Elevated mtDNA diversity in introduced populations of *Cynotilapia afra* (Günther 1894) in Lake Malawi National Park is evidence for multiple source populations and hybridization

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Abstract

Genetic variation in many invasive species shows little or no signs of a founder event, suggesting that high genetic diversity may facilitate establishment success. The rocky-shore, plankton-feeding cichlid fish *Cynotilapia afra* is endemic to Lake Malawi, but naturally absent from many suitable sites. In the 1960s, this species was introduced to the southern areas of the lake, presumably as a result of the aquarium fish trade. It has now become established on a number of rocky areas within the Lake Malawi National Park. Here, we analysed DNA sequence variation in the mitochondrial control region of six native and four introduced populations of *C. afra*, and three populations of the closely-related and hybridizing *Pseudotropheus zebra*. In contrast to previous studies of Lake Malawi rock dwelling cichlids, network analyses suggested that native populations of *C. afra* showed high levels of lineage sorting in mtDNA. Introduced populations showed higher sequence and haplotype diversity than their native counterparts. Our analyses suggested that the elevated gene diversity was largely attributed to the fact that the introduced *C. afra* populations were derived from several genetically distinct and geographically separate populations, and to a lesser extent because of introgressive hybridization with native *P. zebra*. The establishment and spread of *C. afra* may be partly because of its ability to occupy a vacant ecological niche, but it may also have been facilitated by its enhanced genetic diversity.

Keywords: cichlid fish, invasive species, Lake Malawi, mitochondrial DNA, population genetics

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Introduction

Biological invasions provide the opportunity to observe evolution in action as such events may result in significant demographic changes. Furthermore, interactions between the invasive and native species in the invaded community may have both ecological and genetic repercussions, and rapid evolutionary changes can occur before a new equilibrium is reached. Recent molecular evidence suggests that many invasive species do not show the reduced genetic variability expected of popu-

lations with relatively few founders. Genetic diversity may be retained or even enhanced by multiple introductions from genetically divergent source populations (Kolbe *et al.* 2004; Hänfling 2007; Roman & Darling 2007) or by introgressive hybridization with native species (Ellstrand & Schierenbeck 2000).

Two important evolutionary consequences of multiple introductions and introgression have been suggested. Firstly, these processes may dilute founder effects and reduce the risk of inbreeding depression (Novak & Mack 1993; Kolbe *et al.* 2004; reviewed in Dlugosch & Parker 2008). Secondly, the admixture of gene pools may lead to novel gene combinations with advantageous effects in the new environment (Hänfling 2007).

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Likewise, the production of novel gene combinations during hybridization has been proposed as a major factor contributing to natural adaptive radiation, particularly in African cichlid fishes (Seehausen 2004).

The African Great Lakes Victoria, Tanganyika and Malawi have the richest lacustrine faunas in the world and their cichlid fishes are a textbook example of explosive radiation, with a recent estimate of 451–600 species in Lake Malawi, 447-535 in Lake Victoria (and many more in neighbouring lakes) and 162–184 in Lake Tanganyika (Genner *et al.* 2004a). The lakes differ in size, shape, age and water parameters but the differences in their cichlid faunas may also have been influenced by their derivation from different riverine ancestors (Salzburger *et al.* 2005). The cichlid fishes of Lake Tanganyika show a wide range of reproductive strategies not found in the younger and less genetically diverse cichlid faunas of the other two lakes. However, all three lakes are dominated by cichlid fish, which exhibit striking parallels in morphology (Young *et al.* 2009a), behaviour (Blais *et al.* 2009), life histories (Duponchelle *et al.* 2008) and even in opsin amino acid sequences (Sugawara *et al.* 2005).

These cichlid fishes have served as a model system in studies of evolution (see reviews by Kornfield & Smith 2000; Kocher 2004; Seehausen 2006). Several hypotheses have been suggested to explain the explosive speciation of these cichlids: morphology, particularly of the pharyngeal jaw apparatus (Fryer 1959; Liem 1980), sexually-selected colour variation (Kosswig 1947; Dominey 1984; van Oppen *et al.* 1998), habitat specialization and fragmentation (Fryer 1959; Ribbink *et al.* 1983), hybridization (Salzburger *et al.* 2002; Seehausen 2004) and visually-based mate choice in clear water habitats (Seehausen *et al.* 1997; Terai *et al.* 2006). Equally, both allopatric (Fryer 1959; Ribbink *et al.* 1983; van Oppen *et al.* 1997) and sympatric (Seehausen & van Alphen 1997; Shaw *et al.* 2000) speciation have been suggested as significant processes. Notably, rocky shore cichlid fishes show high levels of geographical variation in colour (Fryer 1959; Ribbink *et al.* 1983) associated with strong genetic structuring (van Oppen *et al.* 1997; Rico *et al.* 2003) and partial reproductive isolation (Knight & Turner 2004) suggesting a major role for geographical isolation in diversification and also a significant degree of local endemism and evolutionarily significant betadiversity (Ribbink *et al.* 1983; Reinthal 1993; Genner *et al.* 2004a,b). Differences in parasite fauna are also known among species and habitats (Bray *et al.* 2006; Blais *et al.* 2007; Amin *et al.* 2008). Given the high level of endemism, anthropogenic translocations of fishes within Lake Malawi are a cause for concern (Ribbink *et al.* 1983; Trendall 1988; Streelman *et al.* 2004; Genner *et al.* 2006; Young *et al.* 2009b).

The recent and rapid evolution of cichlid fishes in the younger lakes, such as Malawi and Victoria renders the reconstruction of species trees from molecular data difficult: many rock-dwelling cichlids retain shared ancestral mtDNA polymorphisms, even where morphological differentiation is clear and reproductive isolation demonstrated or suspected (Moran & Kornfield 1993; Parker & Kornfield 1997; Sturmbauer *et al.* 2001). However, mitochondrial DNA sequence data can still be highly informative in a population genetic framework. This approach has long been employed in studies of humans and other organisms (Bandelt *et al.* 1999; Pakendorf & Stoneking 2005; Atkinson *et al.* 2007; Krystufek *et al.* 2007; Rowold *et al.* 2007) and more recently has proved useful with cichlid fishes from Central America (Barluenga *et al.* 2007) and East Africa (Verheyen *et al.* 2003; Genner *et al.* 2007 Koblmüller *et al.* 2007b). Furthermore, mitochondrial DNA has proven to be a particularly powerful genetic marker for estimating source populations of recent invasions by lizards (Kolbe *et al.* 2004), crabs (Hänfling *et al.* 2002; Roman 2006), fish (Lindholm *et al.* 2005; Azzurro *et al.* 2006) and snails (Chuong *et al.* 2008). The aim of this study was to employ population genetic analyses of mitochrondrial DNA to investigate the consequences of human-mediated translocation of a rock-dwelling cichlid fish, *Cynotilapia afra* into the south-western arm of Lake Malawi. It was hoped to provide a population genetic framework to study the evolutionary consequences of introductions, admixture and hybridization in cichlids but also to make inferences about the spread and colonization pathways of introduced cichlids in Lake Malawi National Park relevant to conservation biologists and managers.

Materials and methods

Study species and area

Cynotilapia afra is a small plankton-feeding fish from the 'mbuna' group confined to clear-water rocky habitats (Fryer 1959; Ribbink *et al.* 1983; Konings 2008). It is naturally widely distributed within Lake Malawi (Ribbink *et al.* 1983; Konings 2008). It is believed that the *C. afra* species complex is indigenous to the northernmost 80% of the lake, from the far north at Ikombe to Mbenji Island and Ntekete in the south (Konings 2008). At several localities within this range, populations of *C. afra* occur sympatrically with populations of the closely-related *Pseudotropheus zebra* complex (Kassam *et al.* 2005). Populations of *C. afra* found in the southern part of the lake at Thumbi West Island were reported to have been released by an aquarium fish exporter in the 1960s or 1970s (Ribbink *et al.* 1983).

The species has subsequently spread around the island and has been found on other nearby rocky shores (Fig. 1). It has been suggested that introduced populations of *C. afra* have undergone rapid microevolutionary change as well as introgressive hybridization with *P. zebra* (Streelman *et al.* 2004). Thumbi West Island now forms part of the Lake Malawi National Park (Trendall 1988; Streelman *et al.* 2004), which was established in 1980 as the World's first national park targeted principally on freshwater fish, and was designated a UNESCO World Heritage Site in 1984. It covers 9,400 ha in the southern part of the lake (Hough 1989). Its waters are among the most heavily impacted by translocations, with at least 13 cichlid fish taxa being introduced to Thumbi West Island alone (Genner *et al.* 2006).

Sampling

Samples of *C. afra* were collected from six localities within the native range of the species: Mara Rocks; Nkhata Bay; Likoma Island; Mbenji Island; Chiofu Bay; and Ntekete Rocks and from four sites known to be inhabited by introduced populations: Domwe Island; Otter Point and two locations from Thumbi West Island (Table 1 & Fig. 1). Specimens of *P. zebra* were collected from the same sites where we collected introduced *C. afra* (Fig. 1). Fifteen individuals per site were collected from each population.

A noninvasive sampling method was employed, collecting live fish using monofilament nets while SCUBA diving. A small tissue sample was taken by clipping the posterior (soft-rayed) part of the dorsal fin, which regenerates within a few days. To ensure reliable species identification, all samples were collected from territorial males in full breeding colour and jaw teeth of all specimens were examined using a hand lens, as the possession of unicuspid teeth is diagnostic for the genus *Cynotilapia* (Trewavas 1935; Fryer 1959). All live specimens were released back into the water. Fin clips were preserved in 100% ethanol before molecular analysis in the laboratory.

Molecular analysis

Total genomic DNA was extracted using the Promega WIZARD genomic DNA purification kit. The mitochondrial DNA (mtDNA) of the entire control region was amplified using forward primer HapThr-2 + 4 (5′–CCT-ACTCCCAAAGCTAGGATC-3′) and reverse primer Fish12s (5′-TGCGGAGACTTGCATGTGTAAG-3′), (Joyce *et al.* 2005). Polymerase chain reaction (PCR) was performed in a 25 μL reaction consisting of 2 μL DNA template, 2.5 μL 10X PCR buffer, 2.5 μL dNTPs (1 mM), 1 μL each primer (10 mM stock), 1.25 μL MgCl$_2$ (25 mM stock), 14.5 μL PCR water and 0.25 Units *Taq*. The PCR running conditions were as follows, 3 min at 94 °C, 45 s at 94 °C and 50 s at 56 °C for 34 times, then 1 min 30 s at 72 °C and followed by 10 min of 72 °C. PCR products were purified and sequenced in both directions using the professional sequencing service of MACROGEN Centre (Pathfinder in Genomics Research, 908 World Meridian Centre, Seoul, 153-023 Korea).
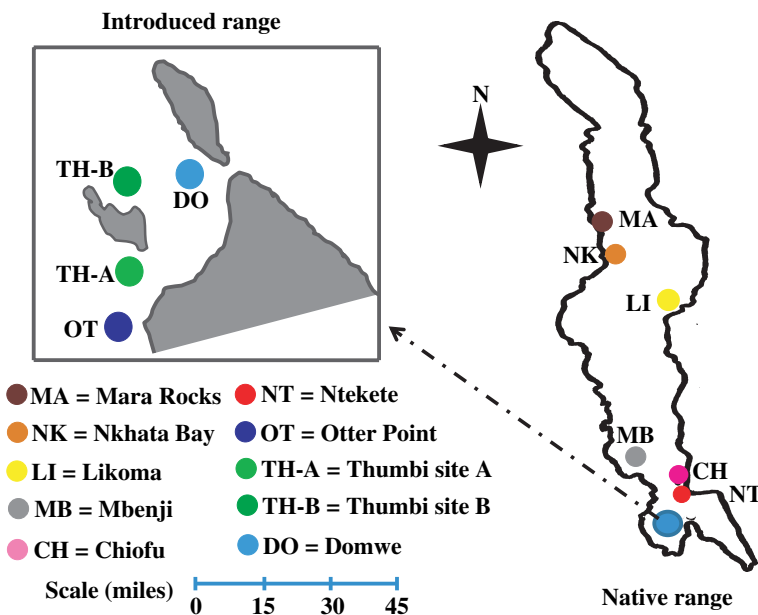

Fig. 1 Locations of sample site for native and introduced populations of *C. afra* in Lake Malawi.

Table 1 Sampled populations, sample sizes and summary statistics. All sequence statistics are based on a full mtDNA control region sequence (910 bp) alignment from 195 sequences

Species	Locality	Co-ordinates	N sequenced	Total haplotypes	Haplotypes unique to population	Haplotype diversity (H)	Nucleotide diversity (π)
Introduced							
C. afra	Domwe Island	13° 59′ 85.5″S, 34° 50′ 32.6″E	15	3	0	0.65	0.0019
	Thumbi West Island (A)	14° 01′ 50.5″S, 34° 49′ 44.4″E	15	6	3	0.76	0.0007
	Thumbi West Island (B)	14° 01′ 08.0″S, 34° 49′ 19.7″E	15	6	3	0.82	0.0030
	Otter Point	14° 02′ 31.6″S, 34° 49′ 37.6″E	15	8	4	0.90	0.0033
Native							
C. afra	Mara Rocks	11° 14′ 44.8″S, 34° 16′ 44.5″E	15	3	2	0.23	0.0003
	Nkhata Bay	11° 36′ 47.1″S, 34° 28′ 23.1″E	15	4	3	0.67	0.0007
	Likoma	12° 01′ 48.9″S, 34° 43′ 46.5″E	15	5	3	0.63	0.0012
	Mbenji	13° 26′ 31.8″S, 34° 29′ 29.1″E	15	4	2	0.47	0.0010
	Chiofu	13° 32′ 01.8″S, 34° 51′ 86.6″E	15	5	5	0.71	0.0011
	Ntekete	13° 37′ 72.4″S, 34° 51′ 25.7″E	15	4	4	0.37	0.0019
P. zebra	Domwe Island	13° 59′ 85.5″S, 34° 50′ 32.6″E	15	8	6	0.91	0.0036
	Thumbi West Island (A)	14° 01′ 50.5″S, 34° 49′ 44.4″E	15	5	1	0.63	0.0016
	Otter Point	14° 02′ 31.6″S, 34° 49′ 37.6″E	15	4	2	0.37	0.0006
Total			195	48	—	—	—

Data analysis

The software package CodonCode Aligner (Ewing *et al.* 1998) was used to edit individual sequences and to align forward and reverse sequences of each individual. The resulting consensus sequences of all individuals were aligned using ClustalW (Thompson *et al.* 1994) in combination with MEGA version 4.1 (Tamura *et al.* 2007) and submitted to GenBank (accessions GQ380500-GQ380547). The alignment was checked by eye and was trimmed to a uniform length of 910 bp including gaps, which were treated as point mutations during analysis. DnaSP version 4.5 (Rozas *et al.* 2003) was used to estimate mtDNA haplotype diversity (H), and nucleotide diversity (π) within populations as well as between-population measures, F_{ST} and D_A (net distance, Nei 1987). Haplotype diversity standard errors (SE) were estimated by a jackknife procedure (Nei & Jin 1989; Lynch & Crease 1990).

To estimate the contribution of genetic introgression between *C. afra* and *P. zebra* within the introduced range, we analysed mtDNA sequences of *P. zebra*, identifying haplotypes shared with *C. afra* and calculating their contribution to nucleotide (π) and haplotype (H) diversity using DnaSP version 4.5 (Rozas *et al.* 2003).

To explore the relationships between the introduced population of *C. afra* and possible source populations, we used two approaches. Genetic distances among

populations (D_A) were calculated using DnaSp version 4.5 (Rozas *et al.* 2003), which were then used in XLSTAT version 2008 (Addinsoft 2008) to configure coordinates for a multidimensional scaling (MDS) approach. Secondly, a network of haplotypes was constructed using a median-joining algorithm in the Network software version 4.5.10 (Bandelt *et al.* 1999).

Results

Within-population genetic diversity

A total of forty-eight haplotypes were found across the whole data set of 195 sequences, 150 from *C. afra* and 45 from *P.zebra* (Table 1). The introduced populations of *C. afra* at Thumbi West Island site B and Otter Point had significantly higher mtDNA haplotype and nucleotide diversity compared with the native populations (Table 1 & Fig. 2). The introduced population of *C. afra* at Otter Point exhibited the highest mtDNA haplotype ($H = 0.90$) and nucleotide ($\pi=0.0030$) diversity, while the native population at Mara Rocks had the lowest mtDNA haplotype ($H = 0.23$) and nucleotide ($\pi=0.0003$) diversity (Table 1 & Fig. 2). In the native *P. zebra* group, the Domwe Island population had the highest haplotype ($H = 0.91$) and nucleotide ($\pi=0.0040$) diversity, while Otter Point population had the lowest ($H = 0.37$, $\pi=0.0006$) diversity (Table 1).
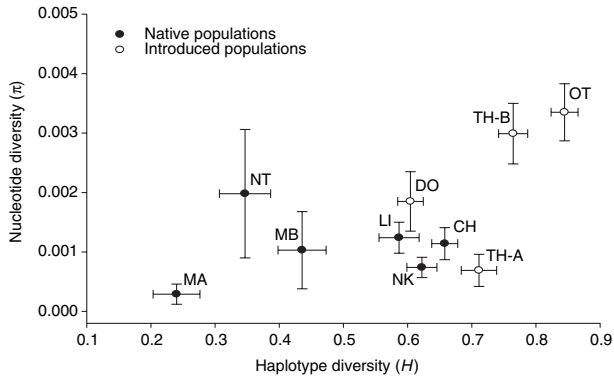
Fig. 2 Haplotype vs. nucleotide diversity (mean and SE) for the native (solid circles) and introduced (open circles) *C. afra* populations. Standard errors of *H* were calculated using a jack-knife procedure. (The abbreviations for populations are given in Fig. 1).

Among-population genetic diversity

The network analysis revealed substantial lineage sorting and no shared haplotypes among most native populations. Only the two samples from the north-eastern part of the lake (Mara Rocks and Nkhata Bay) appeared to be closely related and even shared their most common haplotype (Fig. 3). The native populations Ntekete and Chiofu appear to be particularly distinct and shared haplotypes neither with the introduced populations nor with other native populations. However, two highly divergent haplotypes were found at Ntekete in low frequencies perhaps indicating immigration from a genetically divergent conspecific population or mitochondrial introgression (Fig. 3).

By contrast, haplotypes from most of the introduced populations were widely scattered across the network and a number of haplotypes were shared with other introduced and native populations. Haplotype H7 was common at both Nkhata Bay and Mara Rocks and was shared with three of the introduced samples. Haplotypes H11 and H13, otherwise unique to Likoma Island, were found in the Otter Point population. The Mbenji population shared two haplotypes with introduced populations, but surprisingly, one of these haplotypes was also shared with *P. zebra* (H16). A substantial cluster of haplotypes (top right in Fig. 3) was shared among introduced populations but only rarely found in the native *C. afra* populations. This suggests there may be one or more unsampled source populations.

Only three haplotypes (H7, H16 and H31) were shared between *C. afra* and *P. zebra* in the range of the introduced species (Fig. 3), so that proportionally the genetic diversity contributed through hybridization could be estimated at: $H = 0.52\%$ and $\pi = 1.9\%$. In other words, we estimated that >98% of the mitochondrial diversity in the introduced *C. afra* populations was of conspecific origin, and <2% derived from local hybridization with *P. zebra*.

Pairwise genetic differentiation (both D_A and F_{ST}) was lower for comparisons among introduced populations than native populations (Fig. 4). This is consistent with the MDS plot which shows less scatter for the introduced populations than for the native populations
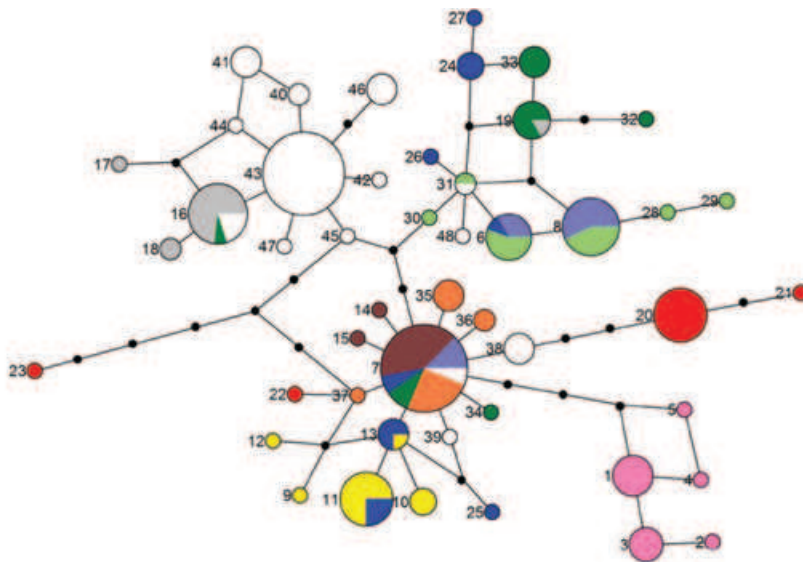

Fig. 3 A parsimony network of *C. afra* and *P. zebra* haplotypes based on mtDNA sequences. Each of the 48 haplotypes is numbered. Each circle represents a single haplotype and its diameter is proportional to the number of individuals found to posses that haplotype. The colour codes represent the source of the haplotype based on geographical location within Lake Malawi as defined in Fig. 1. A black dot (●) represents unsampled haplotypes and the open circle (○)*P. zebra* haplotypes sampled from the range of introduced *C. afra*.
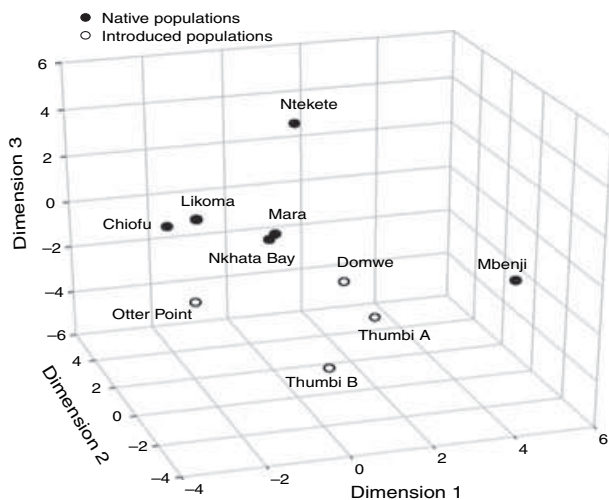
Key

Introduced range   **Native range**
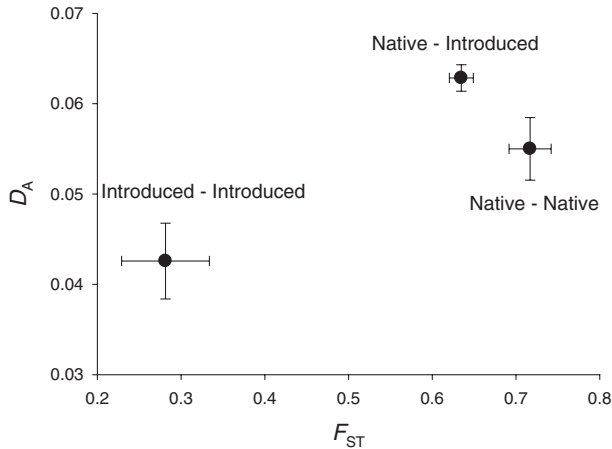
Fig. 4 F_{ST} vs. average pairwise nucleotide differentiation (D_A, mean and SE) for comparisons of categories of *C. afra* populations (Introduced–Introduced, Native–Introduced and Native–Native). Standard errors were calculated by jackknifing the pairwise population comparisons.

Fig. 5 Multidimensional scaling (MDS) plot of genetic distances (D_A) for the native (solid circles) and introduced (open circles) *C. afra* populations.

(Fig. 5). Furthermore, the MDS plot shows that the Ntekete population, although geographically closest to the introduced populations, was genetically distinct from the introduced populations and therefore unlikely to have contributed to the introduction. Relationships among the native populations were not predictable from geographical distances among them. For example, the neighbouring populations at Ntekete and Chiofu were clearly genetically different, while those of the more distant populations at Nkhata Bay and Mara Rocks were genetically very similar (Fig. 1, 5).

Discussion

Here we analysed the full control region of mitochondrial DNA of several native and introduced populations of the cichlid fish *Cynotilapia afra* that was introduced in Lake Malawi National Park in the 1960s. We showed that both introduced and native populations had high mtDNA variation. We also demonstrated evidence of introgressive hybridization with native *Pseudotropheus zebra*.

Population structure of native *C. afra*

Our study has demonstrated a high level of mtDNA sequence variation among the native populations of *C. afra*. This contrasts sharply with the previous studies of Lake Malawi cichlids, where levels of lineage sorting were very low not only among intraspecific populations, but even among species from different genera (Moran & Kornfield 1993; Parker & Kornfield 1997; Sturmbauer *et al.* 2001). This has been attributed to recent contact among populations during low lake level stands (Owen *et al.* 1990; Fryer 1997), recent speciation (Moran & Kornfield 1993), persistently large population sizes and introgressive hybridization on secondary contact (Streelman *et al.* 2004). Our results suggest a different recent history for *C. afra* populations, perhaps with persistent geographical isolation and recent genetic bottlenecks. In contrast to many other mbuna species, *C. afra* appears to be subject to local genetic drift and shows high genetic differentiation across populations. This hypothesis may be investigated further, for example, using microsatellite markers. This finding was unexpected, given that *C. afra* often exists at high population densities, forms large feeding schools in the water column and penetrates to deeper water than many other mbuna (at least 50 m; Konings 2008). These traits would suggest that *C. afra* might readily disperse across stretches of deep water. Nevertheless, the high levels of genetic differentiation found in this study suggest that such potentially favourable conditions for dispersal do not result in frequent gene flow. This conclusion is consistent with the failure of *C. afra* to naturally colonize the southern arms of the lake following the last lake level rise. At the time of the first thorough survey of the southern part of the lake in the late 1970s, no *C. afra* were recorded from any other site in the south-eastern or south-western arms of the lake, apart from sites with likely anthropogenic introduction (Ribbink *et al.* 1983). The southern arms of the lake are relatively shallow and are believed to have been dry during low water stands of the lake around 25,000-70,000 years ago (Scholz & Rosendahl 1988; Cohen *et al.* 2007) or perhaps even as recently as 200 years ago (Owen *et al.* 1990).

Colonization history of introduced populations

All four nonnative *C. afra* populations were characterized by elevated levels of nucleotide and/or haplotype diversity compared with the native populations, which is consistent with a scenario of admixture among genetically divergent source populations (Kolbe *et al.* 2004; Roman & Darling 2007). Our results further show that genetic differentiation among introduced populations was lower than among native populations, which is expected if the same source populations contributed to the gene pools of different introduced populations. The admixture between different source populations could have happened either in the holding facilities of the aquarium trader who received stock from a number of localities and/or within the lake when the fish from different localities escaped or were released.

Our results provide some indication which native populations could have contributed to the gene pool of the introduced populations. Populations from the northern part of Lake Malawi (Mara Rocks, Likoma and Nkhata Bay) and to a smaller extent from the central west coast (Mbenji) share haplotypes with at least one introduced population and are therefore potential source populations. By contrast, haplotypes from two nearby south-eastern populations (Chiofu and Ntekete) were not found in the introduced populations. These localities are close to the introduction site but were not regularly visited by aquarium exporters until recently.

It seems likely that at least one additional genetically differentiated native population not sampled in our study must have contributed to the gene pool of the introduced populations. Given the close relationship among African cichlids and frequent incomplete lineage sorting among species (Parker & Kornfield 1997), there is also a possibility that some of the diversity in introduced populations originates from hybridization with other Mbuna species. However, a phylogenetic comparison of our data with 5125 sequences from African cichlids available on GenBank (data not shown) could not identify a candidate species for such introgression. We suggest that future studies should also investigate the possible contribution from other populations of *Cynotilapia* in the south eastern arm part of the lake, such as *Cynotilapia sp.* 'Chinyankhwazi' and *Cynotilapia sp.* 'Chinyamwezi', as well as other northern populations (e.g. Chisumulu Island). Overall our analysis provides clear evidence that all introduced *C. afra* populations were founded by an admixture from genetically differentiated source populations.

Furthermore, we suggest that multiple introductions could provide an explanation for the genetic differentiation between populations on the northern (Thumbi B) and southern shores of Thumbi West Island (Thumbi A).

Streelman *et al.* (2004) had previously found a similar pattern of divergence in both male colour phenotype and microsatellite allele frequencies, but suggested that this was resulting from rapid evolution following a single colonization event, perhaps associated with hybridization with *P. zebra*. Our study supports the hypothesis that *C. afra* hybridized with *P. zebra*, but indicates that such introgression is unlikely to be a major cause of differentiation among populations from northern and southern shores of Thumbi West. Rather, it seems plausible that *C. afra* was introduced into two sites on Thumbi West Island, but like the Otter Point populations, the northern Thumbi West population was not recorded by Ribbink *et al.* perhaps because it was small and of restricted distribution at the time of their study. This hypothesis may be investigated further, using highly variable genetic markers such as microsatellites in conjunction with recently developed nonequilibrium approaches for population genetic analysis. For example, assignment based methods were successfully employed to identify contemporary dispersal patterns in the Chinese mitten crab (*Eriocheir sinensis*; Herborg *et al.* 2007) and Approximate Bayesian Computation (ABC) have proved useful in distinguishing competing introduction scenario of invasive toads *Bufo marinus* (Estoup *et al.* 2001, 2004), birds (*Zosterops lateralis*, Estoup & Clegg 2003), corn rootworms (*Diabrotica virgifera virgifera*, Miller *et al.* 2005) and fruit flies (Thornton & Andolfatto 2006; Pascual *et al.* 2008).

Our results suggest that *C. afra* may not in fact be spreading out from its original introduction site, as initially feared by conservation biologists. This is consistent with the poor dispersal capacity of *C. afra* as suggested by the marked genetic structure among native populations. It appears that the populations at Otter Point and Thumbi West (A and B sites) probably represent at least three separate introductions. Furthermore, the Domwe Island population does not seem to have been founded by the population from the neighbouring north shore of Thumbi W, but more likely from the population from the southern shore. This suggests anthropogenic transportation, perhaps by some of the many local fishing crews that operate their nets in the area. *Cynotilapia afra* as a mid-water feeder is particularly prone to being caught in the 'Chirimila' nets that target the zooplankton-feeding *Copadichromis* and *Mchenga* locally known as 'utaka' and the *Engraulicypris sardella* 'Usipa' schools.

Genetic variation and establishment success

Our analysis suggests that the establishment of *C. afra* in the southern part of the lake was associated with, and possibly facilitated by, the relatively high genetic

diversity of the founder populations, probably in part because of the multiple origins of the founders. Other studies of plants *Phalaris arundinacea* (Lavergne & Molofsky 2007 and review Ellstrand & Schierenbeck 2000), crabs *Carcinus maenas* (Roman 2006) and lizards *Anolis sagrei* (Kolbe *et al.* 2004) have suggested that increased genetic diversity of introduced species may be associated with invasion success. Multiple introductions or introduction from different origins is likely to offset the effects of genetic drift and loss of variation commonly associated with founder events (Kolbe *et al.* 2004; Hänfling 2007; Roman & Darling 2007).

Of course, it is possible that other factors may facilitate an invasion, such as the availability of a suitable ecological niche. Most Malawian rock-dwelling cichlids feed at least partly on benthic prey, such as loose or attached algae and associated fauna. By contrast, *C. afra* seems to feed exclusively on plankton in the water column (Konings 2008). Unlike other mbuna that are generally solitary, territorial and tightly bound to the substrate, *C. afra* typically forms large feeding schools that rise high up into the water column. It is often extremely abundant in suitable habitats (Ribbink *et al.* 1983), as indeed it is now on Thumbi West Island. A recent study of species distributions and abundances on Thumbi West Island attempted to estimate levels of competition among rocky shore cichlid fish species, finding generally higher levels of competitive exclusion between species that had not naturally co-evolved (Young *et al.* 2009b). A striking exception was *C. afra* which, despite its abundance, appeared to have no impact on any of the indigenous species, suggesting that its niche was largely vacant prior to its introduction.

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