Separated by sand, fused by dropping water: habitat barriers and fluctuating water levels steer the evolution of rock-dwelling cichlid populations in Lake Tanganyika

STEPHAN KOBLMÜLLER,* WALTER SALZBURGER,† BEATE OBERMÜLLER,* EVA EIGNER,* CHRISTIAN STURMBAUER* and KRISTINA M. SEFC*

Department of Zoology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria, †Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

Abstract

The conditions of phenotypic and genetic population differentiation allow inferences about the evolution, preservation and loss of biological diversity. In Lake Tanganyika, water level fluctuations are assumed to have had a major impact on the evolution of stenotopic littoral species, though this hypothesis has not been specifically examined so far. The present study investigates whether subtly differentiated colour patterns of adjacent *Tropheus moorii* populations are maintained in isolation or in the face of continuous gene flow, and whether the presumed influence of water level fluctuations on lacustrine cichlids can be demonstrated in the small-scale population structure of the strictly stenotopic, littoral *Tropheus*. Distinct population differentiation was found even across short geographic distances and minor habitat barriers. Population splitting chronology and demographic histories comply with our expectation of old and rather stable populations on steeper sloping shore, and more recently established populations in a shallower region. Moreover, population expansions seem to coincide with lake level rises in the wake of Late Pleistocene megadroughts ~100 KYA. The imprint of hydrologic events on current population structure in the absence of ongoing gene flow suggests that phenotypic differentiation among proximate *Tropheus* populations evolves and persists in genetic isolation. Sporadic gene flow is effected by lake level fluctuations following climate changes and controlled by the persistence of habitat barriers during lake level changes. Since similar demographic patterns were previously reported for Lake Malawi cichlids, our data furthermore strengthen the hypothesis that major climatic events synchronized facets of cichlid evolution across the East African Great Lakes.

Keywords: climate change, colour pattern variation, demographic history, divergence with gene flow, population structure, *Tropheus moorii*

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Introduction

Cases of intraspecific variation provide good opportunities to study the evolutionary processes responsible for phenotypic and genetic diversification, for example how drift and/or selection counteract the effects of gene flow in the absence of reproductive isolation (e.g. King & Lawson 1995). In this respect, animal body coloration

Correspondence: Kristina M. Sefc, Fax: +43-(0)316-3809875; E-mail: kristina.sefc@uni-graz.at

has received much attention as it responds to natural and sexual selection (e.g. Endler 1983; Seehausen & van Alphen 1999; Galeotti *et al.* 2003; Salzburger 2009), but may also evolve through drift alone (e.g. Brakefield 1990; Hoffman *et al.* 2006). Perhaps even more often, different evolutionary forces act in combination (e.g. Rudh *et al.* 2007). Colour divergence in some species of the haplochromine cichlids of Lakes Victoria and Malawi has been ascribed to sexual selection on male nuptial colour patterns (e.g. Seehausen & van Alphen 1999; Knight & Turner 2004), but may in other instances

be a by-product of reproductive isolation based on other cues (Plenderleith *et al.* 2005; Amorim *et al.* 2008; Seehausen *et al.* 2008), and also of random drift (Arnegard *et al.* 1999). In Lake Tanganyika, many of the endemic (mostly non-haplochromine) cichlid species exhibit allopatric colour variation to various extents (Konings 1998). Interestingly, there is no predictive relationship between the amount of geographic colour pattern diversity and population structure (Duftner *et al.* 2006, 2007; Koblmüller *et al.* 2007, 2009a; Sefc *et al.* 2007a). Allopatric colour pattern variation is most pronounced in the genus *Tropheus* (tribe Tropheini, phylogenetically nested within the Haplochromini; Salzburger *et al.* 2005; Koblmüller *et al.* 2008, 2010) with about 120 described colour morphs (Schupke 2003).

Most *Tropheus* populations are sexually monomorphic, and both sexes are territorial and employ colour signals in intra- and intersexual communication (e.g. Sturmbauer & Dallinger 1995). Studies of admixed populations in the lake (Salzburger *et al.* 2006) and laboratory mate choice experiments (Egger *et al.* 2008, 2010) showed variable degrees of assortative mating preferences between morphs. In particular, females of the morph analyzed in the present study did not distinguish between males of their own morph and the distinctly different 'Nakaku' morph (Fig. 1) in lab experiments. Two more similar, but still clearly distinguishable populations of red *Tropheus* showed no assortative preferences at all (Egger *et al.* 2010). This lack of discrimination makes it questionable whether female mate preferences can account for colour pattern diversification and the maintenance of existing diversity. It has not been tested whether introgression between *Tropheus* morphs could be curbed by reduced competitive success of foreign and hybrid phenotypes (Raeymaekers *et al.* 2010). Divergent selection on coloration and vision (Gray & McKinnon 2006) is unlikely to be strong, as similar habitat structure and light conditions are encountered along the distribution range of *Tropheus*. Alternatively, the current phenotypic differentiation among populations may not have to withstand gene flow, but could be maintained by population isolation. A previous population genetic study on *Tropheus* indeed inferred philopatric behaviour of females based on strong mitochondrial differentiation across short distances of mostly continuous rocky shoreline (Sefc *et al.* 2007a). In contrast, the absence of nuclear differentiation between some of the populations left open the possibility of male-biased gene flow, although the number of nuclear markers in that study was too small to reach a definitive conclusion (Sefc *et al.* 2007a).

Tropheus are highly stenotopic inhabitants of rocky habitat along the entire shore of Lake Tanganyika. Populations along a stretch of largely continuous shoreline differ only slightly and often gradually in colour pattern, while highly distinct morphs are often separated by major habitat barriers such as wide and shallow muddy river estuaries. Mitochondrial introgression between presently allopatric populations (Sturmbauer & Meyer 1992; Baric *et al.* 2003; Sturmbauer *et al.* 2005) was presumably triggered by lake level fluctuations, which extended to several hundred meters below present level repeatedly in the history of the genus (e.g. Scholz *et al.* 2003; Cohen *et al.* 2007) and displaced the littoral populations. As evidenced by several studies, accounting for the history of the physical environment is of utmost importance to understand population differentiation, diversification and hybridization (e.g. Koskinen *et al.* 2002; Froufe *et al.* 2003; Bowie *et al.* 2006; Koblmüller *et al.* 2009b; Fraser *et al.* 2010). Quaternary climate oscillations have played a predominant role in shaping the present geographical distribution and genetic structure of populations and species (Avise 2000; Hewitt 2000). The recurrent Pleistocene glacial cycles not only affected temperate species, but also organisms in the tropics, and both aquatic and terrestrial species were affected in a similar way (e.g. Wilson 2006; Anthony *et al.* 2007; Melo-Ferreira *et al.* 2007; Gatton *et al.* 2008; Cossíos *et al.* 2009; Koblmüller *et al.* 2009b; Xu *et al.* 2009; Faurby *et al.* 2010; Harris & Taylor 2010; You *et al.* 2010). In East Africa, these quaternary climatic oscillations resulted in alternating periods with more humid or more arid climate and associated rises and drops of water levels in large water bodies (Gasse 2000; Scholz *et al.* 2003; Cohen *et al.* 2007). Thus, phylogenetic, phylogeographic and population genetic patterns observed in East and South African cichlids have generally been related to these Pleistocene climatic changes (e.g. Verheyen *et al.* 1996; Markert *et al.* 1999; Rüber *et al.* 2001; Sturmbauer *et al.* 2001, 2005; Joyce *et al.* 2005; Duftner *et al.* 2006; Egger *et al.* 2007; Koblmüller *et al.* 2007, 2009a; Sefc *et al.* 2007a), yet, studies specifically testing for the impact of Pleistocene water level fluctuations on East African cichlids are scarce (Genner *et al.* 2010).

In the present study, we ask, first, whether subtly differentiated colour patterns of adjacent *Tropheus moorii* populations are maintained in isolation or in the face of continuous gene flow, and second, whether the generally presumed influence of water level fluctuations on lacustrine cichlids can be demonstrated in the small-scale population structure of the strictly stenotopic, littoral *Tropheus*. We use nine microsatellite loci and sequences of the mitochondrial control region to investigate population differentiation and demographic history within a single morph of *Tropheus moorii*. This morph is characterized by a yellow to orange blotch on its flank (Fig. 1), which is large and brightly orange in the
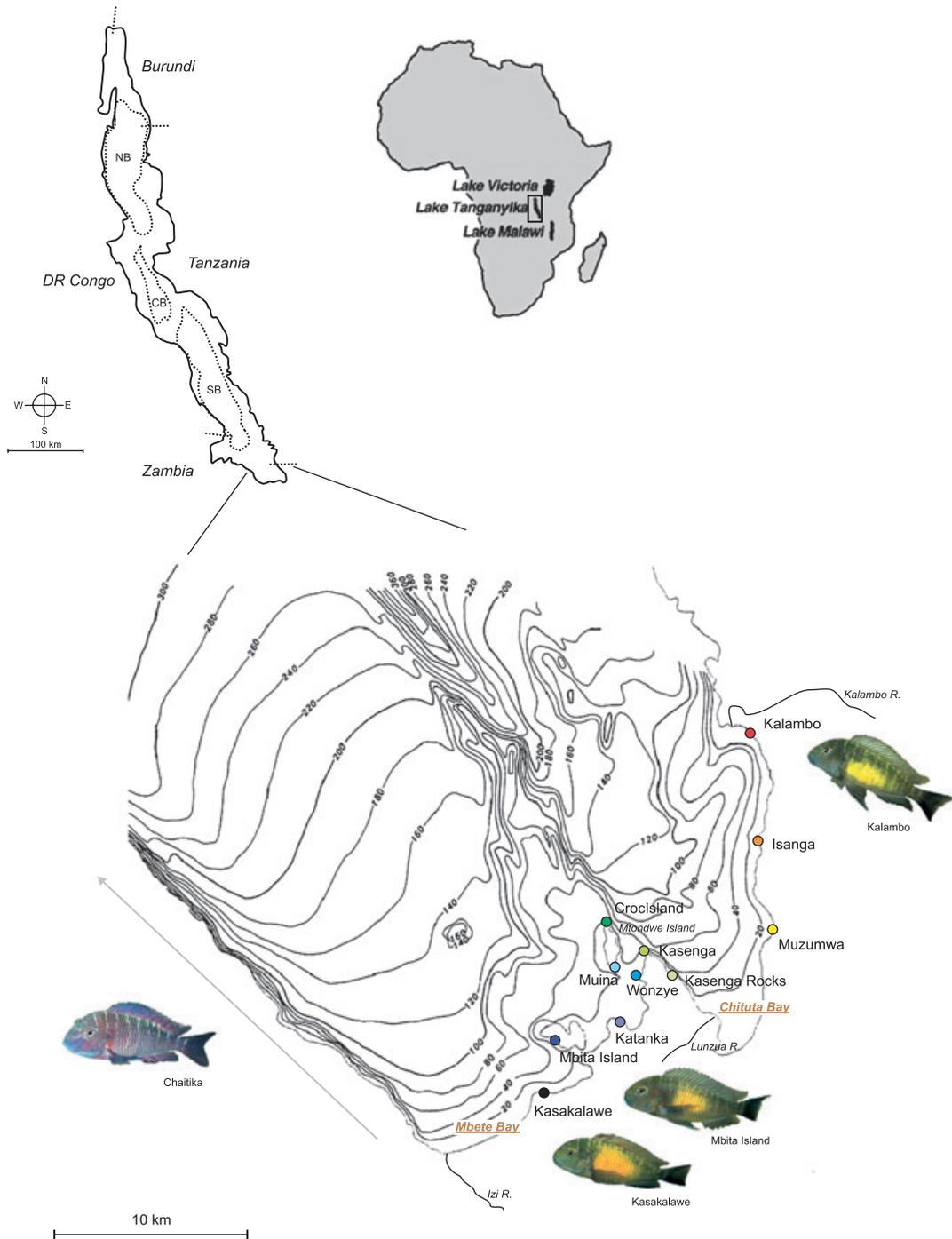
Fig. 1 Map of Lake Tanganyika, East Africa, showing sampling locations along the southern shore. *Dashed lines* indicate the three deepwater basins of the lake (NB, northern basin; CB, central basin; SB, southern basin). Bathymetric lines are given in 20 m intervals. Photographs illustrate body colour variation along the southern shoreline. Furthermore, the blue morph that inhabits the continuous rocky shoreline west of Mbete Bay is shown. *Tropheus* photographs were kindly provided by F. Carnevale and J. Stetka.

western-most population of Kasakalawe, much smaller and more yellowish at nearby Mbita Island, and gradually becoming more yellowish and shifting from a more dorsal to a more ventral position on the flank from Mbita Island eastwards. There is also variation in blotch size and hue within populations. Eleven population samples were taken at intervals of 1.5–10 km along a stretch of coastline including shallow and steep shores,

two islands and a presumable habitat barrier (a sandy bay formed by a river estuary). Philopatric behaviour of both sexes, i.e. differentiation in both mitochondrial and nuclear markers even across short distances of negotiable habitat, would imply a potential role for drift as a promoter of colour pattern diversification, and population isolation as preserver of phenotypic diversity. Expected signatures of lake level fluctuations in current population genetic patterns include (i) an association between population splitting and demographic development on one hand and the dynamics of *Tropheus* habitat during lake level fluctuations on other hand (Arnegard *et al.* 1999; Sefc *et al.* 2007a; Genner *et al.* 2010), and (ii) an association between the predicted shoreline structure during periods of low water level and current population structure. If hydrologic events were the major determinants of population evolution, then populations in the steeper sloping sections of the study area (i.e. east of Chituta Bay) are expected to display signals of comparatively stable demographic history and long-standing isolation, as they may have existed at approximately their current sites for a long period (Arnegard *et al.* 1999) and only been moved vertically along the lake slope by minor water level fluctuations (Sefc *et al.* 2007a). Populations of the shallow shores, on other hand, must have experienced extensive displacements even with rather moderate changes of the water level (Fig. 1), and may have been subjected to admixture, population decline, recolonization and splitting with subsequent population growth in the more recent past (Arnegard *et al.* 1999; Sefc *et al.* 2007a).

Materials and methods

Sampling and laboratory methods

Fin clips of 319 individuals of *Tropheus moorii* were collected from 11 localities in the southern part of Lake Tanganyika (Fig. 1) in 2000 and 2004, and preserved in 96% ethanol. The steep shore populations from Kalambo, Isanga, and Muzumwa are situated east of a large habitat barrier represented by Chituta Bay, while the remaining populations west of Chituta Bay inhabit a rather shallow and slowly sloping region. The sample from Kasakalawe used here is different from that in Sefc *et al.* (2007a), but there was no significant difference in haplotype frequencies between the two samples (not shown). Whole genomic DNA was extracted as described in Duftner *et al.* (2005). Partial mitochondrial control region and partial proline-tRNA was amplified with primers L-Pro-F_Tropheus [5′-AACCCCYRCCCCTAACTCCCAAAG-3′; modified from L-Pro-F (Meyer *et al.* 1994)] and TDK-D (Lee *et al.* 1995). Polymerase chain reactions (PCR) contained 0.5 U Taq DNA polymerase (BioTherm™), 0.25 μM

of each primer, 0.25 μM dNTP mix, 1.5 mM MgCl₂ buffer, and 1 μL of the extracted DNA, in a total volume of 10 μL. PCR was performed on a GeneAmp PCR system 9700 (Applied Biosystems) with an initial denaturation at 94°C for 5 min followed by 45 cycles with denaturation at 94°C for 30 s, primer annealing at 52°C for 30 s and extension at 72°C for 40 s, with a final extension phase at 72°C for 5 min. Purification of PCR products and chain termination sequencing followed Duftner *et al.* (2005). DNA fragments were purified with Sephadex™ G-50 (Amersham Biosciences) and visualized on an ABI 3100 capillary sequencer (Applied Biosystems). Sequences are deposited in GenBank (HQ721469–HQ721783). Electropherograms were controlled and edited in Sequence Navigator (Applied Biosystems), and subsequently aligned in SE-AL (A. Rambaut, http://tree.bio.ed.ac.uk/software/seal/). Sequences are deposited in GenBank (HQ721469 -HQ721783). The alignment consisted of 28 bp of proline-tRNA and 388 bp of mitochondrial control region sequence.

Nine microsatellite loci were genotyped in three multiplex reactions: UNH130 + UNH154 + Pzeb3; UNH908 + Pzeb2; UME002 + UME003 + TmoM11 + TmoM27; primer sequences in Lee & Kocher (1996), Zardoya *et al.* (1996), Van Oppen *et al.* (1997), Carleton *et al.* (2002), Albertson *et al.* (2003). Amplification reactions contained 1 U Taq DNA polymerase (BioTherm™), 0.25 μM dNTP mix, 1.5 mM MgCl₂ buffer, 0.25 μM of each primer except UNH130 (0.125 μM) and UNH154 (0.06 μM) and 1 μL of the extracted DNA, in a volume of 10 μL. Forward primers were labelled with the fluorescent dyes FAM, HEX and NED. PCR cycling started with a denaturation phase at 94°C for 5 min followed by 33 cycles with denaturation at 94°C for 30 s, primer annealing at 54°C for 30 s and extension at 72°C for 50 s. Fragments were separated on an ABI 3100 capillary sequencer (Applied Biosystems), sized against Genescan-500 ROX (Applied Biosystems) and analyzed using GENEMAPPER 3.7 (Applied Biosystems).

Within-population patterns of genetic diversity

Genetic diversity indices for mtDNA sequences [number of haplotypes (H), haplotype diversity (H_D), nucleotide diversity (π)] were calculated in DnaSP (Rozas *et al.* 2003). Microsatellite variability was estimated by the number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosity, using Arlequin v.3.0 (Excoffier *et al.* 2005). Tests for Hardy–Weinberg equilibrium (Markov chains of 100 000 steps following 1000 dememorization steps) and linkage disequilibrium between loci (10 000 permutations) were calculated in Arlequin v.3.0. P values were corrected for multiple testing following the method of Benjamini & Hochberg (1995).

Among-population patterns of genetic diversity

To illustrate the phylogenetic relationships among the sampled individuals, a neighbornet graph based on p-distances between mtDNA haplotypes was calculated in SPLITSTREE v.4.11.3 (Huson & Bryant 2006). Furthermore, a population tree based on microsatellite allele sharing distances (Nei *et al.* 1983) was calculated in POPTREE2 (Takezaki *et al.* 2010) and visualized in FIGTREE 1.2.3 (Rambaut 2009).

Population differentiation was estimated by θ_{ST} (Weir & Cockerham 1984) and Φ_{ST} (Excoffier *et al.* 1992) from mtDNA sequence data and θ_{ST} (Weir & Cockerham 1984) from microsatellite data in Arlequin v.3.0, with significance inference corrected for multiple testing (Benjamini & Hochberg 1995). Since comparisons of traditional F_{ST} derivatives among markers with different levels of polymorphism are problematic (e.g.; Hedrick 2005; Jost 2008; Heller & Siegismund 2009) we also use the estimator of actual differentiation, D_{EST} (Jost 2008) in the software SMOGD v.1.2.5 (Crawford 2010). D_{EST} across loci was calculated as the arithmetic mean, which is considered the most sensible estimate when comparatively few loci and samples are used (see e.g. Heller *et al.* 2010).

Isolation by distance (IBD) among the eight populations west of Chituta Bay was tested by a regression of differentiation estimators scaled as, e.g. $\Phi_{ST}/(1 - \Phi_{ST})$ on linear geographic distance using the program zt (Bonnet & Van de peer 2002). Simple Mantel tests were performed with 10 000 randomizations. Because *T. moorii* would most likely disperse along the shoreline, geographic distances were measured along the coast and along ridges connecting coast with island populations.

Nuclear genetic structure was also investigated by Bayesian model-based clustering in STRUCTURE v.2.3 (Pritchard *et al.* 2000). This analysis determines the most likely number of differentiated clusters (K) represented by the sample and assigns the sampled genotypes to the inferred clusters. The log likelihood of the data [ln Pr[X|K]] was estimated, given different numbers of genetic clusters K, using an admixture model with correlated allele frequencies and using sampling locations as prior information (given the low levels of differentiation among some sampling sites) (Falush *et al.* 2003; Hubisz *et al.* 2009). Five replicate analyses (2×10^5 burn-in cycles, 10^6 MCMC iterations) were run for the whole dataset (testing K values of 1–12) and on two subsets including only western (K = 1–9) or eastern (K = 1–4) samples. Following Evanno *et al.* (2005), we calculated ΔK, which corresponds to the rate of change of the likelihood between successive K values, using the program STRUCTURE HARVESTER (Earl 2009; available from http://ssers.soe.usc.edu/~dearl/software/struct_ harvest/) to identify the best supported values of K. The output files for the best estimates of K were then summarized in CLUMPP (Jakobsson & Rosenberg 2007) using the default parameters.

Demographic history

In order to compare the trajectories of population sizes over time between steep and shallow shorelines, historic population sizes were inferred for all populations separately and for pooled groups of the western, shallow-shore and eastern, steep-shore populations by Bayesian skyline plot (BSP) analyses (Drummond *et al.* 2005) in BEAST 1.4.6 (Drummond & Rambault 2007) and visualized in TRACER 1.4 (Rambaut & Drummond 2008). This coalescent-based approach estimates the posterior distribution of effective population sizes at intervals along a phylogeny, thereby allowing inferences of population size fluctuations over time. The skyline plot model assumes a panmictic population and age estimates might be slightly biased upward by population structure and by gene flow from other populations (Navascues & Emerson 2009). Therefore, the mtDNA haplotypes of a divergent lineage (see 'Results'; lineage 1-A4 in Fig. 2), which have probably introgressed into the populations investigated here, were omitted from the demographic analyses (both from the BEAST and the below described IMa analyses), since they would inflate coalescence times within populations and potentially cause biased population size estimates. In our analyses of the pooled population groups, population structure may indeed cause an upward bias of the estimates.

We applied the model of nucleotide evolution selected in MODELGENERATOR v.0.85 (Keane *et al.* 2006) with a strict molecular clock assuming a substitution rate of 0.0325 and alternatively 0.057 per site per MY (Sturmbauer *et al.* 2001; Koblmüller *et al.* 2009a; note that independent calibrations for recent diversification events in cichlids revealed a very similar substitution rate of 0.0324 per site per MY, Genner *et al.* 2007, 2010). We used default settings for skyline model (constant) and number of groups (10). The various datasets required different run-lengths, but all analyses were run until effective samples sizes for all parameters were >200. Analyses were run twice using different random seeds to test for convergence.

Isolation with migration

For comparisons of population divergence times, gene flow rates and population sizes in the shallow and the steep shore sections, parameters of divergence time (t) and migration rates (m_1, m_2) between populations along with the effective population sizes of the ancestral (θ_A)
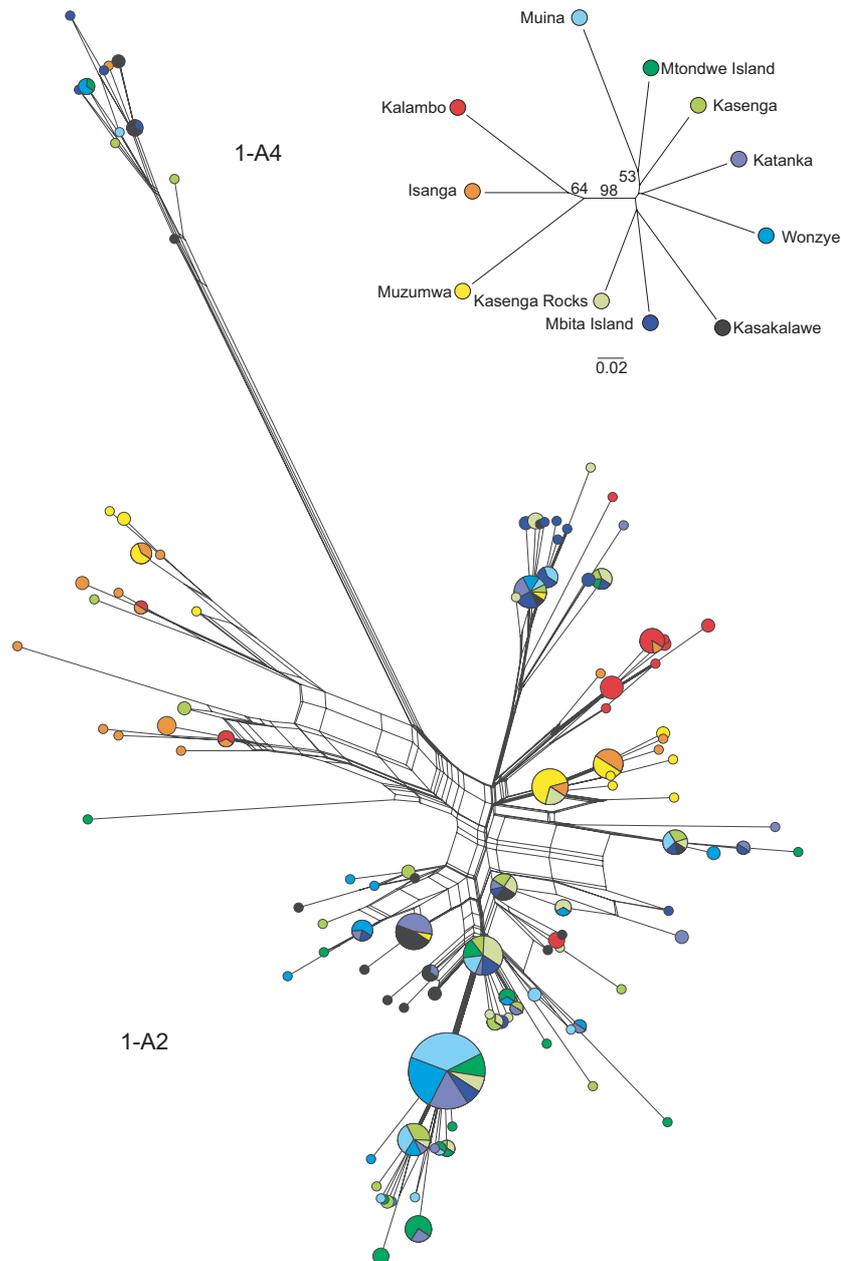
Fig. 2 Phylogenetic relationships among samples and populations of the yellow blotched *Tropheus moorii* morph from Zambia. (a) Neighbornet graph for the Zambian yellow-blotched *Tropheus moorii* computed with uncorrected p-distances and based on 315 sequences of the most variable part of the mitochondrial control region. Each circle represents one haplotype, the diameter of the circle correlates with the number of individuals sharing the same haplotype. See Fig. 2b for population colour codes. Clades 1-A2 and 1-A4 represent two of the mitochondrial lineages defined in Sturmbauer *et al.* (2005). (b) Population tree based on microsatellite allele sharing distances. Bootstrap values >50 are shown at the respective branches.

and the daughter populations (θ_1, θ_2) were estimated by coalescence simulations based on mtDNA sequences in IMa [http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm#IM; Hey & Nielsen (2004, 2007)]. Analyses were conducted with all pairs of populations on the steep shore east of Chituta Bay, with all pairs of four populations in the shallow region west of Chituta Bay (Kasakalawe, Mbita Island, Katanka and Kasenga Rocks), and across Chituta Bay by pooling all samples on either side of the bay. Analyses were restricted to four western populations in order to keep computation time within limits. The selection of the four populations was based on their geographic distribution and the assumption (derived from differentiation estimates) that

they represent the general pattern of genetic structuring in the area. IMa model assumptions include a split of an ancestral population into two daughter populations and the absence of selection, recombination, within-population structure and gene-flow from unsampled populations. Given that gene flow among *Tropheus* populations was found to be low overall, the most probable violation of the above assumptions concerns the presence of population structure in the pooled samples on each side of the bay. The bias introduced by structure is considered to remain small as long as the population differentiation within the sample is low compared to the divergence between the two samples (J. Hey, communication to IM newsgroup; Strasburg & Rieseberg 2010), as is the case in our analysis. A more serious problem may be represented by an increased variance and inaccuracy of parameter estimates due to deviations from the assumed substitution pattern (Strasburg & Rieseberg 2010). The best fitting substitution model for our data is HKY+I+G (MODELGENERATOR v.0.85; Keane *et al.* 2006), which does not greatly differ from the HKY substitution model applied to mitochondrial sequences data by IMa, such that we expect only modest biases resulting from this issue.

Based on the same reasoning as applied above for the BEAST analysis, the mtDNA haplotypes of a divergent lineage were omitted from the IMa analysis. A minimum of three replicate runs per population comparison were performed with >5 000 000 steps (until ESS for each estimated parameter were >50; Hey & Nielsen 2004) and a burn-in time of 1 000 000 steps under a finite-sites model (HKY; Hasegawa *et al.* 1985) with different random number of seeds. The analyses were considered to have converged upon the stationary distribution if independent runs generated similar posterior distributions. The values with the highest posterior probability were scored as best estimates. Credibility intervals for each parameter are represented by the 90% highest posterior density (HPD) interval, which corresponds to the shortest span that includes 90% of the probability density of a parameter. However, it should be noted here that the HPD is strongly dependent on the priors and the upper bound of the 90% HPDs is often close to the fixed parameter maximum, or, when parameter estimates do not approach zero, gets extraordinarily large (also see Wilson 2006). Since all replicate runs yielded similar parameter estimates, we report results from the longest run only. Pairwise population divergence times were used to illustrate the chronology of population splits in a NJ tree computed in MEGA 3.1 (Kumar *et al.* 2004). To translate parameters for population splitting times into absolute values we employed the substitution rate of 0.0325–0.057 per site per MY (Sturmbauer *et al.* 2001; Koblmüller *et al.*

2009a; consistent with the findings of Genner *et al.* 2007, 2010).

Results

Genetic diversity and population differentiation

A total of 117 mitochondrial haplotypes was detected in 315 individuals; amplification failed from the remaining four individuals. Haplotype variability within populations was high with an average of 16.2 haplotypes per population sample, 92.6% mean haplotype diversity and 1.37% mean nucleotide diversity (see Table 1 for population values). Haplotype diversity was slightly but not significantly lower in the populations from the steep shores east of Chituta Bay than in the populations of the shallow region west of the bay (mean across eastern populations: haplotype number $H = 14$, haplotype diversity $H_e = 90.0\%$, nucleotide diversity $\pi = 1.21\%$; mean across western populations: $H = 17$, $H_e = 93.5\%$, $\pi = 1.43\%$; Mann Whitney U tests, all $P > 0.05$). The haplotypes belong to two different lineages (Fig. 2a), which have been described in a previous phylogeographic study by Sturmbauer *et al.* (2005) as mtDNA lineage 1-A2, holding the majority of the haplotypes detected in the present work, and mtDNA lineage 1-A4, which is found at low frequencies across the entire study range (7 individuals from Kasakalawe, 4 from Mbita Island, 2 from Wonzye, 1 from Muina, 1 from Mtondwe Island, 2 from Kasenga and 1 from Isanga). Only three haplotypes were found on both sides of

Table 1 Population sample sizes (N) and genetic diversity of mtDNA sequences

Population	N	H	H_e	π
West of Chituta Bay				
Kasakalawe	32	21	0.960	0.01809
Mbita Island	31	21	0.972	0.01846
Katanka	30	16	0.917	0.01143
Wonzye	26	15	0.945	0.01594
Muina	30	12	0.846	0.00931
Mtondwe Island	28	16	0.934	0.01380
Kasenga	29	19	0.968	0.01711
Kasenga Rocks	29	16	0.938	0.01065
East of Chituta Bay				
Muzumwa	30	13	0.862	0.01006
Isanga	28	18	0.950	0.01671
Kalambo	26	11	0.889	0.00958
Mean (SD)	29.0	16.2	0.926	0.01374
	(1.9)	(3.4)	(0.043)	(0.00363)
Total	315	117	0.976	0.01633

H, number of haplotypes, H_e, gene diversity, π, nucleotide diversity.

Chituta Bay, while haplotype sharing among populations located on the same side of the bay was much more common (Fig. 2a).

The microsatellite markers were moderately to highly polymorphic (Table 2). Per locus and population heterozygosities ranged from 12 to 100% (Table 2), with means of 75% expected and 73% observed heterozygosity across populations. Again, genetic diversity was lower in the eastern steep-shore than in the western shallow-shore populations [means across eastern and western populations: allele number N_A (east) = 10.6, N_A (west) = 12.4, Mann–Whitney U-test: $P = 0.023$; expected heterozygosity H_E (east) = 72.0%, H_E (west) = 75.8%, Mann–Whitney U-test: $P = 0.01$; observed heterozygosity H_O (east) = 70.7%, H_O (west) = 74.6%, Mann–Whitney U-test: $P = 0.182$]. Significant departures from Hardy–Weinberg expectations were detected at Pzeb2 and TmoM11 in the Wonzye sample, at Pzeb2 in Kasenga, and at TmoM27 in Isanga. Since none of these loci deviated from Hardy–Weinberg expectations in

Table 2 Microsatellite diversity in populations of *Tropheus moorii*

Population		Pzeb2	Pzeb3	TmoM11	TmoM27	UME002	UME003	UNH130	UNH154	UNH908	Average
West of Chituta Bay											
Kasakalawe	N_A	18	13	17	10	12	19	16	5	4	12.67
	H_O	0.94	0.84	0.81	0.66	0.87	0.94	0.84	0.47	0.28	0.74
	H_E	0.94	0.87	0.91	0.60	0.86	0.94	0.83	0.44	0.33	0.75
Mbita Island	N_A	21	9	17	12	10	19	18	7	3	12.89
	H_O	0.90	0.94	0.94	0.77	0.84	0.90	0.81	0.32	0.32	0.75
	H_E	0.94	0.79	0.91	0.84	0.84	0.91	0.88	0.32	0.30	0.75
Katanka	N_A	16	13	19	11	12	16	16	5	3	12.33
	H_O	0.93	0.79	0.93	0.70	0.81	0.96	0.89	0.68	0.43	0.79
	H_E	0.93	0.82	0.94	0.79	0.76	0.94	0.84	0.70	0.37	0.79
Wonzye	N_A	17	9	19	7	10	18	16	4	4	11.56
	H_O	**0.81**	0.83	**0.83**	0.79	0.88	1.00	0.92	0.54	0.21	0.76
	H_E	0.95	0.78	0.93	0.76	0.73	0.94	0.93	0.57	0.20	0.75
Muina	N_A	16	11	18	10	8	18	18	6	4	12.11
	H_O	0.86	0.64	0.83	0.68	0.54	0.84	0.84	0.44	0.28	0.66
	H_E	0.92	0.76	0.96	0.84	0.54	0.94	0.94	0.55	0.29	0.75
Mtondwe Island	N_A	19	12	25	10	9	18	15	4	4	12.89
	H_O	0.97	0.83	0.97	0.79	0.79	0.93	0.83	0.50	0.21	0.76
	H_E	0.94	0.84	0.96	0.74	0.79	0.93	0.81	0.55	0.20	0.75
Kasenga	N_A	17	11	21	10	9	20	14	5	3	12.22
	H_O	**0.80**	0.88	0.96	0.83	0.58	0.96	0.81	0.65	0.23	0.74
	H_E	0.94	0.85	0.94	0.77	0.70	0.95	0.83	0.63	0.21	0.76
Kasenga Rocks	N_A	16	10	22	12	10	17	20	5	4	12.89
	H_O	0.90	0.90	0.87	0.70	0.90	0.87	0.87	0.50	0.40	0.77
	H_E	0.93	0.86	0.94	0.70	0.80	0.92	0.90	0.47	0.35	0.76
East of Chituta Bay											
Muzumwa	N_A	19	10	9	8	8	11	14	5	3	9.67
	H_O	0.87	0.72	0.80	0.57	0.70	0.93	0.83	0.43	0.33	0.69
	H_E	0.91	0.69	0.75	0.58	0.76	0.87	0.90	0.44	0.32	0.69
Isanga	N_A	20	12	19	11	9	18	10	4	4	11.89
	H_O	0.85	0.86	0.89	**0.71**	0.71	0.82	0.64	0.43	0.18	0.68
	H_E	0.96	0.84	0.93	0.89	0.56	0.93	0.69	0.59	0.17	0.73
Kalambo	N_A	17	7	13	9	6	18	15	5	3	10.33
	H_O	0.96	0.72	0.85	0.85	0.65	1.00	0.84	0.73	0.12	0.75
	H_E	0.93	0.78	0.86	0.84	0.59	0.94	0.88	0.68	0.18	0.74
Average across populations	N_A	17.82	10.64	18.09	10.00	9.36	17.45	15.64	5.00	3.55	11.95
	H_O	0.89	0.81	0.88	0.73	0.75	0.92	0.83	0.52	0.27	0.73
	H_E	0.94	0.81	0.91	0.76	0.72	0.93	0.86	0.54	0.27	0.75

N_A, number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity. Deviations of H_O from Hardy–Weinberg expectations at a 0.05 significance level after Benjamini–Hochberg correction are indicated by bold print.

more than two samples, we included all data in subsequent analyses. There was no linkage disequilibrium between any of the nine loci.

Mitochondrial and nuclear differentiation among all populations, estimated from haplotype frequencies (mt-θ_{ST}), nucleotide distances (Φ_{ST}) and microsatellite allele frequencies (SSR-θ_{ST}), was highly significant (overall mt-θ_{ST} = 0.067, $P < 0.001$; Φ_{ST} = 0.278, $P < 0.001$; SSR-θ_{ST} = 0.039, $P < 0.001$). Pairwise population comparisons revealed significant nuclear and mitochondrial differentiation in most cases, with the following exceptions among populations located west of Chituta Bay (Tables 3 and 4): None of the markers detected differentiation between two populations separated by 2.7 km of continuous rocky habitat (Kasenga and Kasenga Rocks). One or two of the metrics of population differentiation that we used lacked significance in three pairs of neighbouring populations separated by discontinuous habitat (Katanka and Wonzye, Wonzye and Kasenga, Muina and Mtondwe), in three pairs of island and nearby shore populations (Wonzye and Muina, Wonzye and Mtondwe Island, Mtondwe Island and Kasenga), and in four pairs of populations separated by longer distances of discontinuous habitat (Mbita Is. and Kasenga Rocks, Katanka and Kasenga Rocks, Katanka and Mtondwe Island, Katanka and Kasenga). There was a significant correlation between mitochondrial and nuclear pairwise population differentiation estimates (SSR-θ_{ST} and mt-θ_{ST} matrices: Pearson r = 0.743, $P < 0.001$; SSR-θ_{ST} and mt-Φ_{ST} matrices: r = 0.759, $P < 0.001$; SSR-D_{EST} and mt-D_{EST}: r = 0.452, $P = 0.001$; see Table S1, Supporting information, for pairwise SSR-D_{EST} and mt-D_{EST} estimates). The shoreline distances between sampling sites, disregarding habitat structure, had a significant effect on mitochondrial differentiation (mt-Φ_{ST}: r = 0.603, $P = 0.0101$; mt-D_{EST}: r = 0.497, $P = 0.0103$), but not on microsatellite differentiation (SSR-θ_{ST}: r = 0.357, $P = 0.0761$; SSR-D_{EST}: r = 0.345, $P = 0.0959$).

Impact of the habitat barrier on population differentiation

Pairwise population differentiation values across Chituta Bay (averages of SSR-θ_{ST} = 0.045; mt-θ_{ST} = 0.075; Φ_{ST} = 0.298) were higher than between populations on the same side of the bay (averages of SSR-θ_{ST} = 0.018; mt-θ_{ST} = 0.035; Φ_{ST} = 0.091; Mann–Whitney U-test $P < 0.001$). Hierarchical analyses of microsatellite and mitochondrial variance, with populations grouped into 'east of Chituta' and 'west of Chituta', further confirmed the contribution of population separation across Chituta Bay to total variance (microsatellites: within-group θ_{SC} = 0.023, $P < 0.001$; between-group θ_{CT} = 0.016, $P < 0.01$; mtDNA: within-group θ_{SC} = 0.041, $P < 0.001$; between-group θ_{CT} = 0.028, $P < 0.05$; within-group Φ_{SC} = 0.112, $P < 0.001$; between-group Φ_{CT} = 0.187, $P < 0.01$); the high mt-Φ_{CT} value reflects considerable nucleotide divergence between populations across the bay. Consistent with these findings, the population tree based on SSR data shows a clear split across Chituta Bay contrasting with little statistical support for the splits on each side of the bay (Fig. 2b).

Table 3 Pairwise population differentiation between eleven populations of *Tropheus moorii* based on mtDNA sequences

		West of Chituta Bay, shallow region							East of Chituta Bay, steep shore		
	P	Kasakalawe	Mbita Island	Katanka	Wonzye	Muina	Mtondwe Island	Kasenga	Kasenga Rocks	Muzumwa	Isanga	Kalambo
Kasakalawe	11		0.012*	0.024*	0.029***	0.054***	0.034***	0.012*	0.029**	0.068***	0.026***	0.056***
Mbita Island	11	0.079*		0.029*	0.018*	0.029*	0.029**	0.015*	0.015^{NS}	0.080***	0.039***	0.069***
Katanka	4	0.093**	0.129***		0.023^{NS}	0.038*	0.040**	0.044***	0.051***	0.100***	0.067***	0.097***
Wonzye	6	0.055*	0.100**	0.012^{NS}		0.011^{NS}	0.036*	0.031**	0.041**	0.095***	0.053***	0.083***
Muina	5	0.198*	0.207***	0.058*	0.032^{NS}		0.046**	0.042**	0.040*	0.123***	0.080***	0.111***
Mtondwe Island	7	0.179***	0.210***	0.059*	0.024^{NS}	-0.009^{NS}		0.040***	0.032*	0.102***	0.058***	0.088***
Kasenga	10	0.050*	0.091**	0.020^{NS}	-0.009^{NS}	0.047*	0.043*		0.015^{NS}	0.084***	0.041***	0.071***
Kasenga Rocks	6	0.103**	0.074*	0.026^{NS}	0.049*	0.110**	0.118***	0.027^{NS}		0.068**	0.049***	0.086***
Muzumwa	7	0.237***	0.215***	0.296***	0.283***	0.400***	0.374***	0.221***	0.243***		0.036*	0.125***
Isanga	12	0.213***	0.214***	0.299***	0.265***	0.375***	0.346***	0.191***	0.265***	0.097**		0.069***
Kalambo	7	0.291***	0.261***	0.371***	0.339***	0.455***	0.422***	0.285***	0.335***	0.293***	0.245***	

Number of private haplotypes (P) are given for each population. Above diagonal: θ_{ST} values, below diagonal: Φ_{ST} values.
Significance levels, $P < 0.05$, < 0.01 and < 0.001, after correction for multiple tests, are indicated as *, ** and ***, respectively.

Likewise, the SSR-based Bayesian inference of population structure revealed a peak in ΔK (Evanno *et al.* 2005) for *K* = 2, corresponding to a division between populations east and west of Chituta Bay (Fig. 3a).

Separate analyses of the eastern populations resulted in three genetic clusters corresponding to the three sampling sites (Fig. 3b). In contrast, the western populations received the highest log likelihood values [ln

Table 4 Pairwise population differentiation between eleven populations of *Tropheus moorii* based on nine microsatellites

	N	Kasakalawe	Mbita Island	Katanka	Wonzye	Muina	Mtondwe Island	Kasenga	Kasenga Rocks	Muzumwa	Isanga	Kalambo
Kasakalawe	32											
Mbita Island	31	0.014***										
Katanka	28	0.022***	0.021***									
Wonzye	24	0.026***	0.020***	0.009*								
Muina	25	0.037***	0.040***	0.025***	0.009*							
Mtondwe Island	29	0.016***	0.017***	0.005^{NS}	0.012**	0.021***						
Kasenga	26	0.017***	0.019***	0.001^{NS}	0.007^{NS}	0.013**	0.001^{NS}					
Kasenga Rocks	30	0.009**	0.008*	0.010**	0.007*	0.016***	0.008*	0.006^{NS}				
Muzumwa	30	0.051***	0.057***	0.051***	0.048***	0.058***	0.048***	0.042***	0.037***			
Isanga	29	0.029***	0.043***	0.026***	0.040***	0.047***	0.028***	0.016**	0.023***	0.048***		
Kalambo	26	0.068***	0.083***	0.041***	0.049***	0.049***	0.056***	0.034***	0.052***	0.078***	0.027***	

The number of samples (N) is given for each population. Significance levels of $P < 0.05$, < 0.01 and < 0.001 after correction for multiple tests are indicated by *, ** and ***, respectively.
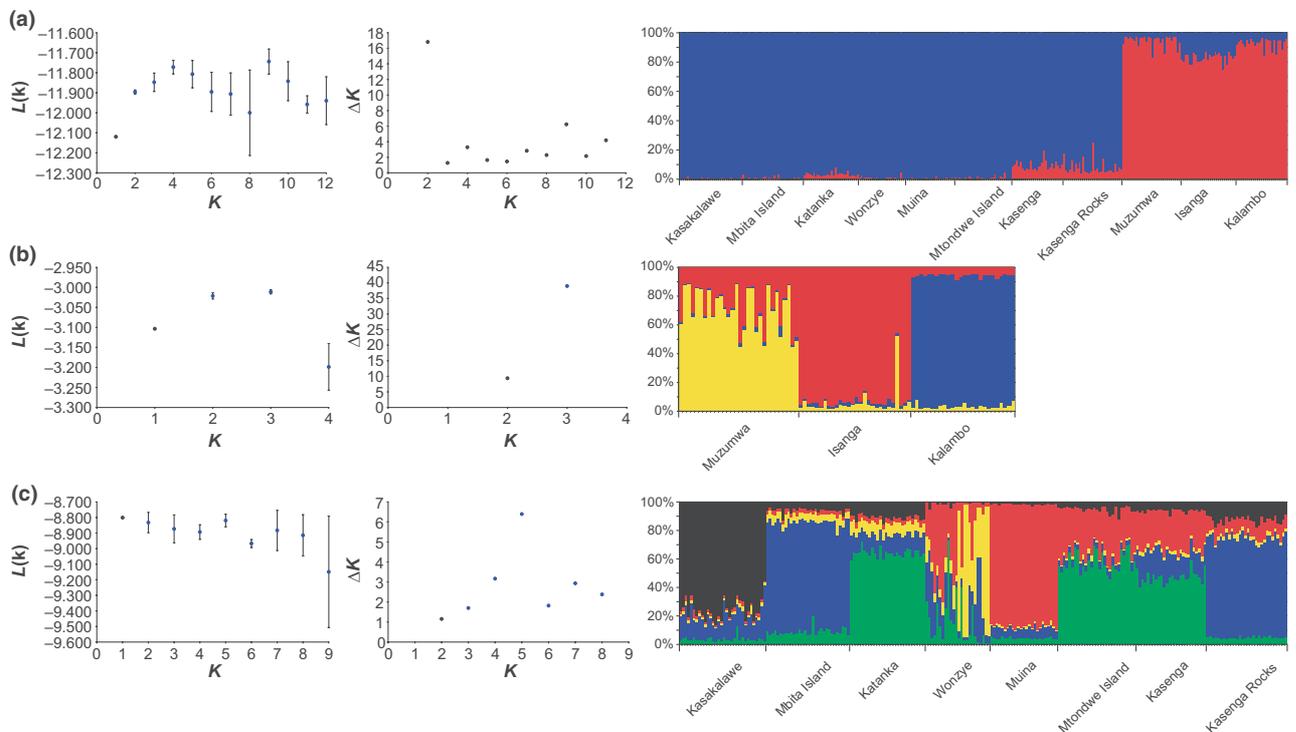
Fig. 3 Bayesian clustering analysis of the yellow-blotched *Tropheus moorii*. (a) the full data, (b) samples east of Chituta Bay, and (c) samples west of Chituta Bay. Left: mean likelihood [L(K) ± SD] over 5 runs assuming K clusters; middle: ΔK, where the modal value of the distribution is considered as the highest level of structuring; right: individual assignment to the most probable number of clusters K as inferred from the ΔK statistic. Note that it is not possible to infer ΔK for K = 1.

Pr[*X* | *K*)] for a *K* value of one, and Δ*K* values of the analyses assuming *K* > 1 peaked at five genetic clusters unrelated to sampling sites (Fig. 3c), suggesting that the differentiation among the western populations is too low to cluster the individuals according to their sampling sites.

Chronology of population splitting, gene flow and demographic history

We used a coalescence-based model of isolation-with-migration to estimate parameters of population size, time since divergence and gene flow for the three eastern and four of the western (Kasakalawe, Mbita Island, Katanka, Kasenga Rocks) populations from the mitochondrial sequence data. The maximum likelihood estimates of population divergence times suggest that the oldest split occurred between the populations west and east of Chituta Bay (Table S2, Supporting information, Fig. 4) and that the western populations diverged much more recently than the eastern populations (Mann–Whitney *U*-test: *P* = 0.02). The split between western and eastern populations was dated to 140–250 KYA. East of Chituta Bay, the Kalambo population diverged from Isanga and Muzumwa between 125 and 220 KYA and Muzumwa split from Isanga between 90 and 150 KYA. A stream inflow and sandy bay south of Kalambo may have contributed to the longstanding isolation of this population. West of the bay, the split of Kasakalawe from the other western populations was approximately 30–55 KYA, while the splits between the remaining three populations happened almost simultaneously about 15–30 KYA (Fig. 4).

Both current and ancestral population size estimates were on average higher for the western than for the eastern populations (Table S2, Supporting information; Mann–Whitney *U*-tests: ancestral population sizes,
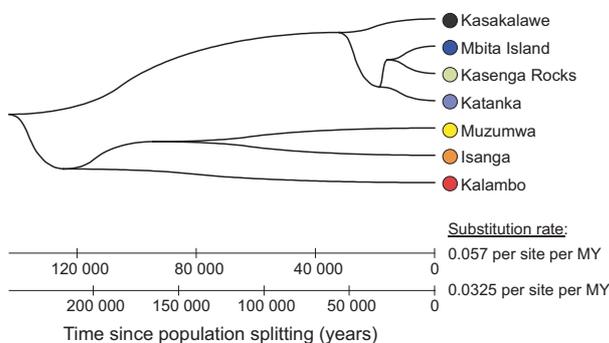
P = 0.02; current population sizes, *P* = 0.289). Estimated migration rate parameters (reflecting the probabilities that individuals migrate, not the number of migrants which also depend on population sizes) were near zero across Chituta Bay and between several of the population pairs located on the same side of the bay (in 4 out of 9 population comparisons). In those five pairs with higher migration rate parameters (ranging from 1.41 to 4.16), asymmetric migration suggested by the maximum-likelihood estimates was not supported by the associated confidence intervals (Table S2, Supporting information). The difference between averaged migration rates among the western and among the eastern populations (1.1 vs. 0.3) was not significant (Mann–Whitney *U*-test *P* = 0.345) and mainly due to the high estimates of migration from Katanka into the other western populations. Disregarding migration estimates out of Katanka, average migration rate estimates among the western populations were similar to those among the eastern populations (0.4 vs. 0.3).

Bayesian reconstructions of the development of population sizes over time reveal population growth on each side of Chituta Bay (Fig. 5a, b). Analyses of pooled eastern and pooled western samples both detected an initial population expansion starting ~50–90 KYA and a period of rapid growth starting ~7–12 KYA, but both, the initial and the more recent, expansion occurred at higher rates west of Chituta Bay (Fig. 5a). Individual population sizes were traced back to the origin of each population as inferred by the IMa analysis (Fig. 5b). Size expansions were detected in all western populations except Mtondwe Island and Kasenga, which have been experiencing population declines, and Wonzye, which has been rather stable, since the establishment of the current populations. East of Chituta Bay, historically stable population sizes were inferred for the Kalambo population, whereas Muzumwa and Isanga have been growing at a slow and fast rate, respectively, since their splitting. In agreement with IMa parameter estimates, larger current population sizes were inferred west of Chituta Bay than east of the bay except for the large current population size estimate at Isanga (Fig. 5b).

Discussion

Habitat barriers curb gene flow in stable environmental conditions

The evolution and persistence of population-specific colour patterns across short geographic distances suggest that gene flow between populations is very low, unless it is countered by strong selection or strong drift within each population (Hey 2006; Nosil 2008). For the stenotopic rock-dwelling *Tropheus*, even rather small

Fig. 4 Chronology of population splitting, shown as a neighbour joining tree based on IMa divergence time estimates. Scale bars present time in years before present, derived from the minimum and maximum divergence rate in Sturmbauer *et al.* (2001) and Koblmüller *et al.* (2009a).
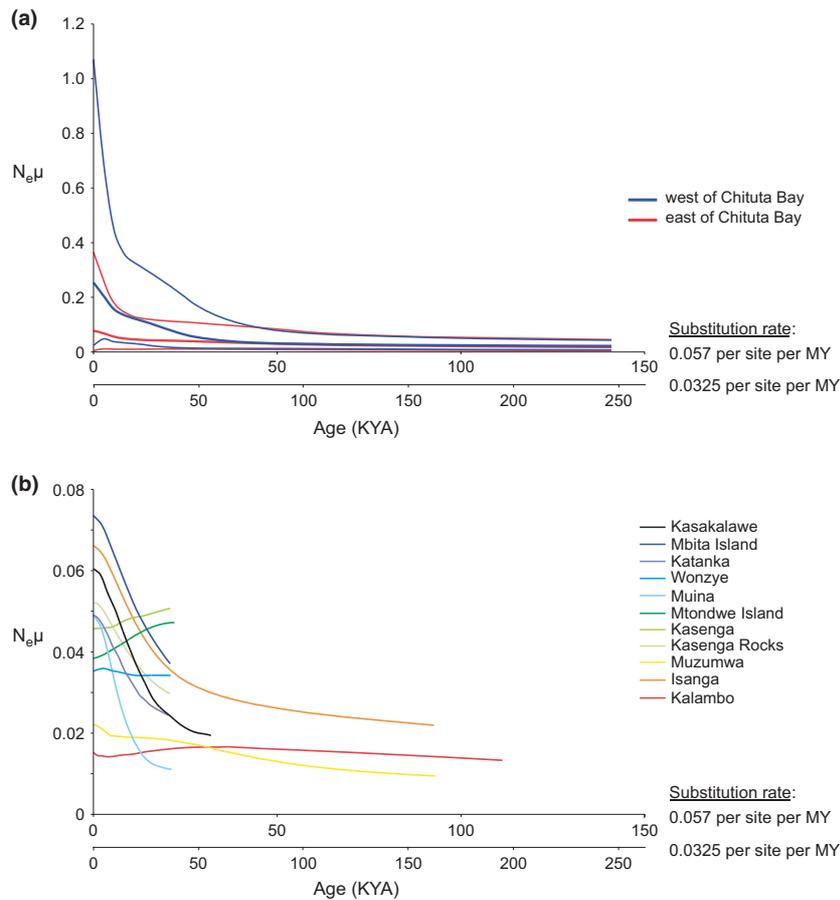
Fig. 5 Demographic histories of Zambian yellow blotched *Tropheus moorii* populations based on the most variable part of the mitochondrial control region. (a) Bayesian skyline plots for samples west and east of Chituta Bay. Thick and thin lines refer to the mean and the 95% HPD intervals, respectively. (b) Bayesian skyline plots (mean estimates only) for all populations separately. See Fig. 1 for population colour codes. The *y*-axis represents the combined parameter of effective population size N_e times the mutation rate μ.

stretches of sandy shore, such as estuaries of brooks or small bays disrupting the rocky shore at frequent intervals, are assumed to represent barriers to dispersal, but gene flow between populations may occur independently of their philopatric tendencies when lake level drops shift the shoreline and displace the littoral populations, as has been happening repeatedly in the history of the species (e.g. Scholz *et al.* 2003; Sturmbauer *et al.* 2005; Egger *et al.* 2007; McGlue *et al.* 2008). Moreover, a previous comparison of mitochondrial and nuclear genetic population differentiation raised the possibility of male-mediated gene flow among adjacent *Tropheus* populations despite their stenotopic habitat preferences, but since data from only three microsatellite markers were available in that study, the issue remained unresolved (Sefc *et al.* 2007a).

The present study, with nuclear data from nine microsatellite loci, provides no evidence of male dispersal preventing the evolution of nuclear genetic structure. Instead, there is significant nuclear and mitochondrial differentiation between most populations (except for some populations in the area of Mtondwe Island). Likewise, the gene flow estimates from the isolation-with-migration (IM) model were generally low. Moreover,

according to the IMa parameter estimates, the difference between the levels of mitochondrial differentiation among the western and among the eastern populations is predominantly due to different divergence times and population sizes and to a lesser extent to different rates of gene flow. Overall, the observed population structure suggests that even small habitat discontinuities curb gene flow among *Tropheus* populations very effectively. High levels of genetic differentiation on small geographic scales were also found in other stenotopic rock-dwelling cichlid species, especially those tied to the substrate by aufwuchs-feeding foraging (Van Oppen *et al.* 1997b, 1998; Arnegard *et al.* 1999; Markert *et al.* 1999; Danley *et al.* 2000; Rico & Turner 2002; Sefc *et al.* 2007a; Wagner & McCune 2009). Notably, a habitat barrier of only 35 m created by the inflow of a cold water stream into Lake Malawi caused significant differentiation in a mbuna species (Rico & Turner 2002). As discussed in several of the above cited studies, this separation of stenotopic littoral species into numerous isolated units, which is often associated with geographic colour pattern variation, lends support to the theory of intralacustrine allopatry contributing to the great species richness of rocky shore cichlids (e.g. Fryer

1996). In the genus *Tropheus*, the current species-level taxonomy, listing four nominal species and two synonyms, falls short of capturing the diversity represented by the numerous, mostly allopatric colour morphs (Schupke 2003; Egger *et al.* 2007).

Most current *Tropheus moorii* populations have been expanding since their split from an ancestral population and currently display high levels of genetic diversity and differentiation. These findings imply that the extant phenotypic diversity among the studied populations does not have to withstand the homogenizing effect of ongoing gene flow. Moreover, they allow considering a role for drift (Arnegard *et al.* 1999; Markert *et al.* 1999) in the evolution of colour pattern diversity among these populations, for example when population admixture during a lake level drop increased the phenotypic variation in the populations, which then colonized the novel habitats emerging during the subsequent water level rise.

The impact of lake level fluctuations on population structure and demography

Given the small amount of ongoing gene flow among populations, we next investigate the effect of historic hydrology on current population structure. An association of population splitting with lake level fluctuations is corroborated by the reconstructed sequence of population divergence. The first split occurred across Chituta Bay, which probably persisted as a barrier to the rock-dwelling *Tropheus* even when the lake level dropped ~200–300 m below present level (Fig. 1). Subsequent population splits occurred much earlier along the steep shores east of Chituta Bay than in the shallow region west of Chituta Bay (Fig. 4). This is consistent with population divergence in the course of rising water levels: along the steep coast, the current stretch of rocky shoreline emerged at a lower lake level, i.e. earlier, than the rocky sections in the shallow region, and steep-shore populations were able to occupy their sites long before shallow-shore populations (see Arnegard *et al.* 1999). Moreover, lake bathymetry suggests that subsequent small fluctuations up to ~100 m below present could have been managed by vertical migration along the steep slope without interfering with population isolation. In the shallow regions, the same fluctuations would have caused horizontal displacement and perhaps contact with neighbouring populations, or extinction of local populations, both followed by recolonization from a common source. The alignment of genetically reconstructed events with historic lake level drops is hindered by the large confidence intervals of the former and the uncertainty about the extent of the latter. Whereas several studies agree on the timing of

major water level drops in Lake Tanganyika, their extent has been a longstanding matter of debate. Most recent studies indicate rather drastic decreases of the water level during the most severe drops in the Late Pleistocene. Thus, a lake level drop of ~435 m was suggested for the megadrought prior to ~100 KYA, and the water level was proposed to have been reduced by ~260 m during the last glacial maximum (LGM) at ~20 KYA (McGlue *et al.* 2008), while previous estimates for the LGM range from 150 to 400 m below the present level (Rüber *et al.* 2001 and references therein). The splits among the eastern steep-shore populations date back to >90 KYA (with large confidence intervals), such that these populations apparently persisted throughout the more recent Pleistocene fluctuations more or less unimpaired, although this is difficult to envision if the lake level dropped by more than 100 m. In contrast, the splits among the shallow-shore populations are more readily lined up with a rising water level after the lowstand during the LGM (Tiercelin & Mondeguer 1991; Cohen *et al.* 1997; Scholz *et al.* 2003; Felton *et al.* 2007; McGlue *et al.* 2008).

Reconstructed demographic population histories also comply with the expected combined effects of lake level fluctuations and bathymetry. Most populations expanded during the last ~50–100 KY, i.e. the period coinciding with the major lake level rise recently proposed for Lakes Malawi and Tanganyika following the East African megadroughts 75–135 KYA (Scholz *et al.* 2003; Cohen *et al.* 2007; McGlue *et al.* 2008). An additional boost of population growth coincides with rising water levels at the end of the last glacial maximum (Tiercelin & Mondeguer 1991; Cohen *et al.* 1997; Scholz *et al.* 2003; Felton *et al.* 2007; McGlue *et al.* 2008). This demographic development is congruent with similarly extensive population expansions of Lake Malawi rock-cichlid populations following a lake lowstand during the East African megadroughts (Genner *et al.* 2010). As in the present study, large current population sizes and recent population expansions have been inferred in other *Tropheus* populations as well as in other littoral cichlids in southern Lake Tanganyika (Koblmüller *et al.* 2007, 2009a; Sefc *et al.* 2007a). Even though these estimates of effective population sizes may suffer upward biases from violations of model assumptions, *Tropheus* populations are certainly very large with census counts of >100 subadult and adult individuals per 400 m^2 at two locations in our study area (Sturmbauer *et al.* 2008).

As predicted from the presumed historical habitat dynamics, the demographic change was much stronger in the western shallow-shore populations than in the steep-shore populations east of Chituta Bay (Fig. 5). Moreover, the high coalescent-based estimates of population sizes

and the high indices of mitochondrial and nuclear genetic diversity for the western populations may in part reflect the effect of recurrent population fusions and introduction of genetic variability from other populations. Similarly, but on a larger geographic scale, populations of the cichlid *Eretmodus cyanostictus* from the rather shallow southern tip of Lake Tanganyika displayed higher levels of genetic diversity than populations from the steep Congolese coast, where the flanks of the rift drop continuously to ~1 400 m below the current lake surface; moreover, like in the present study, genetic divergence was lower among the shallow-shore *Eretmodus* populations (Sefc *et al.* 2007a).

The effects of the changes in shoreline structure during lake level fluctuations are not only evident in the different population histories on steep and shallow coasts, but also in the disparate responses to two major habitat barriers in the area, Mbete and Chituta Bay, despite of their currently similar extension of ~6–7 km of shoreline. Mbete Bay separates two ancient mitochondrial, phenotypically distinct lineages of *Tropheus* (Sefc *et al.* 2007a), whose split dates back to ~370–650 KYA (calculated from net divergence between lineages), whereas populations across Chituta Bay split at ~140–250 KYA (Fig. 4) and phenotypic differentiation is much smaller (Fig. 1). The bathymetry of Lake Tanganyika (Fig. 1) suggests that the shallow sandy habitat corresponding to Chituta Bay might have disappeared and been replaced by steep coast when the lake level dropped by more than 300 m, whereas the shallow sloping shore, which is more likely to remain sandy, would have increased considerably in its width in the area corresponding to Mbete Bay.

The longstanding population isolation across rather short geographic distances in a single body of water may appear astonishing, especially when considering that entire lacustrine species flocks evolved within much shorter timeframes. However, compared with other lacustrine environments and their fish communities, Lake Tanganyika is very old, the geomorphology of its southern basin has been rather stable, and the age of the genus *Tropheus* (Koblmüller *et al.* 2010) exceeds the age of entire endemic fish species flocks counting hundreds of species such as the one of Lake Victoria (Verheyen *et al.* 2003; Stager & Johnson 2008). As long as barriers to dispersal persist throughout time, isolation may do so as well. Similar extents of deep genetic divergence across short distances (e.g. across Mbete Bay) were observed in other stenotopic rock dwellers of Lake Tanganyika (Duftner *et al.* 2006; Sefc *et al.* 2007a) and even in the ecologically more versatile *Neolamprologus caudopunctatus* (Koblmüller *et al.* 2007). We ascribe the low absolute values of microsatellite differentiation estimates between the divergent populations in our

study to allele size homoplasy accumulating over the long period of isolated evolution (Nauta & Weissing 1996; Queney *et al.* 2002; Sefc *et al.* 2007b).

Compared to the phenotypic diversity displayed by haplochromine cichlids in Lakes Victoria and Malawi and by species flocks in postglacial lakes, the phenotypic differentiation between the divergent *Tropheus* populations of our study is rather small and primarily restricted to coloration. When our study populations established themselves in southern Lake Tanganyika, they were already part of a mature community of littoral rock-dwelling cichlids, and therefore did not encounter the ecological opportunities offered to the founders of the species flocks in other lakes. Furthermore, it is well established that intra- and intersexual selection promoted colour pattern diversification in the haplochromine species flocks (e.g. Maan *et al.* 2004; Pauers *et al.* 2004; Dijkstra *et al.* 2007), whereas no such clear evidence exists for *Tropheus* (Egger *et al.* 2008, 2010; Sefc 2008; Steinwender *et al.* unpublished data). With interspecific competition preventing ecological differentiation and without evident selection on colour patterns, phenotypic differentiation among *Tropheus* populations may indeed proceed comparatively slowly.

Conclusions

Climate oscillations drive cycles of population isolation and gene flow

The above conclusions are derived from the overall trends indicated by our results, but we are not oblivious to some specific patterns pertaining to individual populations, such as, e.g. gene flow out of Katanka indicated by differentiation statistics and coalescent analysis, population decline in two western populations (Mtondwe Island and Kasenga) and the different demographic reconstructions of two undifferentiated populations (Kasenga and Kasenga Rocks). While biologically plausible explanations could be constructed for some of these cases, the stochasticity of lineage sorting is an equally likely contributor to idiosyncratic results and large confidence intervals around the IMa and BSP estimates mitigate some of the putative inconsistencies. Based on the current data, we therefore do not elaborate on individual populations but concentrate on broader patterns receiving more general support.

Overall, our data endorse the view that during the evolutionary history of the studied *Tropheus* populations (and probably of other *Tropheus* populations in comparable environmental situations), prolonged periods of allopatric divergence, mediated by philopatric behaviour and sensitivity to dispersal barriers, were sporadically interrupted by gene flow, which was effected by

lake level fluctuations and controlled by the persistence of habitat barriers during lake level changes. The concordance of the here described demographic patterns with those of rock-dwelling cichlids from Lake Malawi (Genner *et al.* 2010) supports the idea that large-scale, climate-driven environmental changes synchronized population differentiation and diversification not only among populations and species within one lake, but also across both lakes (Sturmbauer *et al.* 2001), although the finer details of population structure and demography are certainly subject to species-specific modifications (Meyer *et al.* 1995; Taylor *et al.* 2001; Duftner *et al.* 2006; Koblmüller *et al.* 2007, 2009a; Sefc *et al.* 2007a; Wagner & McCune 2009).

Environmentally determined cycles of allopatric differentiation and hybridization in secondary contact are not uncommon in taxa lacking postzygotic incompatibility, for example when Pleistocene climate oscillations imposed range shifts on both temperate and arctic species (e.g. Kontula & Väinölä 2004; Viñas *et al.* 2004; Melo-Ferreira *et al.* 2007; Koblmüller *et al.* 2009b). In sympatric species, alternating periods of reproductive isolation and hybridization are often associated with fluctuating selection pressure, rendering hybrid fitness higher in some conditions than in others (Grant & Grant 1993). The adaptive value of different colour patterns in *Tropheus* is not known, but the communicative function of body coloration suggests a role in social and sexual interaction (Egger *et al.* 2008, 2010). Indeed, pronounced colour pattern differences between morphs separated by large habitat discontinuities sometimes coincide with assortative mate preferences (Egger *et al.* 2008, 2010), which could prevent hybridization and cause hybrid disadvantage during secondary contact (Salzburger *et al.* 2006). In contrast, a lack of discrimination particularly between more similar morphs (Egger *et al.* 2010) suggests little constraint to gene flow among some phenotypically differentiated populations. Hence, both the evolution and the preservation of phenotypic variation among *Tropheus* populations in a region may be contingent on a sufficiently wide spacing of gene flow-mediating environmental disturbances.

Acknowledgements

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Data accessibility

Data deposited at Dryad: doi: 10.5061/dryad.8832

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Estimates of actual differentiation, D_{EST} (Jost 2008), based on nine microsatellite loci (above diagonal) and mtDNA (below diagonal).

Table S2 Maximum likelihood estimates of the parameters for the effective population sizes of the ancestral (θ_A) and the daughter populations (θ_1, θ_2), migration rates ($m_{2 \to 1}$, $m_{1 \to 2}$), and population divergence time (T) inferred with IMa. Values in parentheses represent the interval of the 90% highest posterior density (HPD).

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