

# Were Population Bottlenecks Associated with the Radiation of the Mbuna Species Flock (Teleostei: Cichlidae) of Lake Malawi?

Paul Moran and Irv Kornfield

Department of Zoology and Center for Marine Studies, University of Maine

Mitochondrial DNA haplotype diversity and frequency distribution were examined within and among four narrowly endemic species and one cosmopolitan species of the rock-dwelling cichlid fish species flock (mbuna) of Lake Malawi in East Africa. The endemics, restricted to very small islands, appear to have originated less than 20,000 yr ago. Relative and absolute levels of genetic diversity were used to examine the possibility that these endemics arose through founder events, as has been suggested for the Hawaiian drosophiloids, to which the East African cichlids have been compared. Three principal results emerged from this study. First, the undescribed species *Pseudotropheus zebra* 'black dorsal' was found to be depauperate of mtDNA haplotype diversity relative to sister taxa, suggesting a severe population bottleneck during, or subsequent to, its recent origin. Second, significant differences in haplotype frequency existed among all five closely related species examined here. Geologic evidence and distributional limits indicate that this divergence in haplotype frequency occurred rapidly, consistent with population bottlenecks. Paradoxically, in three of four species examined there was no apparent reduction in genetic diversity, and two had haplotype diversity values that were high relative to other freshwater fishes. Third, it was found that replicate collections of single species at different sites within the same general locations, without obvious barriers to gene flow, also exhibited significant differences in haplotype frequency. Such apparent fine-scale genetic structuring, whether spatial or temporal, has substantial implications for estimates of effective population size and modeling of speciation processes.

## Introduction

The haplochromine cichlid fish species flocks of the East African Great Lakes are the vertebrate counterpart of the incredibly species-rich Hawaiian drosophiloids. With between 200 and 500 species in each of the three Great Lakes (Malawi, Tanganyika, and Victoria), these groups are among the most species-rich vertebrate faunas known (Fryer and Iles 1972; Greenwood 1981; Ribbink et al. 1983; Eccles and Trewavas 1989). It has been suggested that the African cichlids share with the Hawaiian drosophiloids some of the same biological characteristics that have contributed to rapid speciation. Dominey (1984) hypothesized that, in haplochromine cichlids, as in the drosophiloids, sexual selection, associated with complex "specific mate-recognition systems" (Paterson 1976; Ringo 1977), resulted in the dramatic radiation of these two groups. He also

suggested that limited dispersal in many of the East African cichlids, combined with environmental factors, provided opportunities for founder populations to develop in isolation and further contributed to species proliferation (see Carson and Templeton 1984; Barton and Charlesworth 1984 for reviews of the theory and controversy surrounding speciation via founding events). It is this latter issue that our study seeks to examine. We focus on the mbuna of Lake Malawi, a monophyletic assemblage of endemic rock-dwelling cichlids (Trewavas 1935; Ribbink et al. 1983) (rock-dwelling cichlids of Lake Malawi are *mbuna* [Cichawa] in the territorial waters of Malawi and Mozambique and *vidongo* [Swahili] in Tanzanian waters). Recently, the importance of genetic drift to speciation in these faunas has again been suggested (Keeton et al. 1993, p. 457), although an empirical evaluation is conspicuously absent. To derive full benefit from this system as a model for the study of isolating mechanisms and adaptive radiation, it is important to answer the fundamental question of how these species arose. We have collected data to evaluate whether there is genetic evidence that selected endemic species experienced recent population bottlenecks.

Previous descriptive studies using restriction fragment-length polymorphism (RFLP) and sequence

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Address for correspondence and reprints: Paul Moran, Conservation Biology, Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, Washington 98112-2097. E-mail: paul.moran@sci.nwfsc.noaa.gov.

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analyses of mitochondrial DNA (mtDNA) revealed that, among mbuna species, mtDNA haplotypes are not sufficiently divergent to provide extensive phylogenetic information. Apparently, this group has radiated so rapidly that insufficient time has transpired for the accumulation of mutation along independent species lineages (Kornfield 1978; Meyer et al. 1990; Kocher et al. 1993; Moran et al. 1994). Furthermore, many mbuna species are polymorphic for divergent mtDNA lineages ( $\alpha$  and  $\beta$ ; 1.5% sequence divergence) that predate their isolation from sister taxa (Moran and Kornfield 1993). This retained ancestral polymorphism indicates that mbuna species are so recently derived that there has been inadequate time for complete lineage sorting to have occurred. These previous results are consistent with the geographic and climatic history of the region: some of the mbuna species that are polymorphic for  $\alpha$  and  $\beta$  mtDNA lineages inhabit islands that did not exist 20,000 yr ago, before the last rise in lake level (Scholz and Rosendahl 1988). In some cases, it appears that new habitats, now containing narrowly endemic species, may have become available only as recently as 200 yr ago (Owen et al. 1990). Moreover, the demographic parameters that characterize these species appear to be consistent with conditions that contribute to retention of ancestral polymorphism (Avice et al. 1984). Because of incomplete lineage sorting and a striking lack of cladistically informative morphological characters, no convincing phylogeny has yet been advanced for the mbuna.

This study examines genetic diversity in four narrowly endemic species from the Maleri Islands in the southern part of Lake Malawi: *Pseudotropheus xanstromachus*, *P. zebra* 'black dorsal,' *P. zebra* 'blue,' and *P. zebra* 'red dorsal.' "Narrowly endemic," or simply "endemic," means that a species is restricted to a single island or group of island or to a single mainland rock outcrop (virtually all the Malawi haplochromine cichlids are endemic to the lake [Eccles and Trewavas 1989]). These endemics are distinguished by unique male breeding coloration, characters presumably conferring reproductive isolation (Ribbink et al. 1983). It is unclear, however, whether sympatric sibling species at these restricted locations diverged in situ or resulted from multiple colonizations.

In contrast to the difficulties encountered using mtDNA for phylogenetic analysis in the mbuna, there is substantial population genetic information to be gained from the examination of mtDNA haplotype diversity and haplotype frequency distribution. The patterns of haplotype distribution and levels of diversity may provide critical insight into the demographics associated with mbuna speciation and radiation. This study

extends the results of earlier allozyme studies (Kornfield 1978; McKaye et al. 1984), by providing additional sensitivity due to the fourfold-lower effective population size of mtDNA haplotypes relative to nuclear haplotypes (Nei and Tajima 1981; Birky et al. 1983). This characteristic of mtDNA makes it sensitive not only to historic population sizes, particularly to population bottlenecks, but also to levels of gene flow among local endemics.

In Lake Malawi, these endemics appear to be reproductively isolated by distance and inappropriate habitat when allopatric, or by mate choice when sympatric. Here we focus on mtDNA haplotype diversity and frequency distribution within and among the four endemic species and also compare these endemics to three allopatric populations of one widespread species, *P. zebra* 'BB,' in an effort to elucidate the demographics and population processes that may have been associated with speciation. The ultimate question we wish to address is, Did the Maleri Island endemics arise through founder events? If so, we would expect to find reduced genetic diversity relative to cosmopolitan species that did not experience bottlenecks as well as differences in haplotype frequency among putative sibling species as a result of drift.

## Material and Methods

To assess levels of haplotype diversity and frequency distribution, RFLPs in mtDNA were examined in five species of mbuna (table 1). Purified mtDNA was prepared from 97 individuals by density gradient ultracentrifugation (Lansman et al. 1981; Dowling et al. 1990) and assayed using four restriction enzymes (*Apa*I, *Ava*I, *Ava*II, and *Mbo*I). Earlier work indicated that these four enzymes revealed nearly all the unique haplotypes that were detected using a suite of 18 enzymes (Moran et al. 1994). Restriction fragments were radioactively labeled (Drouin 1980), separated by gel electrophoresis, and visualized by autoradiography (Lansman et al. 1981; Dowling et al. 1990). An individual's mitochondrial DNA haplotype was defined by the composite of restriction fragment profiles (patterns of fragment presence/absence) for the four enzyme digests. These presence/absence data were also used to estimate sequence divergence between haplotypes and nucleotide diversity within populations (the average number of substitutions per base,  $d_{ij}$  and  $d_x$ , respectively; Nei 1987). The results presented here are robust to the limitations ordinarily imposed by fragment analysis because this study focuses primarily on haplotype diversity and frequency distribution, treating nucleotide diversity estimates in only a peripheral way. Further, no attempt is made to estimate phylogenetic relationships among haplotypes. Haplotype frequency differences among species and among collec-

ions within species were examined using a heterogeneity chi-square test with Monte Carlo simulation (Roff and Bentzen 1989). Chi-square tests, haplotype diversity, nucleotide diversity within populations, and nucleotide divergence between populations were calculated using NEAP version 4.0 (McElroy et al. 1992).

Four narrowly endemic mbuna species (Ribbink et al. 1983) were sampled by scuba divers using block nets: *Pseudotropheus xanostomachus*, *P. zebra* 'black dorsal,' *P. zebra* 'blue,' and *P. zebra* 'red dorsal' (table 1). All are restricted to the Maleri Islands in the southern part of Lake Malawi (fig. 1). It has been suggested by the author that allopatric color morphs similar to some of these species may actually be conspecifics (Konings 1989). Although it is difficult to resolve this issue definitively, it appears that convergent coloration may not be uncommon in this fauna (McElroy and Kornfield 1991; and unpublished data). We concur with Ribbink and coworkers (Ribbink et al. 1983) in the view that these taxa represent unique, narrowly endemic species restricted in their distributions to the Maleri Islands. One of the few cosmopolitan species with lakewide distribution, *P. zebra* 'BB,' was collected at three locations: Nkhata Bay (in the north of the lake) and from Mumbo Island and Thumbi Island West (both in the southwest arm).

**Results**

In aggregate, the four restriction enzymes used in this study produced an average total of 30 fragments per haplotype. The number of fragments multiplied by the  $\lambda$ -value (number of bases recognized; Nei 1987) of the

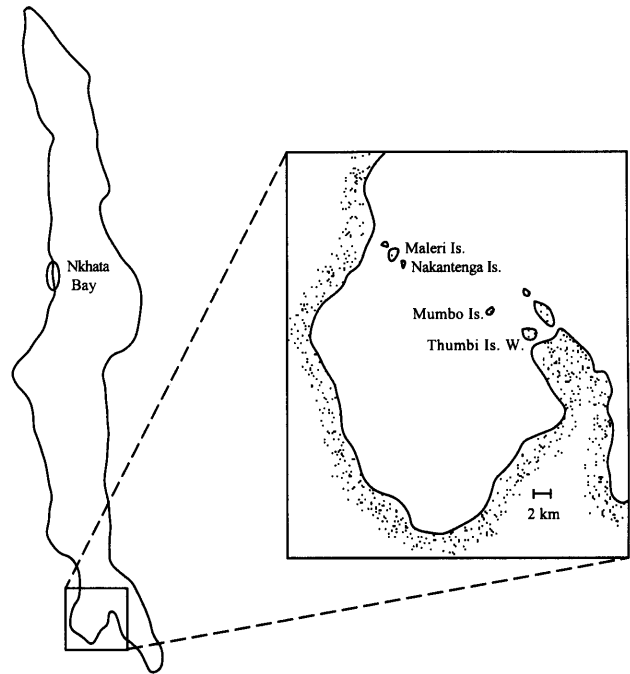


FIG. 1.—Collection locations for eight populations of five mbuna species in Lake Malawi, East Africa.

enzyme that produced them indicates that approximately 140 base pairs were assayed. Various combinations of 32 polymorphic fragments produced 23 different haplotypes among the five species examined (table 2). The level of variability observed using these enzymes does not reflect a random sample of the mtDNA genome because the enzymes were explicitly chosen to reveal high levels of polymorphism (Moran and Kornfield

**Table 1**  
**Mbuna Species of Lake Malawi Sampled for mtDNA Haplotype Diversity and Frequency Distribution within and among Species**

Species Name	Location	Date	N	
<i>Pseudotropheus xanostomachus</i> . . . . .	Maleri Island	Dec. 1990	14	
<i>Pseudotropheus zebra</i> 'black dorsal' . . . . .	Maleri Island	Aug. 1988	5	
		Dec. 1990	24	
		Aug. 1988	6	
<i>Pseudotropheus zebra</i> 'blue' . . . . .	Maleri Island	Aug. 1988	6	
		Nakantenga Island	April 1986	1
			Aug. 1988	6
<i>Pseudotropheus zebra</i> 'red dorsal' . . . . .	Nakantenga Island	Dec. 1990	14	
		Nkhata Bay	Aug. 1987	2
			Nov. 1990	6
			Dec. 1990	10
		Mumbo Island	Aug. 1988	4
Thumbi Island W.	April 1986	4		
<b>Total</b> . . . . .			<b>96</b>	

NOTE.—*Pseudotropheus zebra* 'BB' is a cosmopolitan species; all others are narrow endemics, restricted to one or more of the Maleri Islands (fig. 1).

**Table 2**  
**Frequency Distribution of mtDNA Haplotypes among Species**

SPECIES	$\alpha$ MTDNA LINEAGE							
	CCCC	CCCB	CCCM	DCCC	CCGC	CCGB	CC8C	CC8L
<i>Pseudotropheus xanstromachus</i> . . . . .	0	0	0	0	3	0	0	0
<i>P. zebra</i> 'black dorsal' . . . . .	0	0	0	0	0	0	0	0
<i>P. zebra</i> 'blue' . . . . .	0	0	0	0	0	0	1	1
<i>P. zebra</i> 'red dorsal' . . . . .	4	0	0	0	0	0	1	0
<i>P. zebra</i> 'BB' Nkhata Bay . . . . .	12	1	1	1	0	1	0	0
<i>P. zebra</i> 'BB' Mumbo Island . . . . .	2	0	0	0	0	0	0	0
<i>P. zebra</i> 'BB' Thumbi Island W. . . . .	0	0	0	0	0	0	0	0
Total . . . . .	18	1	1	1	3	1	2	1

NOTE.—Each letter or number of the haplotype designation (column heading) represents the mtDNA genotype revealed by one of following enzymes: *ApaI*, *AvaI*, *Avall*, or *MboI*, respectively. MtDNA lineage assignment ( $\alpha$  and  $\beta$ ) follows Moran and Kornfield (1993).

1993). Consequently, the nucleotide diversity and nucleotide divergence estimates (table 3) are upwardly biased. However, these measures are useful for relative comparisons among these taxa. Haplotype diversity estimates, on the other hand, are not influenced in the same way that genetic distance measures are but are rather a function of the frequency distribution of haplotypes within a population.

Haplotype diversity varied widely among species, from 0.136 in *Pseudotropheus zebra* 'black dorsal' to 0.933 in *P. zebra* 'blue' (fig. 2). Levels of haplotype diversity showed a marked discontinuity among species; *P. zebra* 'black dorsal' was substantially lower in haplotype diversity than any of the other species examined here. Reduced diversity in *P. zebra* 'black dorsal' was also reflected by nucleotide diversity that was at least an order of magnitude lower than any of the other species (table 3).

Significant differences in haplotype frequency were observed among all five mbuna species (table 3). Furthermore, significant frequency differences were also found between collections within species. For example, *P. zebra* 'BB' collected at Thumbi Island W. showed a different frequency distribution than 'BB' at Nkhata Bay ( $P < 0.001$ ). Divergence of frequencies between allopatric populations separated by hundreds of kilometers was not unexpected—previous allozyme studies indicated spatial structuring on this scale (McKaye et al. 1984). However, haplotype frequencies for *P. zebra* 'BB' at Thumbi Island W. were also significantly different from *P. zebra* 'BB' at Mumbo Island, only 5.9 km away ( $P < 0.001$ ). Furthermore, at Nakantenga, an island less than 1 square km in size surrounded by continuous rocky habitat, the three collections of *P. zebra* 'red dorsal'

showed significant heterogeneity in haplotype frequency ( $P = 0.020$ ). Similar heterogeneity was found among collections of *P. zebra* 'BB' at Nkhata Bay ( $P = 0.008$ ). These results are in contrast to the findings for *P. zebra* 'black dorsal,' in which two discrete collections were not significantly different in haplotype frequency. Although the sample sizes are small for the replicate collections, the differences in haplotype frequency are statistically significant and were observed in multiple species (representing independent tests). Given that virtually all collections produced multiple mtDNA haplotypes, there is no reason to believe that samples were biased for family groups. Whether these results represent spatial differences, temporal differences, or perhaps both remains unclear.

## Discussion

Three salient points emerge from the results presented here. First, *Pseudotropheus zebra* 'black dorsal' showed significantly lower haplotype diversity than any of the other species examined, consistent with a severe population bottleneck. Second, significant differences in haplotype frequency were evident among endemic species of the Maleri Islands, yet for three of four species sampled, there was no apparent reduction in genetic diversity. Even though allele frequencies change faster than heterozygosity under drift, the levels of haplotype diversity observed here for the latter three species do not seem consistent with population bottlenecks (see below). We consider the possibility that hybridization played a role in the distribution and diversity of haplotypes. We conclude that, while it is possible that hybridization in the early history of the lake contributed to the diversification of the Malawi ichthyofauna, recent hybridization

HAPLOTYPE FREQUENCIES

β MTDNA LINEAGE

FDME	EDME	FDMG	EDMG	EDSE	DDDF	JD6F	DDDK	CDCC	DDCA	2DCE	3DFE	DDCQ	DDDT	DD9N
1	0	0	0	0	2	0	0	0	7	0	0	1	0	0
27	0	0	0	0	0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	0	0	2	0	1	0	0	0	1	0
13	2	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
0	1	0	1	2	0	0	0	0	0	0	0	0	0	0
41	3	1	1	2	2	1	2	1	9	1	1	1	1	1

is rare at best. Third, statistically significant differences among collections within species at single locations suggests that philopatry (at least among females) is even more extreme than previously recognized. For reasons we describe below, this result is unlikely to be attributable entirely to either temporal variation in a panmictic population or to biased sampling of family groups.

Population Bottleneck in *Pseudotropheus zebra* 'Black Dorsal'

Of 29 *P. zebra* 'black dorsal' individuals collected from the Maleri Islands, all but two had identical haplotypes, and each of these differed by only one restriction site from the common haplotype. No other species produced so few haplotypes, regardless of sample size. For example, a single collection of six *P. zebra* 'blue' individuals revealed five different haplotypes, including members of the α and β mtDNA lineages. Among the five species examined here, only *P. zebra* 'black dorsal' was found to lack the polymorphism found in many

other mbuna species for the divergent α and β mtDNA lineages (Moran and Kornfield 1993). The near monomorphism observed in *P. zebra* 'black dorsal,' combined with an absence of the α lineage, produced a nucleotide diversity estimate ten times lower than the next lowest estimate (table 3). The extreme contrast between *P. zebra* 'black dorsal' and species like *P. zebra* 'blue' strongly suggests that *P. zebra* 'black dorsal' experienced one or more severe population bottlenecks during, or subsequent to, its origin. This degree of bottlenecking may have played a role in the isolation and divergence of this species. One effect of a severe bottleneck, not reflected in the dynamics of mtDNA, is loss of variability due to de facto inbreeding associated with small population sizes. The finding of reduced diversity in one of the four endemic species examined here implies that there are probably many more species among the Lake Malawi mbuna that have experienced bottlenecks. However, whether speciation events are commonly the result of population bottlenecks remains unclear. The

**Table 3**  
**Probability Values for Homogeneity of mtDNA Haplotype Frequencies Appear above the Major Diagonal**

	1	2	3	4	5
1. <i>Pseudotropheus xanstromachus</i> . . . . .	<b>0.983</b>	0.000	0.007	0.000	0.000
2. <i>P. zebra</i> 'black dorsal' . . . . .	1.006	<b>0.093</b>	0.000	0.001	0.000
3. <i>P. zebra</i> 'blue' . . . . .	0.071	1.330	<b>1.528</b>	0.001	0.006
4. <i>P. zebra</i> 'red dorsal' . . . . .	0.505	0.136	0.737	<b>1.113</b>	0.000
5. <i>P. zebra</i> 'BB' . . . . .	0.359	1.566	0.402	0.701	<b>1.222</b>

NOTE.—Nucleotide divergence values occur below the major diagonal. Nucleotide diversity within populations is shown in boldface. Nucleotide divergence and diversity values are listed as percentages.

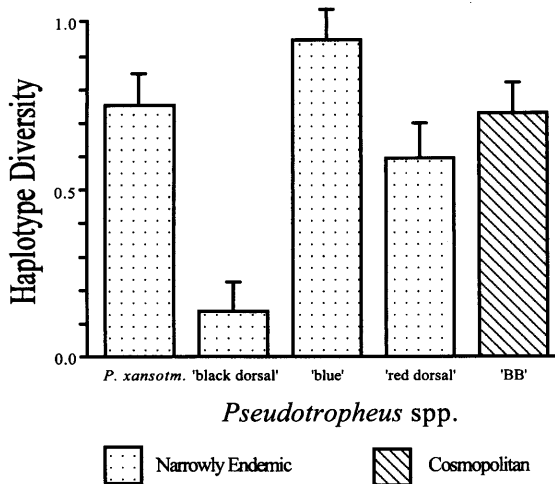


FIG. 2.—Mitochondrial haplotype diversity and standard errors for five species of mbuna. *Pseudotropheus zebra* 'black dorsal' is significantly lower in haplotype diversity than any of the other species examined here, whereas the latter show diversity that is quite high relative to other freshwater fishes (Avisé et al. 1987).

fact that several of the endemic species retain high diversity suggests that, although a bottleneck event may be associated with cladogenesis and may indeed be sufficient to produce isolation, it does not appear necessary in all cases.

The high levels of haplotype diversity observed in *P. xanstormachus* and *P. zebra* 'blue' suggest that these species were founded by relatively large numbers of individuals. However, it may be that localized structure in the endemic populations themselves have confounded these results; that is, diversity estimates may depend on the number of demes sampled. If multiple demes were sampled from each species, we would expect higher diversity and more similar haplotype frequencies among species. While results presented here do seem to demonstrate localized population structuring (e.g., *P. zebra* 'BB' at Nkhata Bay or *P. zebra* 'red dorsal' at Nakantenga Island), local structuring cannot fully explain our results. The species showing the highest diversity, *P. zebra* 'blue,' was represented by a single collection from a single, very circumscribed, location. Further, two replicate samples of *P. zebra* 'black dorsal' did not show differences in haplotype frequency, and both were nearly devoid of genetic diversity. It is clear that the genetic structure of *P. zebra* 'black dorsal' is fundamentally different than the other species and that other endemic species retain high levels of haplotype diversity within demes.

#### Drift without Depression of Haplotype Diversity

The degree of divergence in haplotype frequency among species, in the absence of reduced diversity within most populations, seems paradoxical at first; it is unclear

how drift—the presumed source of these frequency differences—could have occurred if populations were large enough to retain such remarkably high diversity. Assuming that these endemic taxa originated after formation of the habitat to which they are now restricted, these species are less than 20,000 yr old (Scholz and Rosendahl 1988) and may be only 200 yr old (Owen et al. 1990). We find at least three potential explanations for the observation of high diversity in the presence of haplotype frequency differences. First, it may have been that population flush/crash cycles took place, in which localized demes served to generate new diversity (Carson 1975). This scenario might have generated new haplotype diversity and increased the genetic distance between incipient species (depending on mutation rate and population sizes). However, this would not be expected to increase the genetic distance between any two existing haplotypes (Wilson et al. 1985). The  $\alpha$  and  $\beta$  mtDNA lineages, found in all three of the high diversity endemics, differ by more than 1.5% in DNA sequence (Moran and Kornfield 1993), making them older than most, if not all, species of mbuna. Thus, founding populations of three of the endemics (whether a single group or multiple groups) must have contained enough individuals to represent both  $\alpha$  and  $\beta$  lineages. Subsequent crash cycles were apparently not severe enough to eliminate this polymorphism. We believe the presence of this ancestral polymorphism in multiple species provides strong circumstantial evidence against the founder flush hypothesis.

A second explanation for the observation of drift without depression of diversity could have involved local or regional structuring that may have been present in the original source population such that founded populations were drawn from different haplotype frequency distributions, all with comparable levels of diversity. At a coarse level, this explanation seems unlikely, given geographic relationships of these endemic species to potential source populations on the mainland and the probable pattern of recolonization during lake transgression. However, if a single source population were structured to contain multiple demes, then founded populations with equivalent diversities could possess different frequencies because they arose from different demes.

Finally, it may be that the founders of all four endemic species were drawn from a single source population that was extremely diverse. If the endemic species originated from a single, highly diverse source with several haplotypes in high frequency and many rare haplotypes, similar to that observed in *P. xanstormachus*, for example, then even relatively large sets of founders might easily differ from each other in haplotype frequency yet

retain considerable diversity. However, it was unclear how much diversity these species might maintain through time, given finite population sizes.

To further evaluate this single-diverse-source hypothesis, we examined change in haplotype diversity through time analytically. The nonequilibrium formulas describing haplotype diversity within and among species (J. Felsenstein, personal communication),

$$h'_B = h_B(1-v)^2 + (2v-v^2) \quad (1)$$

and

$$h'_W = h_W \left( 1 - \frac{1}{N_f} \right) (1-v)^2 + (2v-v^2), \quad (2)$$

were solved iteratively, where  $h'_B$  and  $h'_W$  are haplotype diversities between and within populations, respectively,  $v$  is the sequence specific mutation rate (Nei 1987; eq. [10.14]), and  $N_f$  is the evolutionarily effective number of females. This assumes a constant mutation rate and an infinite-site model of neutral mutation.

Intuitively, the single-source explanation seemed quite plausible. However, our calculations revealed that the observed level of diversity within species is larger than can be explained without invoking unrealistic mutation rates or effective population sizes. This is essentially independent of diversity in the source population because diversity declines steadily unless effective population size is greater than 100,000 or the mutation rate is two orders of magnitude higher than typically accepted values (1%–2% per million yr) (Brown et al. 1979; Benzen et al. 1989). Our calculations simply confirm the intuitive approximation that evolutionarily effective population size (harmonic mean number of females, in this case) must be larger than the inverse of mutation rate in order for mutation to offset the loss of diversity due to finite population size. One would expect the mutation rate per haplotype ( $v$ ) to be somewhat higher than the more familiar rate per nucleotide ( $\mu$ ) because, as described above, the restriction enzymes used in this study were chosen to reveal a large number of polymorphic sites; that is, the particular sequences assayed had higher per-base mutation rates than might be expected for the molecule as a whole. In spite of this fact, we believe the rate of mutation required to explain the observed levels of diversity is outside the bounds of expectation.

The explanation for this particular finding remains unclear. However, regardless of whether the three high-diversity species arose from a single source population or multiple sources, it is clear that, unlike *P. zebra* 'black dorsal,' these results are not consistent with extreme

bottlenecks. Recent studies of variation at one of the major histocompatibility (*Mhc*) loci in Malawi cichlids (Klein et al. 1993; Ono et al. 1993) are consistent with this idea, although *Mhc* variation in cichlids may be maintained by overdominant selection (Hughes and Nei 1988, 1989).

Some investigators would argue that hybridization among mbuna species played a role in the distribution of genetic diversity. Indeed, these fish hybridize readily under artificial conditions. However, we do not believe hybridization can explain the results reported here. Neither interspecific matings nor hybrids have been reported in the wild among these fishes despite extensive study and in situ observation (Fryer and Isles 1972; Ribbink et al. 1983; unpublished data; P. N. Reinthal, personal communication). Some artificially produced hybrids possess distinctive morphologies (McElroy and Kornfield 1993) that would be readily apparent. Further, in a number of cases in which mbuna have been intentionally transplanted from one area of the lake to another, the fishes have maintained their phenotypic and behavioral integrity, that is, they are still recognizable as discreet biological species (Ribbink et al. 1983; Stauffer and Hert 1991). Perhaps most significantly, numerous pairs of syntopic sibling species show significant differences in allozyme frequencies (Kornfield 1978; McKaye et al. 1984), consistent with reproductive isolation. Recent microsatellite results are also inconsistent with hybridization (unpublished data). Further, the  $\alpha/\beta$  polymorphism was observed in many taxa distributed over a broad geographic area (Moran and Kornfield 1993), implying that hybridization would have to be widespread. Finally, the outcome of a single hybridization event for an mtDNA haplotype can be modeled in much the same manner as the fate of a single mutant (Fisher 1930; Kimura and Ohta 1969). Hence, in the absence of selection, the probability of persistence of a heterospecific mtDNA haplotype is small. Although it is possible that historical conditions may have influenced the frequency of hybridization (Crapon de Caprona 1986), it seems unlikely that hybridization is responsible for the high levels of diversity reported here.

#### Mbuna Species Composed of Localized Demes

The final result, suggesting extreme philopatry, may be quite significant in obtaining a better understanding of the evolutionary biology of this remarkable group. The level of population structuring within a species has a dramatic effect on effective population size and consequently on the expected rates of genetic change (Wright 1931, 1938). Earlier work has indicated limited mobility among members of these obligate rock-dwelling fishes (Hert 1992; unpublished data; but see McKaye and Gray 1984). Given the small size of Nakantenga Island and

continuous distribution of rocky habitat, it appears that the spatial scale of population structuring is on the order of hundreds of meters rather than 10s of kilometers as previously thought. The frequency differences observed among collections are taken to be primarily indicative of spatial heterogeneity. Although temporal variation may have contributed to these results, it is unlikely that the haplotype frequency differences can be attributed entirely to changes through time. We take this view for two reasons. First, field observations indicate fairly large population sizes for all these species, and generations are overlapping. Second, and most important, the high levels of diversity revealed in this study indicate that effective population size must be very large. If spatial structuring is as fine in scale as some of our results suggest, then effective population sizes may be substantially smaller than would be evident from census data alone. Thus, temporal variation in haplotype frequency might be expected, although this is probably variation in small individual demes rather than in a single much larger panmictic population. Although we cannot fully partition intraspecific variance in haplotype frequency into spatial and temporal components, we believe the most likely explanation for these results is that the mbuna, known to exhibit limited dispersal, are indeed highly philopatric and are composed of multiple demes even in the absence of physical barriers to gene flow.

To fully understand the extent and significance of population structuring in mbuna and to obtain estimates of effective population size, further microgeographic sampling will be required. Replicate samples will be required from multiple locations within the ranges of the species of interest. Multiple individuals of a single taxon, for example, *P. zebra* 'BB' at Nkhata Bay should be collected along transects at intervals of several meters. Autocorrelation could be used to examine the scale of population differentiation and to partition variation into spatial and temporal components. This fine-grain analysis will further benefit from the examination of recently developed microsatellite markers (unpublished data).

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