Widespread geographical distribution of mitochondrial haplotypes in rock-dwelling cichlid fishes from Lake Tanganyika

A. MEYER,*† L. L. KNOWLES* and E. VERHEYEN‡

*Department of Ecology and Evolution, †Program in Genetics, State University of New York, Stony Brook, NY 11794-5245, USA and ‡Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1040 Brussels, Belgium

Abstract

The spectacularly diverse cichlid fish species flocks of the East African Rift Lakes have elicited much debate on the potential evolutionary mechanisms responsible for the origin of these adaptive radiations. An historical perspective on population structure may offer insights into the processes driving population differentiation and possibly speciation. Here, we examine mitochondrial DNA (mtDNA) sequence variation in two endemic species of rock-dwelling cichlids, Simochromis babaulti and S. diagramma, from Lake Tanganyika. Phylogeographic analyses were used to infer what factors might have been important in the genetic structuring of Simochromis populations. Patterns of mtDNA differentiation in Simochromis were compared to those of other rock-dwelling cichlids to distinguish between competing hypotheses concerning the processes underlying their evolution. In striking contrast to previous findings, populations of Simochromis, even those separated by up to 300 km, were found to share mitochondrial DNA haplotypes. There is no correspondence between mtDNA genealogies and the geographical distribution of populations. Only S. babaulti, but not S. diagramma was found to have a significant association between genetic and geographical distance. These phylogeographic patterns suggest that the evolutionary effects of abiotic and biotic factors shaping population genetic structure may differ substantially even among closely related species of rock-dwelling cichlids. Physical events and barriers to gene flow that are believed to have had a major impact on the geographical distribution and intralacustrine speciation of Tropheus do not seem to have equally strongly affected its close relative Simochromis. These findings emphasize that no single mechanism can be responsible for the formation of population structure, speciation, and the adaptive radiation of all cichlid fishes.

Keywords: adaptive radiation, cichlids, phylogeography, Simochromis babaulti, speciation

Received 5 May 1995; revision received 2 August 1995; accepted 8 November 1995

Introduction

Alternative explanations for the origin of adaptive radiations such as those of Darwin's finches from the Galapagos islands and cichlid fishes in the East African Great Lakes have elicited considerable interest among evolutionary biologists (reviewed in e.g.: Fryer & Iles 1972; Meyer 1993). It now seems to be established that the Lake Victoria and Lake Malawi species flocks arose through intralacustrine speciation, i.e. within the confines of the lake basin; this is also suspected for the Lake Tanganyika flock, but awaits confirmation (Meyer et al. 1990; reviewed in Meyer 1993). The 200–300 species of Lake Victoria cichlids are likely to have been derived from a single ancestral lineage that probably radiated in less (maybe much less) than 200 000 years (Meyer et al. 1990). Speciation might indeed proceed extremely rapidly in East African cichlids; some researchers believe that several species in the southern end of Lake Malawi arose within the last two hundred years (Owen et al. 1990). One of the debates surrounding the evolutionary history of cichlids is centred around which mode of speciation might have led to the rapid formation of these large species flocks. The relative importance of
intrinsic biological factors (e.g. behaviour, morphology, or ecology) and abiotic factors such as lake level fluctuations and physical barriers to gene flow in the origin of these adaptive radiations is disputed. Clearly, biological differences even among closely related species can be responsible for differences in dispersal rates which in turn is an important factor which influences rates of speciation (e.g. Mayr 1963; Futuyma & Mayer 1980; but see Schliewen et al. 1994). However, dispersal rates and the amount of gene flow exhibited between populations have not been studied extensively in East African cichlid fishes. Mark-recapture studies have been used with success in birds and mammals to estimate gene flow but these methods are of limited use in cichlid fishes. Molecular markers might instead prove to be a powerful tool in this line of research.

The majority of cichlid species in the radiations of Lakes Victoria, Malawi and Tanganyika are maternally mouth-brooding species largely confined to the patchily distributed rocky habitats along the lakes’ shorelines (Fryer & Iles 1972; Ribbink et al. 1983; Cohen et al. 1993). Among biotic factors presumed to reduce gene flow among cichlid populations that are spatially isolated by discontinuities in suitable habitat (Fryer & Iles 1972) are their habitat specificity, site fidelity, limited dispersal abilities (McKay & Gray 1984) and homing behaviour (Hert 1990, 1992). Furthermore, during periods of geographical separation, isolating mechanisms might evolve by sexual selection, e.g. specific mate recognition through differences in the colours of males (Mayr 1984; Dominey 1984; Turner 1994); these would pre-zygotically prevent mating between members of incipient species. These isolating mechanisms might arise as by-products of accumulating genetic changes between geographically separated populations (Lande 1981, 1982). Alternative explanations for speciation in rock-dwelling cichlids have emphasized the role of extrinsic physical factors, including vicariant biogeographic events and geographical isolation by distance or habitat type which would restrict gene flow. Although the processes driving genetic differentiation, and ultimately speciation, are historical in nature, they are reflected in the patterns of distribution of genetic variation within and among populations and may therefore be identified (e.g. Avise 1988; Coyne 1992).

The phylogeographic structure of any species reflects patterns of historical fragmentation, changes in population size and distribution, and species-specific dispersal capabilities (e.g. Avise 1988; Avise & Ball 1990; reviewed in Avise 1994). The magnitude of genetic divergence distinguishing populations from different geographical regions varies across species, reflecting differences in life-history patterns, dispersal characteristics (e.g. Bermingham & Avise 1986; Avise et al. 1987a), and the historical demography of the respective populations (e.g. bottlenecks and episodes of population growth; Avise et al. 1984; Neigel & Avise 1986; Avise et al. 1988; Avise & Ball 1990; Rogers & Harpending 1992). From the extent of sequence divergence and the geographical distribution of genealogical lineages, we can estimate which processes are most likely to have produced the observed patterns of genetic differentiation.

Major partitions in a gene genealogy in which geographically localized populations occupy distinct branches of the gene tree may indicate that gene flow has been historically restricted, possibly by extrinsic (biogeographic) barriers (e.g. Bermingham & Avise 1986; Slatkin 1987; Palumbi & Wilson 1990; Taylor & Dodson 1994; Richardson & Gold 1995). However, another plausible explanation for such a pattern is that the genetic discontinuities resulted from localized extinction of mtDNA genotypes in a widely distributed species with limited dispersal capabilities (Avise et al. 1987a). Alternative explanations for the biogeographic distribution of genetic variation may be tested by comparing phylogeographic patterns across multiple taxa and relating these patterns to taxon-specific life-history characteristics (e.g. Bermingham & Avise 1986; Saunders et al. 1986; Avise et al. 1987b; Avise 1989; Patton & Smith 1989; Reeb & Avise 1990; Vogler & DeSalle 1993).

Even in species whose populations exhibit little or no spatial differentiation, and in which both genetic and life-history data suggest that gene flow has been high, the genetic structure is still greatly influenced by historical demography. For example, in widespread species with large population sizes in which both common and rare mtDNA haplotypes are broadly distributed, the statistical distribution of pairwise divergences among haplotypes (coalence times, Hudson 1990) can suggest that historical population sizes were smaller than contemporary populations, or that they experienced dramatic fluctuations in size (e.g. Avise et al. 1986; Ball et al. 1988). In species which lack biogeographic structure, two plausible explanations for sharing of mitochondrial lineages among populations are (1) ongoing gene flow and (2) retention of ancestral mtDNA lineages (incomplete lineage sorting) (Avise et al. 1984; Neigel & Avise 1986; Nei 1987; Slatkin & Maddison 1989; Takahata 1989). Relatively few migrants per generation would limit geographical differentiation between populations (e.g. Slatkin 1987; Edwards 1993). If the time of divergence is too recent to allow sorting of mitochondrial lineages present in the founder populations, geographically separate populations might share mtDNA haplotypes (Avise 1989; Moran & Kornfield 1993; Bowers et al. 1994).

Despite the critical role of genetic structure of populations in understanding the mechanisms of the spectacular diversification of East African cichlids (Mayr 1963), only two molecular studies on this issue have been undertaken so far. Seventy-five per cent of the studied populations of

Melanochromis from Lake Malawi were each fixed for a particular mitochondrial haplotype (Bowers et al. 1994). Only two mtDNA haplotypes were shared among geographically proximate (a few kilometres) populations of Melanochromis (Bowers et al. 1994). No sharing of mtDNA haplotypes was found between populations separated by about 150 km, among closely related species of the Pseudotropheus tropheops species complex from Lake Malawi (Reinthal & Meyer, 1997), but some was found in Pseudotropheus zebra (Moran & Kornfield 1993). Similarly, in rock-dwelling cichlids of the genus Tropheus from Lake Tanganyika, five of six phylogenetically distinct lineages of Tropheus came from geographically proximate localities and often large coloration and mitochondrial differences separated geographically close populations (Sturmbauer & Meyer 1992). All of these studies are consistent with the explanation that speciation in cichlids has been associated with abiotic, biogeographic processes such as fluctuations in lake levels (e.g. Owen et al. 1990; Sturmbauer & Meyer 1992) and that isolation through distance and/or habitat type are effective barriers to gene flow in these fish.

Phylogeographic patterns need to be replicated across cichlid species within the same lake to test the general applicability of the hypothesis that the same abiotic factors (such as availability of suitable habitat, or changes in lake level) played a predominant role in determining population genetic structure and ultimately speciation in East African cichlids. In this study, we characterize the geographical pattern of generic variation for two additional cichlid species from Lake Tanganyika, Simochromis babaulti and S. diagramma. These two genera are ecologically very similar, scraping 'Aufwuchs' from rocky substrates. Both have lake-wide distributions, but Simochromis tends to be much less abundant than Tropheus (Brichard 1989; Takamura 1984; Sturmbauer 1990; E.V. and A.M., personal observations). Simochromis and Tropheus (Sturmbauer & Meyer 1992) are closely related genera in the tribe Trophini, and both genera currently comprise six species (Poll 1986). But, Tropheus is likely to contain a much larger number of species than Simochromis (Sturmbauer & Meyer 1992).

Materials and methods

Specimens

Twenty-five individuals of Simochromis babaulti (from 11 localities) and 28 individuals of S. diagramma (from 13 localities), were collected during an expedition in 1992 from a total of 17 different localities distributed along an ~440-km section of the eastern shoreline of Lake Tanganyika (Fig. 1) and were preserved in 70% ethanol. S. marginatus, could not be sampled in sufficient numbers (only four individuals were collected from two locations) but was included for comparison.

![Maps of Lake Tanganyika showing localities where S. babaulti and S. diagramma were collected and the distribution of mtDNA haplotypes](image)

Fig. 1 Maps of Lake Tanganyika showing localities where S. babaulti and S. diagramma were collected (on the left with smaller numbers) and the distribution of mtDNA haplotypes. The three shaded areas within the current lake basin indicate the three separate lakes that existed during the geologically recent lake level drop (by 600 m) (Tiercelin & Mondeguee 1991).

Table 1 Polymorphic nucleotide positions that distinguish haplotypes in *S. diagramma* and *S. babaulti*

<table>
<thead>
<tr>
<th>Haplotype designation</th>
<th>Haplotype 1</th>
<th>Haplotype 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAB1</td>
<td>G A T C T C</td>
<td>A C A T C T</td>
</tr>
<tr>
<td>BAB2</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>BAB3</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>BAB4</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>BAB5</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>BAB6</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>BAB7</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>BAB8</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>BAB9</td>
<td>G</td>
<td>T</td>
</tr>
<tr>
<td>BAB10</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>BAB11</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>BAB12</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>BAB13</td>
<td>T</td>
<td>C</td>
</tr>
</tbody>
</table>

Dots indicate identity with DIA1 and BAB1 sequences, respectively; nucleotide positions are shown across the top.

Polymerase chain reaction

A 414-base-pair (bp) segment of the mitochondrial control region was sequenced (Fig. 1, Tables 1 and 2). DNA was extracted from white muscle tissue. DNA sequences were obtained via the polymerase chain reaction (PCR) and both DNA strands were sequenced directly. Detailed protocols and primer sequences used for PCR amplification and direct sequencing have been previously published (Kocher et al. 1989; Meyer et al. 1990; Meyer et al. 1994).

Intra- and interspecific genetic variation was estimated based on pairwise sequence differences. Overall nucleotide diversity (θ, Nei & Li 1979) was calculated as a measure of polymorphism within *S. babaulti* and *S. diagramma*. Phylogenetic relationships among mtDNA haplotypes were estimated with maximum parsimony, using outgroup rooting and the heuristic search algorithm weighting transitions and transversions equally in PAUP Version 3.1.1 (Swofford 1993). Two other members of the tribe Tropheini were used as out-groups: *Petrochromis trewavasae* and *Tropheus duboisi*. For direct comparisons of *Simochromis* phylogenetic patterns to those previously documented for *Tropheus* (Sturmbauer & Meyer 1992), phylogenetic relationships were also examined by weighting transversions nine times over transitions in parsimony analyses as in Sturmbauer & Meyer (1992).

Results

Sequence variation

Of the 414 bp analysed, only 13, 16 and 10 positions varied within *S. babaulti*, *S. diagramma* and *S. marginatus*, respectively; only one of these was at a homologous posi-

<table>
<thead>
<tr>
<th>Location</th>
<th>Haplotype BAB1</th>
<th>Haplotype BAB2</th>
<th>Haplotype BAB3</th>
<th>Haplotype BAB4</th>
<th>Haplotype BAB5</th>
<th>Haplotype BAB6</th>
<th>Haplotype BAB7</th>
<th>Haplotype BAB8</th>
<th>Haplotype BAB9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3(5)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4(1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6(1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8(2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9(1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11(4)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12(5)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13(2)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14(1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17(1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Haplotypes which are shared among localities.

Table 2 Frequency distribution of mtDNA haplotypes among 25 individuals of *S. babaulti* from 11 different localities in Lake Tanganyika (number of individuals sampled per location given in parentheses)
tion between species (Table 1). The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U38808, U38984-U38995, U40532, U50347-U50349). Among the 25 individuals of S. babaulti sampled, nine haplotypes were identified; 13 haplotypes were found among the 28 individuals of S. diagramma (Tables 1–3). Only eight of the 29 variable sites involved transversions, suggesting that the divergence among the haplotypes is recent. None of the mitochondrial haplotypes were shared among species. A mean and standard deviation of 3.61 ± 3.23 (0.87 ± 0.78%), 4.91 ± 2.96 (1.19 ± 0.71%), and 5.67 ± 4.04 (1.37 ± 0.98%) substitutions was inferred among the nine S. babaulti, the 13 S. diagramma, and the three S. marginatus haplotypes, respectively.

The mean number of pairwise substitutions among the haplotypes of the three Simochromis species was 15.67 ± 2.59 (3.78 ± 0.62%). Mean numbers of pairwise substitutions among all S. babaulti individuals were 2.23 ± 2.36 (0.54 ± 0.57%), all S. diagramma specimens 3.17 ± 2.63 (0.76 ± 0.64%). Among all individuals, these species differ by a mean 2.76 ± 2.57 substitutions (0.67 ± 0.62%). Nucleotide diversity indices (π) were quite low: 0.0026 and 0.0037 for S. babaulti and S. diagramma, respectively.

Geographical distribution of mtDNA variation
The number of different mtDNA haplotypes observed was variable among localities; however, when more than one individual was collected from a given location, populations of both S. babaulti and S. diagramma typically contained more than one haplotype (Fig. 1, Tables 2 and 3). Of those, only two of six S. babaulti populations were monotypic for a particular mtDNA haplotype (localities 1 and 8; Fig. 1 and Table 2); none of the six S. diagramma populations were fixed for a single haplotype (Table 3).

Populations of both S. babaulti and S. diagramma not only tended to be polymorphic, but several mtDNA haplotypes were shared among even geographically distant localities separated by as much as 300 km (Fig. 1). Moreover, some haplotypes (e.g. S. babaulti haplotype 1 and S. diagramma haplotype 3) were found in more than one region of the lake that were separated during a geologically recent major drop of 600 m in the water level (Tiercelin & Mondegue 1991), a significant vicariance event. No region of the lake appeared to be genetically depauperate or to possess an excessive diversity of haplotypes for either Simochromis species and genetic and geographical distance do not seem to be strongly related (Fig. 2). Mantel tests of association between geographical and genetic distance matrices (Sokal & Rohlf 1994) show that Simochromis babaulti (N = 276, r = 0.18675, t = 2.144, P < 0.05), but not S. diagramma populations (N = 351, r = 0.03711, t = 0.667, P > 0.05) are significantly structured.

Phylogenetic analyses
The monophyly of each Simochromis species was supported irrespective of the weighting scheme employed in parsimony analyses (Fig. 3) and no haplotypes were shared among them. Hence, the three of Simochromis species appear not to have retained ancient polymorphisms from their common ancestors. The branching topology among the haplotypes did not reflect the biogeographic distribution of populations in either S. babaulti or S. diagramma, although in both species the most basal haplotypes were from the most southern locality sampled (Figs 1 and 3). For both species, haplotypes from the same locality did not tend to be genetically most similar (e.g. S. diagramma

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Frequency distribution of mtDNA haplotypes among 28 individuals of S. diagramma from 13 different localities in Lake Tanganyika (number of individuals sampled per location given in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplotypes</td>
<td>Location</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>1(1)</td>
<td>1</td>
</tr>
<tr>
<td>2(1)</td>
<td>0</td>
</tr>
<tr>
<td>3(1)</td>
<td>0</td>
</tr>
<tr>
<td>5(1)</td>
<td>0</td>
</tr>
<tr>
<td>6(2)</td>
<td>0</td>
</tr>
<tr>
<td>10(3)</td>
<td>0</td>
</tr>
<tr>
<td>12(4)</td>
<td>0</td>
</tr>
<tr>
<td>13(5)</td>
<td>0</td>
</tr>
<tr>
<td>16(6)</td>
<td>0</td>
</tr>
<tr>
<td>17(1)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Haplotypes which are shared among localities.
haplotypes 3, 8 and 9 from locality 10; Figs 1 and 3) (but see above for results of the Mantel test). Prevalence of shared mtDNA haplotypes among geographically distant populations and a lack of strong phylogeographic structure (Figs 1–3; Tables 2 and 3) seem to be characteristic of these species – this is in striking contrast to previous findings for cichlids (e.g. Sturmbauer & Meyer 1992; Moran & Kornfield 1993; Browsers et al. 1994; Reinthal & Meyer, 1997).

Discussion

A major fluctuation in lake level divided Lake Tanganyika into three isolated basins 200 000 (Coulter 1991; Tiercelin & Mondeguer 1991) to 75 000 (Scholz & Rosendahl 1988, 1990) years ago, and the effect of this event is strongly reflected in the biogeographic distribution of some rock-dwelling cichlids (Tropheus: Sturmbauer & Meyer 1992; Eretmodini: Verheyen et al., 1996). Several aspects of the phylogeography of Simochromis merit consideration. The sharing of haplotypes across geographically widely separated localities and the incongruence between phylogenetic patterns and the geographical distribution of populations does not support the hypothesis that discontinuities in available habitat are effective barriers to gene flow for these fishes (Fig. 1). These findings for Simochromis contrast with those of the phylogenetically closely related genus Tropehus for which five of six mitochondrial lineages had a geographically restricted distribution that coincided with the intermittent lake basins during this major drop in lake level (Sturmbauer & Meyer 1992) and in some species of the Tribe Eretmodini (Verheyen et al., 1996). That several Simochromis haplotypes occur along the coasts of all three historical lake basins (see below) is inconsistent with the hypothesis that population genetic structure of all Tanganyikan rock-dwelling cichlids is similarly strongly influenced by historical biogeographic barriers to gene flow (Sturmbauer & Meyer 1992). The
Simochromis situation also contrasts markedly with the patterns of genetic variation documented for other rock-dwelling cichlids from Lake Malawi (Bowers et al. 1994; Reinthal & Meyer, 1997). The lack of spatial differentiation among populations of S. diagramma (but less so in S. babaulti) might suggest extensive contemporary gene flow or alternatively differential extinction of haplotypes between different populations. This interpretation would set Simochromis apart from other rock-dwelling Lake Tanganyika cichlids. This is because their strict habitat requirements, and distributional data on colour variation suggests that much population differentiation and little gene flow seem to exist (Koelings & Dieckhoff 1992; Kohda et al. 1996). For many Tanganyikan cichlids stretches of unsuitable habitats (e.g. sandy beaches, marshes, and estuaries) separate colour morphs and seem to prevent gene flow by inhibiting migration across hostile habitat (Kohda et al. 1996). Furthermore, the current distributional patterns of many cichlid species and colour morphs from Lake Tanganyika reflect the three intermittent palaeo-lakes (Koelings & Dieckhoff 1992). However, the patterns of intralacustrine distribution of many Tanganyikan cichlids are more complex than previously assumed, e.g. the Malagarasi River delta might not be a major geographical barrier to some cichlid species (Snoeks et al. 1994). Moreover, we caution that the apparent lack of population differentiation (in S. diagramma, but not S. babaulti) might be partly due to the small number of samples per population that we were able to collect and the potential importance of incomplete lineage sorting in bringing about the observed patterns still awaits investigation with larger sample sizes.

Populations which are isolated from each other, especially small ones, diverge and approach fixation of haplotypes or monophyly more rapidly than those with gene flow (Neigl & Avise 1986; Avise 1989; Avise & Ball 1990) since incipient divergence among populations may be erased due to the extinction/colonization process (Slatkin 1977). The typically extremely small population size of many rock-dwelling (but maybe not sand-dwelling or open-water?) cichlid species (Ribbink et al. 1983) would seem to suggest that population integrity will constantly be challenged. Explanations for the differences in phylogeography between Simochromis and Tropheus may be found in biological and historical (which might include differential effects of major ancient vicariance events due to biological differences) differences between these species. The phylogeographic effects that the drop in lake level in Lake Tanganyika had on Simochromis might have been smaller due to biological differences between Simochromis and Tropheus, or they might have been obliterated by subsequent gene flow more so in Simochromis than in Tropheus.

Within the genus Tropheus, presumed biological species differed by, on average, 2.7% sequence divergence, with a maximum of 3.8% among 17 individuals from a single locality (Mpulungu) (Sturmbauer & Meyer 1992). A maximum of 14.5% corrected divergence was found within the genus Tropheus if the most basal species, T. daboisi, was included in the comparison (Sturmbauer & Meyer 1992). The three species of Simochromis studied here differ by about 3.8% sequence divergence from each other, but within each Simochromis species the amount of sequence difference was much less, typically around 1%. Assuming similar rates of mitochondrial control region evolution, species of Tropheus and Simochromis might thus differ in age. Simochromis being younger than Tropheus.

Quantitative natural history and ecological data (e.g. migration and dispersal data) with relevance to phylogeography for Simochromis and Tropheus support this notion that biological differences between these genera might provide an explanation for the observed differences in the genetic population structure (Nelissen 1975, 1976; 1977; Brichard 1978, 1989; Kumawara 1986, 1987; Sturmbauer 1990; Yanagisawa & Sato 1990; Sturmbauer & Meyer 1992; Sturmbauer, personal communication). Species of these two genera are quite similar in several biological features, e.g. they are all algae scrapers, and female mouth-brooders. In Simochromis only the males are territorial but in Tropheus sometimes also the females are; the former are polygamous, the latter monogamous. It has been observed that juvenile Tropheus form shoals to a much lesser extent than Simochromis for which large shoals (hundreds of individuals) of females and juveniles have been observed, pointing at a larger capacity for dispersal in Simochromis than in Tropheus species. Simochromis appears to occupy a wider range of habitats: it is mostly found in rocky shore areas, but unlike Tropheus, it has also been seen in estuaries and in areas where rocks are coated with sediment (Koelings & Dieckhoff 1992).

Among the features that most notably differentiate Tropheus from Simochromis is the conspicuous coloration of the former. Simochromis are rather drab in coloration and species tend to have a large geographical distribution throughout the lake, whereas Tropheus has a large number of geographically localized brightly coloured morphs or species (e.g. Marlier 1959; Matthes 1962; Nelissen 1976; Brichard 1989). Coloration differences have been implicated in most models of cichlid diversification; colour most likely serves as a behavioural signal involved in species recognition and sexual selection (Fryer & Iles 1972; Dominey 1984; McElroy & Kornfield 1990; reviewed in Greenwood 1991; Turner & Burrows 1995). Coloration is an important trait for the establishment and maintenance of reproductive isolation in cichlids (Holzberg 1978; Marsh et al. 1981; Ribbink et al. 1983; Dominey 1984; reviewed in Turner 1994; Turner & Burrows 1995). In Tropheus (but possibly not in Simochromis) colour is likely to play an

important role in intermate competition and also female choice (Nelissen 1976). In some Malawi cichlids females base mate choice on sympatrically living male colour morphs (Holzberg 1976; Hert 1992; reviewed in Turner 1994). Male *Tropheus* defend feeding and mating territories more fiercely in intermate competition than *Simochromis* do (Nelissen 1976; Sturmbrauer 1990; Koenings & Dieckhoff 1992). Therefore, coloration diversification would be enhanced by both intermate competition and sexual selection *via* female choice based on male colour (Kosswig 1947; Dominey 1984; Mayr 1984; G. F. Turner, personal communication). This raises the possibility for *Tropheus*, that sexual selection through (female) mate choice for local colour morphs and against the colour morphs of the migrant individual may counteract the potentially mixing effects of gene flow (Lande 1981, 1982; Dominey 1984). This hypothesis deserves to be tested in mate choice experiments.

Generally, habitat specificity, dispersal rates and distances, and discontinuities in suitable habitat are conditions which promote subdivision of populations (e.g. Taylor & Bentzen 1993), however, the same abiotic conditions seem to have different effects on *Simochromis* and *Tropheus*. That phylogeographic patterns can differ dramatically between ecologically similar, closely related species came as a surprise. Differences in the propensity to disperse and differences in mating system and coloration-mediated sexual selection may explain the dissimilar phylogeographies for these cichlids.

Several classes of mechanisms that would restrict gene flow between populations, such as (1) habitat choice, (2) natural selection against migrants, (3) sexual selection against migrants, (4) selection against hybrids, could be largely responsible for speciation in *Simochromis* and possibly other rock-dwelling cichlids in Lake Tanganyika. While the results of this study do not entirely rule out several alternatives, they, nonetheless, suggest that abiotic as well as abiotic processes might be important in structuring populations of rock-dwelling cichlids and may bring about substantial differences in genetic structure among species which are, in turn, likely to affect rates and modes of speciation. These data point out that closely related species of cichlid fishes have remarkably different capabilities for dispersal and responses to abiotic factors that affect gene flow. Future studies using molecular markers to learn about gene flow and speciation in cichlids need to expand into work on sand-dwelling and open water species since they are likely to experience different, if any, barriers to gene flow than the rock-dwelling cichlids. Future work on the molecular ecology and speciation of cichlids will also need to incorporate a nuclear view (through, for example, the characterization of single-microsatellite loci) to be able to, e.g. differentiate female (studied through mtDNA) and male dispersal. Our findings suggest that hypotheses regarding modes of speciation in cichlids may need to be expanded beyond allopatric models of simple isolation by distance or habitat type. Behaviour (which should include work on the mechanisms and genetics of mating preferences and male traits such as coloration), coloration, and life history of the fish themselves need to be considered in addition to abiotic factors if we hope to understand the multitude of underlying mechanisms responsible for the spectacular diversification of cichlids.

Acknowledgements
We thank E. Mayr, C. Sturmbrauer, and C.-I. Wu for helpful discussions and D. Futuyma, M. Kretzmann, E. Mayr, L. Rüber, O. Seehausen, G. Turner, and three referees for thoughtful comments on the manuscript. Particular thanks to C. Sturmbrauer and the other members of the 1992 expedition. We are grateful to the National Science Foundation, USA (grants BSR-9119867 and BSR-9107838 to A.M.), the Max-Planck-Society, Germany (to A.M.), and the North Atlantic Treaty Organization (collaboration grant CRG-910911 to A.M. and E.V.) and the Belgium government (FBR grant 2-90004-90 and FBR-MI grant 30-33 to E.V.) for financial support. Fieldwork was carried out in collaboration with the Tanzanian Fisheries Research Institute and the University of Burundi; we thank Prof. P. O. J. Bwambodi and G. Ntakimazi for collaboration and permits for research and collection.

References

MITOCHONDRIAL DNA OF LAKE TANGANYIKA CICHLIDS


Yanagisawa Y, Sat o T (1990) Active browsing of mouthbrood ing females of *Tropheus duboisi* and *Tropheus moorii* (Cichlidae) to feed the young and/or themselves. *Environmental Biology of Fishes*, 27, 43-50.

We wish to dedicate this article to the late Prof. P. H. Greenwood of the British Museum of Natural History, who, for many years, led the way in the study of systematics and evolutionary biology of East African cichlid fish. Humphrey Greenwood's fundamental contributions to the taxonomy of cichlids made it possible also for molecular approaches to revisit these problems in speciation and the formation of species schools. This paper is part of a continuing collaboration between the laboratories of Axel Meyer and Erik Verheyen on the evolution of East African cichlid fish. Axel Meyer is an Associate Professor in the Department of Ecology and Evolution at Stony Brook University and interested in molecular phylogenetics and evolutionary biology. Lacey Knowles is a graduate student in the same department and is interested in questions having to do with phylogeography, genetics of speciation, and molecular evolution. Erik Verheyen is a staff scientist at the Natural History Museum at Brussels and is working on several projects involving the evolution and phylogeny of African animals.