

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

Correspondence: Axel Meyer. Tel.: +1-516-632-7434. Fax: +1-516-632-7626. E-mail: ameyer@life.bio.sunysb.edu

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 (McKaye & 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 1989; 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 1989; 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 (coalescence 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 genetic 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 Tropheini, 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 \approx 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.

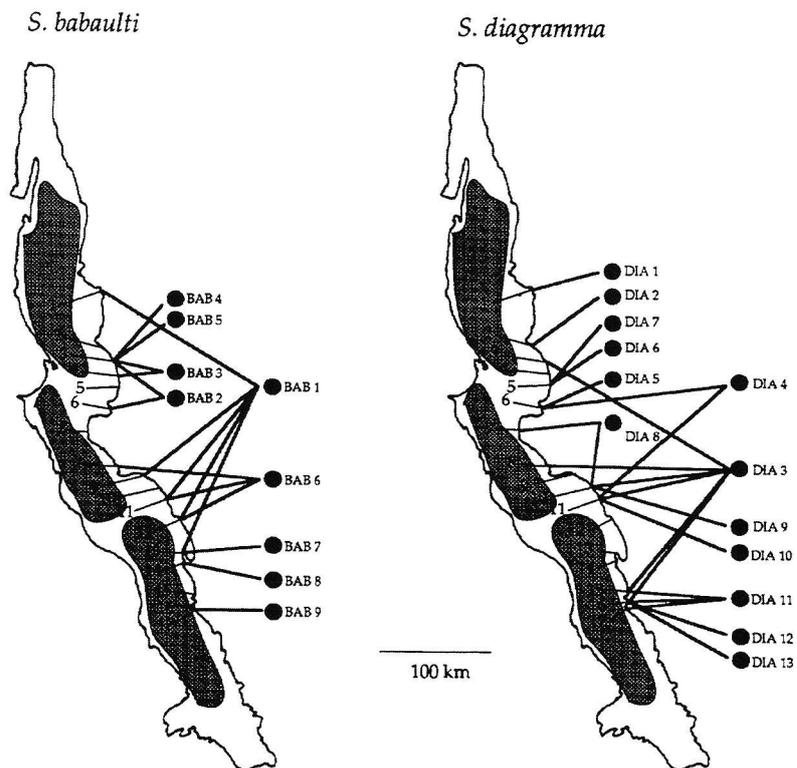


Fig. 1 Maps of Lake Tanganyika showing localities where *S. babaulti* and *S. diagramma* were collected (on the left with smaller numbers) and the distribution of mtDNA haplotypes. The three shaded areas within the current lake basin indicate the three separate lakes that existed during the geologically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

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

Haplotype designation	6	9	2	2	5	5	6	6	7	2	1	5	6	7	8	8	Haplotype designation	9	7	5	9	3	6	6	5	6	8	3	4	5	
DIA1	G	A	T	C	T	C	C	G	T	T	A	C	T	C	A	G	BAB1	A	C	A	T	C	T	C	T	A	T	T	A	A	
DIA2	T	BAB2	.	.	.	A	T	
DIA3	.	.	C	T	.	.	T	BAB3	T	T	
DIA4	T	BAB4	.	.	.	A	.	.	T	
DIA5	T	G	G	BAB5	T	
DIA6	.	.	C	.	.	.	T	.	.	A	BAB6	G	
DIA7	.	.	C	.	.	.	T	BAB7	A	.	.
DIA8	.	G	C	.	.	.	T	.	.	A	BAB8	G	.
DIA9	.	.	C	T	.	.	T	.	C	A	BAB9	G	T	T	.	A	C	.	A	.	C	.	.	.	
DIA10	A	.	C	T	.	.	T	A															
DIA11	C	.	T															
DIA12	.	.	.	T	.	T	T	A	C	.	G	.	C	.	.	.															
DIA13	.	.	.	T	.	T	T	A	C	.	.	C	.	.	A	.															

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 (π , 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-

Location	Haplotypes								
	BAB1*	BAB2*	BAB3*	BAB4	BAB5	BAB6*	BAB7	BAB8	BAB9
1(2)	2	0	0	0	0	0	0	0	0
3(5)	0	2	1	1	1	0	0	0	0
4(1)	0	0	1	0	0	0	0	0	0
6(1)	0	1	0	0	0	0	0	0	0
8(2)	0	0	0	0	0	2	0	0	0
9(1)	1	0	0	0	0	0	0	0	0
11(4)	1	0	0	0	0	3	0	0	0
12(5)	1	0	0	0	0	4	0	0	0
13(2)	1	0	0	0	0	0	1	0	0
14(1)	0	0	0	0	0	0	0	1	0
17(1)	0	0	0	0	0	0	0	0	1

*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 ($0.87 \pm 0.78\%$), 4.91 ± 2.96 ($1.19 \pm 0.71\%$), and 5.67 ± 4.04 ($1.37 \pm 0.98\%$) 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 ($3.78 \pm 0.62\%$). Mean numbers of pairwise substitutions among all *S. babaulti* individuals were 2.23 ± 2.36 ($0.54 \pm 0.57\%$), all *S. diagramma* specimens 3.17 ± 2.63 ($0.76 \pm 0.64\%$). Among all individuals, these species differ by a mean 2.76 ± 2.57 substitutions ($0.67 \pm 0.62\%$). 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* popula-

tions 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 3 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)

Location	Haplotypes												
	DIA1	DIA2	DIA3*	DIA4*	DIA5	DIA6	DIA7	DIA8*	DIA9	DIA10	DIA11*	DIA12	DIA13
1(1)	1	0	0	0	0	0	0	0	0	0	0	0	0
2(1)	0	1	0	0	0	0	0	0	0	0	0	0	0
3(1)	0	0	1	0	0	0	0	0	0	0	0	0	0
5(2)	0	0	0	0	0	1	1	0	0	0	0	0	0
6(2)	0	0	0	1	1	0	0	0	0	0	0	0	0
7(1)	0	0	0	0	0	0	0	1	0	0	0	0	0
8(1)	0	0	1	0	0	0	0	0	0	0	0	0	0
10(3)	0	0	1	0	0	0	0	1	1	0	0	0	0
11(4)	0	0	2	1	0	0	0	0	0	1	0	0	0
15(5)	0	0	3	0	0	0	0	0	0	0	2	0	0
16(6)	0	0	3	0	0	0	0	0	0	0	1	1	1
17(1)	0	0	0	0	0	0	0	0	0	0	1	0	0

*Haplotypes which are shared among localities.

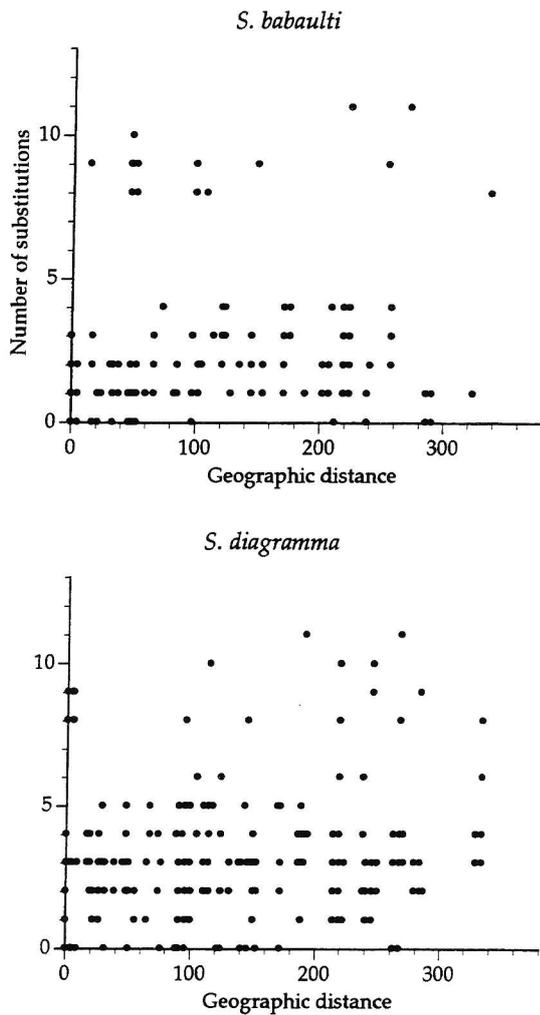


Fig. 2 Scatter plots of all pairwise comparisons of all specimens of *S. babaulti* and *S. diagramma*. Graphs show the numbers of substitutions in a 414-bp portion of the mitochondrial control region and the distance (in kilometres) that separates the localities at which the particular specimens from that pairwise comparison were caught.

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; Browers *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 &

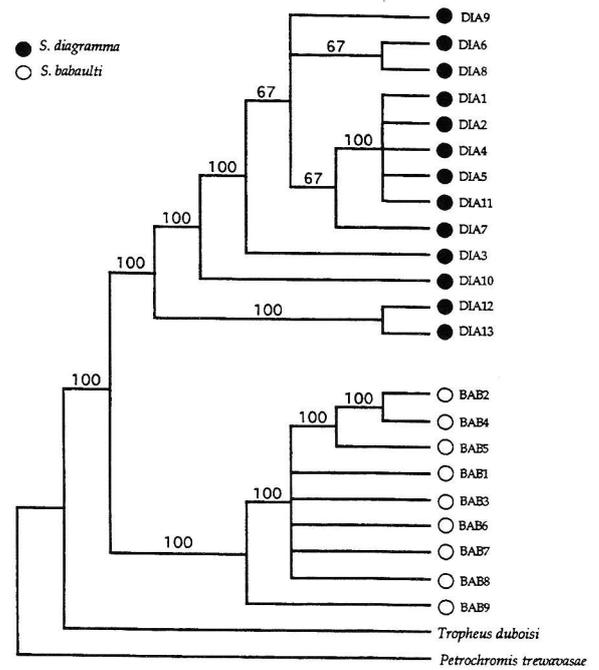


Fig. 3 Majority rule consensus tree of the six minimum length trees (tree lengths of 64 steps and a consistency index of 0.79) for all *S. babaulti* and *S. diagramma* mtDNA haplotypes based on a maximum parsimony analysis (Swofford 1993) using *Tropheus duboisi* and *Petrochromis trewavasae* as out-groups.

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 *Tropheus* 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 (Koenings & 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 (Koenings & 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 (Neigel & 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. duboisi*, was included in the comparison (Sturmbauer & Meyer 1992). The three species of *Simochromis* studied here differ by about 3.8% sequence difference 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 (Koenings & 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 intermale competition and also female choice (Nelissen 1976). In some Malawi cichlids females base mate choice on sympatrically living male colour morphs (Holzberg 1978; Hert 1992; reviewed in Turner 1994). Male *Tropheus* defend feeding and mating territories more fiercely in intermale competition than *Simochromis* do (Nelissen 1976; Sturmbauer 1990; Koenings & Dieckhoff 1992). Therefore, coloration diversification would be enhanced by both intermale 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 biotic 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 find-

ings suggests 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. Sturmbauer, 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. Sturmbauer 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 (FJBR grant 2-90004-90 and FJBR-MI grant 30-35 to E.V.) for financial support. Fieldwork was carried out in collaboration with the Tanzanian Fisheries Research Institut and the University of Burundi, we thank Profs P. O. J. Bwathondi and G. Ntakimazi for collaboration and permits for research and collection.

References

- Avise JC (1989) Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution*, **43**, 1192–1208.
- Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman and Hall, NY.
- Avise JC, Ball RM Jr (1990) Principles of genealogical concordance in species concepts and biological taxonomy. In: *Oxford Surveys in Evolutionary Biology* (eds Futuyma D, Antonovics J), pp. 45–68. Oxford University Press, Oxford.
- Avise JC, Arnold J, Ball RM *et al.* (1987a) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.
- Avise JC, Ball RM, Arnold J (1988) Current vs. historical population sizes in vertebrates species with high degree of gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. *Molecular Biology and Evolution*, **5**, 331–344.
- Avise JC, Helfman GS, Saunders NC, Hales LS (1986) Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. *Proceedings of the National Academy of Sciences of the USA*, **83**, 4350–4354.
- Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution*, **20**, 99–105.
- Avise JC, Reeb CA, Saunders NC (1987b) Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). *Evolution*, **41**, 991–1002.

- Ball RM, Freeman S, James FC *et al.* (1988) Phylogeographic population structure of red-winged blackbirds assessed by mitochondrial DNA. *Proceedings of the National Academy of Sciences of the USA*, **85**, 1558–1562.
- Bermingham E, Avise JC (1986) Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, **113**, 939–965.
- Bowers N, Stauffer JR, Kocher TD (1994) Intra- and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malawi, Africa. *Molecular Phylogenetics and Evolution*, **3**, 75–82.
- Brichard P (1978) Un cas d'isolement de substrats rocheux au milieu de fonds de sable dans le nord du lac Tanganyika. *Revue Zoologique Africainique*, **92**, 518–524.
- Brichard P (1989) *Cichlids of Lake Tanganyika*. TFH Publications, Neptune City.
- Cohen A, Soreghan AJ, Scholz CA (1993) Estimating the age of formation of lakes: an example from Lake Tanganyika, East-African rift system. *Geology*, **21**, 511–514.
- Coulter GW (1991) The benthic fish communities. In: *Lake Tanganyika and Its Life* (ed. Coulter GW), pp. 151–199. Oxford University Press, London.
- Coyne JA (1992) Genetics and speciation. *Nature (London)*, **555**, 511–515.
- Dominey WJ (1984) Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiian *Drosophila*. In: *Evolution of Fish Species Flocks* (eds Kornfield I, Echelle AA), pp. 231–249. University of Maine Press, Orono.
- Edwards SV (1993) Mitochondrial gene genealogy and gene flow among island and mainland populations of a sedentary songbird, the grey-crowned babbler (*Pomatostomus temporalis*). *Evolution*, **47**, 1118–1137.
- Fryer G, Iles TD (1972) *The Cichlid Fishes of the Great Lakes of Africa. Their Biology and Evolution*. Oliver and Boyd, Edinburgh.
- Futuyma DJ, Mayer GG (1980) Non-allopatric speciation in animals. *Systematic Zoology*, **29**, 254–271.
- Greenwood PH (1991) Speciation. In: *Cichlid Fishes: Behavior, Ecology and Evolution* (ed. Keenleyside MHA), pp. 86–102. Chapman & Hall, London.
- Hert E (1990) Factors in habitat partitioning in *Pseudotropheus zebra* (Pisces: Cichlidae), an introduced species to a species-rich community of Lake Malawi. *Journal of Fish Biology*, **36**, 853–856.
- Hert E (1992) Homing and home-site fidelity in rock-dwelling cichlids (Pisces: Teleostei) of Lake Malawi, Africa. *Environmental Biology of Fishes*, **33**, 229–237.
- Holzberg S (1978) A field and laboratory study of the behavior and ecology of *Pseudotropheus zebra* (Boulenger), an endemic cichlid of Lake Malawi (Pisces: Cichlidae) *Zeitschrift für zoologische Systematik und Evolutionsforschung*, **16**, 171–187.
- Hudson R (1990) Gene genealogies and the coalescent process. In: *Oxford Surveys in Evolutionary Biology* (eds Futuyma D, Antonovics J), pp. 1–44. Oxford University Press, Oxford.
- Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers: *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196–6200.
- Kohda M, Yanagisawa Y, Sato T *et al.* (1996) Geographic colour variations in cichlid fishes at the southern end of Lake Tanganyika. *Environmental Biology of Fishes*, in press.
- Koenings A, Dieckhoff W (1992) *Tanganyika Secrets*. Cichlid Press, Miami.
- Kosswig C (1947) Selective mating as a factor for speciation in cichlid fish of East African Lakes. *Nature (London)*, **159**, 604–605.
- Kuwamura T (1986) Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. *Journal of Ethology*, **4**, 129–146.
- Kuwamura T (1987) Distribution of fishes in relation to the depth and substrate at Myako, east-middle coast of Lake Tanganyika. *African Study Monographs*, **7**, 1–14.
- Lande R (1981) Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences USA*, **78**, 3721–3725.
- Lande R (1982) Rapid origin of sexual isolation and character divergence in a cline. *Evolution*, **36**, 213–223.
- Marlier G (1959) Observations sur la biologie littorale du lac Tanganyika. *Revue Zoologique et Botanique africainique*, **59**, 164–183.
- Marsh AC, Ribbink AJ, Marsh BA (1981) Sibling species complexes in sympatric populations of *Petrotilapia trewavas* (Cichlidae, Lake Malawi). *Zoological Journal of the Linnean Society*, **71**, 253–264.
- Matthes H (1962) Poissons nouveaux ou intéressants du lac Tanganyika et du Ruanda. *Annales Musee Royale Africainique Centrale Series Scientifique Zoologique*, **8**, 27–58.
- Mayr E (1963) *Animal Species and Evolution*. Oxford University Press, London.
- Mayr E (1984) Evolution of fish species flocks: a commentary. In: *Evolution of Fish Species Flocks* (eds Kornfield I, Echelle AA), pp. 3–11. University of Maine Press, Orono.
- McElroy DM, Kornfield I (1990) Sexual selection, reproductive behavior, and speciation in the mbuna species flock of Lake Malawi (Pisces: Cichlidae). *Environmental Biology of Fishes*, **28**, 273–284.
- McKaye KR & Gray WN (1984) Extrinsic barriers to gene flow in rock-dwelling cichlids of Lake Malawi: macrohabitat heterogeneity and reef colonization. In: *Evolution of Fish Species Flocks* (eds Kornfield I, Echelle AA), pp. 169–183. University of Maine Press, Orono.
- Meyer A (1993) Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology and Evolution*, **8**, 279–284.
- Meyer A, Kocher RD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature (London)*, **347**, 550–553.
- Meyer A, Morrissey JM, Scharl M (1994) Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature (London)*, **368**, 539–542.
- Moran P, Kornfield I (1993) Retention of an ancestral polymorphism in the mbuna species flock (Pisces: Cichlidae) of Lake Malawi. *Molecular Biology and Evolution*, **10**, 1015–1029.
- Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5269–5273.
- Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Processes and Theory* (eds Nevo E, Karlin S), pp. 515–534. Academic Press, NY.
- Nelissen M (1975) Contribution of the ethology of Simochromis diagramma (Günther) (Pisces, Cichlidae). *Acta Zoologica et Pathologica Antverpiensia*, **61**, 31–46.

- Nelissen M (1976) Contribution to the ethology of *Tropheus moorii* Boulenger (Pisces, Cichlidae). *Revue Zoologique africainique*, **90**, 17–29.
- Nelissen M (1977) *Pseudosimochromis*, a new genus of the family Cichlidae (Pisces) from Lake Tanganyika. *Revue Zoologique Africainique*, **91**, 730–731.
- Owen RB, Crossley R, Johnson TC, Tweddle D, Kornfield I, Davison S, Eccles DH, Engstrom DE (1990) Major low levels of Lake Malawi and implications for speciation rates in cichlid fishes. *Proceedings of the Royal Society (London) Series B*, **240**, 519–553.
- Palumbi SR, Wilson AC (1990) Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. *Evolution*, **44**, 403–415.
- Patton JL, Smith MF (1989) Population structure and the genetic and morphologic divergence among pocket gopher species (genus *Thomomys*). In: *Speciation and its Consequences* (eds Otte D, Endler JA), pp. 284–304. Sinauer, Sunderland.
- Poll M (1986) Classification des cichlidae du lac Tanganyika: tribus, genres et especies. *Mémoires de la Classe des Sciences Académie Royale de Belgique*, **45**, 1–163.
- Reeb CA, Avise JC (1990) A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. *Genetics*, **124**, 397–406.
- Reinthal PN, Meyer A (1997) Molecular phylogenetic tests of speciation models in African cichlid fishes. In: *Molecular Evolution and Adaptive Radiations* (eds Givnish TJ, Sytsma KJ). Cambridge University Press, Cambridge, in press.
- Ribbink AJ, Marsh BA, Ribbink AC, Sharp BJ (1983) A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African Journal of Zoology*, **18**, 149–310.
- Richardson LR, Gold JR (1995) Evolution of the *Cyprinella lutrensis* species group. III. Geographic variation in the mitochondrial DNA of *Cyprinella lutrensis* – the influence of Pleistocene glaciation on population dispersal and divergence. *Molecular Ecology*, **4**, 163–171.
- Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.
- Saunders NC, Kessler LG, Avise JC (1986) Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphenus*. *Genetics*, **112**, 613–627.
- Schliwen U.K, Tautz D, Pääbo S (1994) Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature (London)*, **368**, 629–632.
- Scholz CA, Rosendahl BR (1988) Low lake stands in Lake Malawi and Tanganyika. *Science*, **240**, 1645–1648.
- Scholz CA, Rosendahl BR (1990) Coarse-clastic facies and stratigraphic models from Lake Malawi and Tanganyika. *American Association of Petroleum Geologists Memoir*, **50**, 151–168.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.
- Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.
- Slatkin M, Maddison WP (1989) A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics*, **123**, 603–613.
- Snoeks J, Rüber L, Verheyen E (1994) The Tanganyika problem: comments on taxonomy and distribution patterns of its cichlid fauna. *Advances in Limnology*, **44**, 357–374.
- Sokal RR, Rohlf FJ (1994) *Biometry*, 3rd edn. Freeman, San Francisco.
- Sturmbauer C (1990) *Vergleichende Untersuchungen zur Physiologie und Ethologie herbivorer Fische*. PhD thesis, University of Innsbruck, Austria.
- Sturmbauer C, Meyer A (1992) Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature (London)*, **358**, 578–581.
- Swofford DL (1993) *PAUP. Phylogenetic Analysis Using Parsimony*, Version 3.1.1. Illinois Natural History Survey, Champaign, IL.
- Takahata N (1989) Gene genealogy in three related populations: consistency probability between gene and population trees. *Genetics*, **122**, 957–966.
- Takamura K (1984) Interspecific relationships of aufwuchs-eating fishes in Lake Tanganyika. *Environmental Biology of Fishes*, **10**, 225–241.
- Taylor EB, Bentzen P (1993) Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. *Evolution*, **47**, 813–832.
- Taylor EB, Dodson JJ (1994) A molecular analysis of relationships and biogeography within a species complex of Holarctic fish (genus *Osmerus*). *Molecular Ecology*, **3**, 235–248.
- Tiercelin J-J, Mondeguer J (1991) The geology of the Tanganyika through. In: *Lake Tanganyika and Its Life* (ed. Coulter GW), pp. 7–48. Oxford University Press, London.
- Turner GF (1994) Speciation mechanisms in Lake Malawi cichlids: a critical review. *Advances in Limnology*, **44**, 139–160.
- Turner GF, Burrows MT (1995) A model of sympatric speciation by sexual selection. *Proceedings of the Royal Society (London) Series*, **B260**, 287–292.
- Verheyen E, Rüber L, Snoeks J, Meyer A (1996) Mitochondrial phylogeography of rock dwelling cichlid fishes reflects lake level fluctuations in Lake Tanganyika. *Philosophical Transactions of the Royal Society London, Ser. B*, **351**, (in press).
- Vogler AP, DeSalle R (1993) Phylogeographic patterns in coastal North American tiger beetles (*Cincindela dorsalis say*) inferred from mitochondrial DNA sequences. *Evolution*, **47**, 1192–1202.
- Yanagisawa Y, Sato T (1990) Active browsing of mouthbrood ing femals 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.
