

# Hybridization and contemporary evolution in an introduced cichlid fish from Lake Malawi National Park

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## Abstract

Rapidly evolving systems offer the chance to observe genetic and phenotypic change in real time. We exploit a well-characterized introduction of cichlid fish into Lake Malawi National Park to document a short history of habitat colonization and the evolution of genes and colour pattern. In the early 1960s, a fish exporter introduced individuals of *Cynotilapia afra* to a single site (Mitande Point) of Thumbi West Island and, as late as 1983, the species was confined to this location. In 2001, *C. afra* had colonized the entire perimeter of Thumbi West. In July of that year, we sampled *C. afra* individuals from six sites around the island and scored variation in dorsal fin colour as well as allelic diversity at six microsatellite loci. We found that, in two decades, *C. afra* had diverged into genetically distinct, phenotypically different northern and southern populations. We observed a high proportion of hybrids between the introduced *C. afra* and the native *Metriaclima zebra* on the southern coast of Thumbi West, and speculate that hybridization is facilitated by low water clarity at these windward sites. The short history of *C. afra* at Thumbi West is a microcosm of contemporary evolutionary divergence and may provide the opportunity to study the process from start to finish in genetic detail.

**Keywords:** cichlid, colour pattern, hybridization, introduced species, rapid evolution

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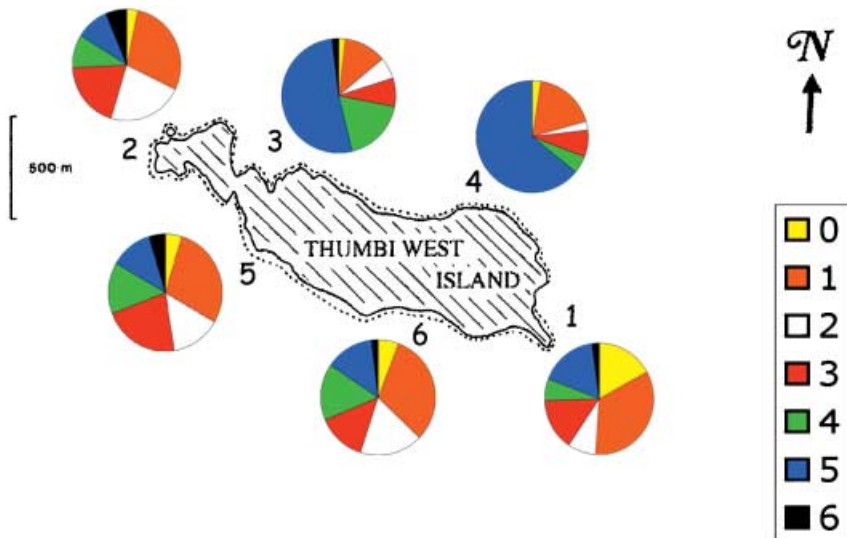
## Introduction

Thousands of cichlid species have evolved within the great lakes of Tanganyika, Malawi and Victoria in the last 2 million years (Turner *et al.* 2001). Cichlids are noteworthy because populations are often genetically subdivided on very small geographical scales (Van Oppen *et al.* 1997; Rico & Turner 2002) and sites can harbour a variety of endemic colour forms (Arnegard *et al.* 1999; Smith & Kornfield 2002). Colour pattern is thought to evolve in concert with the visual environment and novelties are sometimes produced by hybridization (Seehausen *et al.* 1997; Smith & Kornfield 2002; Smith *et al.* 2003). The role of hybridization in cichlid evolution has been debated, and new data suggest that the

phenomenon is more common than previously believed. Recent synthesis points to introgression as a source of genetic diversity and novel phenotypes, especially after changes in environment (Rüber *et al.* 2001; Salzburger *et al.* 2002; Smith & Kornfield 2002). Given that environmental change (e.g. the rise and fall of water level) is a frequent occurrence in East African lakes (Sturmbauer *et al.* 2001), hybridization may be a natural evolutionary force contributing to cichlid biological diversity. As humans continue to perturb these lacustrine environments, the opportunity for human-induced evolution through hybridization increases (e.g. Seehausen *et al.* 1997).

The potential for human-induced 'contemporary evolutionary and conservation biologists (Thompson 1998; Palumbi 2001; Stockwell *et al.* 2003). Reports have documented extremely rapid rates of phenotypic evolution, or reproductive isolation, following human introductions (Hendry

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**Fig. 1** Frequency of dorsal fin colour patterns in *Cynotilapia afra*, superimposed on a map of Thumbi West Island, Lake Malawi. Note the position of sampling sites 1–6; site 1 is Mitande Point, where *C. afra* was introduced in the 1960s. Each differently coloured wedge represents the frequency of individuals with the corresponding number of blue bars interrupting the black-banded fin (e.g. yellow = 0 blue bars; also see Table 2). Analysis of genetic structure suggests that *C. afra* has diverged into northern (sites 2, 3, 4) and southern (sites 1, 5, 6) populations at Thumbi West (text and Fig. 2).

*et al.* 2000; Huey *et al.* 2000; Koskinen *et al.* 2002). In turn, a great deal of effort has been expended to describe the ecological circumstances that might favour introduced, or invasive species (Mooney & Cleland 2001; Lee 2002). A disputed hypothesis suggests that translocated species might use genetic and phenotypic variation gained via hybridization as a stimulus for subsequent success (Ellstrand & Schierenbeck 2000). That introduced species might exploit hybridization as an evolutionary springboard is consistent with the growing appreciation of the process in adaptation (Grant & Grant 1996; Burke & Arnold 2001; Rieseberg *et al.* 2003). In this report, we use a well-characterized cichlid species introduction in Lake Malawi to document hybridization and contemporary evolution.

In the 1960s, an aquarium fish exporter based at Cape Maclear introduced roughly 20 cichlid species from northern Lake Malawi to a single site (Mitande Point: Fig. 1, site 1) at Thumbi West Island, presently located in Lake Malawi National Park (Ribbink *et al.* 1983). In the early 1980s, Ribbink *et al.* (1983) undertook a survey of the rock-dwelling cichlids (i.e. mbuna) throughout the lake and commented that the introduced *Cynotilapia afra* was still confined to Mitande Point. The translocated stock of *C. afra* is of unknown origin, but may be from Mara Rocks (Stauffer *et al.* 1996) or Likoma Island (Munthali & Ribbink 1998), each hundreds of kilometres from Mitande Point.

The introduced *C. afra* had black dorsal fins (Stauffer *et al.* 1996). Stauffer and colleagues observed blue barring in the black dorsal fin of *C. afra* specimens for the first time in 1991–92, despite 68 collections from Thumbi West between 1983 and 1990. This variation in colour (Fig. 1 of Stauffer *et al.* 1996), as well as intermediate dentition, led to the conclusion that *C. afra* was hybridizing with the native *Metriaclima zebra* (Stauffer *et al.* 1996). No genetic evidence was presented to substantiate this claim.

In July of 2001, we investigated the distribution of introduced mbuna at Thumbi West Island (M.R. Kidd, manuscript in preparation). *Cynotilapia afra* had dispersed around the perimeter, with pockets of varying abundance (Table 1). We noticed that all collecting sites harboured individuals with variably coloured dorsal fins. We sampled individuals of *C. afra* from six sites around the island and scored dorsal fin coloration as well as polymorphism at six microsatellite loci. We sought to answer three questions: (i) Do differences in colour pattern exist at sites around Thumbi West? (ii) Does population genetic structure exist around the island? (iii) Is there genetic evidence of hybridization? Our results offer a glimpse into the early stages of genetic and phenotypic differentiation among cichlid populations.

## Materials and methods

### *Cynotilapia afra* at Thumbi West Island

Thumbi West Island is located in the southern end of Lake Malawi in Lake Malawi National Park. It is approximately 1800 m long and is 500 m wide at its widest point (Fig. 1). The island extends lengthwise from the east to west such that its southern coast faces strong trade winds that blow from May to August. These winds stir the deeper, cooler waters and create higher levels of gross primary productivity than in the northern (and deeper) region of the lake (Eccles 1974; Munthali & Ribbink 1998). Thumbi West Island has a rocky shore around the majority of its circumference with small interrupting intervals of sand and reeds (Trendall 1988).

*Cynotilapia afra* is a small, planktivorous species native to the northern part of Lake Malawi, introduced to Thumbi West Island in the 1960s. In July 2001, we sampled 32–51 male individuals of *C. afra* from six sites around the island

**Table 1** Summary statistics for genetic polymorphism at six microsatellite loci and dorsal fin colouration in *Cynotilapia afra* at six sites around Thumbi West Island, Lake Malawi

Site	Density	$H_O$	$H_E$	Microsatellite loci — Allelic richness						Total	Colour (1 SD)
				<i>c-ski1</i>	<i>UV</i>	2014	973	2191	<i>clc5</i>		
1	20	0.807	0.918	14	5.9	23	27.7*	23.4	25.4	119.4	2.2 (1.8)
2	2	0.835	0.907	9.8	6	25.8	25.4	20.7	22.4*	110.1	2.6 (1.6)
3	15	0.812	0.868	6.7	6.5	20.9*	24.5	16.5	18.9	94	3.9 (1.3)
4	3	0.885	0.908	12.5	5.9	21.9	22	19.5	17.6	99.4	3.9 (1.4)
5	3	0.885	0.933	12	7.8	28.3	27	29.6	22.4	127.1	2.7 (1.6)
6	7	0.835	0.920	9.6	7.6	20.7	27.5	27.4	22	114.8	2.6 (1.6)

Density was calculated as the number of individuals within 1 m on each side of two 30.5-m transects (MR Kidd, in preparation).  $H_O$  is observed heterozygosity averaged over loci (SD range 0.06–0.12).  $H_E$  is expected heterozygosity averaged over loci (SD range 0.05–0.10). Cells with asterisks indicate population–locus pairs in violation of Hardy–Weinberg equilibrium ( $\alpha = 0.05$ ,  $P < 0.0014$ ). Allelic richness for each population–locus pair is presented in columns 5–10. Total is the sum of allelic richness estimates across loci for each population. Colour is the mean number of blue bars interrupting the black dorsal fin (for 32–51 individuals per population).

(Fig. 1). Fishes were collected with monofilament nets while SCUBA diving. Fin clips were taken from each individual and stored in 95% ethanol prior to genetic analysis.

#### *Cynotilapia afra* dorsal fin colouration

Immediately upon collection, the number of blue bars interrupting the black dorsal fin of *C. afra* males was scored. Scores ranged from 0, a purely black-banded dorsal fin, to 6, a black fin with six vertical blue bars. Bars were counted if they made complete or incomplete interruptions. We analysed dorsal fin colour data in two ways. First, we used one-way analysis of variance (ANOVA) to compare the mean number of blue bars in the dorsal fin of *C. afra* individuals from the six collection sites. We followed the ANOVA with a Tukey's *post hoc* multiple comparisons test to identify specific sites that differed from one another. Second, we used exact tests, implemented in GENEPOP vs 3.1d (Raymond & Rousset 1995), to evaluate pairwise differences among sites in the frequency of dorsal fin colour patterns. Significance values for exact tests were Bonferroni-corrected for multiple comparisons ( $\alpha = 0.05$ ,  $P < 0.0033$ ).

#### Population genetics of *Cynotilapia afra*

DNA was extracted following standard phenol–chloroform procedures. Six microsatellite loci were examined in 32 individuals from each of the six collection sites. A tetranucleotide repeat (CAAT) was scored in intron 3 of the cichlid UV-sensitive opsin gene (hereafter *UV*; Carleton *et al.* 2000) using primers F: agctgctgggtgctctga, R: ctgcaacctgcagaggaa; a CA repeat was scored in the 3' UTR (untranslated region) of the cichlid chloride channel gene *clc5* (Miyazaki *et al.* 1999) using primers F: agggatgaaggatccaggagt, R: aggacagcgtgcatagttc; a CT repeat 5' of the cichlid *c-ski1* proto-oncogene (Huang *et al.* 1999; Strelman *et al.* 2003) was scored using

primers F: gtcagtcacattcctggctg, R: ttctatgctcctgcggtttt; and CA repeats were scored in anonymous loci UNH973 (GenBank accession no. G68260), UNH2191 (BV005710) and UNH2014 (primers F: tgcaactgagagctgacatga, R: cagacttcacttcaccaatca). These markers are physically unlinked; each is mapped to different linkage groups of the tilapia (Lee *et al.*, manuscript in preparation) or mbuna (Albertson *et al.* 2003; Strelman unpublished data) genomes. Whenever possible, loci were amplified by polymerase chain reaction in triplex and then electrophoresed on 4% acrylamide gels using an ABI 377 DNA sequencer (Applied Biosystems). Fluorescently labelled polymerase chain reaction fragments were detected with GENESCAN (version 3.1.2, Applied Biosystems) and alleles were scored by eye.

We used GENEPOP version 3.1d (Raymond & Rousset 1995) to conduct: (i) probability tests of Hardy–Weinberg equilibrium for each locus at each of the six sites and (ii) per locus exact tests of genic differentiation (allele frequency differences) for each pairwise comparison of sites. Allelic richness per locus and site was estimated using FSTAT version 2.9.3.2 (Goudet 1995). We tested the hypothesis that allelic richness differed between the northern (sites 1, 5, 6) and southern (sites 2, 3, 4) sides of Thumbi West using a permutation test (10 000 iterations) in FSTAT. Mean  $F_{ST}$  (Weir & Cockerham 1984) across loci, among pairs of sites, was calculated using MSA 2.65 (Dieringer & Schlötterer 2003). Statistical support for  $F_{ST}$  was derived from 10 000 permutations of alleles. All significance values were Bonferroni-corrected for multiple tests. We tested the correlation between genetic differentiation ( $F_{ST}/1 - F_{ST}$ ) and geographical distance between sites (measured around the perimeter of Thumbi West) using Mantel tests (5000 iterations) implemented in GENEPOP version 3.1d. Similarly, we used Mantel tests (5000 iterations) to evaluate the association between genetic differentiation (in this case,  $F_{ST}$ ) and 'colour pattern distance' between sites. 'Colour pattern

distances' were generated by computing chord distances (Cavalli-Sforza & Edwards 1967), between sites, in dorsal fin colour pattern frequencies (see above), using PHYLIP version 3.5c (Felsenstein 1993).

Next, we used factorial correspondence analysis in the program GENETIX version 4.0 (Belkhir 1999) to plot *C. afra* individuals (sampled from each of the six sites around Thumbi West Island) in multivariate space. This technique is an appropriate method with which to visualize genetic relationships among populations with complex histories.

#### Hybridization with *Metriaclima zebra*

We wanted to test the hypothesis (Stauffer *et al.* 1996) that the introduced *C. afra* had hybridized with the native *M. zebra* at Thumbi. These two species have similar body colouration, but are distinguished by abundance (*M. zebra* are at least 10 times more abundant at most sites), body size (*M. zebra* males are larger), dorsal fin colour (*M. zebra* males at Thumbi West lack barring in their blue dorsal fin) and dentition (*M. zebra* have bicuspid teeth in the first tooth row, *C. afra* have unicuspid teeth in the first tooth row). Collections of 32–60 *M. zebra* males were made at each of the six sites at Thumbi West. We sorted fishes into *C. afra* and *M. zebra* in the field based on size and dorsal fin colour, and later confirmed these designations in the laboratory by examination of teeth in dried specimens. We observed a small frequency (<1%) of 'intermediate' individuals, characteristic of first- or second-generation hybrids, as defined phenotypically in Stauffer *et al.* (1996).

As part of a separate project (Streelman, unpublished results), four microsatellite markers (*LIV*, *c-ski1*, UNH2191, *clc5*; also typed in *C. afra*) were genotyped in the *M. zebra* samples. Using MSA 2.65 (Dieringer & Schlötterer 2003), we calculated mean  $F_{ST}$  across loci, among pairs of sites for the *M. zebra* collections. We also estimated  $F_{ST}$  between *C. afra* and *M. zebra* sampled at the same site. Statistical support for  $F_{ST}$  was derived from 10 000 permutations of alleles. We used GENEPOP version 3.1d (Raymond & Rousset 1995) to perform per locus exact tests of allele frequency

differences for *C. afra* vs. *M. zebra* collected at common sites. Significance values were Bonferroni-corrected for multiple tests.

We used the program STRUCTURE version 2.0 to calculate admixture proportions (Pritchard *et al.* 2000; Beaumont *et al.* 2001) for *C. afra* individuals at each of the six sites around Thumbi West. In this analysis, we included a source population drawn from *M. zebra* sampled at Domwe Island and Otter Point (Streelman, unpublished). These sites are located roughly 2.5 and 1.5 km north and south of Thumbi West, respectively; *C. afra* has not been observed at either locale. In essence, we forced the model to consider these individuals as pure *M. zebra* and then estimated the proportion of *M. zebra* genes in *C. afra* genomes. STRUCTURE was used to generate 95% probability intervals for individual admixture proportions. Finally, we used permutation (1000 iterations, RESAMPLING STATS EXCEL ADD-IN version 2.0) to test the hypothesis that mean admixture proportions differed at northern (sites 1, 5, 6) vs. southern (2, 3, 4) sites.

## Results

#### *Cynotilapia afra* dorsal fin colouration

Fishes at the six sites, collected in 2001, differed in colour pattern (ANOVA,  $P = 7.5 \times 10^{-8}$ , Fig. 1). The mean number of blue bars interrupting the black dorsal fin was greater at sites 3 and 4 than at other locations (Table 1). Similarly, the frequency of colour patterns differed among sites at Thumbi West (Table 2), with more than half of all individuals at sites 3 and 4 having five or more blue bars in the dorsal fin. Both methods of testing differences in colour pattern (i.e. means and frequencies) provided identical insight: fishes from sites 1, 2, 5 and 6 had colour patterns similar to one another, and distinct from individuals from sites 3 and 4. Because the dorsal fins of *C. afra* at Thumbi were free of interrupting blue bars until 1991 (Stauffer *et al.* 1996), we suggest that this colour polymorphism (and divergence among sites) has evolved in the last decade.

Site/ pattern	Number of blue bars in the dorsal fin						
	0	1	2	3	4	5	6
Site 1	0.170	0.34	0.085	0.149	0.064	0.17	0.021
Site 2	0.032	0.29	0.226	0.194	0.097	0.097	0.065
Site 3	0.02	0.12	0.06	0.08	0.18	0.52	0.02
Site 4	0.026	0.179	0.026	0.077	0.051	0.641	0
Site 5	0.048	0.286	0.143	0.214	0.143	0.119	0.048
Site 6	0.059	0.314	0.176	0.137	0.157	0.137	0.02

**Table 2** Frequency of *Cynotilapia afra* dorsal fin colour patterns at six sites around Thumbi West Island, Lake Malawi

Pattern refers to the number (0–6) of blue bars in the black-banded dorsal fin of *C. afra* males, collected in 2001 (also see pie charts, Fig. 1).

Population genetics of *Cynotilapia afra*

Three of 36 population-locus pairs departed from Hardy–Weinberg equilibrium (Table 1). Departures showed no clear trend with respect to locus or population. Fishes from northern sites (2, 3 and 4) had reduced allelic richness when compared to those from the south (1, 5, and 6; Table 1,  $P = 0.051$ ). Forty-one of 90 pairwise exact tests of allele frequency difference were significant after correction for multiple tests (data not shown). In the majority of cases, results from exact tests are mirrored in pairwise  $F_{ST}$  values averaged over loci (Table 3). For instance, when  $F_{ST}$  is significantly different from zero (e.g. sites 3 or 4 vs. sites 5 or 6), five or six of six loci tested showed differences in allele frequency according to exact tests. The situation is more complicated for comparisons of sites 1 and 2. Significant allele frequency differences were observed at loci *c-ski1*, *UV* and *UNH2191*; *clc5* showed nominally significant allele frequency differences, but *UNH2014* and *UNH973* did not. Mean  $F_{ST}$  is significantly different from zero (0.021) for sites 1 vs. 2 if loci *UNH2014* and *UNH973* are excluded from analysis. Genetic differentiation ( $F_{ST}/1 - F_{ST}$ ) is not correlated with physical distance between sampling sites ( $P = 0.213$ ). Nor is genetic differentiation ( $F_{ST}$ ) correlated with colour pattern distance ( $P = 0.26$ ; results are no different if  $F_{ST}/1 - F_{ST}$  is used; also see Table 3).

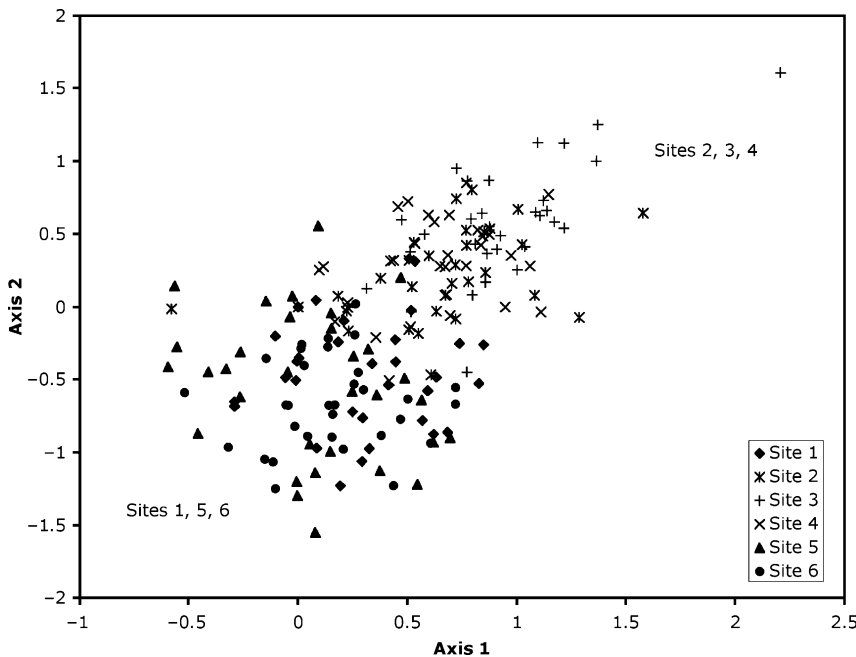
Factorial correspondence analysis of *C. afra* individuals suggests differentiation between populations on the northern vs. southern perimeter of Thumbi West Island (Fig. 2). The factorial correspondence analysis plot shows strong separation between the clustering of *C. afra* at sites 1, 5 and 6 from those at sites 2, 3 and 4. Taken together (Table 3,

**Table 3** Pairwise comparisons of genetic and colour pattern divergence in *Cynotilapia afra* at six sites around Thumbi West Island, Lake Malawi

	2	3	4	5	6
$F_{ST}$					
1	0.012†	0.03*	0.017*	0.009	0.009
2		0.011	0.009	0.021*	0.021*
3			0.013	0.042*	0.04*
4				0.019*	0.017*
5					0.002
MCT					
1	NS	0.0003	0.0008	NS	NS
2		0.006	0.02	NS	NS
3			NS	0.002	0.0003
4				0.009	0.002
5					NS

In the top matrix, we report mean  $F_{ST}$  values across loci. Significance of  $F_{ST}$  (indicated by asterisk) was determined by 10 000 permutations of alleles (Dieringer & Schlötterer 2003) and corrected for multiple tests ( $\alpha = 0.05$ ,  $P < 0.0033$ ). In the bottom matrix, we report  $P$ -values from Tukey’s multiple comparisons tests (MCT), following one-way ANOVA (which tested differences in mean number of blue bars in the dorsal fin of *C. afra* individuals from the six sites). Exact tests of colour pattern frequencies yielded identical results to the MCTs (data not shown). A map showing the location of sampling sites is presented in Fig. 1. † $F_{ST}$  between *C. afra* at sites 1 and 2 is significantly different from zero (0.021) if markers *UNH973* and *UNH2014* are excluded from analysis.

Fig. 2), the genetic data demonstrate that individuals from the north (sites 2, 3 and 4) are diverging from fishes from the south (sites 1, 5 and 6). Given that *C. afra* was confined to Mitande Point in the early 1980s (Ribbink *et al.* 1983), it



**Fig. 2** Factorial correspondence analysis (FCA) plot of *Cynotilapia afra* individuals from six sites around Thumbi West Island, Lake Malawi. The first two factorial axes capture 48% of variation in the groupings. Factorial correspondence analysis discriminates between *C. afra* populations from the north (sites 2, 3, 4) vs. those from the south (sites 1, 5, 6).

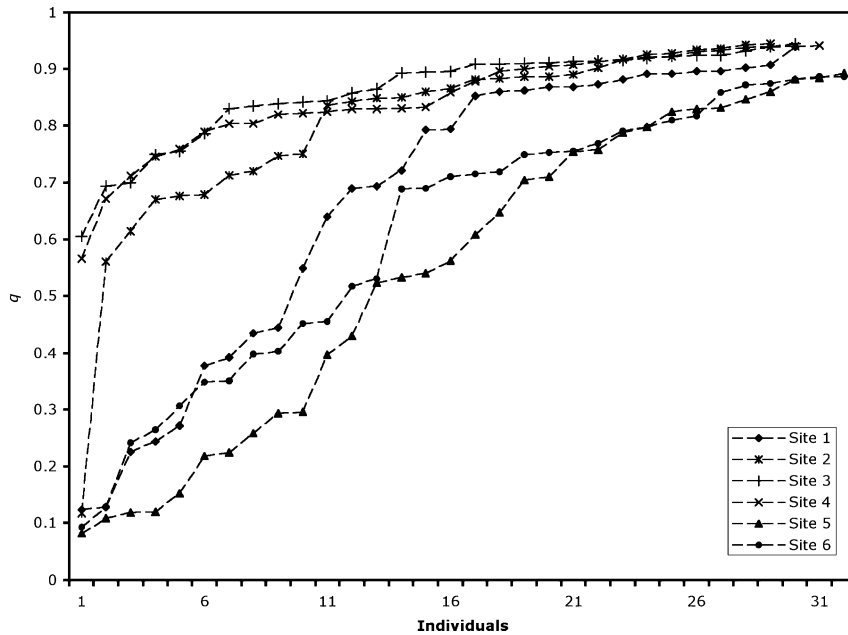


Fig. 3 Admixture proportions for *Cynotilapia afra* individuals from six sites around Thumbi West Island, Lake Malawi. We plot  $q$ , the proportion of each genome derived from *C. afra*, ranked and sorted by collection site. Northern sites (2, 3 and 4) have higher  $q$  than southern sites (also Table 4).

follows that this differentiation occurred after or coincident with the colonization of Thumbi West Island, in the last two decades.

#### Hybridization with *Metriaclicma zebra*

The native *M. zebra* does not show significant population structure (all mean  $F_{ST} < 0.01$ ) among sites at Thumbi West when considering the four microsatellite loci typed in common with *C. afra* (*UV*, *c-ski1*, UNH2191, *clc5*) or with the addition of two other markers (Streelman, unpublished). Significant estimates of mean  $F_{ST}$  are observed for comparisons of *C. afra* vs. *M. zebra* at common sites around Thumbi West (values range from 0.019 to 0.056; all corrected  $P < 0.006$ ), with the lowest value being at site 5. In fact, at site 5, the only locus exhibiting significantly different allele frequencies between *C. afra* and *M. zebra* is *UV* ( $UV F_{ST}$  between *C. afra* and *M. zebra* at site 5 is 0.06).

Analysis of admixture proportions shows higher values of mean  $q$  (in this case, the proportion of an individual's genome derived from *C. afra*) and lower variance at northern (2, 3, 4) vs. southern (1, 5, 6) sites (Table 4; Fig. 3). At sites 5 and 6, one third of individuals examined have  $q$  scores less than 0.5, whereas at site 3 and 4, no individual has a  $q$  score less than 0.5. On the south side of the island (sites 1, 5 and 6), a total of 38 individuals (40%) have confidence intervals around  $q$  that do not include 1. By contrast, on the northern coast (sites 2, 3 and 4), a total of seven individual (7%) have confidence intervals around  $q$  that do not include 1. Permutation tests support the conclusion that mean  $q$  is higher on the northern vs. southern coast of Thumbi West ( $P < 0.001$ ).

Table 4 Mean admixture proportions in *Cynotilapia afra* at six sites around Thumbi West Island, Lake Malawi

Site	Mean $q$ (1 SD)
1	0.664 (0.269)
2	0.800 (0.170)
3	0.860 (0.084)
4	0.850 (0.089)
5	0.546 (0.281)
6	0.610 (0.244)

In this case,  $q$  is the proportion of an individual's genome derived from *C. afra*. Northern sites (2, 3 and 4) have higher values of  $q$  than southern sites (also see Fig. 3).

## Discussion

### *Divergence has occurred rapidly in introduced Cynotilapia afra*

We have used a well-documented species introduction to study the early stages of population differentiation in cichlid fishes. *Cynotilapia afra* was introduced to Mitande Point of Thumbi West Island in the 1960s (Munthali & Ribbink 1998) and individuals were still confined to that site in the early 1980s (Ribbink *et al.* 1983). Blue barring in the black dorsal fins of *C. afra* was first noticed at Mitande in 1991 despite extensive sampling between 1983 and 1990 (Stauffer *et al.* 1996). Our observations and genetic analysis from 2001 suggest that *C. afra* colonized the entire island of Thumbi West some time during the last two decades and diverged into northern and southern populations with

differently coloured dorsal fins. Our findings add to a growing list of examples that highlights rapid rates of microevolution (Reznick *et al.* 1997; Huey *et al.* 2000; Koskinen *et al.* 2002) and genetic divergence (Hendry *et al.* 2000), often in conjunction with human introductions. Both Huey *et al.* (2000) and Hendry *et al.* (2000) report the divergence of phenotypes and/or the evolution of reproductive isolation in 10–15 generations. Assuming a generation time of 10–12 months for *C. afra*, the differentiation of allele frequency and colour pattern on the north and south of Thumbi West Island has occurred in a similar time interval.

We are uncertain about the evolutionary forces contributing to genetic and phenotypic differentiation in *C. afra*. Pairwise estimates of population structure are not correlated with geographical distance. This indicates that genetic divergence between *C. afra* populations is not explained solely by physical distance from the introduction site, but our test may be compromised by low power (i.e. fewer than 10 populations, Peterson & Denno 1998). Nor is pairwise genetic differentiation correlated with differences in the frequency of dorsal fin colour patterns. The lack of correspondence here seems mostly to be the result of comparisons involving site 2 (Table 3), which harbours fishes from the north that look like those from the south.

Without detailed reconstructions of colonization history and the phenotypic distributions of colour morphs, we cannot know which came first (e.g. did colour differentiation occur before, during or after colonization?). It may be that founding populations, which colonized the north vs. the south of Thumbi West from Mitande Point, were sampled unevenly from a pre-existing pool of colour variants. It is also possible that dorsal fin phenotypes have been selected by ecological conditions in newly colonized habitats. For at least 4 months of the year, the strong southern trade winds mix the deeper and shallower water layers and increase primary productivity in the shallower southern arm of Lake Malawi. These winds generate currents and wave action that push turbid water up against the south side of Thumbi West. In July 2001, we noticed that the water on the south side of the island (sites 5 and 6) was considerably cloudier than on the protected northern coast (sites 3 and 4). The importance of male colour pattern to cichlid mate choice is well established (Seehausen & van Alphen 1998), as is the potential for mate preference to vary with changing water clarity (Seehausen *et al.* 1997). It is notable then, that *C. afra* individuals do not experience seasonal turbidity in their native habitat, where they mate assortatively with respect to *Metriaclima zebra* (Stauffer *et al.* 1996; Munthali & Ribbink 1998).

The population of *C. afra* at site 2 may help to resolve the role of sampling effects vs. selection on dorsal fin colour pattern. Site 2 is the furthest from the point of introduction at Mitande, harbours the smallest density of *C. afra* (Table 1), and was probably the last to be colonized. It is possible that colonists

to site 2 were drawn unequally from the distribution of colour morphs on the northern perimeter. Alternatively, selection in the new habitat has acted against a subset of migrating individuals (i.e. those with more blue barring in the dorsal fin). Site 2, like site 1 (Mitande Point), is located at the tip of Thumbi West Island, and may represent an intermediate visual environment (neither protected nor fully exposed). These questions set the agenda for future field research: water clarity and mating success among male colour morphs should be measured seasonally at sites around the island.

#### *The history (and future) of hybrids at Thumbi West Island*

We have genetically confirmed the hypothesis (Stauffer *et al.* 1996) that introduced *C. afra* hybridized with the native *M. zebra* at Thumbi West Island. Notably, admixture proportions are significantly different for northern vs. southern populations separated by less than 1 km. Northern populations (2, 3, 4) comprised individuals with predominantly *C. afra* genomes (i.e. high  $q$ ) while southern sites were a mixture of individuals with *C. afra*, *M. zebra* or mosaic genomes. It was difficult to predict from our field observations the number of *C. afra* individuals carrying *M. zebra* alleles (i.e. in the vast majority of cases, these 'hybrid' individuals resembled *C. afra* phenotypically).

The absolute values of  $q$  should be interpreted with caution for the following reasons (Pritchard *et al.* 2000). First, our analysis was conducted with only four microsatellite loci. Second, mbuna species carry numerous alleles at most microsatellite loci and many of these are expected to be shared ancestral polymorphisms. It is likely then, that  $q$  in Fig. 3 is a slight underestimate of the true proportion of the genome drawn from *C. afra*. Importantly, none of these caveats affect our conclusions regarding the comparative distribution of  $q$  on the north vs. the south of Thumbi West.

It is intriguing to speculate why there are more hybrids on the southern perimeter of Thumbi West. Stauffer *et al.* (1996) suggested that hybridization at Mitande Point was galvanized by differences in the abundance of *C. afra* and *M. zebra*, causing *C. afra* females to mate with the numerically dominant and physically larger *M. zebra* males. Because the initial abundance of *C. afra* was low at all sites and *M. zebra* seems to be uniformly distributed, this explanation is not sufficient to account for differences in hybridization on each side of the island. It is possible that features of the visual environment facilitate hybridization and the success of hybrids on the southern coast (see above). Differences in numerical abundance and physical size, in regions of low water clarity, may continue to drive hybridization between *C. afra* and *M. zebra* at sites 1, 5 and 6.

We are unsure how hybridization has contributed to dorsal fin colour variation in *C. afra* at Thumbi West. Throughout its range, most populations of *C. afra* are polymorphic for dorsal fin colour pattern. Stauffer *et al.* (1996) report blue

barring in the black fins of *C. afra* specimens for the first time in 1991. It is possible that barring is the result of hybridization with *M. zebra*, as Stauffer *et al.* (1996) suggest, or that this represents the re-expression of a phenotype initially absent in the translocated stock (i.e. that hybridization occurs but is not the cause of fin colour variation). In support of the latter, we find the greatest number of individuals with blue barring on the northern side of Thumbi West, where rates of hybridization are lowest. We do not understand the genetic basis of dorsal fin colour pattern in *C. afra* (nor in *C. afra* × *M. zebra* hybrids), although our results imply that it may be more complex than proposed by Stauffer *et al.* (1996). Because cichlid species can be raised and hybridized in the laboratory, this should be a focus of future effort.

### *Cynotilapia afra* at Thumbi West as a microcosm of evolutionary divergence

It is challenging to study evolution because biologists are usually left to infer mechanism from the products left behind. It is for this reason that most examples of 'evolution in action' are serendipitous (e.g. Grant & Grant 2002). The case of *C. afra* at Thumbi West Island is significant because we have the opportunity to study evolutionary divergence from start to finish in genetic detail. It is clear that *C. afra* populations on each side of the island are experiencing markedly different evolutionary trajectories. This story, as it develops, may possess all of the ingredients of cichlid evolutionary models (Kornfield & Smith 2000): habitat colonization, contact between two previously isolated populations, hybridization, selection with gene flow, and divergence of genes and phenotype. Monitoring how things unfold may help us to understand the ecological foundations of differentiation and the importance of human disturbance to the evolutionary process.

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