Published in final edited form as:

Ecol Lett. 2017 May 1; 20(5): 651–662. doi:10.1111/ele.12766.

Shifting barriers and phenotypic diversification by hybridization

Kristina M. Sefc^{1,*}, Karin Mattersdorfer¹, Angelika Ziegelbecker¹, Nina Neuhüttler¹, Oliver Steiner², Walter Goessler², and Stephan Koblmüller¹

¹Institute of Zoology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria

²Institute of Chemistry - Analytical Chemistry, University of Graz, Universitätsplatz 1, 8010 Graz, Austria

Abstract

The establishment of hybrid taxa relies on reproductive isolation from the parental forms, typically achieved by ecological differentiation. Here, we present an alternative mechanism, in which shifts in the strength and location of dispersal barriers facilitate diversification by hybridization. Our case study concerns the highly diverse, stenotopic rock-dwelling cichlids of the African Great Lakes, many of which display geographic color pattern variation. The littoral habitat of these fish has repeatedly been restructured in the course of ancient lake level fluctuations. Genetic data and an experimental cross support the hybrid origin of a distinct yellow-colored variant of *Tropheus moorii* from ancient admixture between two allopatric, red and bluish variants. Deficient assortative mating preferences imply that reproductive isolation continues to be contingent on geographic separation. Linking paleolimnological data with the establishment of the hybrid variant, we sketch a selectively neutral diversification process governed solely by rearrangements of dispersal barriers.

Keywords

environmental fluctuations; climate change; *Tropheus*; Cichlidae; Lake Tanganyika; hybrid speciation; carotenoid coloration; color pattern; mate choice; genetic admixture

Introduction

Interspecific hybridization and genomic admixture among divergent populations can generate novel phenotypic traits much faster than evolutionary processes relying on mutation and selection (Stelkens & Seehausen 2009; Heliconius Genome Consortium 2012; Nichols et al. 2015). A challenge associated with diversification by hybridization lies in the isolation of a homoploid hybrid lineage from its parental taxa (Buerkle et al. 2000). Ecological

* **correspondence:** Kristina M. Sefc, Universitätsplatz 2, 8010 Graz, Austria; Tel. +43-316-3805601, fax: +43-316-3809875, kristina.sefc@uni-graz.at.

Statement of authorship: K.M.S. conceived the study and designed the experiments; K.M. and S.K. conducted fieldwork; K.M. generated the genetic data; A.Z. and N.N. conducted the mate choice experiments; O.S., W.G. and K.M.S. carried out the HPLC analysis; K.M.S., K. M. and S. K. analyzed the data; K.M.S. wrote the manuscript.

Data accessibility statement: The AFLP dataset will be deposited in dryad and DNA sequences will be deposited in Genbank; accession numbers and data DOI will be included in the article. Carotenoid HPLC profiling data are included in the supplementary information.

differentiation is generally considered essential to reduce both gene flow and competition between homoploid hybrids and their sympatric parent taxa (Gross & Rieseberg 2005; Rosenthal 2013). Occasionally, hybrid traits induce assortative mating and thus confer behavioral isolation of early generation hybrids (Melo et al. 2009; Selz et al. 2014b). Of course, reproductive isolation and competition avoidance could effectively be achieved by geographic separation between hybrids and their progenitors (Buerkle et al. 2000; Duenez-Guzman et al. 2009), but since hybrid taxa necessarily originate in sympatry or parapatry with the parental forms, geographic isolation can only be established in a second step. Several studies identified hybrid taxa whose ecological distinctness enabled them to achieve geographic isolation from the parental lineages by colonizing a different, sometimes extreme habitat type (Nolte et al. 2005; Gompert et al. 2006; Rieseberg et al. 2007).

Alternatively, when habitats are repeatedly restructured e.g. as a consequence of climate oscillations (Nores 1999; Hewitt 2011), shifts in the strength and location of dispersal barriers may not only facilitate hybridization in secondary contact but furthermore establish geographic isolation between parent and hybrid taxa. More specifically, a rearrangement of dispersal barriers may generate a spatially confined admixture zone that includes only a fraction of each of the two parents' ranges and is separated from the rest of the parents' ranges by new barriers. In this 'shifting barriers' model, the hybrid population can become stabilized irrespective of immediate ecological differentiation or behavioral isolation from the parental forms (Fig. 1).

Richly structured habitats undergoing periodic restructuring are for instance found in the shallow littoral zone of large lakes, where lake level fluctuations entail major alterations in the course and structure of the shoreline (Colman 1998; Danley et al. 2012; Vogel et al. 2015). Along the vast shorelines of the East African lakes Tanganyika and Malawi, patches of rocky habitat are isolated from each other by sand beaches and estuaries of rivers and streams. These habitat interruptions impose strong barriers to the dispersal of stenotopic rock-dwelling organisms and have shaped the phylogeographic pattern of genetic and sometimes also phenotypic population differentiation, both of which can be pronounced even across short geographic distances (e.g., (van Oppen et al. 1997; Arnegard et al. 1999; Taylor et al. 2001; Egger et al. 2007; Wagner & McCune 2009; Koblmüller et al. 2011).

Changes in the extent and distribution of rock and sand habitat have repeatedly been brought about by lake level fluctuations. The most recent severe fluctuations, which included drops of several hundred meters below present level, occurred during the last glacial maximum (LGM, ~12 KYA) and during a period of severe droughts (the early Late Pleistocene megadroughts) between 75 and 125 KYA (Cohen et al. 1997; Cohen et al. 2007; McGlue et al. 2008; Lyons et al. 2015). Effects of the associated shoreline changes on the rock-dwelling fish are evident in the populations' demographic history, genetic diversity and genetic structure (Genner et al. 2010; Koblmüller et al. 2011; Nevado et al. 2013; Winkelmann et al. 2017).

In Lake Tanganyika, cichlid fish of the endemic genus *Tropheus* are common, highly stenotopic inhabitants of the shallow rocky littoral (typically in depths of 1 to 10 m). Populations display distinct genetic and color pattern differentiation even across minor

habitat barriers (Schupke 2003; Egger et al. 2007; Sefc et al. 2007; Koblmüller et al. 2011; Konings 2013; Nevado et al. 2013; Sefc et al. 2017; Sturmbauer et al. 2017), but show only little if any genetically determined eco-morphological differentiation (Kerschbaumer et al. 2011). The rapid genetic differentiation across small barriers, which took place since the establishment of the present high-stand shoreline following the LGM, and the absence of differentiation in the absence of barriers (Koblmüller et al. 2011) suggest that gene flow is controlled by barriers rather than by distance. Moreover, correlations between genetic variation and lake bathymetry indicate that introgression among populations depends on lake level fluctuation-associated changes in habitat structure rather than continuous gene flow (Koblmüller et al. 2011; Nevado et al. 2013; Sefc et al. 2017).

While *Tropheus* represents one of the most extreme examples of color pattern diversity with >100 distinct color variants (Schupke 2003), rich genetic and chromatic differentiation has also evolved in other stenotopic rock-dwelling cichlid species (Arnegard et al. 1999; Smith et al. 2003; Wagner & McCune 2009). Signatures of genomic admixture suggested a potential hybrid origin of color variants in some species (Smith et al. 2003; Egger et al. 2007; Koblmüller et al. 2010). Based on these facts, we propose that hybridization played a role in the pronounced color pattern diversification of stenotopic littoral cichlids and that both secondary contact and subsequent isolation were facilitated by rearrangements of habitat barriers in the course of lake level fluctuations.

Three color morphs of *T. moorii* (Boulenger 1998) (a *Tropheus* ‘morph’ unites related populations with similar color patterns) are distributed allopatrically along ~150 km of shoreline in southern Lake Tanganyika (Fig. 2). Littoral populations in this region of the lake were greatly displaced, and possibly brought into secondary contact, by the lake level drops of >400 m and ~260m below present level during the early late Pleistocene megadroughts and the last glacial maximum, respectively (Cohen et al. 1997; Cohen et al. 2007; McGlue et al. 2008). The yellow morph, which is located between the bluish and red morph, has indeed been suggested to be a hybrid between its neighboring morphs (Egger et al. 2007), but the phylogenetic test which prompted this suggestion has later been shown to yield high rates of false positive signals, when divergence has in fact been dichotomous (Schneider et al. 2016). Here, we readdress the origin of the yellow-morph *Tropheus* and use it as example to build empirical support for the ‘shifting barriers’ model of phenotypic diversification through hybridization (Fig. 1). We conducted population genetic analyses to distinguish the hypothesized hybrid origin of the yellow-morph *Tropheus* from the alternative scenario of dichotomous divergence, related the inferred admixture event to shifts of dispersal barriers during ancient lake level fluctuations, and assessed the efficacy of geographic population separation for preventing gene flow. Experimental crosses explored the causal relationship of phenotypic diversification with the hybridization event, i.e. whether the new, yellow body color of the admixed populations could arise instantaneously from hybridization between the parent morphs rather than in response to subsequent, perhaps adaptive, evolution. Integumentary carotenoid profiles were compared among populations to explore the biochemical novelty of the new hybrid phenotype. Finally, to address the potential of the outlined diversification process to initiate speciation rather than remain contingent on geographic isolation, we tested behavioral reproductive isolation between hybrid and parent morphs. Our results support a scenario in which shifting habitat barriers facilitated

secondary contact and filled in for ecological and behavioral differentiation to ensure reproductive isolation and maintain the phenotypic diversity between hybrid and parent forms.

Material and Methods

Sampling and genetic data analysis

For genetic analyses, populations of *T. moorii* were sampled from 12 locations in southwestern Lake Tanganyika between 2005 and 2009 (Fig. 2, Table S1). DNA extraction from alcohol-preserved fin clips employed proteinase K digestion and ammonium acetate precipitation. Mitochondrial control region sequence data and AFLP data (18 selective primer pairs) were generated as in (Sefc et al. 2017). The aligned mtDNA data matrix comprised 800 bp. Estimates of diversity, differentiation and net divergence in mtDNA sequences were calculated as described in (Sefc et al. 2017).

A Bayesian inference tree was inferred in MrBayes 3.2 (Ronquist et al. 2012). Posterior probabilities were obtained from MCMC simulations (2 independent runs; 10^6 generations each; trees sampled every 1000 generations; burn-in 10%) run under the best-fitting model of molecular evolution as identified by the Bayesian Information Criterion in jModelTest 0.1.1 (Posada 2008). Chain stationarity and parameter convergence were checked using Tracer 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>). Demographic parameters were estimated in IMA2 (Hey 2010) (mutation model: HKY; 2 replicate runs with different random number seeds; burn-in: 100,000 steps; effective sample size for each estimated parameter 200).

Population genetic analyses of the AFLP data (1045 polymorphic markers) were carried out as in (Sefc et al. 2017). Analyses in STRUCTURE v. 2.3.3 (Pritchard et al. 2000) were run with five replicates for each K ranging from 1 to 13, and the most likely number of clusters was determined following the method of (Evanno et al. 2005). A multidimensional scaling plot of AFLP variation was based on Jaccard distances, as implemented in the R-package Vegan (R Core Team 2013; Oksanen et al. 2015). The Neighbor-Joining tree was based on (Nei & Li 1979) distances.

Four nuclear loci (TMO-4C4, Rag1 exon3, Rag1 intron2, Phpt1) were sequenced and searched for SNP polymorphisms (Appendix S1).

Experimental crosses between red- and bluish-morph *Tropheus*

In January 2011, two red-morph females (population *T. m.* 'Chimba') and two bluish-morph males (population *T. m.* 'Chaitika') were introduced into a 720 liter tank. Over a period of two years, both females mated repeatedly with one of the two males and produced 60 F1 offspring.

Carotenoid extraction and HPLC analysis

The fish used for integumentary carotenoid extraction originate from stocks kept at the Institute of Zoology at Graz University. Fish dying in our facility were conserved at -20°C, provided that they showed no signs of decay, damage or sickness-induced phenotypic

alterations. Fish were fed standard flake food (JBL Novo Malawi). Most population samples were comprised of individuals that belonged to different batches and had died at different points in time, yet individuals within a population had similar carotenoid composition (Fig. S1), suggesting that the variance in sampling conditions inherent to this approach did not distort the analysis. The bluish morph was represented by population Chaitika (n=7). Ilangi (n=7) and Chilanga (n=6) represented the admixed populations, and the red morph was represented by populations from Kachese (n=1), Ndole (n=3), Chipimbi (n=6) and Moliro (n=5). As coloration of *T. moorii* is sexually monomorphic, population samples comprised both males and females.

Skin sample area was delimited dorsally by the lateral line and comprised the sections between pectoral fin and the anal opening ('rump') and between the front edge of the anal fin and the base of the caudal fin ('caudal peduncle'; Fig. S2). Scales and muscle tissue were carefully removed. The fin sample was taken from the anterior part (up to spine #8) of the dorsal fin (Fig. S2). Samples were collected in 1.5 ml polypropylene (PP) tubes and incubated for 16 hours in 500 μ l acetone on an Eppendorf thermoshaker at 10°C / 750 rpm. After 3 min centrifugation at 14,000 rpm, aliquots were transferred into 250 μ l PP vials for HPLC analysis. The extracts were protected from light at all times. All samples were prepared within one week and analyzed immediately after extraction. The order, in which fish were analyzed, was randomized such that individuals from the same population were processed in different batches (Table S2).

Carotenoids were separated on an Agilent 1260 Infinity HPLC system equipped with a Zorbax SB C18 150 x 4.6 mm, 5 μ m column (Agilent, Waldbronn, Germany) using a flow rate of 1.0 ml min⁻¹ and an injection volume of 10 μ l. The column was operated at 30°C. Acetonitrile (gradient grade) was used to elute the free carotenoids from the column. After each run, the column was flushed with 100 % tetrahydrofuran for 5 minutes to remove strongly retained compounds, especially the carotenoid esters. The carotenoids were detected at a wavelength of 475 nm. Typical chromatograms are shown in Fig. S3. Signal areas between 2 to 20 min were integrated (perpendicular dropline between two adjacent peaks) and recorded for each chromatogram. The relative signal intensity per peak was scored as percentage of the total signal area of the extract. The sample preparation and the chromatographic conditions were attuned to the detection of non-esterified carotenoids. Saponification was omitted in order to avoid the risks of artefact formation and degradation (Rodriguez-Amaya 2001). A presence/absence data matrix of the scored carotenoid peaks per fish was used to calculate Jaccard distances among individual fish, which were displayed graphically by non-metric multidimensional scaling (NMDS) using the R package vegan (Oksanen et al. 2015). Differences among morphs and populations were tested by permutations (function 'envfit' in vegan).

Mate choice experiments

Mating preferences of yellow morph females (population Ilangi) were tested in two 2-way choice experiments with males from Ilangi paired with males from Nakaku (bluish morph) or Moliro (red morph), respectively. Within each experiment, individuals were used only once and ten successful trials were completed. The experimental setup was identical to

experiment 1 in (Hermann et al. 2015). Briefly, each trial consisted of a 4-day acclimation period, during which males and females were separated by mesh partitions, and a 4-day period with two “access sessions” per day, during which females could interact with one male at a time and behavior was scored for 30 minutes following the first interaction of the pair. Female courtship rate (FC) with each stimulus male was determined as the counts of T-positions (courting fish nuzzling each other’s genital papilla) and female quiver bursts per minute observation time. A ‘relative female courtship’ index (RFC) for each female was calculated as $(FC_{\text{homotypic}} - FC_{\text{heterotypic}}) / (FC_{\text{homotypic}} + FC_{\text{heterotypic}})$, such that positive values of RFC signify a bias towards positive assortative courtship in a trial. An intercept-only model was used to test whether the average of the RFC values of females within an experiment deviated significantly from zero, which would indicate a population bias towards positive or negative assortative courtship preferences.

Results

Genomic admixture in the yellow morph populations

The STRUCTURE analysis of the AFLP data identified two genetic clusters corresponding to the red and the bluish color morph, respectively, and genomic admixture in geographically intermediate, yellow and orange-colored populations (Fig. 3a; Fig. S4). Congruently, multidimensional scaling of AFLP variation (Fig. 3c) and a Neighbor-Joining tree based on the genetic distances among individuals (Fig. S5) placed the yellow morph intermediate to the bluish and the red morph. These analyses also revealed a geographic cline in the admixture proportions along the distribution of the yellow-morph populations (Fig. 3, Fig. S5). Based on the genetic structure apparent in the STRUCTURE and the multidimensional scaling plot, we refer to admixed populations (Linangu, Ilangi, Chilanga and Chisanze) in contrast to pure red and pure bluish morph populations, where heteromorphic ancestry coefficients from STRUCTURE averaged to only 1.1%. Consistent with the signal in the AFLP data, admixed populations shared a nuclear SNP polymorphism at the nuclear Tmo-4C4 locus with the bluish population, whereas the pure red morph populations (except Kachese) were fixed for one of the two alleles (Fig. 3b). Among the three other investigated loci, Phpt1 was monomorphic and the polymorphisms at the Rag intron and exon loci were shared among all populations (Appendix S1).

Mixing the yellow-morph color pattern

Two observations suggest that hybridization between bluish and red morph *Tropheus* could have given rise to the yellow morph color pattern. First, experimental crosses between red and bluish morph *Tropheus* (female *T. m.* ‘Chimba’ x male *T. m.* ‘Chaitika’) produced yellow colored F1 offspring, which resembled one of the extant admixed populations (Fig. 3d). Second, increasing red morph ancestry in the admixture cline by and large coincides with increasing redness of the admixed populations (Fig. 2, Fig. 3).

Integumentary carotenoid pigment composition

The red and yellow integument of *Tropheus* is rich in carotenoids (Mattersdorfer et al. 2012). To explore whether the novel, yellow coloration of the admixed populations represents a biochemical novelty in terms of the metabolic generation of carotenoid

compounds that are not produced by the parental populations, the chromatographic profiles of free (i.e., non-esterified) carotenoids in skin (rump and caudal peduncle) and dorsal fin tissue extracts were compared among the three morphs. Across all extracts, 25 different chromatographic signals were distinguished by retention time (Table S2, Fig. S2). Most signal peaks were shared between the admixed populations and at least one of the parental morphs (Fig. 4). Only one minor peak (<2% relative intensity) was restricted to an admixed population and was detected exclusively in the dorsal fin tissue of five *T. m.* 'Ilangi' fish (Fig. 4). Multidimensional scaling of presence/absence data per signal peak and tissue sample grouped most individuals by population, with significant differences among morphs and among populations within morphs (Fig. S1).

Support for ancient admixture

The geographic signal in the admixture proportions argues against incomplete nuclear lineage sorting after the split between the red and the bluish morph as alternative to admixture in secondary contact. Moreover, the known effect of habitat barriers on *Tropheus* dispersal (see Introduction) makes it unlikely that the nuclear admixture pattern reflects divergence under an 'isolation by distance' model of continuous gene flow. Consistent with the existence of a major habitat barrier between red and bluish morph *Tropheus* in the form of the Lufubu River estuary, the genetic data imply that the admixture between the two morphs was restricted to a period in the past. Substantial ongoing introgression from the parental lineages predicts allelic correlations, but linkage disequilibrium (LD; Table S1) among AFLP loci was not higher in the admixed populations (mean of % locus pairs in LD = 0.044%) than in the pure red and bluish populations (0.046%). Furthermore, ongoing gene flow would erode genetic differentiation, but populations were significantly differentiated from each other in AFLP markers and in mitochondrial control region haplotypes (Table S1), and multidimensional scaling of AFLP variation separated the admixed from the pure populations, as well as many populations within morphs, from each other (Fig. 3c).

Moreover, admixed-population *Tropheus* did not share mitochondrial haplotypes with red or with bluish morph *Tropheus* (Fig. 5). Admixed-population mtDNA haplotypes grouped exclusively with haplotypes of the pure red morph populations, whereas bluish morph *Tropheus* represented a highly divergent mtDNA lineage. While this contrasts with the evidence of nuclear admixture, the retention of only one parental mtDNA lineage after ancient introgression is not uncommon (Gompert et al. 2006; Mavárez & Linares 2008; Medina et al. 2013). To investigate whether the discrepancy between nuclear and mtDNA patterns could be caused by male biased dispersal, we calculated microsatellite-based relatedness coefficients between males and between females in a bluish morph population (Nakaku) using data for 53 males and 100 females at 9 microsatellite loci (Koch et al. 2008). Opposite to the expectation for male-biased dispersal, average relatedness was slightly higher among males than among females (males, mean $\pm$ standard deviation of $r_{QG} = -0.001 \pm 0.151$; females, $r_{QG} = -0.011 \pm 0.145$; $t = 2.18$, $p = 0.029$).

The net divergence between the two major mitochondrial lineages amounts to 5.3% (Fig. 5), which translates into a divergence time between red and bluish morph *Tropheus* of 0.85 – 1.5 million years (1.78 – 3.11% substitution rate, Nevado et al. 2009). Using IMA2, highest

posterior probability estimates of divergence times between individual populations and between pure red and admixed morphs varied within a range from ~100 to ~170 KYA (3.11% substitution rate) or from ~175 to ~300 KYA (1.78% substitution rate; Table S3). We note that lacking an appropriate molecular clock calibration for recent substitution rates in the genus *Tropheus*, the calculated values are likely to overestimate actual divergence times (Ho et al. 2011). Migration parameter estimates between the admixed and the red morph populations were low and, when asymmetric, most often suggested higher mitochondrial gene flow from pure red into the admixed *Tropheus* than vice versa (Table S3).

Lack of assortative mating preferences

Mating preferences of yellow morph females (population ‘Ilangi’) were assessed in two separate two-way choice experiments, one against bluish and one against red males. Several females courted preferentially with one of the two stimulus males, but across all females in an experiment, courtship was not biased towards the own or the heterotypic morph (Fig. 6). Given that courtship among heterotypic *Tropheus* in two-way choice experiments predicted interbreeding in experimental secondary contact (Sefc et al. 2015), the present results indicate that premating reproductive isolation between the yellow and its parental morphs has not yet evolved.

Discussion

The proposed ‘shifting barriers’ model of phenotypic diversification by hybridization (Fig. 1) receives empirical support from this and previous studies in *Tropheus moorii*. First, *Tropheus* populations are strictly structured by habitat barriers (Sefc et al. 2007; Koblmüller et al. 2011; Nevado et al. 2013; Sefc et al. 2017), and perturbations of the littoral habitat structure during major lake level fluctuations are evident in the paleolimnological record (Cohen et al. 1997; Cohen et al. 2007; McGlue et al. 2008). Second, our data revealed admixture of two old genetic lineages in the hypothesized hybrid populations and strong genetic differentiation between the admixed and the parental populations, consistent with spatially confined, ancient hybridization. Third, the experimental creation of yellow-colored F1 demonstrated that the novel body coloration could have been a direct consequence of hybridization. Finally, although phenotypic diversification concerned a potential mate choice trait, the lack of assortative mating preferences in the hybrid population demonstrates the dependency of reproductive isolation on geographic separation and suggests that speciation may not readily ensue from the described process.

Ancient admixture followed by geographic isolation

To appreciate the proposed scenario of population admixture and isolation driven by shifting barriers, it is important to remember that *Tropheus* are strictly restricted to the shallow rocky littoral and that even minor habitat discontinuities interfere with gene flow (Koblmüller et al. 2011; Sefc et al. 2017). During lake level fluctuations, the narrow band of *Tropheus* habitat follows the receding or expanding shoreline, while wave action, changes in shoreline inclination and substratum and the hydrologic regimes of the tributaries restructure the distribution of sand and rock habitat along the shallow littoral (Cohen et al. 1997). Previous studies in *Tropheus* revealed that population splits, introgression and population size

changes are connected to lake level fluctuation-induced habitat changes (Egger et al. 2007; Koblmüller et al. 2011; Nevado et al. 2013; Sefc et al. 2017; Sturmbauer et al. 2017).

The following scenario links *Tropheus* population structure with paleolimnologic reconstructions to infer a possible course of events. The genetic distinctness between the red and bluish morph mitochondrial lineages suggests that the two morphs have been diverging for an estimated ~0.85 – 1.5 million years. A major barrier to dispersal, which may have effectuated this longstanding divergence, is the large Lufubu River, whose wide sandy estuary currently separates the two lineages. The large size of the river and the location of its inflow within a steeply sloping shore section, whose course is only little modified by lake level fluctuations (Fig. 2), suggest that the Lufubu estuary can have persisted as a dispersal barrier throughout changing environmental conditions. However, extreme transformations of the shoreline, which certainly affected the Lufubu barrier, occurred during the early Late Pleistocene megadroughts between 75-125 KYA, when the lake dropped >400 m below present level (McGlue et al. 2008). These lowstands caused the shoreline to recede northwards beyond the present Lufubu River estuary (Fig. 2), thereby possibly obliterating the dispersal barrier, and dramatically displaced the littoral populations. As predicted by the ‘shifting-barriers’ model, the concurrence of changing habitat structure and shifting population distributions could have shaped a spatially confined admixture zone between red and bluish *Tropheus*. With shifts to more humid conditions and the rising of the lake level, population isolation was retained or even reinforced, as the littoral habitat expanded (Koblmüller et al. 2011; Sefc et al. 2017) and the resuscitated tributaries contributed to the fragmentation of the rocky littoral. Importantly, the chronology of population splitting within the bluish morph *Tropheus* is consistent with a north-to-south re-colonization of their present distribution range (Sefc et al. 2017), as expected after a northwards retreat during a lake lowstand.

Our data do not allow us to date the admixture between the red and bluish morph. Introgression of the bluish *Tropheus* lineage is unlikely to predate the divergence between the pure red and the admixed populations, which was estimated to 100-300 KYA and – accounting for a likely bias in the molecular dating of recent events (Ho et al. 2011) - may have been associated with the fluctuations during the early Late Pleistocene megadroughts. On the other hand, introgression between populations must have ceased sufficiently long ago for genetic differentiation and complete mitochondrial lineage sorting to occur in the admixed populations, whose high genetic diversity suggests large effective population sizes and therefore slow genetic drift. Putting together genetic and paleolimnological evidence, we presume that the admixture between the bluish and the red morph *Tropheus* happened during the megadrought lake level fluctuations, after which the bluish morph *Tropheus* inhabiting the shoreline south of the Lufubu River estuary once more remained isolated from the populations north of the estuary. The bathymetric relief (Fig. 2) shows that bluish *Tropheus* could persist south of the Lufubu River estuary through the next major lake level drop of minus 260 m during the last glacial maximum (McGlue et al. 2008). In contrast, this drop displaced populations in the shallow area northwest of the Lufubu estuary and may have triggered the gene flow between the pure red and the admixed populations that has been indicated by the IMA2 parameter estimates.

Increase of color diversity is due to hybridization

Generally, the experimental recreation of a putative hybrid phenotype is strong evidence that the phenotype indeed resulted from hybridization (Rieseberg et al. 2003; Mavarez et al. 2006; Medina et al. 2013). In our study, laboratory crosses between red and bluish *Tropheus* produced yellow-colored offspring, comparable to one of the natural admixed populations (Fig. 3d). While we did not expect to exactly recreate one of the extant yellow wild-type color patterns, the experimental F1 demonstrates that the yellow color phenotype can arise instantaneously during the admixture between the two parental lineages and that the increase in phenotypic diversity can be directly related to the hybridization event. This has important bearings on predictions regarding the relationship between color pattern and environmental variation in the *Tropheus* color pattern radiation. In various taxa, color trait variation represents adaptation to environmental conditions (Endler 1980; Marchetti 1993), while in others, including some hybrid species, habitat choice is effected adaptively according to given phenotypic properties (Gompert et al. 2006; Rieseberg et al. 2007). In contrast, the ‘shifting barriers’ model does not include an interaction between the properties of the inhabited habitat and the color phenotype, as hybrid phenotype and distribution range are independently determined by the parental genotypes and the rearrangement of dispersal barriers, respectively. This does not preclude selection on color patterns subsequent to the admixture event. On the whole, however, the heterogeneity in terms of rock sizes, shoreline inclination and aspect of the rocky patches occupied by populations of the same *Tropheus* color morphs (Konings 2013) suggests that a given color pattern works under a range of environmental conditions.

Novel hybrid colors result from a mix of the parental carotenoids

Dietary carotenoids are either directly deposited in the integument or enzymatically transformed before deposition, and variation in carotenoid body color can reflect either different types or different concentrations of carotenoids, or both (Sefc et al. 2014). Studies in plants revealed that it is not uncommon for hybrids to produce novel chemical compounds not present in their parents (Orians 2000). In accordance with these observations, feathers of hybrid birds contained appreciable amounts of several carotenoid compounds that were not detected in the parental forms (Hudon et al. 2015). The ability of hybridization to turn out novel, transgressive phenotypes constitutes a major contribution to evolution (e.g., (Rieseberg et al. 2007; Stelkens & Seehausen 2009; Selz et al. 2014b; Nichols et al. 2015). In our study system, the yellow body coloration of the admixed populations appears novel compared to the color impression given by the parental morphs, but insofar as carotenoids were distinguishable by the employed chromatographic separation, the admixed populations do not produce novel carotenoid compounds.

Rapid diversification does not translate into rapid speciation

A final question is whether the here described process of diversification has the potential to culminate in speciation, in which case the persistence of the hybrid taxon should eventually become independent of its geographic separation from the parental lineages. Distinctiveness of hybrids in traits directly related to mate choice, such as odor, calls and color, can provide for assortative mating and hence contribute to reproductive isolation between hybrids and

their parental forms (Doherty & Gerhardt 1983; Mavarez et al. 2006; Segura et al. 2011; Selz et al. 2014b). It is known that color patterns mediate assortative mating preferences in various cichlid species (Maan & Sefc 2013; Selz et al. 2014a), and in experimental cichlid hybrids, a correlation has been observed between assortative mating and color distinctiveness from parental species (Selz et al. 2014b). The yellow morph *T. moorii* ‘Ilangi’ in our mate choice tests are clearly distinct from the color patterns of both parental morphs, yet there was no evidence for color-assortative mating preferences. This implies that neither the distinct hybrid color pattern that emerged during admixture nor allopatric divergence following the admixture event have yet established sexual barriers, and that geographic separation continues to be indispensable for reproductive isolation between hybrid and parent populations. Indeed, the geographic separation and the independence from ecological differentiation, both of which facilitate rapid diversification in our model, may on the other hand delay the evolution of assortative mating preferences and protract the initiation of speciation.

Conclusion

The evolutionary history of the yellow *Tropheus* hybrid morph adverts to a possibly decisive role of shifting barriers in diversification through hybridization, but how taxonomically and geographically widespread could this mechanism be? Its key components are fragmented habitats subject to periodic rearrangements of dispersal barriers, and stenotopic organisms that are restricted in their dispersal by these barriers in all life stages. Water level fluctuations have repeatedly re-structured the marine littoral as well as the littoral of large ancient lakes, but in many aquatic species, planktonic larval dispersal interferes with population isolation (Cheang et al. 2012; Koblmüller et al. 2015). Mouthbrooding interferes with larval dispersal in *Tropheus* and many other stenotopic rock-dwellers in Lakes Malawi and Tanganyika, many of which display pronounced allopatric color pattern variation among eco-morphologically undifferentiated populations (Konings 1998; Genner & Turner 2005; Maan & Sefc 2013). Lake level fluctuations have been proposed as drivers of diversity among lacustrine cichlids by splitting formerly continuous populations (Rossiter 1995) or generating ecological opportunities (Winkelmann et al. 2017). The ‘shifting barriers’ model of diversification through hybridization represents another way in which lake level fluctuations can have promoted cichlid diversification.

Periodic rearrangements of dispersal barriers have also affected terrestrial organisms on continental shelf archipelagos, where the connectivity among individual islands varied in the course of sea level changes (Comes et al. 2008; Esselstyn & Brown 2009). Similarly, Pleistocene climatic oscillations repeatedly established and extinguished habitat bridges for stenotopic organisms such as cold-adapted species in alpine areas, or amphibians in arid regions (Guralnick 2007; Galbreath et al. 2009; Dufresnes et al. 2016). In all these cases, environmental fluctuations may have created spatially confined contact zones and then sequestered hybrid and parent taxa on separate (sky) islands or in otherwise fragmented habitat patches. With the continuous refinement of methods for the identification and dating of ancient hybridization, future work will provide more data to appraise the importance of habitat structure rearrangements for diversification by hybridization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Lawrence Makasa, Danny Sinyinza and all the other people at the Mpulungu Station of the Ministry of Agriculture and Cooperatives, Republic of Zambia for their help and hospitality during fieldwork. We thank Walter Salzburger for the oligonucleotide primers used in the analysis of the nuclear SNP loci, Ad Konings, Peter Berger and Wolfgang Gessl for *Tropheus* photographs, and Kai Winkelmann and Martin Genner for the line drawing of Lake Tanganyika used in Fig. 2. The work was supported by the Austrian Science Fund (FWF) grant number P20883-B16 to KMS.

References

- Arnegard ME, Markert JA, Danley PD, Stauffer JRI, Ambali AJ, Kocher TD. Population structure and colour variation of the cichlid fish *Labeotropheus fuelleborni* Ahl along a recently formed archipelago of rocky habitat patches in southern Lake Malawi. *Proc R Soc Lond B Biol Sci.* 1999; 266:119–130.
- Boulenger GA. Report on the collection of fishes made by Mr. J.E.S. Moore in Lake Tanganyika during his expedition 1895-96. *Transactions of the Zoological Society of London.* 1898; 15:1–37.
- Buerkle CA, Morris RJ, Asmussen MA, Rieseberg LH. The likelihood of homoploid hybrid speciation. *Heredity.* 2000; 84:441–451. [PubMed: 10849068]
- Cheang CC, Tsang LM, Ng WC, Williams GA, Chu KH, Chan BK. Phylogeography of the cold-water barnacle *Chthamalus challengerii* in the north-western Pacific: effect of past population expansion and contemporary gene flow. *J Biogeogr.* 2012; 39:1819–1835.
- Cohen AS, Lezzar K, Tiercelin J, Soreghan M. New palaeogeographic and lake-level reconstructions of Lake Tanganyika: implications for tectonic, climatic and biological evolution in a rift lake. *Basin Research.* 1997; 9:107–132.
- Cohen AS, Stone JR, Beuning KRM, Park LE, Reinthal PN, Dettman D, et al. Ecological consequences of early Late Pleistocene megadroughts in tropical Africa. *Proc Natl Acad Sci USA.* 2007; 104:16422–16427. [PubMed: 17925446]
- Colman SM. Water-level changes in Lake Baikal, Siberia: tectonism versus climate. *Geology.* 1998; 26:531–534.
- Comes HP, Tribsch A, Bittkau C. Plant speciation in continental island floras as exemplified by *Nigella* in the Aegean Archipelago. *Philos Trans R Soc Lond B Biol Sci.* 2008; 363:3083–3096. [PubMed: 18579477]
- Danley PD, Husemann M, Ding B, DiPietro LM, Berverly EJ, Peppe DJ. The impact of the geologic history and paleoclimate on the diversification of East African cichlids. *Int J Evol Biol.* 2012
- Doherty JA, Gerhardt HC. Hybrid tree frogs: vocalizations of males and selective phonotaxis of females. *Science.* 1983; 220:1078–1080. [PubMed: 17754555]
- Duenez-Guzman EA, Mavárez J, Vose MD, Gavrillets S. Case studies and mathematical models of ecological speciation. 4. Hybrid speciation in butterflies in a jungle. *Evolution.* 2009; 63:2611–2626. [PubMed: 19545268]
- Dufresnes C, Litvinchuk SN, Leuenberger J, Ghali K, Zinenko O, Stöck M, et al. Evolutionary melting pots: a biodiversity hotspot shaped by ring diversifications around the Black Sea in the Eastern tree frog (*Hyla orientalis*). *Mol Ecol.* 2016
- Egger B, Koblmüller S, Sturmbauer C, Sefc KM. Nuclear and mitochondrial data reveal different evolutionary processes in the Lake Tanganyika cichlid genus *Tropheus*. *BMC Evol Biol.* 2007; 7:137. [PubMed: 17697335]
- Endler JA. Natural selection on color patterns in *Poecilia reticulata*. *Evolution.* 1980; 34:76–91. [PubMed: 28563214]

- Esselstyn JA, Brown RM. The role of repeated sea-level fluctuations in the generation of shrew (Soricidae: *Crocodyra*) diversity in the Philippine Archipelago. *Mol Phylogenet Evol.* 2009; 53:171–181. [PubMed: 19501180]
- Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol.* 2005; 14:2611–2620. [PubMed: 15969739]
- Galbreath KE, Hafner DJ, Zamudio KR. When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). *Evolution.* 2009; 63:2848–2863. [PubMed: 19663994]
- Genner MJ, Turner GF. The mbuna cichlids of Lake Malawi: a model for rapid speciation and adaptive radiation. *Fish Fish.* 2005; 6:1–34.
- Genner MJ, Knight ME, Haesler MP, Turner GF. Establishment and expansion of Lake Malawi rock fish populations after a dramatic Late Pleistocene lake level rise. *Mol Ecol.* 2010; 19:170–182. [PubMed: 20002582]
- Gompert Z, Fordyce JA, Forister ML, Shapiro AM, Nice CC. Homoploid hybrid speciation in an extreme habitat. *Science.* 2006; 314:1923–1924. [PubMed: 17138866]
- Gross BL, Rieseberg LH. The ecological genetics of homoploid hybrid speciation. *J Hered.* 2005; 96:241–252. [PubMed: 15618301]
- Guralnick R. Differential effects of past climate warming on mountain and flatland species distributions: a multispecies North American mammal assessment. *Global Ecol Biogeogr.* 2007; 16:14–23.
- Heliconius Genome Consortium. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature.* 2012; 487:94–98. [PubMed: 22722851]
- Hermann CM, Brudermann V, Zimmermann H, Vollmann J, Sefc KM. Female preferences for male traits and territory characteristics in the cichlid fish *Tropheus moorii*. *Hydrobiologia.* 2015; 748:61–74. [PubMed: 25983339]
- Hewitt GM. Quaternary phylogeography: the roots of hybrid zones. *Genetica.* 2011; 139:617–638. [PubMed: 21234647]
- Hey J. Isolation with migration models for more than two populations. *Mol Biol Evol.* 2010; 27:905–920. [PubMed: 19955477]
- Ho SY, Lanfear R, Bromham L, Phillips MJ, Soubrier J, Rodrigo AG, et al. Time-dependent rates of molecular evolution. *Mol Ecol.* 2011; 20:3087–3101. [PubMed: 21740474]
- Hudon J, Wiebe KL, Pini E, Stradi R. Plumage pigment differences underlying the yellow-red differentiation in the Northern Flicker (*Colaptes auratus*). *Comp Biochem Physiol B Biochem Mol Biol.* 2015; 183:1–10. [PubMed: 25575737]
- Kerschbaumer M, Postl L, Koch M, Wiedl T, Sturmbauer C. Morphological distinctness despite large-scale phenotypic plasticity - analysis of wild and pond-bred juveniles of allopatric populations of *Tropheus moorii*. *Naturwissenschaften.* 2011; 98:125–134. [PubMed: 21161156]
- Koblmüller S, Steinwender B, Weiß S, Sefc KM. Gene flow, population growth and a novel substitution rate estimate in a subtidal rock specialist, the black-faced blenny *Tripterygion delaisi* (Perciformes, Blennioidei, Tripterygiidae) from the Adriatic Sea. *J Zool Syst Evol Res.* 2015; 53:291–299. [PubMed: 26617435]
- Koblmüller S, Egger B, Sturmbauer C, Sefc KM. Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. *Mol Phylogenet Evol.* 2010; 55:318–334. [PubMed: 19853055]
- Koblmüller S, Salzburger W, Obermüller B, Eigner E, Sturmbauer C, Sefc KM. Separated by sand, fused by dropping water: habitat barriers and fluctuating water levels steer the evolution of rock-dwelling cichlid populations in Lake Tanganyika. *Mol Ecol.* 2011; 20:2272–2290. [PubMed: 21518059]
- Koch M, Hadfield JD, Sefc KM, Sturmbauer C. Pedigree reconstruction in wild cichlid fish populations. *Mol Ecol.* 2008; 17:4500–4511. [PubMed: 18986496]
- Konings, A. *Tropheus in their Natural Habitat*. Cichlid Press; El Paso, Texas: 2013.
- Konings, A. *Tanganyika Cichlids in their Natural Habitat*. Cichlid Press; El Paso, Texas: 1998.

- Lyons RP, Scholz CA, Cohen AS, King JW, Brown ET, Ivory SJ, et al. Continuous 1.3-million-year record of East African hydroclimate, and implications for patterns of evolution and biodiversity. *Proc Natl Acad Sci USA*. 2015; 112:15568–15573. [PubMed: 26644580]
- Maan ME, Sefc KM. Colour variation in cichlid fish: developmental mechanisms, selective pressures and evolutionary consequences. *Semin Cell Dev Biol*. 2013; 24:516–528. [PubMed: 23665150]
- Marchetti K. Dark habits and bright birds illustrate the role of the environment in species divergence. *Nature*. 1993; 362:149–152.
- Mattersdorfer K, Koblmüller S, Sefc KM. AFLP genome scans suggest divergent selection on colour patterning in allopatric colour morphs of a cichlid fish. *Mol Ecol*. 2012; 21:3531–3544. [PubMed: 22625655]
- Mavarez J, Salazar CA, Bermingham E, Salcedo C, Jiggins CD. Speciation by hybridization in *Heliconius* butterflies. *Nature*. 2006; 441:868–871. [PubMed: 16778888]
- Mavárez J, Linares M. Homoploid hybrid speciation in animals. *Mol Ecol*. 2008; 17:4181–4185. [PubMed: 19378399]
- McGlue MM, Lezzar KE, Cohen AS, Russell JM, Tiercelin J, Felton AA, et al. Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical East Africa. *J Paleolimnol*. 2008; 40:635–653.
- Medina I, Wang JJ, Salazar C, Amézquita A. Hybridization promotes color polymorphism in the aposematic harlequin poison frog, *Oophaga histrionica*. *Ecol Evol*. 2013; 3:4388–4400. [PubMed: 24340180]
- Melo MC, Salazar C, Jiggins CD, Linares M. Assortative mating preferences among hybrids offer a route to hybrid speciation. *Evolution*. 2009; 63:1660–1665. [PubMed: 19492995]
- Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci U S A*. 1979; 76:5269–5273. [PubMed: 291943]
- Nevado B, Mautner S, Sturmbauer C, Verheyen E. Water-level fluctuations and metapopulation dynamics as drivers of genetic diversity in populations of three Tanganyikan cichlid fish species. *Mol Ecol*. 2013; 22:3933–3948. [PubMed: 23837841]
- Nevado B, Koblmüller S, Sturmbauer C, Snoeks J, Usano-Aleman J, Verheyen E. Complete mitochondrial DNA replacement in a Lake Tanganyika cichlid fish. *Mol Ecol*. 2009; 18:4240–4255. [PubMed: 19780975]
- Nichols P, Genner MJ, van Oosterhout C, Smith A, Parsons P, Sungani H, et al. Secondary contact seeds phenotypic novelty in cichlid fishes. *Proc Biol Sci*. 2015; 282
- Nolte AW, Freyhof J, Stemshorn KC, Tautz D. An invasive lineage of sculpins, *Cottus* sp., (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. *Proc R Soc Lond B Biol Sci*. 2005; 272:2379–2387.
- Nores M. An alternative hypothesis for the origin of Amazonian bird diversity. *J Biogeogr*. 1999; 26:475–485.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara R, et al. *Vegan: Community ecology package*. R package version 2.2-1. 2015
- Orians CM. The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions. *Am J Bot*. 2000; 87:1749–1756. [PubMed: 11118409]
- Posada D. jModelTest: phylogenetic model averaging. *Mol Biol Evol*. 2008; 25:1253–1256. [PubMed: 18397919]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*. 2000; 155:945–959. [PubMed: 10835412]
- R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2013.
- Rieseberg LH, Kim S, Randell RA, Whitney KD, Gross BL, Lexer C, et al. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica*. 2007; 129:149–165. [PubMed: 16955330]
- Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, et al. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*. 2003; 301:1211–1216. [PubMed: 12907807]

- Rodriguez-Amaya, DB. A Guide to Carotenoid Analysis in Foods. ILSI press; Washington, DC: 2001.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 2012; 61:539–542. [PubMed: 22357727]
- Rosenthal G. Individual mating decisions and hybridization. *J Evol Biol.* 2013; 26:252–255. [PubMed: 23323999]
- Rossiter A. The cichlid fish assemblages of Lake Tanganyika: ecology, behaviour and evolution of its species flock. *Advances in Ecological Research.* 1995; 26:187–252.
- Schneider K, Koblmüller S, Sefc KM. hext, a software supporting tree-based screens for hybrid taxa in multilocus data sets, and an evaluation of the homoplasy excess test. *Methods Ecol Evol.* 2016; 7:358–368.
- Schupke P. African Cichlid II. Tanganyika I. *Tropheus*. Aqualog, Germany. 2003
- Sefc KM, Mattersdorfer K, Hermann CM, Koblmüller S. Past lake shore dynamics explain present pattern of unidirectional introgression across a habitat barrier. *Hydrobiologia.* 2017
- Sefc KM, Hermann CM, Steinwender B, Brindl H, Zimmermann H, Mattersdorfer K, et al. Asymmetric dominance and asymmetric mate choice oppose premating isolation after allopatric divergence. *Ecol Evol.* 2015; 5:1549–1562. [PubMed: 25937900]
- Sefc KM, Baric S, Salzburger W, Sturmbauer C. Species-specific population structure in rock-specialized sympatric cichlid species in Lake Tanganyika, East Africa. *J Mol Evol.* 2007; 64:33–49. [PubMed: 17160645]
- Sefc KM, Brown AC, Clotfelter ED. Carotenoid-based coloration in cichlid fishes. *Comp Biochem Physiol A Mol Integr Physiol.* 2014; 173:42–51.
- Segura DF, Vera MT, Rull J, Wornoayporn V, Islam A, Robinson AS. Assortative mating among *Anastrepha fraterculus* (Diptera: Tephritidae) hybrids as a possible route to radiation of the *fraterculus* cryptic species complex. *Biol J Linn Soc.* 2011; 102:346–354.
- Selz OM, Pierotti MER, Maan ME, Schmid C, Seehausen O. Female preference for male color is necessary and sufficient for assortative mating in 2 cichlid sister species. *Behav Ecol.* 2014a; 25:612–626.
- Selz O, Thommen R, Maan M, Seehausen O. Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. *J Evol Biol.* 2014b; 27:275–289. [PubMed: 24372872]
- Smith PF, Konings A, Kornfield I. Hybrid origin of a cichlid population in Lake Malawi: implications for genetic variation and species diversity. *Mol Ecol.* 2003; 12:2497–2504. [PubMed: 12919487]
- Stelkens R, Seehausen O. Genetic distance between species predicts novel trait expression in their hybrids. *Evolution.* 2009; 63:884–897. [PubMed: 19220450]
- Sturmbauer C, Bärger C, Van Steenberge M, Koblmüller S. A separate lowstand lake at the northern edge of Lake Tanganyika? Evidence from phylogeographic patterns in the cichlid genus *Tropheus*. *Hydrobiologia.* 2017
- Taylor MI, Rüber L, Verheyen E. Microsatellites reveal high levels of population substructuring in the species-poor Eretmodine cichlid lineage from Lake Tanganyika. *Proceedings of the Royal Society London Series B.* 2001; 268:803–808.
- van Oppen MJH, Turner GF, C R, Deutsch JC, Ibrahim KM, Robinson RL, et al. Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proc R Soc Lond B Biol Sci.* 1997; 264:1803–1812.
- Vogel H, Russell JM, Cahyarini SY, Bijaksana S, Wattrus N, Rethemeyer J, et al. Depositional modes and lake-level variability at Lake Towuti, Indonesia, during the past~ 29 kyr BP. *J Paleolimnol.* 2015; 54:359–377.
- Wagner CE, McCune AR. Contrasting patterns of spatial genetic structure in sympatric rock-dwelling cichlid fishes. *Evolution.* 2009; 63:1312–1326. [PubMed: 19154384]
- Winkelmann K, Rüber L, Genner MJ. Lake level fluctuations and divergence of cichlid fish ecomorphs in Lake Tanganyika. *Hydrobiologia.* 2017

Figure 1.

Phenotypic diversification by hybridization in the shifting barriers model. Initially, two phenotypically distinct, ecologically equivalent taxa are separated by a dispersal barrier (1). In the following, rearrangements of dispersal barriers may be associated with changes in the spatial distribution of taxa, such as shifts along mountain or lake slopes, driven by environmental fluctuations. In stage (2), a rearrangement of dispersal barriers creates a spatially confined zone of secondary contact, in which hybridization gives rise to a novel phenotype (3). The new dispersal barriers provide for isolation between hybrid and parent taxa, allowing each of them to retain their original ecological niche within their respective distribution ranges. Following stage 3, further developments depend on the occurrence, timing and extent of further habitat rearrangements and on the evolution of assortative mating. Possible outcomes of renewed secondary contact between hybrid and parent taxa (4) include sympatric coexistence after completed speciation (5a), further phenotypic diversification by hybridization (5b) or the complete assimilation of one taxon if the admixture zone extends across this taxon's full range (not depicted). For simplicity, the graph does not include minor barriers within each taxon's range, which are expected to create additional structure. The linear distribution of taxa, as drawn in this example, is not a condition for the application of the model.

Figure 2.

Tropheus moorii populations sampled from southern Lake Tanganyika. Along the studied shoreline, rocky sections alternate with sandy beaches. A particularly strong habitat barrier is imposed by the wide sandy estuary of the Lufubu River, which separates the distribution ranges of the bluish and the yellow-morph *Tropheus*. Note that *T. moorii* are sexually monomorphic. *T. m.* 'Chipimbi' were included only in carotenoid analysis. *T. m.* 'Funda' (no photo) are indistinguishable from *T. m.* 'Nakaku'; *T. m.* 'Katete' and *T. m.* 'Ndole' (no photo) resemble *T. m.* 'Moliro' and *T. m.* 'Chimba', respectively. Photographs: Ad Konings, Peter Berger, Wolfgang Gessl.

Figure 3.

Genetic admixture in the yellow morph *Tropheus* and experimental recreation of the hybrid color pattern. (a) AFLP-based assignment probabilities for K= 2 genetic clusters, determined in STRUCTURE. (b) Frequency distribution of the G/T polymorphism (red/blue) at a SNP locus in TMO-4C4. (c) Multidimensional scaling plot of AFLP variation. (d) Resemblance between the genetically admixed *Tropheus moorii* 'Ilangi' and experimental F1 hybrids between bluish- and red-morph *T. moorii*.

Figure 4.

Carotenoid chromatograms. This schematic representation depicts the relative signal intensity per HPLC peak, averaged across samples, against the average retention time of the peak. Standard deviations are indicated by the thin vertical lines extending from each bar. The arrow marks a signal restricted to the 'Ilangi' population.

Figure 5.
Bayesian inference tree based on mtDNA sequence data.

Figure 6.

Female courtship preferences in two-way choice experiments. Relative female courtship (RFC) values indicate the rate of female courtship directed towards own-population males in relation to courtship directed towards the alternative male. Each bar represents one mate choice trial. The results of the intercept-only models show that across trials within an experiment, RFC did not differ significantly from zero.