

Genetic population structure as indirect measure of dispersal ability in a Lake Tanganyika cichlid

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Abstract Diversification and speciation processes are influenced by intrinsic (ecological specialization, dispersal) and extrinsic (habitat structure and instability) factors, but the effect of ecological characteristics on dispersal is difficult to assess. This study uses mitochondrial control region sequences to investigate the population structure and demographic history of the endemic Lake Tanganyika cichlid *Neolamprologus caudopunctatus* with a preference for the rock-sand interface along two stretches of continuous, rocky shoreline, and across a sandy bay representing a potential dispersal barrier. Populations along uninterrupted habitat were not differentiated; whereas, the sandy bay separated two reciprocally monophyletic clades. The split between the two clades between 170,000 and 260,000 years BP coincides with a period of rising water level following a major lowstand, and indicates that clades remained isolated throughout subsequent lake level fluctuations. Low long-term effective population sizes were inferred from modest genetic diversity estimates, and may be due to recent population expansions starting from small population sizes 45,000–60,000 years BP. Comparisons with available data from specialized rock-dwelling species of the same area suggest that habitat structure and lake level fluctuations determine phylogeographic patterns

on large scales, while fine-scale population structure and demography are modulated by species-specific ecologies.

Keywords Demography · Differentiation · Evolution · Haplotype network · Lake level fluctuations · Lamprologini · Mitochondrial DNA · *Neolamprologus caudopunctatus*

Introduction

Comparative studies of phylogeography and genetic differentiation in sympatric taxa permit the appraisal of historical and geographical influences on population structure and diversification (Bermingham and Moritz 1998; Avise 2000; Arbogast and Kenagy 2001). Additionally, investigations of closely related species that differ in their habitat and dietary preference, dispersal ability, and other relevant traits, can reveal species-specific responses to habitat structure and historic events, and have potential to illustrate the interaction between extrinsic and intrinsic factors on evolutionary processes (e.g., Schneider et al. 1998; Lourie et al. 2005).

Cichlid species flocks of the East African Great Lakes (Tanganyika, Malawi and Victoria) feature rapid speciation rates, and a wealth of ecological and behavioral diversity in a confined geographic space [see Kocher (2004) and Salzburger and Meyer (2004); and references therein], and provide ample opportunity for comparative studies of ecology and evolution within and between lakes (Kocher et al. 1993; Sturmbauer et al. 2001; Pereyra et al. 2004). Endemic flocks of hundreds of cichlid species in each of the lakes (Meyer 1993) represent excellent model systems to

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examine modes and mechanisms of speciation (Sturmbauer 1998; Seehausen and van Alphen 1999; Kornfield and Smith 2000; Danley and Kocher 2001; Streebman and Danley 2003; Seehausen 2004; Genner and Turner 2005), as well as roles and interactions of diversifying forces at intraspecific level (Sturmbauer et al. 1997; Arnegard et al. 1999; Salzburger and Meyer 2004; Seehausen and Schluter 2004).

Several phylogenetic and population genetic investigations demonstrated that geologically or climatologically induced lake level fluctuations (Scholz and Rosendahl 1988) played a major role in the evolutionary history of lacustrine cichlids and influenced diversification patterns, species distribution, and intraspecific population structure in each of the Great Lakes (McKaye and Gray 1984; Sturmbauer and Meyer 1992; Verheyen et al. 2003; Rüber et al. 1998, 2001; Sturmbauer et al. 2001, 2005; Sturmbauer 1998; Koblmüller et al. 2004, 2005; Brandstätter et al. 2005; Duftner et al. 2005). Rossiter (1995) compared the fluctuating lake level with a “species pump” causing recurrent cycles of population fusion and fragmentation, thus promoting diversification among populations. In particular, populations inhabiting rocky shore sections in more shallow and moderately sloping basin areas are highly affected by lake level changes, when the shore line shifts with rising or dropping water levels.

While the impact of lake level fluctuations on the diversification of benthic cichlid communities is well evidenced, it is less well understood how species-specific characteristics such as ecological specialization, breeding behavior, mobility and territoriality affect dispersal across habitat discontinuities and, consequently, diversification potential. Direct tracking of dispersing individuals is difficult, if not impossible to undertake in these lakes, so that indirect evidence via genetic data is a major source of information. Population genetic studies of rock-dwelling cichlids (mbuna) of Lake Malawi demonstrated that habitat discontinuities in form of sandy beaches, marsh or deep water act as migration barriers and curb gene flow even on a very small geographic scale; whereas, gene flow is unconstrained along continuous habitat (van Oppen et al. 1997; Arnegard et al. 1999; Markert et al. 1999; Danley et al. 2000). Habitat discontinuities also restrict dispersal of rock-dwelling non-mbuna species (Pereyra et al. 2004), while deepwater species of the genus *Copadichromis* (Taylor and Verheyen 2001) and open-water cichlids of the genus *Diplotaxodon* (Shaw et al. 2000) show no or very little geographic population substructure. Evidence of constrained dispersal supports models of micro-allopatric diversification in the

particularly species-rich mbuna clade (Rico and Turner 2002), but other modes of speciation, e.g. through disruptive sexual or natural selection, have been proposed to explain the great diversity in taxa showing less genetic population-structure (Turner 1994; Turner and Burrows 1995; Danley and Kocher 2001).

With an age of 9–12 million years, Lake Tanganyika is by far the oldest of the three Great Lakes (Cohen and Soreghan 1993). About 200 species in 54 genera (with more awaiting scientific description; Poll 1986; Snoeks 2000; Turner et al. 2001) descended from nine distinct lineages that colonized the emerging lake (Salzburger et al. 2002; Koblmüller et al. 2005). Dispersal behavior apparently varies considerably among the four rock-dwelling species studied to date in Lake Tanganyika (*Eretmodus cyanostictus*, Rüber et al. 1998, 2001; Taylor et al. 2001; Sefc et al. unpublished; *Variabilichromis moorii*, Duftner et al. 2006; *Tropheus moorii* and *Ophthalmotilapia ventralis*, Sefc et al., unpublished). Major habitat barriers curb gene flow in all four species, but various degrees of philopatry entail different levels of population differentiation and isolation by distance along continuous rocky coastlines in *V. moorii*, *E. cyanostictus* and *T. moorii*; whereas, gene flow is high in *O. ventralis* (Sefc et al., unpublished). Heterogeneous patterns of population structure among Lake Tanganyika rock-dwellers are consistent with the phylogenetic, ecological and behavioral diversity of this old species assemblage.

Many Lake Tanganyika cichlids display intraspecific geographical variation in coloration and high levels of interspecific morphological and ecological diversity, but the data available so far suggest that population structure and dispersal behavior are not necessarily correlated with the number of species or morphs in individual clades. In the present study we investigate the population structure of *Neolamprologus caudopunctatus*, a member of the species-rich, substrate breeding Tanganyikan cichlid tribe Lamprologini (Sturmbauer et al. 1994; Stiassny 1997; Schelly et al. 2006), which accounts for about 40% of the lake’s cichlid species.

Our sample sites span both continuous rocky shore and a large sandy bay, which has previously been identified as important dispersal barrier for strictly rock-dwelling cichlids (Duftner et al. 2006, Sefc et al. unpublished). Comparative data from multiple species with different habitat preferences, ecological requirements and behaviors along the same stretch of coastline will increase our understanding of the interaction between extrinsic (habitat structure and lake level fluctuations) and intrinsic (dispersal behavior, ecological specialization) factors in determining population structure and consequently the potential for

micro-allopatric diversification in the course of lacustrine cichlid radiations.

Materials and methods

Species information

Neolamprologus caudopunctatus inhabits the intermediate habitat of the rock-sand interface, but is occasionally found over purely sandy or rocky bottom. Populations show very little geographical color variation across the species' distribution range in the southern half of the lake, between Kapampa (Congo) and Kala (Tanzania) (Kohda et al. 1996; Konings 1998). The small fish (TL < 6 cm) are light beige with a yellow to orange colored dorsal fin and lack any obvious sexual dimorphism. Large plankton-feeding schools occur from shallow water to a depth of more than 25 m. The diet of this species consists of all kinds of invertebrates, which are picked up from the substrate or from mid water. Breeding takes place in rock crevices, under pieces of rock at sandy bottom, or even in empty gastropod shells, with clutch sizes between 100 and 250 eggs (Konings 1998).

Sampling and DNA extraction

One hundred forty individuals of *Neolamprologus caudopunctatus* were sampled from seven localities in the southern part of Lake Tanganyika: Wonzye ($N = 30$), 08°43' S, 31°08' E; Mbita Island ($N = 24$), 08°46' S, 31°06' E; Katoto ($N = 1$), 08°48' S, 31°01' E; Funda ($N = 3$), 08°46' S, 30°59' E; Katukula ($N = 28$), 08°43' S, 30°57' E; Tongwa ($N = 26$), 08°40' S, 30°53' E; Nakaku ($N = 28$), 08°39' S, 30°52' E (Fig. 1). The sandy habitat stretch of Mbita Bay at the estuary of the Izi river separates five sampling sites along a continuous rocky shore line (Katoto, Funda, Katukula, Tongwa, Nakaku) from the remaining two localities (Mbita Island, Wonzye). Mbita Island is connected to the mainland by a submerged ridge of continuous rocky substrate (currently at a maximum depth of 25 m). Samples were collected during field trips in October 2001 and March 2003. Whole genomic DNA was extracted from fin clips preserved in 96% ethanol applying proteinase K digestion followed by protein precipitation with ammonium acetate.

Amplification and sequencing

We used the primers L-Pro-F (Meyer et al. 1994) and TDK-D (Lee et al. 1995) to amplify the most variable

part of the mtDNA control region. The PCR reactions were prepared for a total volume of 17 μ l containing 0.085 μ l of *Taq* DNA polymerase (BioTherm™), 1.7 μ l of each primer (10 μ M), 1.7 μ l 10 \times dNTP mix, 1.7 μ l 10 \times MgCl₂ buffer, 1.62 μ l enzyme diluent, 6 μ l high performance liquid chromatography (HPLC) water, and 2.5 μ l of the extracted DNA. Amplification was performed on an Air-Thermo-Cycler (Idaho Technologies) under the following conditions: an initial denaturation phase at 94°C for 15 sec followed by 43 cycles with denaturation at 94°C for 0 sec, primer annealing at 52°C for 0 sec, and extension at 72°C for 15 sec. The PCR-products were purified with ExoSAP-IT (Amersham Biosciences) prior to being added as template for chain termination sequencing with the primer L-Pro-F following the protocol described in Duftner et al. (2005). Ambiguous sequences were resolved by sequencing with the reverse primer TDK-D. Electropherograms of unique haplotypes were double-checked for accurate base calling. DNA fragments were purified with Sephadex™ G-50 (Amersham Biosciences) following the manufacturer's instruction and subsequently visualized on an ABI 3100 sequencer (Applied Biosystems). All sequences are available from GeneBank under the accession numbers DQ628768–DQ628907 (see Appendix).

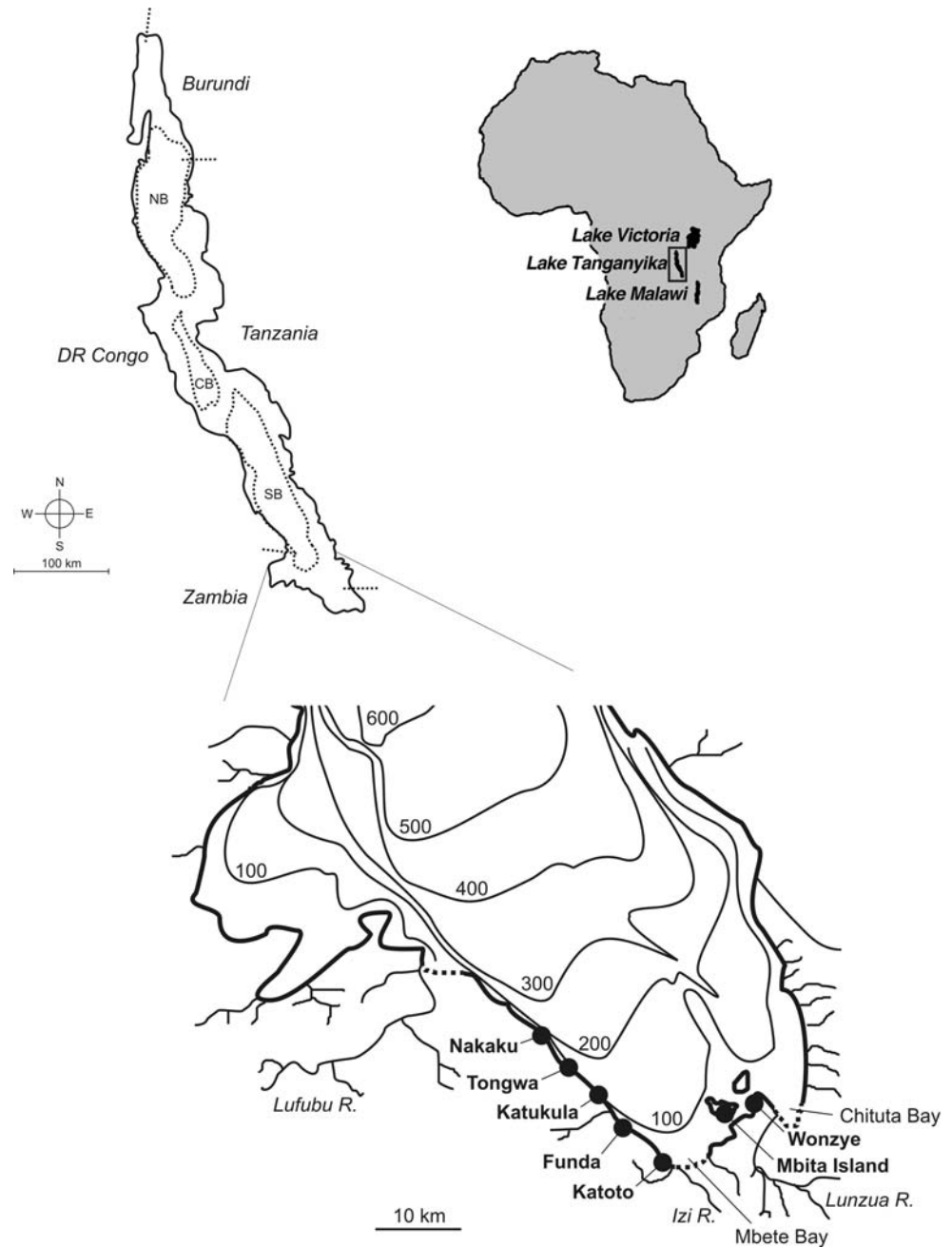
Data analysis

DNA sequences were individually aligned by eye using the SEQUENCE NAVIGATOR software (Applied Biosystems). The alignment comprised 140 individuals of *N. caudopunctatus* with a total sequence length of 327 base pairs (bp). In order to visualize the phylogeographic relationships among the samples, we applied two network construction algorithms, the method of statistical parsimony implemented in the computer program TCS (Clement et al. 2000) and the median-joining network approach (Bandelt et al. 1999) in the computer program NETWORK (version 4.112 available at <http://www.fluxus-engineering.com/sharenet.htm>).

Locations with sufficiently large sample sizes ($N = 24$ – 30 individuals; Wonzye, Mbita Island, Katukula, Tongwa, Nakaku) were included in population analyses. Diversity indices were calculated in DNASP 4.0 (Rozas et al. 2003) and population differentiation was estimated as θ_{ST} (Weir and Cockerham 1984) and Φ_{ST} (Excoffier et al. 1992) in ARLEQUIN v.2.0 (Schneider et al. 1999). Type I errors in pair-wise population comparisons were controlled using the method of Benjamini and Hochberg (1995).

Our data indicated genetic continuity between populations on each side of Mbita Bay, and samples

Fig. 1 Map of Lake Tanganyika, East Africa, with location of sampling localities 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 approximated according to Fig. 1 in Gasse et al. (1989). Sandy or muddy beaches are indicated by dashed lines



were accordingly pooled into an eastern and a western clade for further analysis of divergence time and demographic history. Divergence estimates [mean pair-wise sequence divergence (D_{XY}) and net divergence (D_A)] were calculated in DNASP 4.0 (Rozas et al. 2003). Additionally, we used coalescence simulations in IM [available at <http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm#IM>; based on a method originally developed by Nielsen and Wakeley (2001)] to obtain parameters of divergence time ($T = T_{div}/\mu$) and population size ($\theta = 4N_e\mu$) under the assumption of population

size changes but setting migration rates to zero. Four runs with >7,000,000 steps and a burn-in time of 1,000,000 steps under a finite-sites model (HKY; Hasegawa et al. 1985) were performed to ensure convergence of parameter estimates. Population size changes were examined by coalescence simulations using the program FLUCTUATE (Kuhner et al. 1998) employing HKY mutation parameter estimates obtained from PAUP* (Swofford 2001). Twenty short chains of 20,000 steps and 5 long chains of 2,000,000 steps were run three times for each group of populations

to ensure convergence of the estimates. Mismatch distributions were calculated in DNASP 4.0 (Rozas et al. 2003), and time to the start of population growth was inferred from the parameter $\tau = 2 \mu t$. The fit of the observed haplotype mismatch distributions to the distributions based on growth parameter estimates was evaluated by a Kolmogorov–Smirnov test.

To detect potential bottleneck effects in the recent past we applied a test for heterozygosity excess under the IAM (Cornuet and Luikart 1996), implemented in the program BOTTLENECK (Piry et al. 1997), and investigated the frequency spectrum of haplotype classes (Luikart et al. 1998).

To compare the population structure of several rock-dwelling cichlids across Mbete Bay, we conducted a hierarchical AMOVA (Excoffier et al. 1992) using this data set and the data sets used in Duftner et al. (2006) and Sefc et al. (unpublished).

Results

Genetic variability within populations was moderate with an average of 8 haplotypes, 0.5 % mean nucleotide diversity and 79% mean haplotype diversity (Table 1). The total sample ($n = 140$) included 31 different haplotypes in two distinct clades (Fig. 2). Haplotype networks constructed by two alternative algorithms (statistical parsimony in TCS and median joining in NETWORK) were identical and revealed complete population subdivision across the approximately 7 km of sandy shoreline of Mbete Bay. Samples collected at locations west of Mbete Bay (Nakaku, Tongwa, Katukula, Funda, Katoto) were separated by two fixed substitutions from specimens collected east of the bay (Mbita Island, Wonzye). Within each clade, the most frequent haplotypes were closely related and shared among populations, and numerous unique or low-frequency haplotypes were derived by one or a few mutations from the central haplotypes.

Complete lineage sorting between clades naturally entailed highly significant differentiation estimates in population comparisons across Mbete Bay; whereas, populations on either side of the bay were not significantly differentiated from each other (Table 2; note that Funda and Katoto were not included in this analysis due to small sizes < 5 individuals).

Given the lack of differentiation within each clade, populations on each side of Mbete Bay were pooled for the estimation of demographic population parameters. The conversion of parameter estimates for divergence time, population size and growth used a substitution rate of 6.5 – 8.8% per million years calculated for the most variable part of the mitochondrial control region (Sturmbauer et al. 2001) that includes the 327 bp fragment analyzed here ($\mu = 1.063 \times 10^{-5}$ – 1.4388×10^{-5} per site). Pair-wise net sequence differences ($D_A = 0.01699$) and coalescence simulations allowing for population size changes in IM dated the divergence between clades to ~170,000–260,000 years BP (193,181–261,538 years BP calculated from D_A ; 170,000–250,000 years BP in IM simulations). Unimodal mismatch distributions with τ estimates of 1.291 and 1.323 for the eastern and western clade, respectively, were consistent with recent population expansions in each clade starting at ~45,000–60,000 years BP (44,864–62,244 and 45,976–60,739 years BP, respectively; Fig. 3). Exponential growth parameter estimates from FLUCTUATE ($g = 985$, 82 in the eastern and $g = 940$, 72 in the western clade) translate to growth rates of 3.2×10^{-5} – 4.3×10^{-5} and 3.1×10^{-5} – 4.1×10^{-5} per generation, and present-day effective population sizes were estimated as $N_e = 570,000$ – 770,000 in the eastern clade and $N_e = 1,360,000$ –1,850,000 in the western clade (θ values averaged over three FLUCTUATE runs were 0.025 and 0.06 for east and west, respectively). Simulations in IM were consistent with FLUCTUATE in indicating a two times higher population size in the western clade, but absolute numbers for each clade were considerably

Table 1 Sample size (N) and genetic diversity of mtDNA control region sequences in five populations of *N. caudopunctatus*. For each population the number of haplotypes

(h), nucleotide diversity (π), gene diversity (H_e), and average number of nucleotide differences (k) are given

	N	h	π	H_e	k
Wonzye	30	6	0.00329	0.729	1.076
Mbita Island	24	8	0.00480	0.764	1.569
Katukula	28	8	0.00680	0.847	2.222
Tongwa	26	7	0.00342	0.757	1.117
Nakaku	28	12	0.00554	0.870	1.812
Mean (s.d)	27.2 (2.28)	8.2 (2.28)	0.00477 (0.001477)	0.793 (0.06139)	1.559 (0.4828)
Total	140	31	0.01297	0.905	4.242

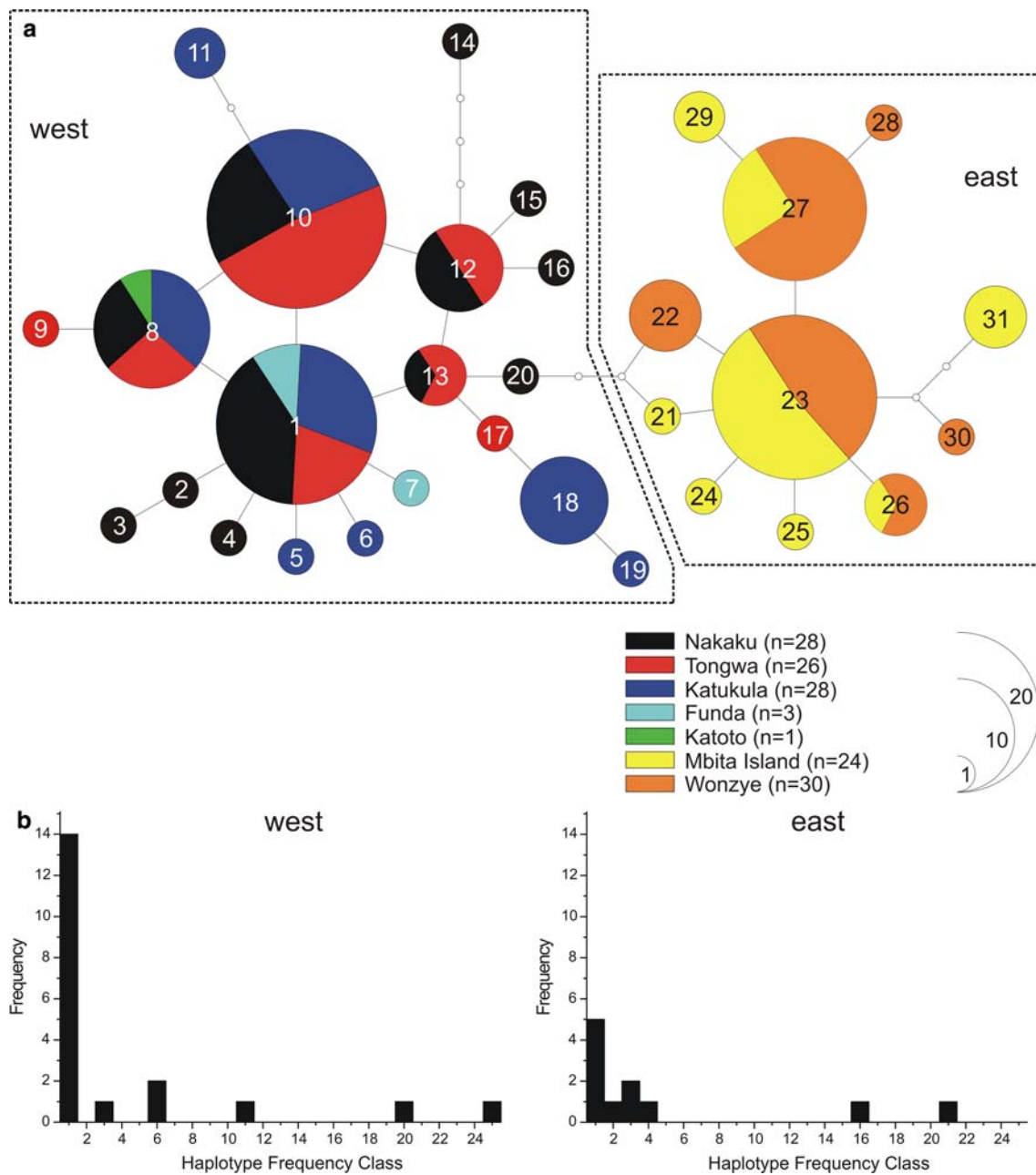


Fig. 2 (a) Haplotype network of *N. caudopunctatus*. Circle diameters are proportional to the number of individual sequences per haplotype. Small open circles represent unsam-

pled haplotypes. (b) Haplotype frequency distribution in populations east and west of Mbeti Bay. Values along the x-axis are the absolute frequencies for each haplotype class

lower than FLUCTUATE estimates (east, $N_e = 300,000$ – $410,000$; west, $N_e = 820,000$ – $1,100,000$).

Despite the low genetic diversity and evidence of population expansion, a recent population bottleneck was refuted by L-shaped haplotype frequency distributions (Fig. 2b) and conformity of gene diversity estimates with equilibrium expectations under the IAM in both clades (west, observed $H_e = 0.838$, $H_{eq} = 0.856$, $p = 0.26$; east, observed $H_e = 0.753$, $H_{eq} = 0.753$, $p = 0.38$).

Discussion

Correlations between habitat preferences, habitat structure and population differentiation in lacustrine cichlids were first described in the haplochromines of Lake Malawi (Van Oppen et al. 1997; Arnegard et al. 1999; Markert et al. 1999; Danley et al. 2000; Pereyra et al. 2004). The accumulating data on population structure in Lake Tanganyika cichlids suggest a higher diversity in patterns of differentiation in the

Table 2 Pair-wise population differentiation values between five populations of *N. caudopunctatus*. Number of private haplotypes (p) are given for each population. θ_{ST} values are shown above diagonal, Φ_{ST} values below diagonal

	p	Wonzye	Mbita Island	Katukula	Tongwa	Nakaku
Wonzye	3	-	0.040	0.213***	0.257***	0.201***
Mbita Island	5	0.043	-	0.194***	0.239***	0.182***
Katukula	5	0.777***	0.744***	-	0.040	0.013
Tongwa	2	0.842***	0.810***	0.066	-	0.019
Nakaku	7	0.791***	0.758***	0.044	0.000	-

Significance levels <0.001 are indicated as ***; all other values were not significantly different from zero after correction for multiple comparisons (Benjamini and Hochberg 1995)

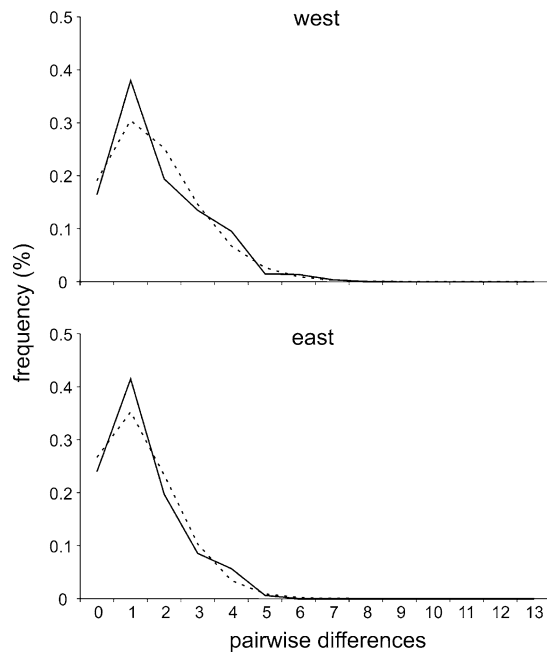


Fig. 3 Mismatch distributions in populations east and west of Mbet Bay. Solid lines represent observed mismatch distributions among haplotype sequences; dashed lines represent expected distributions based on parameter estimates

phylogenetically older and more diverse species assemblage. In analyses of populations sampled along the south-western shoreline of Lake Tanganyika, a 7 km wide stretch of shallow sandy/muddy coast in Mbet Bay was identified as a major barrier to nuclear and mitochondrial gene flow for the strictly rock-dwelling cichlids *Variabilichromis moorii* (tribe Lamprologini; Duftner et al. 2006), *Tropheus moorii* (tribe Tropheini), *Eretmodus cyanostictus* (tribe Eretmodini) and, to a lesser degree, *Ophthalmotilapia ventralis* (tribe Ectodini; Sefc et al., unpublished) (see Table 3). However, patterns of population structure differed among species along the mostly rocky shoreline northwest of Mbet Bay (Table 3), and included isolation by distance in *E. cyanostictus*, significant differentiation regardless of geographic distance in *V. moorii* and *T. moorii*, and panmixis in *O. ventralis*

(Duftner et al. 2006; Sefc et al. unpublished). In the present study of *Neolamprologus caudopunctatus*, an ecologically more flexible inhabitant of the rock-sand interface, mitochondrial control region sequences revealed a high level of genetic differentiation—that is, complete lineage sorting—across Mbet Bay, indicating that the large sandy bay affects (female) dispersal of *N. caudopunctatus* as much or even more than dispersal of the rock-dwelling species. In contrast to the situation observed in *E. cyanostictus*, *T. moorii* and *V. moorii* (Duftner et al. 2006; Sefc et al. unpublished), no genetic structure was detected among populations along ~20 km of mostly rocky shoreline west of Mbet Bay and between the two populations of Mbita Island and Wonzye separated by ~7 km of interspersed rocky and sandy shoreline and shallow water between the shore and the island. The lack of differentiation in the absence of major habitat discontinuities is shared with *O. ventralis*, but gene flow is probably due to different behaviors in the two species. In the strictly rock-dwelling *O. ventralis*, dispersal is mediated by swarms of mouthbrooding mothers and schools of juveniles (Konings 1998), which may bridge unsuitable habitat stretches during their migrations in open inshore water. Although *N. caudopunctatus* prefers the intermediate habitat of the rock-sand interface, individual breeding pairs were observed to occupy snail shells on pure sandy bottom not too far from their preferred habitat, but in contrast to *O. ventralis*, swarms of juveniles and non-breeding adults remained stationary for periods of two months or more (Schaedelin, pers. communication). Their tolerance for suboptimal habitat may enable *N. caudopunctatus* to maintain genetic continuity across minor habitat interruptions, but unlike *O. ventralis*, (female) dispersal behavior apparently does not include long-distance migration across major barriers such as Mbet Bay.

The split between populations east and west of Mbet Bay was dated to 170,000 to 260,000 years BP. Given the relatively frequent occurrence of water level changes in the history of the lake, and the large confidence intervals around genetic estimates of population

Table 3 Population differentiation in different rock-dwelling cichlid species across Mbete Bay illustrated by a hierarchical AMOVA. Φ_{SC} refers to the amount of molecular varianceamong populations along continuous rocky shorelines east and west of Mbete Bay, respectively; Φ_{CT} corresponds to the amount of molecular variance across Mbete Bay

	Φ_{SC}	Φ_{CT}	Φ_{ST}
<i>N. caudopunctatus</i>	0.046*	0.773***	0.784***
<i>V. moorii</i> ^a	0.533***	0.099	0.579***
<i>E. cyanostictus</i> ^b	0.101***	0.334***	0.402***
<i>O. ventralis</i> ^b	-0.009	0.390***	0.396***
<i>T. moorii</i> ^b	0.114***	0.587***	0.635***

Data: ^a Duftner et al. (2006); ^b Sefc et al. (unpublished)

Significance levels <0.05 and <0.001 are indicated as * and *** respectively; all other values were not significantly different from zero

divergence times, the alignment of demographic and hydrologic events is problematic. Still, it is compelling, that the divergence time estimate coincides with a period of rising lake levels at 190,000 – 260,000 years BP following a lowstand of about 350 m below present level (Cohen et al. 1997; note that estimates of the exact magnitude of lake level drops differ between studies, e.g. Scholz and Rosendahl 1988; Gasse et al. 1989; Lezzar et al. 1996; Cohen et al. 1997; Scholz et al. 2003). Lake level fluctuations not only entail changes of habitat characteristics in the deeper lake basins, but also trigger cycles of population fusion and fragmentation through displacement of benthic communities (Rossiter 1995; Sturmbauer 1998; Sturmbauer et al. 2001). The population structure of *N. caudopunctatus* suggests that a single population existed during the -350 m water lowstand, and that this population was split into the extant western and eastern clade, when the lake level rose and the sub-populations colonized newly available habitat on either side of Mbete Bay. Given that our data demonstrate gene flow along mostly continuous habitat, a permanent dispersal barrier must have emerged to prevent further exchange during all subsequent lake level fluctuations (Cohen et al. 1997; Scholz et al. 2003). A longstanding barrier to gene flow in the region of Mbete Bay is also reflected in the population structure of other specialized rock dwellers studied so far, but mitochondrial sequence data suggest that the population splits might date back to different points in time in different species. While *O. ventralis* shows evidence of past and possibly ongoing exchange of haplotypes across Mbete Bay (Sefc et al. unpublished), population divergence across the bay is high in the philopatric rock dwellers *Eretmodus cyanostictus*, *Tropheus moorii* and *Variabilichromis moorii* (Duftner et al. 2006; Sefc et al. unpublished).

The demographic history of *N. caudopunctatus*, as inferred from mitochondrial sequence polymorphisms, differs from other rock dwelling species studied so far in the same area. Nucleotide and haplotype diversity estimates within each population of *N. caudopunctatus*

were lower than in populations of *V. moorii*, *T. moorii* and *E. cyanostictus*, and similar to populations of *O. ventralis*. Recent population expansions starting from small population sizes around 45,000–60,000 years BP were inferred from the haplotype genealogies of both the eastern and the western clade, and could explain the comparatively low long-term effective population sizes implied by modest genetic diversity, although the data bear no signal of a genetic bottleneck. In comparison, population growth in *T. moorii* and *E. cyanostictus* dates back at least twice as long (Sefc et al. unpublished), possibly indicating species-specific responses to common environmental factors.

Differences in demographic histories and population sizes influenced rates of genetic drift and lineage sorting and complicate comparisons of genetic differentiation between species. For example, shared haplotypes between populations of the highly structured *V. moorii* across Mbete Bay in contrast to reciprocal monophyly between eastern and western clades of the less philopatric *N. caudopunctatus* do not necessarily imply higher or more recent gene flow in *V. moorii*, but could equally reflect differential retention of ancestral polymorphism due to different long-term effective population sizes. Despite such potential caveats, comparative analyses of phylogeography and population structure have proven extremely powerful in the discrimination of general and species-specific factors shaping the evolutionary histories of organisms. For example, phylogeographic reconstructions of ecologically diverse seahorses in Southeast Asia revealed a consistent signature of Pleistocene isolation of marine basins in all four species, while species-specific phylogeographic patterns were congruent with expectations based on individual ecologies (Lourie et al. 2005). Similarly, phylogeographic patterns of lizard and frog species in the Wet Tropics of Australia demonstrated common effects of Pleistocene habitat fragmentation overlaid by species-specific ecological characteristics (Schneider et al. 1998). Spatial genetic structure was found to be correlated with ecological

specialization in carabid beetle species (Brouat et al. 2003) and with habitat preferences in Atlantic surgeon fishes (Rocha et al. 2002). In contrast, genetic structure and reconstructed population history were not concordant across ecologically similar, co-distributed species of fruit bats in Malaysia and Thailand and indicated complex relationships between ecology and biogeography (Campbell et al. 2006). As becomes apparent from available data on the cichlids of the East African Great Lakes, population substructure and consequently differentiation and diversification processes in these fishes are the result of compound interactions between multiple factors, which combine to create differentiated responses to shared or analogous environmental conditions. So far, studies on representatives of the highly diversified species assemblage of Lake Tanganyika essentially demonstrated more disparities than commonalities. Clearly, more species will have to be analyzed until generalities in patterns of population differentiation will be discernible to allow the identification of key ecological and life-history traits responsible for shaping population structure.

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Appendix A

Haplotype numbers with according GenBank accession numbers, sampling localities and sample identifications.

Ht. Nr.	Acc. Nr.	Locality	Sample ID
1	DQ628768–DQ628787	Nakaku	2974, 2976, 2985, 2989, 2992, 3050, 3058, 3386
		Tongwa	2899, 2900, 2916, 2922
		Katukula	2870, 2872, 2883, 2885, 2892, 3374
		Funda	3346, 3347
2	DQ628788	Nakaku	2988
3	DQ628789	Nakaku	2991
4	DQ628790	Nakaku	3049
5	DQ628791	Katukula	2880

Ht. Nr.	Acc. Nr.	Locality	Sample ID
6	DQ628792	Katukula	2886
7	DQ628793	Funda	3444
8	DQ628794–DQ628804	Nakaku	2987, 3052, 3056
		Tongwa	2901, 2904, 2918
		Katukula	2868, 2888, 2889, 3296
		Katoto	3449
9	DQ628805	Tongwa	2903
10	DQ628806–DQ628830	Nakaku	2973, 2975, 2977, 2990, 3057, 3387
		Tongwa	2895, 2898, 2902, 2906, 2907, 2908, 2911, 2913, 2914, 2915, 2917, 2921
		Katukula	2869, 2871, 2879, 2882, 2887, 3298, 3299
11	DQ628831–DQ628832	Katukula	2874, 2881
12	DQ628833–DQ628838	Nakaku	3051, 3059, 3060
		Tongwa	2893, 2905, 2919
13	DQ628839–DQ628841	Nakaku	3053
		Tongwa	2909, 2912
14	DQ628842	Nakaku	3054
15	DQ628843	Nakaku	2972
16	DQ628844	Nakaku	3055
17	DQ628845	Tongwa	2897
18	DQ628846–DQ628851	Katukula	2865, 2866, 2867, 2877, 3372, 3373
19	DQ628852	Katukula	2873
20	DQ628853	Nakaku	2986
21	DQ628854	Mbita Island	3302
22	DQ628855–DQ628858	Wonzye	3164, 3168, 3185, 3393
23	DQ628859–DQ628879	Mbita Island	3085, 3091, 3092, 3100, 3102, 3103, 3105, 3108, 3112, 3119, 3300
		Wonzye	3175, 3162, 3165, 3182, 3188, 3189, 3190, 3191, 3305, 3392
24	DQ628880	Mbita Island	3106
25	DQ628881	Mbita Island	3110
26	DQ628882–DQ628884	Mbita Island	3087
		Wonzye	3163, 3184
27	DQ628885–DQ628900	Mbita Island	3089, 3101, 3107, 3389
		Wonzye	3158, 3159, 3167, 3183, 3186, 3187, 3194, 3195, 3303, 3304, 3390, 3391
28	DQ628901	Wonzye	3161
29	DQ628902–DQ628903	Mbita Island	3084, 3301
30	DQ628904	Wonzye	3160
31	DQ628905–DQ628907	Mbita Island	3090, 3095, 3109

References

- Arbogast BS, Kenagy GJ (2001) Comparative phylogeography as an integrative approach to historical biogeography. *J Biogeogr* 28:819–825
- Arnegard ME, Markert JA, Danley PD, Stauffer JR Jr, Ambali AJ, Kocher TD (1999) 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* 266:119–130
- Avise JC (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, Massachusetts and London, UK
- Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289–300
- Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Mol Ecol* 7:367–369
- Brandstätter A, Salzburger W, Sturmbauer C (2005) Mitochondrial phylogeny of the Cyprichromini, a lineage of open-water cichlid fishes endemic to Lake Tanganyika, East Africa. *Mol Phylog Evol* 34:382–391
- Brouat C, Sennedot F, Audiot P, Lebois R, Rasplus J (2003) Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization. *Mol Ecol* 7:1731–1745
- Campbell P, Schneider CJ, Adnan AM, Zubaid A, Kunz TH (2006) Comparative population structure of *Cynopterus* fruit bats in peninsular Malaysia and southern Thailand. *Mol Ecol* 15:29–47
- Clement M, Posada D, Crandall KA (2000) TCS a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1659
- Cohen AS, Soreghan MJ (1993) Estimating the age of ancient lakes: an example from Lake Tanganyika, East African rift system. *Geology* 21:511–514
- Cohen AS, Lezzar KE, Tiercelin JJ, Soreghan M (1997) New palaeographic and lake-level reconstructions of Lake Tanganyika: implications for tectonic, climatic and biological evolution in a rift lake. *Basin Res* 9:107–132
- Cornuet JM, Luikart G (1996) Description and evaluation of two tests for detecting recent bottlenecks. *Genetics* 144:2001–2014
- Danley PD, Markert JA, Arnegard ME, Kocher TD (2000) Divergence with gene flow in the rock-dwelling cichlids of Lake Malawi. *Evolution* 54:1725–1737
- Danley PD, Kocher TD (2001) Speciation in rapidly diverging systems: lessons from Lake Malawi. *Mol Ecol* 10:1075–1086
- Duftner N, Koblmüller S, Sturmbauer C (2005) Evolutionary relationships of the Limnochromini, a tribe of benthic deepwater cichlid fish endemic to Lake Tanganyika, East Africa. *J Mol Evol* 60:277–289
- Duftner N, Sefc KM, Koblmüller S, Nevado B, Verheyen E, Sturmbauer C (2006) Distinct population structure in a phenotypically homogeneous rock-dwelling cichlid fish from Lake Tanganyika. *Mol Ecol* (in press) Early online issue available: doi:10.1111/j.1365-294X.2006.02949.x
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to the human mitochondrial DNA restriction data. *Genetics* 131:479–491
- Gasse F, Lédée V, Massault M, Fontes J-C (1989) Water-level fluctuations of Lake Tanganyika in phase with oceanic changes during the last glaciation and deglaciation. *Nature* 342:57–59
- Genner MG, Turner GF (2005) The mbuna cichlids of Lake Malawi: a model for rapid speciation and adaptive radiation. *Fish Fish* 6:1–34
- Hasegawa M, Kishino T, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* 22:160–174
- Koblmüller S, Salzburger W, Sturmbauer C (2004) Evolutionary relationships in the sand dwelling cichlid lineage of Lake Tanganyika suggest multiple colonization of rocky habitats and convergent origin of biparental mouthbrooding. *J Mol Evol* 58:79–96
- Koblmüller S, Duftner N, Katongo C, Phiri H, Sturmbauer C (2005) Ancient divergence in bathypelagic Lake Tanganyika deepwater cichlids: mitochondrial phylogeny of the tribe Bathyatini. *J Mol Evol* 60:297–314
- Kocher TD (2004) Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Rev Gen* 5:288–298
- Kocher TD, Conroy JA, McKaye KR, Stauffer JR (1993) Similar morphologies of cichlid fishes in Lakes Tanganyika and Malawi are due to convergence. *Mol Phylog Evol* 4:420–432
- Kohda M, Yanagisawa Y, Sato T, Nakaya K, Nimura Y, Matsumoto K, Ochi H (1996) Geographical color variation in cichlid fishes at the southern end of Lake Tanganyika. *Env Biol Fish* 45:237–248
- Konings A (1998) *Tanganyika cichlids in their natural habitat*. Cichlid Press, El Paso
- Kornfield I, Smith PF (2000) African cichlid fishes: model systems for evolutionary biology. *Ann Rev Ecol Syst* 31:163–198
- Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics* 149:429–434
- Lee W-J, Conroy J, Hunting Howell W, Kocher TD (1995) Structure and evolution of teleost mitochondrial control regions. *J Mol Evol* 41:54–66
- Lezzar KE, Tiercelin JJ, De Batist M, Cohen AS, Bandora R, Van Rensbergen C, Le Turdu C, Mifundu W, Klerck J (1996) New seismic stratigraphy and Late Tertiary history of the North Tanganyika basin, East African rift system deduced from multichannel and high-piston core evidence. *Basin Res* 8:1–28
- Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). *Mol Ecol* 14:1073–1094
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J Hered* 89:238–247
- Markert JA, Arnegard ME, Danley PD, Kocher TD (1999) Biogeography and population genetics of the Lake Malawi cichlid *Melanochromis auratus*: habitat transience, philopatry and speciation. *Mol Ecol* 8:1013–1026
- McKaye KR, Gray WM (1984) Extrinsic barriers to gene flow in rock-dwelling cichlids of Lake Malawi: macrohabitat heterogeneity and reef colonization. In: Echelle AA, Kornfield I (eds) *Evolution of fish species flocks*. University of Maine at Orono Press, Orono, Maine, pp. 169–183
- Meyer A (1993) Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends Ecol Evol* 8:279–284
- Meyer A, Morrissey JM, Scharl M (1994) Recurrent origin of sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature* 368:539–541
- Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov Chain Monte Carlo Approach. *Genetics* 158:885–896

- Pereyra R, Taylor MI, Turner GF, Rico C (2004) Variation in habitat preference and population structure among three species of the Lake Malawi cichlid genus *Protomelas*. *Mol Ecol* 12:2691–2697
- Piry S, Luikart G, Cornuet J-M (1997) BOTTLENECK: A program for detecting recent effective population size reductions from allele frequency data. Laboratoire de Modélisation et de Biologie Evolutive, INRA-URLB, Montpellier, France
- Poll M (1986) Classification des cichlidae du lac Tanganika. Tribus, genres et espèces. *Acad R Belg Mem Cl Sci* 45:1–163
- Rico C, Turner GF (2002) Extreme microallopatric divergence in a cichlid species from Lake Malawi. *Mol Ecol* 11:1585–1590
- Rocha LA, Bass AL, Robertson DR, Brown BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Mol Ecol* 11:243–251
- Rossiter A (1995) The cichlid fish assemblages of Lake Tanganyika: ecology, behaviour and evolution of its species flocks. *Adv Ecol Res* 26:187–252
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19:2496–2497
- Rüber L, Verheyen E, Sturmbauer C, Meyer A (1998) Lake level fluctuations and speciation in rock-dwelling cichlid fish in Lake Tanganyika, East Africa. In: Grant P (eds) *Evolution on islands*. Oxford University Press, Oxford, UK, pp 225–240
- Rüber L, Meyer A, Sturmbauer C, Verheyen E (2001) Population structure in two sympatric species of the Lake Tanganyika cichlid tribe Eretmodini: evidence for introgression. *Mol Ecol* 10:1207–1225
- Salzburger W, Meyer A, Baric S, Verheyen E, Sturmbauer C (2002) Phylogeny of the Lake Tanganyika cichlid species flock and its relationships to Central- and East African haplochromine cichlid fish faunas. *Syst Biol* 51:113–135
- Salzburger W, Meyer A (2004) The species flock of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften* 91:277–290
- Schelly R, Salzburger W, Koblmüller S, Duftner N, Sturmbauer C (2006) Phylogenetic relationships of the lamprologine cichlid genus *Lepidiolamprologus* (Teleostei: Perciformes) based on mitochondrial and nuclear sequences, suggesting introgressive hybridization. *Mol Phylogeny Evol* 38:426–438
- Schneider CJ, Cunningham M, Moritz C (1998) Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rain forests of Australia. *Mol Ecol* 7:487–498
- Schneider S, Roessli D, Excoffier L (1999) Arlequin, Version 2.0: a Software for Genetic Analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva
- Scholz CA, Rosendahl BR (1988) Low lake stands in Lakes Malawi and Tanganyika, East Africa, delineated with multifold seismic data. *Science* 240:1645–1648
- Scholz CA, King JW, Ellis GS, Swart PK, Stager JC, Colman SM (2003) Paleolimnology of Lake Tanganyika, East Africa, over the past 100 k yr. *J Paleolimn* 30:139–150
- Seehausen O (2004) Hybridization and adaptive radiation. *Trends Ecol Evol* 19:198–207
- Seehausen O, van Alphen JJM (1999) Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecol Lett* 2:262–271
- Seehausen O, Schluter D (2004) Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc R Soc Lond B* 271:1345–1353
- Shaw PW, Turner GF, Idid MR, Robinson RL, Carvalho GR (2000) Genetic population structure indicates sympatric speciation of Lake Malawi pelagic cichlids. *Proc R Soc Lond B* 267:2273–2280
- Snoeks J (2000) How well known is the ichthyodiversity of the large East African lakes? *Adv Ecol Res* 31:17–38
- Stiassny MLJ (1997) A phylogenetic overview of the lamprologine cichlids of Africa (Teleostei, Cichlidae): a morphological perspective. *S Afr J Sci* 93:513–523
- Streelman JT, Danley PD (2003) The stages of vertebrate evolutionary radiation. *Trends Ecol Evol* 18:126–131
- Sturmbauer C (1998) Explosive speciation in cichlid fishes of the African Great Lakes: A dynamic model of adaptive radiation. *J Fish Biol* 53(Suppl. A):18–36
- Sturmbauer C, Meyer A (1992) Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature* 358:578–581
- Sturmbauer C, Verheyen E, Meyer A (1994) Mitochondrial phylogeny of the Lamprologini, the major substrate spawning lineage of cichlid fishes from Lake Tanganyika in Eastern Africa. *Mol Biol Evol* 11:691–703
- Sturmbauer C, Verheyen E, Rüber L, Meyer A (1997) Phylogeographic patterns in populations of cichlid fishes from rocky habitats in Lake Tanganyika. In: Stepien CA, Kocher TD (eds) *Molecular systematics of fishes*. Academic Press, San Diego, California, pp 97–111
- Sturmbauer C, Baric S, Salzburger W, Rüber L, Verheyen E (2001) Lake level fluctuations synchronize genetic divergence of cichlid fishes in African lakes. *Mol Biol Evol* 18:144–154
- Sturmbauer C, Koblmüller S, Sefc KM, Duftner N (2005) Phylogeographic history of the genus *Tropheus*, a lineage of rock-dwelling cichlid fishes endemic to Lake Tanganyika. *Hydrobiologia* 542:335–366
- Swofford DL (2001) PAUP* 4.0b10: Phylogenetic analysis using parsimony. Sinauer, Sunderland, Massachusetts
- Taylor MI, Rüber L, Verheyen E (2001) Microsatellites reveal high levels of population substructuring in the species-poor Eretmodine cichlid lineage. *Proc R Soc Lond B* 268:803–808
- Taylor MI, Verheyen E (2001) Microsatellite data reveals weak population substructuring in *Copadichromis* sp. “viriginalis kajose”, a demersal cichlid from Lake Malawi, Africa. *J Fish Biol* 59:593–604
- Turner GF (1994) Speciation mechanisms in Lake Malawi cichlids: a critical review. *Adv Limn* 44:139–160
- Turner GF, Burrows MT (1995) A model of sympatric speciation by sexual selection. *Proc R Soc Lond B* 260:287–292
- Turner GF, Seehausen O, Knight KE, Allender CJ, Robinson RL (2001) How many species of cichlid fishes are there in African lakes? *Mol Ecol* 10:793–806
- Van Oppen MJH, Turner GF, Rico C, Deutsch JC, Ibrahim KM, Robinson RL, Hewitt GM (1997) Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proc R Soc Lond B* 264:1803–1812
- Verheyen E, Salzburger W, Snoeks J, Meyer A (2003) Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science* 300:325–329
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370