Extreme microallopatric divergence in a cichlid species from Lake Malawi

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Abstract

We demonstrate significant population structuring on an extremely small spatial scale between adjacent demes of a Lake Malawi haplochromine cichlid species of the mbuna group, *Pseudotropheus callainos*, separated by only 35 m of habitat discontinuity. This substantiates further the notion that intralacustrine allopatric divergence may help to explain the high level of species richness of the mbuna in comparison to other Malawian cichlids, as well as of the Malawian haplochromines as a whole.

Keywords: cichlids, Lake Malawi, microstallites, population structure

Introduction

The fastest large-scale adaptive radiations in recorded evolutionary history have occurred in Lakes Victoria and Malawi in East Africa. At the last count, Lake Malawi was estimated to contain 659 haplochromine cichlid species, all but two endemic to the lake (Turner *et al.* 2001) and 50 other fish species, about half of which are endemic (Ribbink 1994). Lake Victoria has very similar levels of species richness and endemism (Seehausen 2002). These lakes almost certainly harbour the largest number of vertebrate species endemic to any comparably sized area on the planet. Almost all the endemic species are haplochromine cichlid fishes, and the species-rich Malawian and Victorian lineages are related very closely to each other (Meyer 1993; Albertson *et al.* 1999). In each lake, a great proportion of this diversity is represented by small (< 25 cm), brightly coloured rock dwellers known as ‘mbipi’ (L. Victoria: Seehausen 1996) and ‘mbuna’ (L. Malawi: Konings 2001). Why have these ecologically similar lineages undergone such remarkable evolution in both lakes?

Fryer (1959) suggested that allopatric speciation in mbuna could take place within the present-day boundary of Lake Malawi because these fishes are rarely found away

from rocky shore habitats, which are distributed patchily within the lake. Allozyme studies have shown genetic differentiation among populations isolated by deep-water trenches or by shorelines of hundreds of kilometres (McKaye *et al.* 1984). Microsatellite studies have demonstrated population structuring over distances of a few hundred metres of habitat discontinuities (Van Oppen *et al.* 1997a; Arnegard *et al.* 1999; Markert *et al.* 1999). Here, we test whether the population might even be on a much smaller scale than demonstrated hitherto, investigating two samples of an mbuna separated by a waterfall which flows on the southern edge of a sandy beach only 35 m wide located in the Bay of Ruarwe in Lake Malawi.

Materials and methods

The study species was the ‘pearly’ race of *Pseudotropheus* (*Maylandia*) *callainos*. At Ruarwe, both sexes of this species are pure white, apart from several yellow ‘eggspots’ on the anal fin (Konings 2001). This allows it to be distinguished easily from all other sympatric cichlids. At Nkhata Bay and other sites south of Ruarwe, populations are colour polymorphic, with most individuals pale blue, but with some white and blotched individuals. Although it had been suggested that these might represent a distinct allopatric species, formerly called *Pseudotropheus* ‘zebra cobalt’ (Ribbink *et al.* 1983), it is now believed generally that the two are probably conspecific (Konings 2001). As with many other Malawian cichlids, the generic placement

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Fig. 1 Partial view of the Bay of Ruarwe on the northwestern shore of Lake Malawi where the sampling area is located (not to scale). Samples of 50 individuals were collected from three sites (1–3 from north to south). The shore between the three sites is a mosaic of rocky and small sandy patches, the longest uninterrupted sandy beach being about 35 m long represented by the shaded area. There is an inflowing cold-water stream represented by the thick arrow, which flows from the highlands throughout the year on the southern edge of the beach.

of the species is uncertain. Although many recent publications follow Stauffer *et al.* (1997) in assigning the species to the genus *Metriaclima*, this is now considered (Condé & Géry 1999) to be a junior synonym of *Maylandia*, which itself may still be best considered as a subgenus of *Pseudotropheus*.

The sampling area is located on the northwestern shore of Lake Malawi at the northern end of the bay of Ruarwe. Samples of 50 individuals were collected from each of three sites. The shore between the three sites is a mosaic of rocky and small sandy patches, the longest uninterrupted sandy beach being about 35 m long. There is an inflowing cold-water stream (approx. 18 °C, about 5 °C less than the water of the lake in September), which flows from the highlands throughout the year on the southern edge of the beach. The beach is surrounded by rock cliffs and was inundated up to 1980 when the lake level dropped to an extent that has left it exposed for the last 20 years (Fig. 1). The slope from the shoreline is about 45° and there are no bottom rocks in depths above 50 m. Two population samples were collected at either side of this beach (sites 2 and 3) while a third sample (sample 1) was collected at about 700 m from the north edge of the beach (site 1). Sample collection and preservation was as described by Van Oppen *et al.* (1997a, 1998). The shoreline between sample sites 1 and 2 is uninterrupted rocky habitat (Fig. 1).

Samples were prepared and screened for variation at six polymorphic microsatellite loci as described by Van

Oppen *et al.* (1997b). The six loci (Kellogg *et al.* 1995; Van Oppen *et al.* 1997b) include four perfect dinucleotide repeats (*Pzeb1*–3 and *UNH002*), and two imperfect dinucleotide repeats, *Pzeb4* and *Pzeb5*. Allele frequencies, expected (H_E) and observed (H_O) heterozygosities, were calculated using genepop 3.1b (Raymond & Rousset 1995). Samples were tested for linkage disequilibrium and departure from Hardy–Weinberg equilibrium by the Markov chain method. Heterogeneity in allele frequency distribution for all loci, and all pairwise comparisons, was tested based on an assumption of no differentiation.

arlequin 1.1 (Schneider *et al.* 1997) was used to calculate pairwise fixation indices, based on allele frequency variation using an amova framework to estimate weighted F -statistics (θ) over all loci (Excoffier *et al.* 1992). The significance of genetic subdivision was assessed using 1000 permutations. Bonferroni corrections were applied to all pairwise tests using a global significance level of 0.05 ($k = 18$) (Rice 1989).

Results and discussion

All three samples were highly genetically diverse, showing high heterozygosities at most loci. A total of 36, 32, 20, 13, seven and four alleles were observed at *Pzeb1*, *UNH-002*, *Pzeb2*, *Pzeb3*, *Pzeb4* and *Pzeb5*, respectively (Table 1). Significant deviations from Hardy–Weinberg equilibrium

	<i>Pzeb1</i>			<i>Pzeb2</i>			<i>Pzeb3</i>		
	NA	H_O	H_E	NA	H_O	H_E	NA	H_O	H_E
Locus									
Site 1	35	0.82*	0.97	19	0.67*	0.89	11	0.58	0.65
Site 2	35	0.81*	0.95	19	0.66*	0.89	11	0.56	0.62
Site 3	21	0.75*	0.91	13	0.64*	0.84	8	0.52	0.60
	<i>Pzeb4</i>			<i>Pzeb5</i>			UNH-002		
Locus									
Site 1	7	0.55	0.63	2	0.24	0.28	25	0.73*	0.92
Site 2	7	0.56	0.64	2	0.15	0.16	23	0.66*	0.91
Site 3	4	0.47	0.52	4	0.20	0.24	18	0.61*	0.91

*Sequential Bonferroni-adjusted P significantly different from zero.

Table 1 Number of alleles (NA), observed (H_O) and expected (H_E) heterozygosity. Significant deviations from Hardy–Weinberg equilibrium are under $H_O = HWE$ and $H_I =$ heterozygote deficit

in the form of heterozygote deficits were present in three loci (*Pzeb1*, *Pzeb2* and UNH-002). Heterozygote deficiencies were assumed to be due mainly to nonamplifying alleles because true breeding null alleles were found in four of the six loci used in this study in a pedigree test using the closely related species *M. zebra* (Van Oppen *et al.* 1997a, 1998). No significant heterozygote deficits were found at the loci that had no null alleles in the pedigree test. Null alleles would be of significance only if our estimates for population structure were merely artefacts caused by null alleles. An analysis carried out with only the loci that have no heterozygote deficits (*Pzeb* 3, 4 and 5) yielded the same level of significance on the F_{ST} estimates as the analysis carried with all loci ($\theta = 0.103$, $P < 0.001$). Therefore, the presence of null alleles reported by Van Oppen *et al.* (1997a, 1998) in three of the loci clearly had a negligible effect on the estimates of population differentiation. Exact tests for genotypic linkage disequilibrium confirmed the absence of physical linkage at these loci, as reported previously (Van Oppen *et al.* 1997a).

Significant ($P < 0.001$) heterogeneity in allele frequency distribution was found for all loci, but one (*Pzeb5*), between samples collected at opposite sides of the beach and waterfall (samples 2 and 3) and between the sample collected south of the waterfall and that collected 700 m north from the end of the beach (samples 1 and 3) (Figs 1 and 2). F_{ST} estimates were also significantly different from zero between these samples ($\theta = 0.048$, $P < 0.001$ and $\theta = 0.052$, $P < 0.001$, respectively). Neither estimate of differentiation was significant for the comparison of the samples from sites 1 and 2 (Fig. 1), which were separated by 700 m of uninterrupted rocky shoreline ($\theta = 0.003$, $P = 0.978$).

Our F_{ST} estimates for the populations north and south of the waterfall were approximately three times higher than those estimated by Van Oppen *et al.* (1997a) for the same species at Nkhata Bay, where populations were separated by sand and deep-water barriers approximately 20–40

times wider. This suggests that the cold-water inflow may be a more significant barrier than the 35-m sandy beach.

The absence of mbuna (excluding a few species specialized to live over soft-bottom habitats) elsewhere than on the rocky shores of Lake Malawi and the presence of colour variants with restricted ranges within the lake led Fryer (1959) to suggest that these fishes were split into genetically isolated populations by habitat barriers such as sandy beaches or deep-water trenches. Experiments by Hill & Ribbink (1978) demonstrated that mbuna had poor ability to cope with rapid changes in water pressure, as would result from changing water depth. This would render them physiologically incapable of rapidly traversing deep-water barriers if they have to remain near the bottom. Our study suggests that temperature discrepancies of as low as 5 °C may also act as barriers.

Ribbink *et al.* (1983) suggested that restricted distributions of mbuna phenotypes were not only the result of stenotopy, but also behavioural philopatry. Several studies have reported that tagged adult mbuna of both sexes and several species tend to remain in relatively small areas for many months or even years (Hert 1992; Robinson 1995). Territorial males of several species returned to their home range from distances up to 2.5 km when translocated experimentally along continuous rocky shores, demonstrating a surprising homing ability (Hert 1992). Even when released 500 m offshore in open water, five of 10 males returned within a week, compared to 10 of 12 returning the same distance along rocky shores. Thus, it appears that adult fish are sedentary, but capable of rapid long-distance movement, even across major habitat barriers when required. However, when released 1 km away on a sandy beach on the other side of a deep-water barrier none of 20 fish returned, although 11 of 17 fish returned the same distance along rocky shores (Hert 1992). This may suggest that lack of dispersal across some habitat barriers is due

Fig. 2 Allele frequency distributions at loci *Pzeb* 2, 3 and 4 of *M. callainos* 'pearly' from the three samples collected in the Bay of Ruarwe in Lake Malawi. Significant ($P < 0.001$) heterogeneity in allele frequency distribution was found for all loci, bar one (*Pzeb*5), between samples collected at either side of the beach and waterfall (samples 2 and 3) and between the sample from south of the waterfall and that collected 700 m north of the beach (samples 1 and 3). To illustrate the extent of differentiation among samples, only loci which show significant differences and have a small number of alleles are shown (y axis = frequency; x axis = allele size in base pairs).

mainly to behavioural choice rather than physiological inability. Dispersal of juveniles has not been studied directly but unlike many marine teleosts, mbuna produce small numbers of large benthic-living offspring and seem

to lack a dispersal phase (Hert 1992). Molecular evidence of high levels of population structure caused by relatively small barriers, such as we have shown, suggests that juveniles may disperse as little as do adults. Knight *et al.* (1999) suggest that males disperse further than females, perhaps in search of a vacant territory. Although much remains to be learned about the mechanisms and patterns of dispersal in mbuna, such knowledge is clearly important for the study of within-lake allopatric speciation.

Lake Malawi is about 600 km long and up to 80 km wide. Our results show the development of significant genetic divergence between populations 35 m apart separated by an identifiable, but small, habitat discontinuity. As this barrier is so small and apparently trivial, there are probably hundreds of such barriers throughout the lake.

We do not debate the evidence for sympatric speciation or allopatric speciation by land barriers in the evolution of cichlid species. However, it is arguable that neither of these mechanisms predict a greater species richness among rocky shore fishes than other cichlids. Fryer's (1959) intralacustrine allopatry theory can make this prediction. However, three further avenues of research remain to be pursued. First, Fryer suggested that rocky habitats provided equally strong barriers to the movement of sandy shore species as do sandy bays for mbuna. This has yet to be tested. If confirmed, we would expect that the species richness of sandy shore lineages be as great as that of the mbuna. Second, support for the importance of intralacustrine habitat barriers in accelerating speciation would be enhanced by comparable studies in Lake Victoria. These are currently lacking. Recent studies have shown that the mbipi are also species-rich (Seehausen 1996), although again this depends largely on the demonstration of assortative mating among sympatric colour forms (Seehausen & van Alphen 1998), extrapolated to allopatric taxa, which have not yet been tested. Third, it has been suggested that sympatric speciation may be driven by disruptive sexual selection acting on male courtship colour (Turner & Burrows 1995; Higashi *et al.* 1999). This has suggested an alternative explanation for the species richness of rocky shore cichlids. Seehausen *et al.* (1997) pointed out that if the transmitted light spectrum is wide, female cichlids will be readily able to distinguish among hues of male courtship dress, and in Lake Victoria clear-water habitats tend to have more species than turbid habitats. Perhaps a synergy of periods of allopatric divergence followed by strong disruptive sexual selection in sympatry may also account for this diversity.

In summary, Fryer's intralacustrine allopatry theory remains an attractive candidate explanation to explain a considerable proportion of the species richness of the cichlid fauna of the African Great Lakes, and particularly to explain much of the variation in species richness among lineages within a lake.

Our results demonstrate that population structuring in mbuna can be on a much finer geographical scale than proposed previously. Although our study populations do not represent distinct biological species, the demonstration of genetic differentiation on such a small scale indicates the potential role that geographical isolation within the lake may play in allopatric speciation. However, at present this theory still does not provide a satisfactory answer to the key question: what is it about haplochromine cichlid fishes that leads them to speciate so much more rapidly than the many other species that are also split into many genetically isolated populations for long periods?

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