheini in relation to those observed among lineages of the Lamprologini (Sturmbauer et al., 1994) indicate that the Tropheini must thus have undergone radiation at a much later stage than the Lamprologini. According to a tentative calibration of the control region of East African cichlid fishes a Kimura-distance of 7.01% would translate into an age between 807 ka and 1092 ka (Sturmbauer et al., 2001).

Discussion

A stunningly rapid pace of speciation and cladogenesis, particularly at early stages of adaptive radiation, has been repeatedly demonstrated for African cichlid fishes (Meyer et al., 1990; Sturmbauer & Meyer, 1992, 1993; Sturmbauer et al., 1994; Verheyen et al., 1996, 2003; Rüber et al., 1998, 1999; Nagl et al., 1998, 2000; Salzburger et al., 2002b). Despite their rapid pace, speciation events in cichlid fishes were assumed to proceed in a bifurcating and tree-like fashion (Coulter, 1994; Ribbink, 1994; Turner, 1994). Bush phylogenies may be either interpreted as 'soft polytomy' – an analytical artefact, e. g., due to a lack of resolution of the gene segment analyzed and the phylogenetic method used –, or a 'hard polytomy' – generated by a truly simultaneous branching (Maddison, 1989, see Jackman et al., 1999 for discussion). Our phylogenetic analysis suggests a truly bush-like manner of cladogenesis in the Tropheini. The contemporaneous formation of five major lineages is independently supported by three findings. First, the internal branches interrelating these lineages were extremely short leading to a substantial degree of conflict in the resulting tree topologies, despite the excellent overall phylogenetic signal in the data set. Second, these alternative topologies turned out to be equally supported in the four-cluster likelihood mapping analyses, the Wilcoxon signed rank test and the Shimodaira-Hasegawa test. Third, the relative phylogenetic distances among the lineages were strikingly similar. Given the strength of the overall phylogenetic signal in the data set, such a lack of phylogenetic signal in the ancestral branches can only be interpreted as the consequence of a rapid cladogenesis event.

This leads us to suggest that the observed pattern of evolution is the consequence of a contemporaneous origin of several lineages of the Tropheini, and not an

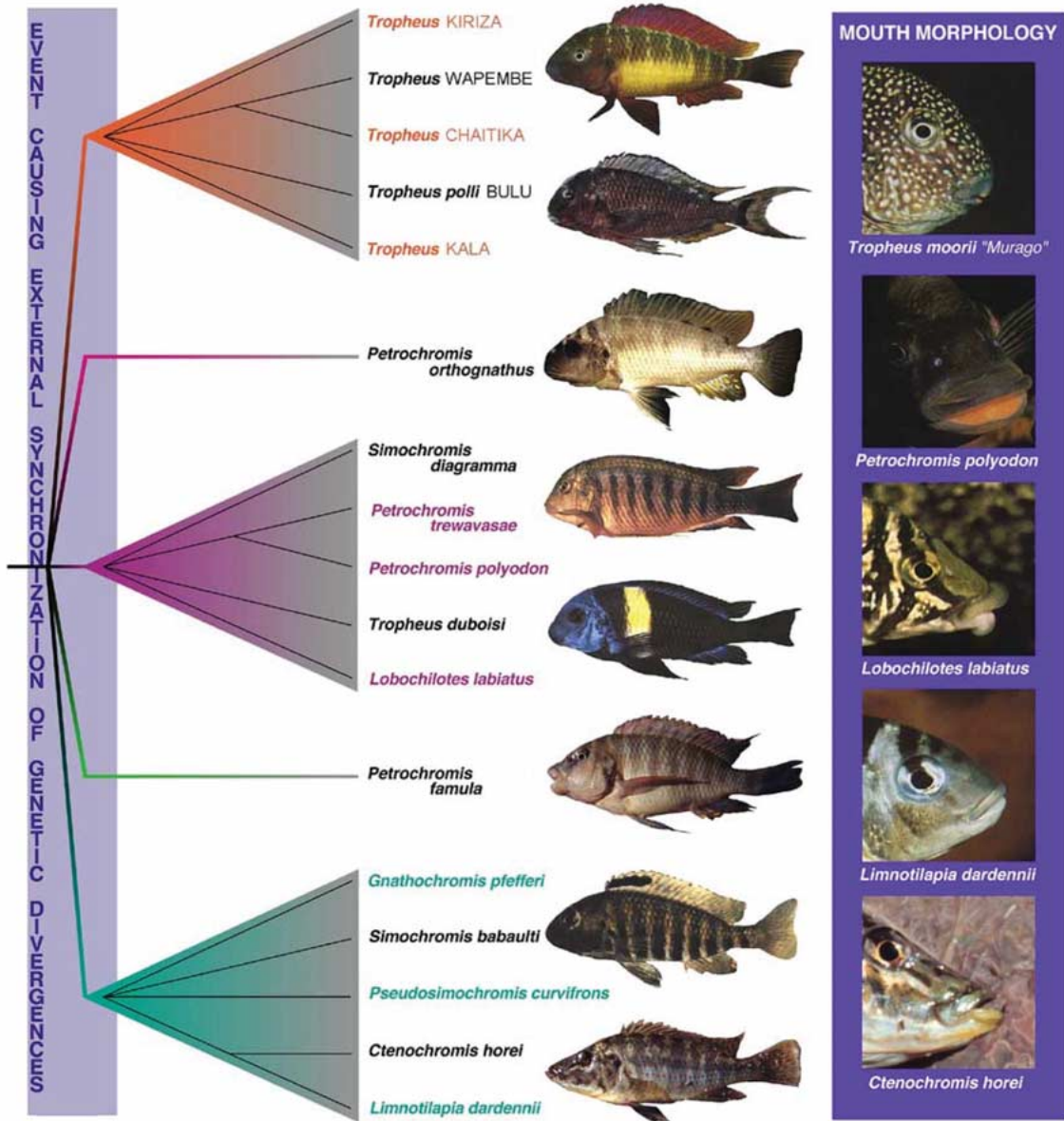


Figure 5. Phylogenetic hypothesis for the radiation of the Tropheini based upon a strict consensus tree of parsimony, neighbor-joining and maximum likelihood, indicating that five distinct lineages arose contemporaneously, triggered by an environmental change. The depicted species (names are in black) and mouth morphologies underline the extreme degree of eco-morphological diversity within and among the five lineages. The paraphyletic placement of members of the genera *Tropheus*, *Simochromis* and *Petrochromis* further points to a high probability of recurrent evolution of corresponding morphologies under this mode of speciation, due to the action of similar selective forces in geographically isolated habitats of the same type.

artifact resulting from the limited power of resolution of the phylogenetic analyses. Concerted cladogenesis can only be induced by a synchronized vicariance event, triggered by external influences enforcing dramatic habitat changes. According to various models of allopatric speciation temporary spatial isolation of

populations is caused by the discontinuous distribution of suitable habitats in an ecosystem, and the influence of external events changing habitats and/or their distribution (Sturmbauer, 1998). In the case of African cichlid fishes, lake level fluctuations were shown to be powerful modulators of habitat distribu-

tion (Sturmbauer, 1998; Sturmbauer et al., 2001). Taking into account that Lake Malawi rose by about 100 m within the last 200 years (Owen et al., 1990), one of the most striking characteristics of lake level fluctuations is their rapidity. The onset of spatial and hence genetic isolation is thus effectively synchronized by a rapid rise of the lake level, enforcing an almost concurrent split of lowstand populations into several units. Two recent studies showed that such synchronous population subdivisions are clearly mirrored in the observed patterns of genetic relatedness of cichlid populations in Lakes Victoria, Malawi and Tanganyika (Sturmbauer et al., 2001; Baric et al., 2003). Populations arising from the same vicariance event show equal genetic distances to each other. In relation to the pace of molecular evolution of mitochondrial genes, a bush-phylogeny interrelating all lineages arising from such a synchronized dispersal incident is the inevitable consequence (Fig. 5). It may also be noted here that the degree of genetic variation of all lineages arising from the same vicariance event depends on the genetic diversity of the founder population at the time of subdivision. This diversity defined the starting point of lineage sorting during the process of coalescence.

An equal age of lineages does not imply that subsequent speciation events in each of the founder populations occurred concurrently. Speciation events may happen at different times in any of those populations, since they depend on local selective forces. Extant species belonging to different lineages that were once formed contemporaneously are almost equidistant in terms of genetic distances to each other, while species originating in the same lineage may show smaller and different genetic distances to each other. When several populations are formed at the same time – e.g. by a major rise of the lake level –, several lineages may subsequently undergo speciation. Explosive speciation via synchronization of genetic divergence seems to be more likely at advanced stages of adaptive radiation, since the involved species must have particular biological characteristics providing the potential to undergo further diversification. Their ability for active dispersal over ecological barriers must be limited, so that major dispersal events can only be induced externally by habitat shifts such as lake level fluctuations. It should be noted that in some cases populations may also be split by a decrease of the lake level, for example when islands become exposed.

A series of molecular phylogenetic analyses suggested *Astatotilapia burtoni* as the closest relative of the Tropheini. This species is widely distributed in

swampy areas and rivers in and around Lake Tanganyika and does not seem to have a reduced ability for dispersal at all. Our phylogeny suggests that the evolution of a reduced ability for active dispersal due to specialization to patchy niches may represent a key innovation allowing to undergo speciation via synchronization of genetic divergence. This feature must have evolved at the very beginning of the radiation in the common ancestor of the Tropheini. The ancestral species has most likely gone extinct due to competition of better adapted descendants (see Sturmbauer, 1998 p. 23).

Our phylogenetic analyses further confirmed *Gnathochromis pfefferi* as a member of the Tropheini (Kocher et al., 1995, Salzburger et al., 2002b) and also suggested the placement of the haplochromine cichlid *Ctenochromis horei* in this tribe (see also Nishida, 1997). Another striking observation from our phylogeny – corroborating previous results based on allozyme data (Nishida, 1997) – is its frequent conflict with the present taxonomic assignments suggesting that several genera may be paraphyletic, so that corresponding trophic specializations may have evolved repeatedly within a single lake. This was observed for the genus *Tropheus* in which *T. duboisi* was consistently placed outside the lineage comprising its congeneric allies, as well as for the genera *Petrochromis* and *Simochromis* (Fig. 5). All these genera were defined primarily on the basis of their particular trophic morphology (Boulenger, 1898; Yamaoka, 1983; Poll, 1986). Such a high frequency of recurrent evolution of convergent morphologies can best be explained as an inherent feature of the proposed mode of speciation, since geographically isolated habitats of the same type should create similar selective forces on their species communities. Moreover, natural selection acted on the same set of colonizing species, so that the initial conditions were likely to be similar in all founder populations. Particular ecological and morphological adaptations, which provide effective resource partitioning, may have been independently favored by natural selection in isolated communities. Under these particular environmental conditions, Stephen J. Gould's mind experiment of 'replaying life's tape' (Gould, 1990) has been performed by nature to indeed produce similar evolutionary outcomes. As a consequence, morphology-based taxonomic analyses interpreting similar pharyngeal morphologies as synapomorphs, justifying the placement of eco-morphologically equivalent taxa in a single genus, may often be misled.

Ancient incomplete lineage sorting was recently suggested as explanation for the para- or polyphyletic placement of inserted retroposons (SINE) among lineages of cichlid fishes in Lake Tanganyika (Takahashi et al., 2001). Incongruence of genetic characters can have two causes: homoplasy and ancestral polymorphism. O'Uigin et al. (2002) argued in a model study on anthropoid primates that homoplasy is a more likely contributor to incongruence in more ancient cladogenesis events, while the persistence of ancestral polymorphism is more likely in younger evolutionary splits. When ancestral polymorphisms undergo lineage sorting, a gene phylogeny that is incongruent to the species phylogeny might be the result. After the divergence of each lineage, alternative nuclear alleles or mitochondrial haplotypes might become fixed stochastically. It is important to note that it is the relative number of incongruent character state changes, which might mislead phylogenetic analyses. Mitochondrial genes tend to evolve more rapidly than nuclear genes and lineage sorting occurs faster due to maternal inheritance, so that the time window of phylogenetic resolution is shorter than for nuclear loci. Our analysis of the Tropheini points to a great degree of conflict. This conflict, however, is mainly due to the lack of phylogenetically informative characters in the ancestral branches. Ancient incomplete lineage sorting, however, might also contribute to the observed conflicts in the phylogenetic analysis of the Tropheini, and it should thus be seen as another indicator for extremely rapid cladogenesis.

All morphologically distinguishable taxa were also genetically distinct in our mitochondrial phylogeny. This observation is in clear contrast to analyses on Lake Malawi and Victoria cichlids (Moran & Kornfield, 1993; Parker & Kornfield, 1997; Nagl et al., 1998). However, this is congruent to the age estimate for the genus *Tropheus*, which may be five token times the age of the entire Lake Victoria cichlid species flock and about the same age as the Lake Malawi cichlid flock (Sturmbauer & Meyer, 1992; Verheyen et al., 2003).

The scenario of speciation proposed here does not rule out the occurrence of sympatric speciation within any geographically isolated population. Species arising from sympatric or micro-allopatric speciation events are more closely related to each other, but they exhibit the same genetic distances to any species of a different population that arose from the primary vicariance event. Previous works on sticklebacks reported a similar evolutionary scenario, in which species gradu-

ally colonized newly emerging lakes during glacial retreat and repeatedly evolved eco-morphologically equivalent pairs of sympatric species (Schluter & McPhail, 1992; Taylor & McPhail, 2000). The crucial difference to the scenario proposed here is the relatively slow succession of repeated colonization events and their origin from more than one refugee population. In the Tropheini, recurrent evolution of equivalent species pairs is not connected to repeated colonization events.

Our data suggest that speciation via synchronization of genetic divergence is likely to be an important promoter of species multiplication in flocks of African cichlid fishes. We are convinced that this pattern of speciation will also be found in other organisms, whenever the ancestral species are already adapted to discontinuous habitats, which rapidly shift due to externally induced events.

Acknowledgements

We wish to thank J. Snoeks and L. Makasa for species identification. We are further grateful to L. Rüber, G. and R. Rieger, P. Ladurner, M. Taylor, J. and W. Wieser for comments on the manuscript. C. S. and S. B. were supported by the Austrian Science Foundation (grants 12339 and 15239) and the Jubiläumsfonds der Österreichischen Nationalbank, E. V. by the Belgian Science Foundation (grant FKFO-MI 30.35) and the Belgian Federal Services for Scientific, Cultural and Technical Affairs (grant DWTC 31.34), and W. S. by a DOC-fellowship of the Austrian Academy of Sciences and a Marie Curie Fellowship of the EU.

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