THE ROYAL SOCIETY

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

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Extremely fine-scale genetic partitioning has recently been detected among populations of Lake Malawi's rock-dwelling cichlids through the study of microsatellite loci. Understanding the mechanisms of genetic differentiation that operate in this rapidly speciating group requires further investigation of the geographic patterns of gene flow and the congruence between morphological and genetic divergence. In pursuit of this goal, genetic variation at four microsatellite loci and variation in male breeding coloration were examined in several populations of *Labeotropheus fuelleborni* from southern Lake Malawi. Significant genetic differentiation exists among populations (overall $F_{ST}=0.063$; $p=0.0002$). While migration appears unrestricted within continuous rocky patches, deep waters and sandy bays more than 2 km wide act as strong barriers to gene flow. Dispersal of *L. fuelleborni* appears to follow a stepping-stone model in which the distribution of habitats often constrains migration to one dimension. It is hypothesized that clinal colour variation in the study area has resulted from the secondary contact of divergent lineages, although reproductive isolation between colour variants is not apparent. Relative to shoreline populations, reduced levels of gene flow among populations inhabiting isolated, deep-water islands provides greater opportunities for drift, adaptation to local conditions, or sexual selection to effect genetic differentiation in this species.

Keywords: genetic differentiation; microsatellites; habitat heterogeneity; isolation by distance; haplochromine cichlid

1. INTRODUCTION

Lake Malawi is thought to have formed some 2 Ma ago (Banister & Clarke 1980) and presently contains an endemic flock of several hundred haplochromine cichlid species (Ribbink *et al*. 1983*a*; Eccles & Trewavas 1989). A large part of this species flock's taxonomic diversity is represented by the colourful, rock-dwelling species known as 'mbuna'. Morphological, ecological, and genetic lines of evidence suggest that the mbuna form a monophyletic group (Trewavas 1935; Fryer 1959*a*; Oliver 1984; Meyer 1993; Moran *et al*. 1994). Virtually all of the more than 200 described and putative species of mbuna are restricted to rocky habitats less than 40 m deep (Ribbink *et al*. 1983*a*). Relatively low levels of isozyme variation (Kornfield 1978; McKaye *et al*. 1982; McKaye *et al*. 1984) and mitochondrial DNA sequence divergence (Kocher *et al*. 1993; Moran *et al*. 1994; Bowers *et al*. 1994; Moran & Kornfield 1995) among mbuna taxa indicate the recent origin of this group.

Several hypotheses have been proposed to explain the diversity, narrow endemicity, and recent radiation of mbuna species. Classic models emphasize adaptive divergence of populations on isolated rocky habitat patches, which are created and destroyed by changes in lake level (Trewavas 1947; Fryer 1959*b*). The reorganization of habitat patches by fluctuating water levels in Lake Malawi at different spatial and temporal scales has been confirmed by historical observations and geological evidence (Hill & Ribbink 1978; Crossley *et al*. 1984; McKaye & Gray 1984; Scholz & Rosendahl 1988; Owen *et al*. 1990). Sexual selection may also have an important role in mbuna lineage splitting (Holzberg 1978; McElroy & Kornfield 1990; McKaye 1991; Deutsch 1997). Dominey (1984) suggested that sexual selection could accelerate the divergence of mate recognition systems among isolated mbuna populations and may account for the rapidity of speciation in Lake Malawi. Others have

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explored models of sympatric speciation (McKaye *et al*. 1984; Turner & Burrows 1995).

An understanding of the spatio-temporal scales and patterns of population subdivision among the mbuna is needed to evaluate the robustness of these speciation models. An early genetic study of mbuna demonstrated significant allozyme variation among populations of *Pseudotropheus zebra* (Boulenger) separated by hundreds of kilometres (McKaye *et al*. 1984). Genetic structure among mbuna populations at smaller scales has been demonstrated by examining variation in mitochondrial DNA (Bowers *et al*. 1994; Moran & Kornfield 1995). Recently, highly variable simple sequence repeat (SSR) markers (Tautz 1989) have been used to detect genetic differentiation among adjacent mbuna populations separated by stretches of sandy substrate ranging from a few kilometres to less than one kilometre long (van Oppen *et al*. 1997; Markert 1998).

Labeotropheus fuelleborni Ahl is present at virtually every rocky outcropping in the lake (Ribbink *et al*. 1983*a*). This species rarely occurs deeper than 7 m and is most abundant at 1 or 2 m in depth, where surge is often an important physical characteristic of the environment (Ribbink *et al*. 1983*a*,*b*; Konings 1990). *Labeotropheus fuelleborni* was not among the mbuna species which colonized artificial reefs at 6–9 m depths during five years of observation (McKaye & Gray 1984). Although *L. fuelleborni* can physiologically compensate for depths as great as 25 m (Ribbink *et al*. 1983*b*), it may be outcompeted by other algal-grazing fishes on moderately shallow, submerged reefs if it has no access to the surge zone. Kornfield (1978) briefly reported that isozyme variation among three allopatric populations of this species was comparable to that noted for heterospecific mbuna from a single locality.

The present study is the first detailed investigation of genetic variability in *L. fuelleborni*. We examined male breeding coloration and genetic variation at SSR loci in populations of *L. fuelleborni* from several localities in southern Lake Malawi. These sites currently form an ecological archipelago of rocky habitat patches. Based on several lines of evidence, Owen *et al*. (1990) estimated that a 120 m draw-down of Lake Malawi occurred between the years 1500 and 1850, which would have made all of our field sites inaccessible to fish within this time period. As the lake refilled to its present level, the sites with the deepest rocky zones were the earliest to become available for colonization, while shallower rocky patches became available more recently. Our purpose is to determine the geographic scale and patterns of genetic subdivision among *L. fuelleborni* populations in this region of the lake and to explore the influences that habitat distribution may have on the population structure of this species.

2. MATERIALS AND METHODS

(a) *Sample collection*

A total of 580 *L. fuelleborni* individuals were sampled from 15 rocky localities in the vicinity of the Nankumba Peninsula (figure 1). Descriptions of many of the collection sites are reported by Ribbink *et al*. (1983*a*), and sample sizes at each locality are listed in table 1. Fish were captured by chasing them into monofilament nets with the aid of SCUBA. Most of the collection localities shown in figure 1 are single rocky habitat patches that are isolated from other such patches by sand. Exceptions to this are cases in which populations were sampled at two different points within contiguous rocky patches, with no obvious intervening barriers to migration (i.e. Ilala Gap to Mvunguti NW, Mvunguti SE to Tsano Rock and two sites within Shallow Reef). Only a single *L. fuelleborni* individual was captured from Mazinzi Reef, a well-studied submerged reef (*ca*. 3–13 m deep) that has historically lacked a persistent *L. fuelleborni* population (Ribbink *et al*. 1983*a*). This individual was excluded from the inter-population comparisons described below.

Our collecting license (G. R. No. 684658) limited us to 300 specimens from Lake Malawi National Park (Mphande Island, Nkhudzi Hills and the sites between and including Tsano Rock and Mumbo Island). Within the park, a *ca*. 0.5–1 cm^2 fin clip was removed from one of the unpaired fins of each individual and preserved in 70–100% ethanol (undenatured), and the fish were released at the collection site. Outside the park, fin clips were similarly collected, but the fish were kept as vouchers in 10% formalin. Tissue samples were stored at *ca*. −15 °C in ethanol until being transported to the United States for genetic analysis.

(b) *Surveys of habitat characteristics*

At each site, SCUBA divers characterized the rocky substrate and measured the depth at the rock–sand interface, which serves as an indication of the relative length of time each patch has been available for colonization. The shoreline lengths of, and distances between, rocky patches were determined with the aid of a GPS unit (Trimble, Sunnyvale, CA, USA) and nautical maps. Distance to the nearest rocky source of *L. fuelleborni* migrants, whether or not a collection site in this study, serves as a measure of the degree of isolation of each site. Patch areas were estimated from simple geometric formulae (e.g. a curvilinear, inclined plane for a rocky shoreline patch and a frustum of a right circular cone for a rocky island).

(c) *Assessment of male breeding coloration*

A previously described colour dimorphism (Ribbink *et al*. 1983*a*), present in several mbuna lineages, was observed within some of the populations in the study area. The common black-barred (BB) *L. fuelleborni* morph was present at every site, whereas the calico-like orange-blotch (OB) morph, if observed at a particular site, was uncommon in females and extremely rare in males (see footnote to table 1). Measurement of inter-population colour variation was based on the breeding coloration of BB males. Coloration of the body and fins was assessed in 20–54 territorial BB males *in situ* at each of 12 field sites. Although overall body coloration, the degree of body barring, and pigmentation in many of the fins were not quantifiable, two characters were amenable to scoring: (i) the presence or absence of yellow pigmentation in the gular region of the head (Ribbink *et al*. 1983*a*), and (ii) the relative area (estimated as fifths) of the dorsal fin clearly demarcated as a red-orange patch. Geographic variation in these two characters was interpreted graphically.

(d) *Molecular techniques*

DNA was extracted from fin clips by proteinase-K digestion, phenol–chloroform extraction, and ethanol precipitation as outlined in Kellogg *et al*. (1995). Four SSR loci (UNH001,

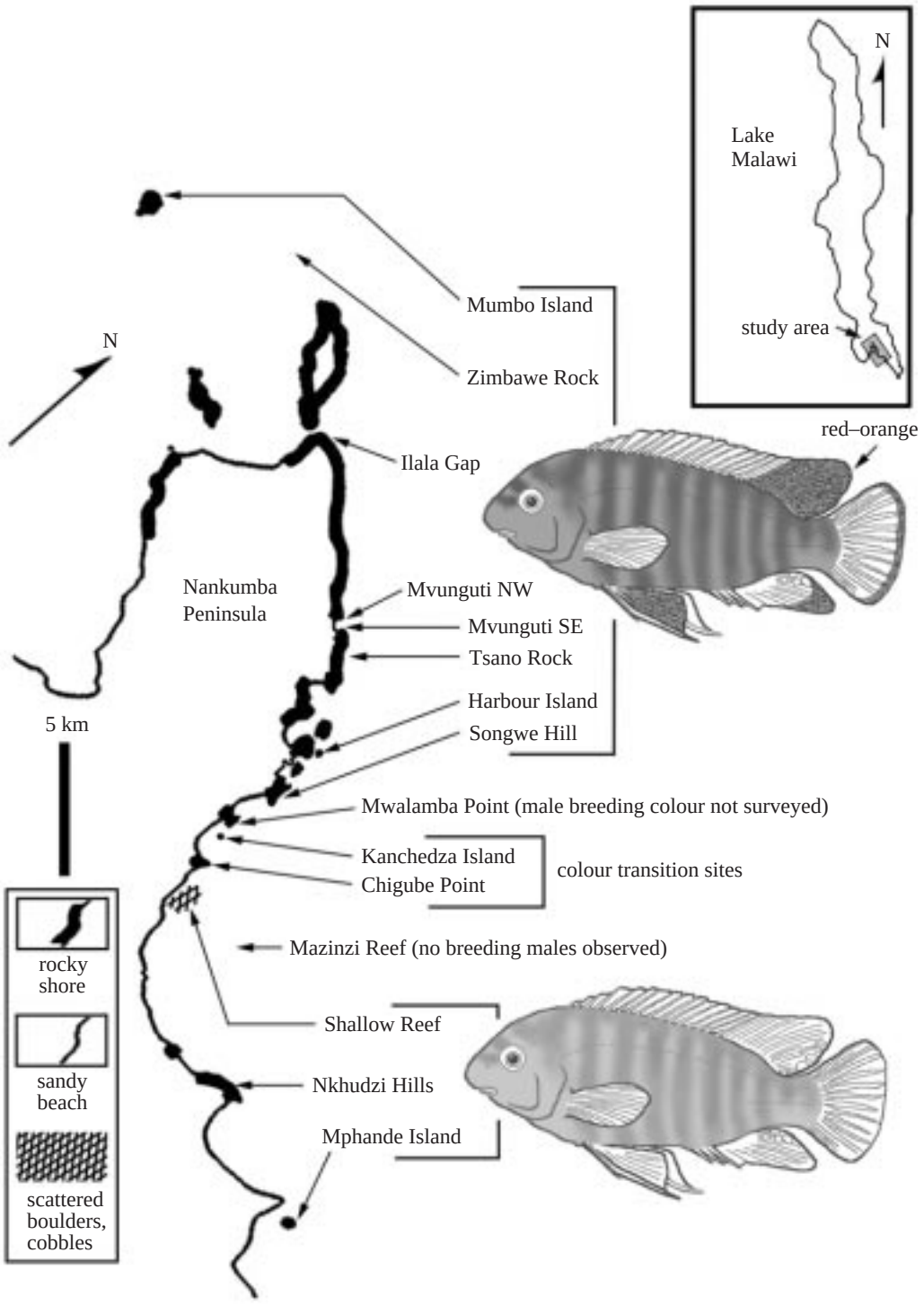

Figure 1. Fifteen localities in southern Lake Malawi from which *Labeotropheus fuelleborni* individuals were sampled. The legend indicates the nature of the substrate along the shore of the Nankumba Peninsula and at nearby islands and reefs. Blue body and grey bar coloration in males is darker in the north-west than in the south-east, and strong red–orange fin pigmentation (stippling) in males from the north-west is more weakly expressed in the south-east.

UNH002, UNH050, and UNH231) were amplified using the polymerase chain reaction (PCR). Primer sequences and annealing temperatures are provided by Markert (1998). All four loci are perfect dinucleotide repeats. The PCR products were resolved by electrophoresis on a 6% denaturing polyacrylamide gel using an ABI 373A DNA sequencer. Gels were run for 8.25 h at 30 W with internal size standards in each lane. Allele sizes were estimated using GeneScan software (Applied Biosystems, Foster City, CA, USA). Fragment size estimates at each locus were sorted by size, binned into size categories differing by *ca.* 2 bp, and ranked. This resulted in clear stepwise increments in bin ranks when they were plotted against allele size estimates. Bin edges (i.e. the lowest and highest estimated fragment size in each bin), alleles from neighbouring bins, and the few bin outliers were re-run on single gels to confirm allele size assignments.

Table 1. *Genetic differentiation between adjacent pairs of* L. fuelleborni *populations in the vicinity of the Nankumba Peninsula*

(For each comparison the table indicates: the respective number of individuals sampled; the distance between the sites in kilometres; the nature of the intervening substrate; estimates of pairwise F_{ST}s and p-values for the null hypothesis F_{ST} not >0; the results of exact tests for identical allelic distributions at each locus; and the estimated number of migrants (N_m) exchanged between populations per generation. Significant p-values at the Bonferroni-corrected alpha probability level ($p<0.0028$) are indicated by asterisks (*). Footnotes refer to the numbers of rare OB morphs included in the comparisons; all other individuals displayed the common BB colour morph.)

comparison	sample sizes	distance (km)	nature of intervening substrate	F_{ST} (p-value)	exact test UNH001	exact test UNH002	exact test UNH050	exact test UNH231	N_m
Mumbo Island–Zimbawe Rock	40, 40	5.4	deep (>50 m) water over sand	0.150 ($p=0.0004$)*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	1.02
Zimbawe Rock–Ilala Gap	40, 35	6.4	deep (>50 m) water over sand	0.141 ($p=0.0004$)*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	1.12
Nkhudzi Hills–Mphande Island	48[a], 39[b]	5.6	wide, shallow sandy bay	0.054 ($p=0.0005$)*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	1.80
Mumbo Island–Ilala Gap	40, 35	10.4	deep (>50 m) water over sand	0.036 ($p=0.0004$)*	$p<0.0001$*	$p=0.0001$*	$p=0.0018$*	$p<0.0001$*	1.78
Shallow Reef–Nkhudzi Hills	62, 48[a]	6.4	wide, shallow sandy bay	0.036 ($p=0.0004$)*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	2.04
Songwe Hill–Mwalamba Point	53[c], 14	1.8	small, shallow sandy bay	0.031 ($p=0.0004$)*	$p=0.0056$	$p=0.0087$	$p=0.0382$	$p=0.2624$	3.25
Kanchedza Island–Chigube Point	37, 28	1.2	small, shallow sandy bay	0.024 ($p=0.0004$)*	$p=0.0205$	$p=0.0717$	$p=0.0244$	$p=0.0065$	5.62
Mvunguti NW–Mvunguti SE	18, 45	0.6	350 m wide, steeply sloping sandy bay	0.023 ($p=0.0004$)*	$p=0.0109$	$p=0.0145$	$p=0.0478$	$p=0.0008$*	3.34
Chigube Point–Shallow Reef	28, 62	1.5	sandy shoreline	0.019 ($p=0.0004$)*	$p=0.0043$	$p<0.0001$*	$p=0.0008$*	$p<0.0001$*	3.38
Harbour Island–Songwe Hill	85[d], 53[c]	2.2	alternating sandy and rocky shoreline	0.018 ($p=0.0004$)*	$p=0.0006$*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	5.66
Mwalamba Point–Kanchedza Island	14, 37	0.6	shallow (<5 m) sandy bay	0.013 ($p=0.0608$)	$p=0.0146$	$p=0.2735$	$p=0.4256$	$p=0.3902$	5.01
Ilala Gap–Tsano Rock	35, 35[e]	8.1	rocky coast; 350 m wide sandy bay	0.010 ($p=0.0008$)*	$p=0.0058$	$p=0.1100$	$p=0.0709$	$p<0.0001$*	3.44
Tsano Rock–Harbour Island	35[e], 85[d]	3.7	mostly rocky coast; >32 m deep channel	0.008 ($p=0.0004$)*	$p<0.0001$*	$p=0.0186$	$p=0.0336$	$p=0.0023$*	5.04
ShallowReef N–Shallow Reef S	49, 13	0.8	scattered rocks in a sand &gravel matrix	0.005 ($p=0.2244$)	$p=0.8146$	$p=0.0151$	$p=0.1012$	$p=0.3783$	2.78
Ilala Gap–Mvunguti NW	35, 18	6.6	continuous rocky shoreline	0.005 ($p=0.1180$)	$p=0.7873$	$p=0.0124$	$p=0.3169$	$p=0.0551$	2.86
Mvunguti SE–Tsano Rock	45, 35	0.9	continuous rocky shoreline	0.001 ($p=0.3450$)	$p=0.0342$	$p=0.7780$	$p=0.3904$	$p=0.9179$	10.4
Mumbo Island–Mphande Island	40, 39[b]	42.4	entire study region	0.079 ($p=0.0005$)*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	$p<0.0001$*	1.29

[a–e]The following numbers of individuals displayed the rare OB colour morph: (a) 1 out of 48 individuals collected from Nkhudzi Hills; (b) 1 out of 39 individuals collected from Mphande Island; (c) 7 out of 53 individuals collected from Songwe Hill; (d) 7 out of 85 individuals collected from Harbour Island; and (e) 2 out of 35 individuals collected from Tsano Rock.

(e) *Data analysis*

Allele frequencies and fixation indices (*F*-statistics) were estimated using FSTAT, version 1.2 (Goudet 1995), which estimates *F*-statistics using the method of Weir & Cockerham (1984) and calculates p-values for these estimates using permutation algorithms. The probability that F_{IS} (within-population heterozygote deficiency) significantly differs from zero was estimated by permuting alleles within populations 5000 times. There was evidence of within-population heterozygote deficiency, so the p-values for the overall and pairwise F_{ST} (between-population heterozygote deficiency) estimates were calculated by permuting genotypes among populations (5000 and 2000 permutations, respectively). In order to reduce the likelihood of making type I (false-positive) errors among the 18 comparisons (17 between populations and one overall), a Bonferroni correction was applied, which set the threshold for acceptance of a significantly positive F_{ST} to $p<0.0028$ (Sokal & Rohlf 1987).

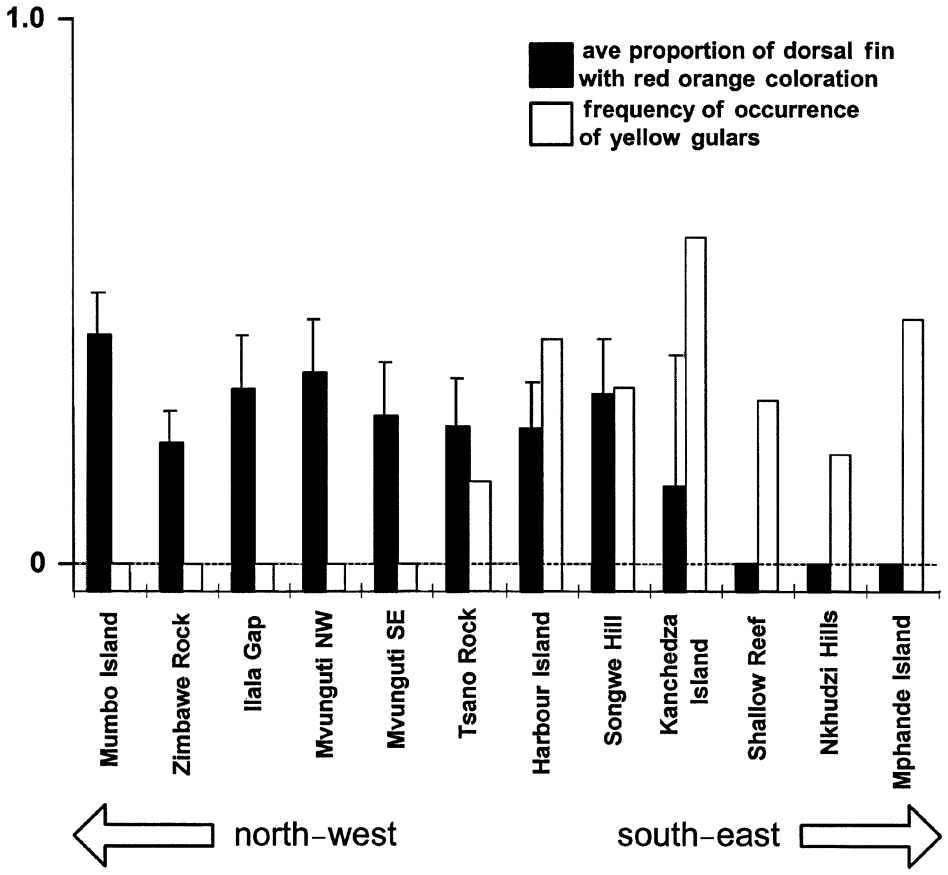

Figure 2. Variation in the coloration of breeding males among sites. Black bars indicate the average proportion of red–orange coloration in the dorsal fin (error bars are standard deviations of the mean), and white bars indicate the frequency of males displaying yellow, rather than white, gular patches.

Linkage disequilibrium was tested within every site for all possible pairs of loci using GENEPOP, version 3.1 (Raymond & Rousset 1995*a*). Genetic differentiation among populations was also evaluated using the exact tests provided by this software, which are more reliable than permutation procedures when sample sizes are small and allele frequencies at highly polymorphic loci are low (Raymond & Rousset 1995*b*; Rousset & Raymond 1997). Overall and between-population probability values for these tests were estimated using a Markov chain method (dememorization number = 5000; 100 batches; and 2000 iterations per batch). Bonferroni corrections were applied. Isolation by distance was investigated by testing for a significant correlation between $F_{ST}/(1-F_{ST})$ and the distance between populations using the Mantel test (5000 permutations), also provided by GENEPOP (Manly 1985; Rousset 1997). Values of $M = (1/4)(1/F_{ST}-1)$, which are equivalent to the number of migrants that would yield the observed levels of population differentiation assuming an island model, were calculated from pairwise F_{ST} estimates (Slatkin 1993), and $\log_{10}M$ was regressed against $\log_{10}$(distance between populations). Although pairwise values of M and distance are not independent, qualitative patterns of dispersal can be inferred from geographic patterns of population subdivision by examining this relationship (Slatkin 1993; Hellberg 1995).

The number of migrants (N_m) exchanged between sites per generation was also estimated using the private alleles method of Barton & Slatkin (1986). In addition, the effective number of alleles (n_e), or the reciprocal of expected homozygosity, of each population was calculated from observed allele frequencies (Hartl & Clark 1989). Relationships between n_e and habitat characteristics were examined by linear regression (Sokal & Rohlf 1987).

3. RESULTS

(a) *Geographic variation in male breeding coloration*

Clinal variation in the breeding coloration of BB males is evident among the *L. fuelleborni* populations surveyed. Breeding males at the south-eastern sites (Mphande Island, Nkhudzi Hills, and Shallow Reef) display a light powder-blue body with faint bars along the flank, whereas males with a deeper blue body and grey to black bars occur to the north-west (figure 1). Between Songwe Hill and Mumbo Island, territorial males always show a distinct red–orange patch that is largely restricted to the posterior, rayed portion (around one-third) of the dorsal fin (figure 2). Although close examination of captured males from the three southernmost sites often revealed a faint yellow–pink cast to the powder-blue dorsal fin or faintly dusky interradial membranes in the rayed portion of the dorsal fin, a clearly demarcated red–orange patch was never observed in underwater observations of these populations. A similar pattern exists for gular coloration. Yellow gular pigmentation was sometimes observed in males from the south-eastern half of the study region, whereas the gular patch always appeared white in the north-western populations (figure 2).

A transition in dorsal fin coloration is observed at Kanchedza Island, with some males having a faint orange patch in a blue dorsal fin and others having completely blue dorsal fins. Bimodality in the frequency distribution of the coarse categories of dorsal fin coloration was not observed for the Kanchedza Island population. Four centrally located populations (at Tsano Rock, Harbour Island, Songwe Hill, and Kanchedza Island) contain

males with red–orange dorsal fin coloration and males with yellow gular patches (figure 2). In these populations, males with white gular patches, a character associated with the north-western populations, may show a tendency to express more red–orange dorsal fin pigmentation, also a north-western trait ($p=0.029$; one-sided Mann–Whitney *U*-test corrected for ties (Sokal & Rohlf 1987)). This test remained marginally significant ($p<0.05$) when no adjustment for ties was made, suggesting a possible correlation between these traits.

(b) *Evidence of population structure*

A high degree of genetic polymorphism is evident at the four SSR loci: 36 alleles at UNH001; 29 alleles at UNH002; 30 alleles at UNH050; and 38 alleles at UNH231. Observed heterozygosity, averaged across all loci and sites, is 0.826. Genetic differentiation among *L. fuelleborni* populations is indicated by a significant ($p=0.0002$) overall F_{ST} ($=0.063$) and a significant outcome for Fisher's combined exact test of genic differentiation for all four loci ($p<0.00001$). Tests of linkage disequilibrium confirm statistical independence among loci, a necessary assumption of these multilocus comparisons. Fine-scale genetic structure is also observed in comparisons of adjacent pairs of allopatric populations. Populations separated by deep (>50 m) water or by distances greater than 2 km across sandy substrates demonstrate significant genetic differentiation from one another based on multilocus F_{ST} values and on exact tests for each of the four loci (table 1). By contrast, gene flow appears to be unrestricted along continuous rocky shorelines as long as 6.6 km and across the 800 m stretch of scattered boulders and cobbles (*ca.* 3–4 m deep) that constitutes the intermediate habitat of Shallow Reef.

A concern in studies of population structure based on variation at highly polymorphic SSR loci is that large population samples are needed for the accurate estimation of allele frequencies. Relative to other measures of population structure, F_{ST} performs well with differing sample sizes and large numbers of alleles (Ruzzante 1997). Pairwise comparisons of *L. fuelleborni* populations across deep waters or sandy bays more than 2 km wide involved samples of 35–85 individuals (mean=50). F_{ST} estimates based on sample sizes in this range and four SSR loci appear to be acceptable for the detection of genetic partitioning between populations (Ruzzante 1997).

There is some evidence of population differentiation across smaller (<2 km) dispersal barriers. The steeply sloping sandy bay (350 m wide) at Mvunguti Village in Lake Malawi National Park, for example, is the only observed barrier to *L. fuelleborni* dispersal along 8.1 km of rocky coastline between Ilala Gap and Tsano Rock (figure 1). The significant multilocus F_{ST} ($p=0.0004$) and the significant difference in allelic distributions at locus UNH231 ($p=0.0008$) estimated between populations on either side of this barrier (Mvunguti NW and Mvunguti SE) appear to account for the genetic differences detected between the Ilala Gap and Tsano Rock populations (table 1). For the most part, conclusions of genetic differentiation at these smaller scales are tenuous due to modest sample sizes or a lack of congruence between results based on F_{ST} and exact tests.

The presence of null alleles (i.e. alleles that do not amplify due to mutations in the PCR primer site) is also a potential problem for tests of population differentiation based on distributions of alleles at SSR loci (Pemberton *et al.* 1995). The proportion of individuals in which no alleles could be scored ranged from 0.86% at UNH050 to 1.7% at UNH002. Unscorable alleles may result from the presence of true null alleles or methodological problems such as failed DNA extraction. Instances of unscorable alleles in this study were often associated with inadequate tissue samples that had been taken from small individuals. Nevertheless, significant within-population heterozygote deficiencies were found at UNH001, UNH002, and UNH231, suggesting the presence of true null alleles at these loci. Van Oppen *et al.* (1997) report a true-breeding null allele at UNH002 for other mbuna species. When UNH050 is considered alone, our general findings of genetic differentiation are confirmed by a significant overall F_{ST} ($p=0.0002$) and by significant exact tests ($p<0.002$ in all cases) for all pairs of adjacent populations separated by deep-water troughs or sandy bays greater than 2 km wide.

Examining histograms of allele frequencies provides further evidence of genetic partitioning among *L. fuelleborni* populations. The pooled set of allele frequencies from the six northern sites (Mumbo Island to Tsano Rock) reveals 'private' alleles that are unique to this region (figure 3). Populations to the south-east of Tsano Rock also contain alleles that are absent or rare in other regions. The most common UNH002 allele in Madzidzi Bay (Songwe Hill to Nkhudzi Hills) was extremely rare elsewhere; 94.3% of all individuals possessing this allele were sampled from populations within Madzidzi Bay. The *L. fuelleborni* population from Mphande Island, the south-eastern terminal site, contains a private allele at UNH001 (figure 3), and the second most frequent alleles at UNH001 (figure 3) and UNH231 (data not shown) in the Mphande Island population are very rare elsewhere. The six combined northern populations exhibit more unique alleles at UNH001 and UNH002 than the six populations sampled from Madzidzi Bay to the south-east, even though sample sizes within these two regions are similar. These patterns holds true for the two loci (UNH050 and UNH231) not illustrated in figure 3.

(c) *The distribution of habitats and population differentiation*

The genetic differentiation of *L. fuelleborni* populations fits a model of isolation by distance. The parameter $F_{ST}/(1-F_{ST})$ is significantly correlated with the distance ($p=0.0004$; one-sided Mantel test), or $\log_e$ distance ($p=0.0004$), between sites. These relationships are also significant ($p=0.011$ and $p=0.010$, respectively) when populations containing the north-western colour variant are considered alone. Figure 4 illustrates a plot of $\log_{10}M$ versus $\log_{10}$(separation distance). The variable M is defined as $(1/4)(1/F_{ST}-1)$ (Slatkin 1993). The slope of the log–log regression of M and distance for all pairs of populations is -0.66 ($p<0.001$; $r^2=37.1\%$). In addition, the regression line was estimated only for the north-western populations, based on flank and dorsal fin coloration (figure 4). In this case, the slope is -0.79

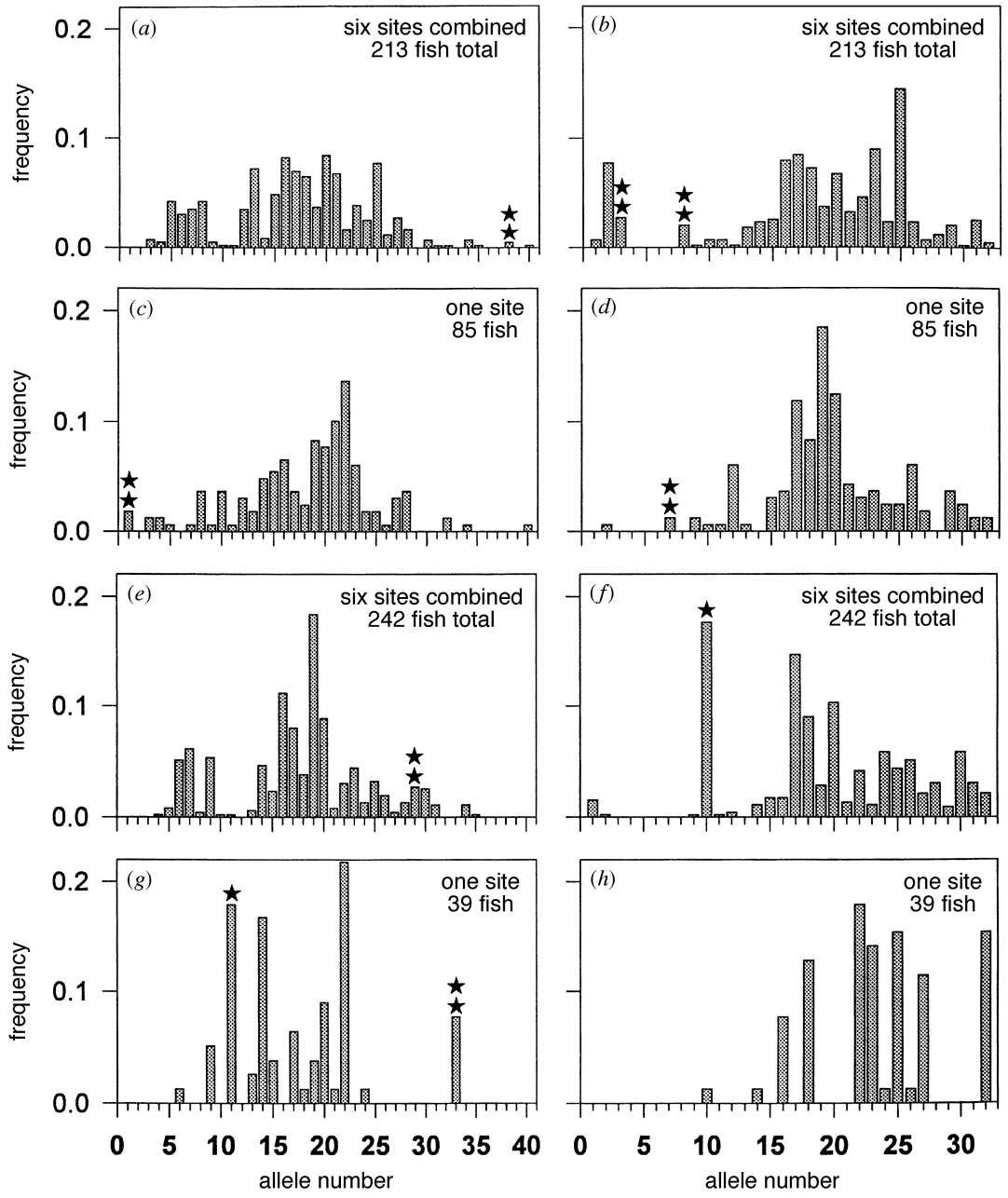

Figure 3. Histograms of allele frequencies at two SSR loci ((*a*, *c*, *e*, *g*)UNH001 and (*b*, *d*, *f*, *h*)UNH002) from four different regions or sites within the study area. Allele frequencies were pooled among the six northern sites (Mumbo Island to Tsano Rock) and among sites in Madzidzi Bay (bounded by Songwe Hill in the north and Nkhudzi Hills in the south) as a means of summarizing allele frequency data. Alleles that are unique to one of these regions or sites are indicated by two stars, and common alleles that are extremely rare elsewhere are indicated by a single star.(*a*, *b*) northern sites; (*c*, *d*) Harbour Island; (*e*, *f*) Madzidzi Bay; (*g*, *h*) Mphande Island.

($p=0.001$; $r^2=35.4\%$). The regression of $\log_{10}M$ versus $\log_{10}$(separation distance) is expected to yield a slope of -1.0 in a one-dimensional stepping-stone model and a slope of -0.5 in a two-dimensional array of stepping-stones (Slatkin & Maddison 1990; Slatkin 1993). Deviation from the two-dimensional expectation may reflect constrained migration in one dimension between shoreline patches throughout much of the study region.

The distribution of habitat patches may also have an influence on the effective number of alleles (n_e) within populations. Increasing distance to the nearest source of migrants is associated with a decrease in n_e ($p=0.037$; $r^2=31.5\%$; figure 5). In addition, n_e increases linearly with increasing $\log_e$-transformed patch area ($p=0.010$; $r^2=33.4\%$; data not shown). These relationships cannot be interpreted independently however, as larger rocky patches tend to be located closer to neighbouring patches. The depth at which the rocky habitats intersect the sandy lake floor serves as a relative measure of the length of time they have been available for colonization. The regression of n_e on depth at the rock–sand interface for all sites was not significant (figure 6). When Zimbawe Rock and Mumbo Island (the two most isolated sites based on the deep-water troughs surrounding them) are excluded

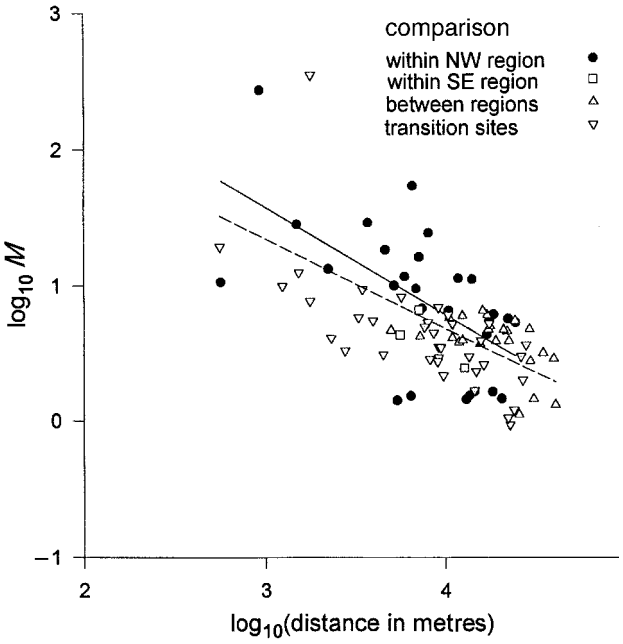

Figure 4. Plot of $\log_{10}M$ versus $\log_{10}$(distance of separation between populations). The variable M is defined as $(1/4)(1/F_{ST}-1)$. Points indicated in the legend are pairwise comparisons in which: both sites were part of the north-western region (Songwe Hill to Mumbo Island; filled circles); both sites fell in the south-eastern region (Shallow Reef to Mphande Island; open squares); one site fell within each region (open triangles pointing up); or at least one of the two sites was from the colour transition zone (Mwalamba Point included; open triangles pointing down). The slope of the regression line for all comparisons (dashed line) is -0.66 ($r^2=37.1\%$), and the slope of the regression line for comparisons from the north-western region only (solid line) is -0.79 ($r^2=35.4\%$).

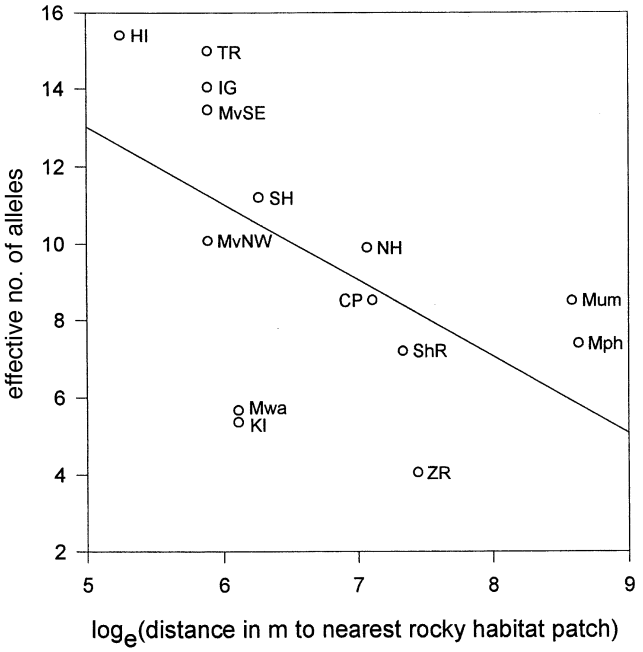

Figure 5. Relationship between effective number of alleles (n_e) and distance to the nearest source of *L. fuelleborni* migrants. The estimated regression line is indicated ($p=0.037$; $r^2=31.5\%$). Site abbreviations for the data points are: CP (Chigube Point), HI (Harbour Island), IG (Ilala Gap), KI (Kanchedza Island), Mph (Mphande Island), Mum (Mumbo Island), MvSE (Mvunguti SE), MvNW (Mvunguti NW), Mwa (Mwalamba Point), NH (Nkhudzi Hills), SH (Songwe Hill), ShR (Shallow Reef), TR (Tsano Rock), and ZR (Zimbawe Rock).

the regression between n_e and depth becomes significant ($p<0.001$; $r^2=74.1\%$). Populations at these two sites appear to have lower effective numbers of alleles than expected based on the length of time they have been available to *L. fuelleborni* migrants. The relationship between n_e and depth is confounded by an association of depth with the geographic position of sites. Nevertheless, figure 6 illustrates that populations at Mumbo Island and Zimbawe rock show reduced genetic variability relative to the other north-western populations at deep-water, mainland sites.

4. DISCUSSION

Significant genetic partitioning exists among allopatric populations of *L. fuelleborni* in the vicinity of the Nankumba Peninsula. Waters deeper than 50 m and sandy bays greater than 2 km wide present strong barriers to gene flow. In some cases, populations separated by slighter geographic barriers could be distinguished from one another genetically, but evidence of population structure at these smaller scales was weaker. Fine-scale population structure has similarly been determined for several other mbuna species in Lake Malawi using SSR markers (van Oppen *et al.* 1997; Markert 1998). *Labeotropheus fuelleborni* has a more cosmopolitan distribution than the other mbuna species examined using microsatellite

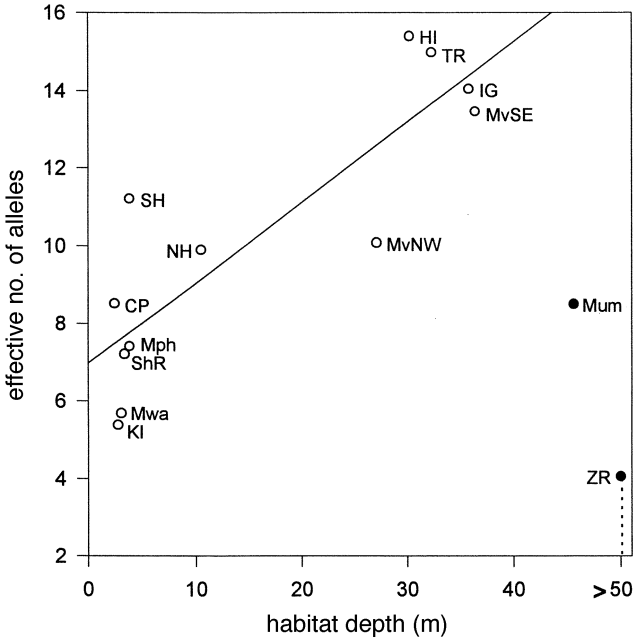

Figure 6. Plot of effective number of alleles (n_e) versus depth at which the rocky habitat patches intersect the sandy lake floor, a measure of the length of time sites have been available for colonization. The estimated regression line, excluding the Mumbo Island and Zimbawe Rock populations (filled circles), is shown ($p<0.001$; $r^2=74.1\%$). Site abbreviations are the same as those given in figure 5.

loci. Although the shallow habitat preference of *L. fuelleborni* may effectively prevent migration along deeper corridors, the ability of this species to use transient 'stepping-stone' patches may enhance its migration along the shore relative to many other mbuna species. For example, *L. fuelleborni* was present on a small ($<700\,m^2$) wave-washed patch of cobbles (<1.2 m deep), which is located on the shore of Madzidzi Bay and contains submerged tree stumps. Other rock-dwelling mbuna taxa were absent from this small rocky patch (personal observation). It is perhaps plausible that forced migration from extremely shallow, intervening rocky habitats due to annual drops in lake level might actually enhance overall gene flow between distant populations. The determination of fine-scale genetic partitioning for *L. fuelleborni*—a widespread species that can use these types of ephemeral habitats—supports the generality of this pattern of population structure among Lake Malawi's rock-dwelling mbuna. Maternal mouthbrooding (Barlow 1991; van Oppen *et al*. 1997), physiological constraints on vertical migration (Marsh & Ribbink 1981; Ribbink 1986), and philopatry (Hert 1992) probably contribute to the striking genetic differentiation that has been observed among mbuna populations.

Genetic partitioning among allopatric *L. fuelleborni* populations in the south-eastern arm of Lake Malawi occurs concomitantly with clinal variation in male breeding coloration, but the degree of colour variation is not fully concordant with the genetic differentiation between populations. There are several possible explanations for this lack of congruence. Drift may operate independently on the different genes responsible for features of male coloration. Similar colour phenotypes, however, seem to be shared among genetically dissimilar *L. fuelleborni* populations at the most isolated sites (e.g. Zimbawe Rock and Mumbo Island). Stabilizing selection on colour genes due to similar light environments, for example, could lead to the same colour phenotypes in genetically differentiated populations, even if drift operated on unlinked regions of the genome. Alternatively, variation in natural and/or sexual selection pressures on colour genes (e.g. Endler 1980; Reznick *et al*. 1990) or varying environmental effects on the expression of colour (e.g. Kodric-Brown 1989) are potential causes of clinal colour variation. The above influences, however, may not fully account for the divergent coloration exhibited by the south-eastern populations, given (i) the short time span during which the shallowest, southern sites have been available to *L. fuelleborni*; (ii) the high levels of migration (more than one migrant per generation) estimated between most pairs of adjacent populations; and (iii) the apparent ecological similarity of the (shallow and periodically turbid) sites across the colour transition zone. An alternative explanation is that the northern and southern *L. fuelleborni* colour forms originated from different ancestral populations that have diverged over a longer period of time. The presence of common alleles in the southern populations that are either absent or extremely rare in the northern populations would be unexpected if recolonization of rocky patches along the Nankumba Peninsula occurred strictly from the north-west to the south-east. The soundness of this line of reasoning, however, relies on assumptions concerning the weak influences of drift and mutation over the brief histories of the southern populations.

Nevertheless, alternative migration routes may have contributed to the 'private' alleles and divergent colour phenotypes currently present in the south-eastern populations. Figure 7 illustrates one such alternative. When the level of Lake Malawi was 50 m lower than it is today, historical eastern-shore populations of *L. fuelleborni* were separated from Madzidzi Bay by a much shorter shoreline distance (figure 7). Two deep-water reefs were located with the aid of local fishermen, which may have served as 'stepping stones' for the migration of *L. fuelleborni* into Madzidzi Bay. One of these, Jerusalem Reef, was explored using SCUBA. The depth of the rocky habitat at this site ranges from 30 m to greater than 47 m. Only two mbuna forms were observed during a single survey of Jerusalem Reef, tentatively identified as a royal blue *Cynotilapia* sp. with black bars and a gold-brown *Metriaclima* (*Pseudotropheus*) sp. with dark brown bars. Given its current widespread distribution, *L. fuelleborni* is likely to have occurred on Jerusalem Reef when the lake was some 30–50 m lower than its present level and this site was a rocky island with a surge zone. Migration of *L. fuelleborni* from the historical Jerusalem Reef population to Mphande Island or Madzidzi Bay may have been facilitated along an ascending ridge, as depicted in a bathymetric map by Owen *et al*. (1990), which probably contains numerous erosion-resistant rocky patches.

Regardless of their origin, preliminary observations of colour variation support the hypothesis that the powder-blue form is not reproductively isolated from the darker, north-western form. A marginally significant correlation between gular and dorsal fin colour traits exists among four centrally located populations. While this finding could be the result of assortative mating or pleiotropic genetic effects, the presence of intermediate and variable dorsal fin coloration at Kanchedza Island and Chigube Point indicates that the specific mate recognition systems (Paterson 1985) of the north-western and south-eastern variants have not diverged enough for reproductive isolation to occur. Although there is weak evidence of genetic partitioning among populations in this colour transition zone, relatively high migration rates between adjacent patches ($N_m = 3$–6 migrants per generation) in this area would be expected to lead to rapid introgression of alleles responsible for male coloration.

The geographic pattern of *L. fuelleborni* population structure fits a stepping-stone dispersal model, in which migration may occur in two dimensions but often only takes place between adjacent shoreline patches (Slatkin & Maddison 1990; Slatkin 1993; Hellberg 1995). The linear relationship between $\log_{10}M$ and $\log_{10}$distance, although not by itself sufficient evidence, is also consistent with the hypothesis that genetic drift and gene flow have begun to approach equilibrium in the north-western populations since the last major fluctuation in lake level some 200–500 years ago (Owen *et al*. 1990). A strong pattern of isolation by distance would not be expected under non-equilibrium conditions (Slatkin 1993). By modelling genetic differentiation among isolated mbuna populations, van Oppen *et al*. (1997) concluded that equilibrium would not be reached during the same period, assuming

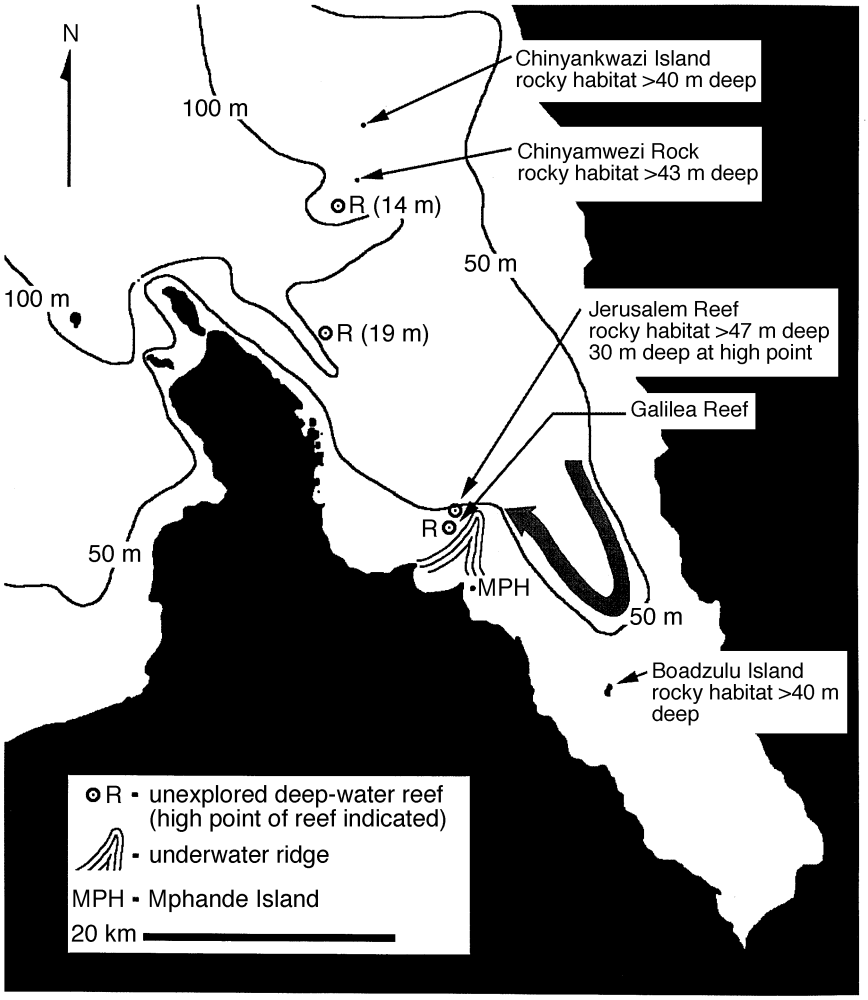

Figure 7. South-east arm of Lake Malawi. Approximate 50 m and 100 m bathymetric isoclines are shown, along with several deep-water rocky patches that became available to mbuna before Mphande Island and the present sites in Madzidzi Bay. The wide arrow indicates a possible historical shoreline migration route. V-shaped segments indicate a steep series of bathymetric isoclines that delimit an ascending, underwater ridge formation, as depicted by Owen *et al.* (1990).

that estimated census numbers of territorial males approximate effective population sizes and that the generation time is three years for Lake Malawi haplochromines. A more rapid establishment of genetic equilibrium, however, could result from (i) higher migration rates (Crow & Aoki 1984), (ii) lower effective population sizes due to high variance in male reproductive success or overlapping generations (Hartl & Clark 1989), and/or (iii) shorter maturation times. Direct estimates of these parameters from demographic and behavioural studies in the wild would allow the time required for equilibration of drift and gene flow in *L. fuelleborni* populations to be more accurately modelled. In addition, simulations are needed to better understand how relationships between gene flow and distance can be detected with microsatellite loci (Hellberg 1995).

In general, the levels of gene flow among *L. fuelleborni* populations inhabiting inshore islands and shoreline rocky outcrops appear too high for genetic differentiation via drift to occur. This may partly explain why *Labeotropheus* Ahl, with only two presently described species (Fryer 1956), is among the most depauperate of mbuna genera. Members of the monotypic mbuna genera *Cyathochromis* Trewavas and *Genyochromis* Trewavas also have unusually widespread distributions compared to other mbuna (Ribbink *et al.* 1983*a*; Konings 1990). It is similarly hypothesized that relatively high rates of gene flow have prevented prolific speciation in these genera as well. Conversely, rates of *L. fuelleborni* migration between Mumbo Island or Zimbawe Rock and neighbouring rocky habitats (N_m=1.02–1.78) approach levels low enough for the loss or fixation of alleles (Wright 1931; Allendorf 1983). Genetic diversity has been reduced in these populations relative to adjacent coastal populations in the north-western part of the study area, even though no obvious divergence in male breeding coloration was detected from field observations. Given the greater potential for selection or drift to effect genetic and morphological differentiation in these isolated, island populations, phenotypic variation at finer scales or in different traits would not be unexpected.

Extremely fine-scale population structure appears to be a general phenomenon common to all rock-dwelling mbuna species in Lake Malawi. Allopatric *L. fuelleborni* populations exhibit significant genetic differentiation when they are separated by deep water or at least 2 km of sandy substrate. Conversely, populations inhabiting continuous rocky habitats show no evidence of interdemic genetic partitioning. The geographic pattern of population structure along an archipelago of habitat patches that was formed within the last 200–500 years supports a model of isolation by distance in which migrants are exchanged between neighbouring patches, often in one dimension. Dramatic fluctuations in lake level have reorganized the spatial distribution of available habitats, which may have allowed distant lineages to come into

closer proximity than predicted by the lake's present topology. While relatively high rates of *L. fuelleborni* migration among shoreline patches act as a strong force against genetic divergence, offshore island populations that have become isolated by deep waters currently experience greatly reduced rates of gene flow. It is in these populations that drift, adaptation to local conditions, or sexual selection may have the greatest effects on gene frequencies. Determining how these potential forces contribute to genetic differentiation and lineage splitting requires further attention.

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