Molecular Evolution and Adaptive Radiation

Edited by

Thomas J. Givnish and Kenneth J. Sytsma
University of Wisconsin – Madison

1997

CAMBRIDGE
UNIVERSITY PRESS

12 Molecular phylogenetic tests of speciation models in Lake Malawi cichlid fishes

Peter N. Reinthal and Axel Meyer

The adaptive radiations of cichlids (Teleostei: Cichlidae) in the East African Great Lakes – Victoria, Tanganyika, and Malawi (Figure 12.1) – are extremely species-rich and bear testimony to the evolutionary success of these fishes. Each lake is occupied by a species flock of at least several hundred cichlid species (Fryer and Iles 1972; Lewis et al. 1986), most of which are endemic to that lake. Each species flock is thought to be a monophyletic assemblage (Greenwood 1984) and contains a sweeping array of morphologically and behaviorally specialized fishes occupying several different ecological roles (Fryer and Iles 1972). Given the unparalleled evolutionary and ecological success of each of these adaptive radiations, their evolutionary origin and ecological maintenance have been much debated and studied (e.g. Mayr 1963, 1984; Fryer and Iles 1972; Coulter 1991; Greenwood 1991; Meyer 1993b). Yet, despite a long history of research, the precise nature of the phylogenetic relationships among endemic cichlid faunas has remained largely unresolved. Few morphological synapomorphies characterize members of each radiation; convergent and parallel evolution in form and ecology, and the masking of informative morphological characters by autapomorphies have also hindered attempts to elucidate relationships among cichlids.

Guilds of ecologically similar, specialized species – such as scale-scrapers, mollusc-crushers, algae-scrapers, or paedophages – are found in all three species flocks (e.g., Fryer and Iles 1972). Similar morphological and behavioral solutions to similar ecological problems have thus arisen in each lake (Stiassny 1981; Greenwood 1983). For example, the Victoria endemic *Macropleurodus bicolor* and the Malawi endemic *Chilotilapia rhoadesii* both exhibit similar, highly derived dentitions, jaw structures, and feeding behaviors whereby they prey on gastropods, crushing their shells with their oral jaws (Greenwood 1983).

The similarity in morphological and behavioral specializations in separate basins gives rise to the much debated question of their origin through common ancestry or convergence/parallelism. If each kind of specialization arose only once in East African cichlids, it would indicate the polyphyly of the species flocks, with each of several different lineages having a geographic distribution that extends beyond the boundaries of a single lake (Stiassny 1981; Greenwood 1983). The remarkable morphological and behavioral similarities among guild members from different lake basins have led authors to suggest that, if such species were found in the same basin, they would be placed in the same genus (Fryer and Iles 1972; Greenwood 1983). Such interpretations, if correct, would mean that each species flock is polyphyletic.

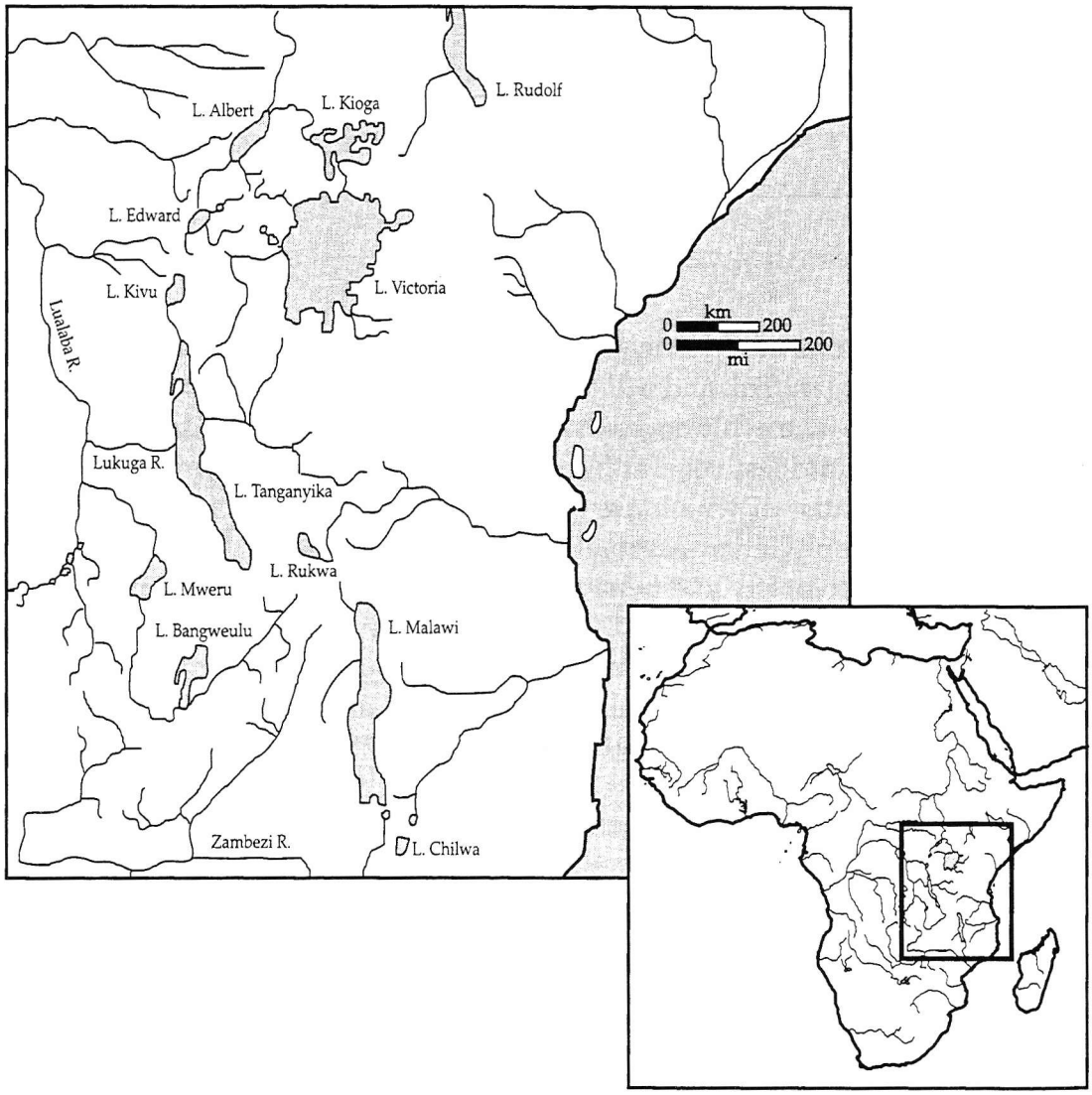


Figure 12.1. Geography of East Africa, including major river systems and the Great Lakes of the rift zone (redrawn after Fryer and Iles 1992).

The lack of morphological synapomorphies that characterize members of each species flock has called into question the utility of even the most careful of phylogenetic reconstructions based on morphology (see Stiassny 1991 for discussion), and highlights the need for other kinds of characters with which to reconstruct the evolutionary history of the East African cichlids. Variation in DNA sequences has proven to be a useful source of phylogenetic information, independent of homoplasy-ridden morphology (Meyer et al. 1990). Based on such data, it now appears well established that cichlid species flocks from Lake Victoria and Lake Malawi are each monophyletic and arose quite recently within the confines of their lakes in a true intralacustrine manner (Meyer et al. 1990, 1991; Sturmbauer and Meyer 1992; Meyer 1993b; Meyer et al. 1994). DNA studies on the question of the between-flock relationships (Meyer et al. 1990; Kocher et al. 1993) demonstrated that the striking morphological

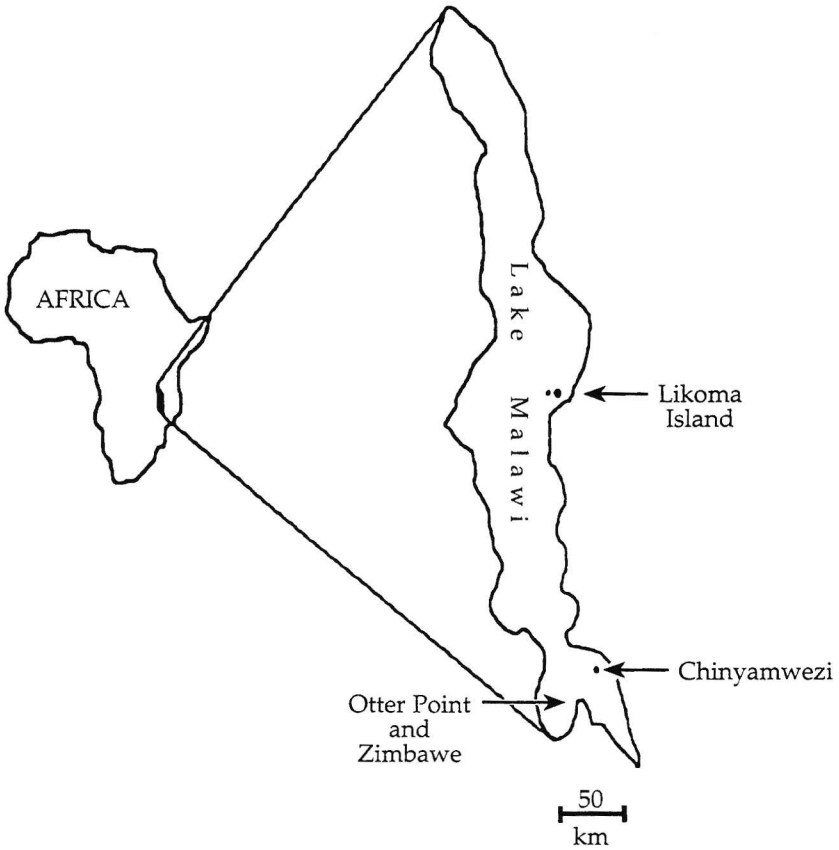


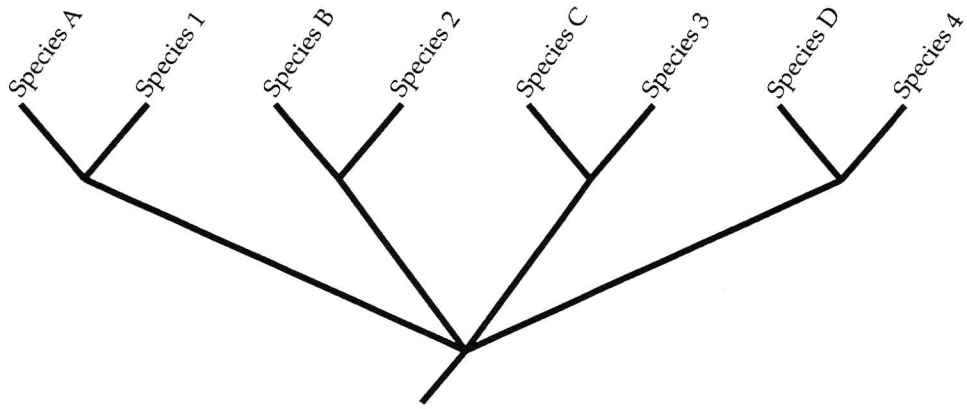
Figure 12.2. Lake Malawi, showing locations where cichlid specimens were collected. See Table 12.1 for a list of the ecologically equivalent pairs of species at Likoma Island and Otter Point.

similarities between certain members of flocks in different lakes (e.g., *Macropleurodus/Chilotilapia*) did, in fact, evolve convergently.

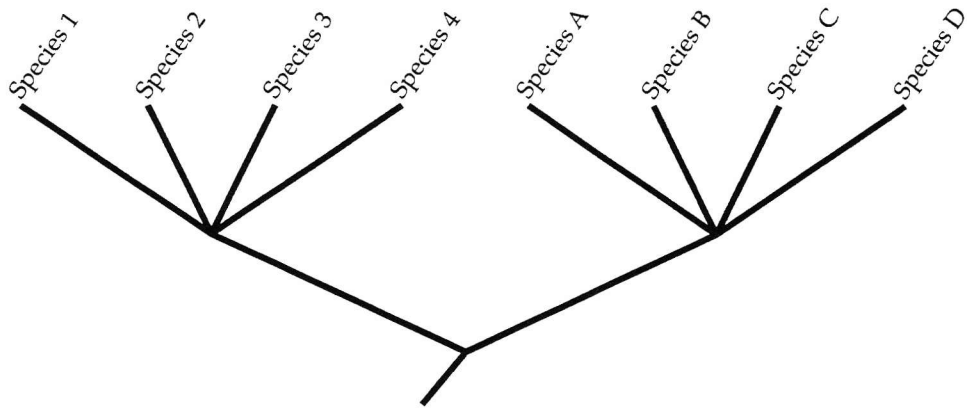
Lake Tanganyika contains the oldest and genetically most diverse cichlid species flock (Nishida 1991). This flock is composed of several morphologically and genetically distinct lineages (Nishida 1991; Sturmbauer and Meyer 1992, 1993; Sturmbauer et al. 1994). The monophyly of the Lake Tanganyika flock has not been clearly established (Nishida 1991; Sturmbauer and Meyer 1992; Kocher et al. 1993; Meyer 1993b; Moran et al. 1994; Sturmbauer et al. 1994; Sültmann et al. 1995; Meyer et al. 1996). Several ancestral lineages are likely to have colonized the Tanganyika basin and relationships among its lineages are still unknown (Sturmbauer and Meyer 1992, 1993; Kocher et al. 1993; Meyer 1993; Sturmbauer et al. 1994; Meyer et al. 1994; Sültmann et al. 1995). It is known, however, that several lineages of Tanganyika cichlids have been able to leave the confines of that lake's basin. For example, a small number of lamprologine cichlids – a lineage otherwise found only in Lake Tanganyika – live in the Zaire river. These lamprologines had previously been assumed to be representatives of basal lineages that, early in the evolution of the Tanganyika flock, colonized that lake and gave rise to about 30% of all cichlid species now found in it. However, mitochondrial DNA data (Sturmbauer et al. 1994) suggest that riverine lamprologine

species are recently derived, and are likely to have left the lake when the Lukaga River began to flow from Lake Tanganyika into the Zaire River.

It is apparent from the phylogeny of the three species flocks (reviewed by Meyer 1993b and Meyer et al. 1994) that the endemic haplochromine cichlids of Lake Tanganyika are closely related to the lineages of East African riverine haplochromines that first colonized Lake Malawi and more recently Lake Victoria (Nishida 1991; Sturmbauer and Meyer 1992). The haplochromine species flocks endemic to Lakes Malawi and Victoria are much younger than the Lake Tanganyika species flock, and therefore genetically more similar to each other than either is to the Tanganyika flock. There are several East African haplochromine lineages that are not endemic to lakes (e.g., *Astatoreochromis, Astatotilapia, Serranochromis*) which are more closely related to the



A. Specialist Colonization



B. Generalist Colonization

Figure 12.3. Alternative phylogenies expected under two alternative speciation models. Species A – D represent those from Otter Point, while species 1 – 4 represent those from Likoma Island from the middle of Lake Malawi. Species 1 and A, 2 and B, 3 and C, and 4 and D are the ecologically equivalent pairs of species. (**A**) Under the specialist-colonization hypothesis, ecological and morphological specialization precedes colonization and geographically distinct specialists would be more closely related to each other than they are to other members found at the same locality. (**B**) Under the generalist colonization hypothesis, all species found at a locality would be descended from the same generalist ancestor, and hence be more closely related to each other than to corresponding specialists from other localities.

haplochromines endemic to Lakes Malawi and Victoria than to those endemic to Lake Tanganyika (Meyer et al. 1990, 1991; Meyer et al. 1994; Sültmann et al. 1995).

Possibility of sympatric speciation and radiation

Molecular systematics can not only provide insights into relationships among broad groups of organisms, but can also permit tests of alternative mechanisms of speciation that may have generated closely related taxa within individual flocks. Speciation is a central issue in evolutionary biology (Slatkin 1987; Lynch 1989; Otte and Endler 1989; Coyne 1992) but only few phylogenetic tests of models of speciation have been conducted (e.g., Otte and Endler 1989; Harrison 1991; Coyne 1992; Mayden 1992). The intralacustrine origins of the haplochromine species flocks does not necessarily imply that they each arose through sympatric speciation. All three large East African lakes underwent repeated lake-level fluctuations that could have fragmented single basins into several separate or satellite lakes that persisted for thousands of years. These major lake-level changes shaped the current distribution of genetic variation and are likely to have been a major influence on speciation in these fishes by restricting gene flow to individual basins (Sturmbauer et al. 1994; Johnson et al. 1996; Meyer et al. 1996; Verheyen et al. 1996). In this paper, we present a case study involving an endemic group of Lake Malawi cichlids to demonstrate how phylogenetic information might be used to test conflicting theories for speciation and the origin of convergence.

Lake Malawi contains an estimated 500 to 1,000 endemic cichlid species, and hosts more species of fish than any other lake in the world (Fryer and Iles 1972; Ribbink et al. 1983; Lewis et al. 1986). One monophyletic lineage of rock-dwelling cichlids known as "mbuna" is a diverse assemblage of more than 200 species; they are restricted to rocky habitats, have localized geographic distributions and very limited dispersal (Hert 1992), and are believed to have evolved extremely rapidly (Fryer 1959, 1977; Kornfield 1978; Ribbink et al. 1983; Lewis et al. 1986; Owen et al. 1990). The eight mbuna species examined in this paper (Table 12.1) are species belonging to the *Pseudotropheus tropheops* species complex. This complex consists of at least 38 known species and is expected to be assigned to a new genus (D. S. Lewis and P. N. Reinthal, unpubl. data). The informal species names used here are those of Ribbink et al. (1983). The status of these taxa as distinct species is supported by genetic, morphological, behavioral, colorational, and

Table 12.1. Species pairs from the *Pseudotropheus tropheops* species complex from Otter Point and Likoma Island, with a summary of the ecological strategies that differentiate sympatric species and unite pairs of allopatric species.

Species Pairs		Ecological Strategy		
Otter Point	**Likoma Island**	**Depth**	**Sediment**	**Habitat**
P. t. "orange chest"	*P. t.* "red cheek"	Shallow	Free	Rock surfaces
P. t. "broad mouth"	*P. t.* "yellow chin"	Shallow	Rich	Rock/Sand
P. cf. *gracilior*	*P. t.* "dark"	Deep	Medium	Rock surfaces
P. cf. *microstoma*	*P. t.* "membe"	Deep	Rich	Rock/Sand

ecological differences (Ribbink et al. 1983; P. N. Reinthal, unpubl. data). Although presumptive data have been presented for hybridization between separate mbuna genera elsewhere (Stauffer et al. 1996), hybridization has never been observed in the field and species translocated within Lake Malawi remain distinct and do not interbreed (Ribbink et al. 1983; P. N. Reinthal, unpubl. data).

Each of the eight species examined has a very restricted geographic distribution, limited to a small part of the shore of Lake Malawi. This kind of distribution is typical of the vast majority of endemic cichlids in all three lakes, especially among rock-dwelling taxa (Reinthal 1993). Only open-water species tend to occur throughout a whole lake basin (e.g., Fryer and Iles 1972; Coulter 1991). At each of two localities from Lake Malawi – Likoma Island in the north, and Otter Point about 200 km to the south (Figure 12.2) – four species were found to be the major representatives of the *Pseudotropheus tropheops* species complex. The four species at a given locality show some ecological differentiation. More important, however, each species corresponds to an ecologically equivalent species at the other locality. Both members of each pair have extremely similar ecological habits (Table 12.1) and morphological adaptations (P. N. Reinthal, unpubl. data). At least two evolutionary models, involving initial colonization by a generalist or specialist, could account for the presence of species with equivalent ecological strategies at separate locations (Figure 12.3).

Allopatric differentiation

Like other East African lakes, Lake Malawi experienced major and minor lake-level fluctuations during its geological history (Sturmbauer and Meyer 1992; Johnson et al. 1996; Sturmbauer et al., submitted). Most recently between 1 and 25 thousand years ago, a large drop in lake level severely disrupted mbuna habitat availability and use (Fryer 1977; Scholz and Rosendahl 1988; Owen et al. 1990). The specialist colonization model (Figure 12.3A) assumes that speciation and ecological specialization occurred in an ancestral lake community during periods of low water levels, when species were concentrated in smaller areas and competition was intensified (McKaye and Marsh 1983). During these high competition situations, habitat preferences and morphological specializations might have been established (Fryer 1959). Ecotypic and morphologically specialized species would colonize newly available appropriate habitat in different localities as lake level rose again. Parallel specialists (sister species under this model) at disjunct localities might later differentiate through breeding preferences as a result of limited dispersal capabilities (imposed by stenotopy, philopatry, and mouth brooding) and sexual selection or genetic drift in populations isolated by non-rocky habitat (Kosswig 1947; Lande 1981; Dominey 1984; McKaye et al. 1990; Ribbink 1991). Male coloration appears to be an important factor in both male-male competition for territories and female mate choice. Both of these factors would be consistent with rapid evolution under models of sexual selection. If sexual selection were to result in different color patterns in allopatric populations, ecological equivalents would be classified as distinct taxa. The same scenario would be true if coloration were to change rapidly as a result of founder events. Both sexual selection and drift are consistent with micro-allopatric models of speciation, but require that ecological

specializations evolve prior to colonization events. In either case, the testable prediction of the specialist colonization model is that allopatric pairs of species with similar ecological and morphological specializations would be more closely related to each other than to other members of their respective local communities (Figure 12.3A).

Sympatric differentiation

Alternatively, the generalist colonization model (Figure 12.3B) assumes that habitat and open niches became available as the lake level rose, and that ancestral colonists of different new communities were not specialists but generalists. We define a generalist colonist as a species that utilized a wider range of habitats than is now observed for individual taxa. Ecological specialization, morphological differentiation, and speciation would then have occurred subsequent to colonization via sympatric specialization to different microhabitats. Different communities would evolve in parallel in separate localities. This model would result in the formation of similar communities of sympatric but ecologically distinct species. Under this "generalist colonization model," a species' closest relatives would be found at the same locality irrespective of their particular ecological strategy or morphological adaptations (Figure 12.3B). The ecologically equivalent species at separate geographic localities within the lake would be relatively distantly related to each other, having evolved parallel adaptations. The generalist colonization model predicts that genetic variation correlates with locality rather than morphological or ecological similarity between species and would suggest repeated convergent evolution within a single lake basin.

Striking examples of convergence between endemic species of cichlid flocks from different East African lakes have been demonstrated before based on mitochondrial DNA-based phylogenetic analyses at the between-lake level, but not before at the within-lake level (see Meyer et al. 1990; Kocher et al. 1993; Meyer 1993b). In this paper we examine the possibility that ecological and morphological specializations in mbunas have evolved sympatrically at very local scales.

Materials and methods

Populations sampled, ecological and morphological data

Eight species of the *Pseudotropheus tropheops* species group were studied at Likoma Island and Otter Point in Lake Malawi (Figure 12.2). Underwater observations and transect studies revealed that each species has distinct and restricted patterns of habitat use and resource utilization; each species had an apparent equivalent at the other locality (Table 12.1). Ecological preferences characterized also included depth, habitat type, and sediment restrictions. Stomach contents analyses demonstrated that different species at each locality eat algal species in different proportions, and thus are ecologically separated along this axis as well (P. N. Reinthal, in prep.).

Amplification and sequencing of mtDNA

Nucleotide sequences of the mitochondrial genome (mtDNA) were obtained for 29 individuals of nine species of the *P. tropheops* group. We analyzed four species from

Otter Point, four species with equivalent ecological strategies from Likoma Island, and the only *P. tropheops* species found at Chinyamwezi Rock, a location in the southeast arm of Lake Malawi (Figure 12.2). In addition, *P. tropheops* "orange chest" is also found at Zimbawe Rock, an island about 7 km from Otter Point; individuals from both sites were examined to determine geographic variation within a species. Representatives of two other mbuna genera (*Labeotropheus fuelleborni, Labidochromis* sp.) and another Malawi haplochromine (*Chilotilapia rhoadesi*) were sequenced as outgroup taxa. A 427 base-pair segment coding for part of the threonine transfer RNA gene, all of the proline tRNA gene, and the most variable part of the control region were sequenced after amplification, using the primer sequences and protocol of Kocher et al. (1989) with minor modifications (Meyer et al. 1990; Sturmbauer and Meyer 1992). Total DNAs were extracted from frozen or ethanol (75%) preserved muscle tissues following standard procedures (Kocher et al. 1989; Meyer et al. 1990).

Table 12.2. Ten variable mtDNA sites, showing genetic variability for the species examined from the *Pseudotropheus tropheops* species complex from Otter Point, Zimbawe, and Chinyamwezi in southern Lake Malawi and Likoma Island in the north. The informal species names are those of Ribbink et al. (1981); all individuals (number shown) of a species from a given site were genetically identical.

			Site									
			64	73	105	185	253	268	275	276	348	356
Locality	**Species**	**No.**	1	2	3	4	5	6	7	8	9	10
Otter Point	*P. t.* "broad mouth"	2	T	A	C	C	C	T	C	T	T	C
	P. cf. *gracilior*	2	T	A	C	C	C	T	C	T	T	C
	P. cf. *microstoma*	2	T	G	C	C	C	T	T	T	T	T
	P. t. "orange chest"	4	T	A	C	C	C	T	C	T	T	C
Zimbawe	*P. t.* "orange chest"	3	T	A	C	C	C	T	C	T	T	T
Chinyamwezi	*P. t.* "chinyamwezi"	3	T	A	C	T	T	T	C	C	C	T
Likoma Island	*P. t.* "red cheek"	3	T	A	C	C	C	T	C	T	T	C
	P. t. "yellow chin"	3	T	A	T	T	C	T	C	C	C	T
	P. t. "dark"	3	C	A	C	T	T	C	C	C	C	T
	P. t. "membe"	4	T	A	C	T	T	T	C	C	C	T

Phylogenetic analysis

The genealogical relationships among the mtDNA sequences were estimated under maximum parsimony using PAUP 3.0s (Swofford 1991). Given the high degree of similarity among taxa, there were no ambiguities in the alignment. Exhaustive searches were conducted. Geographic and ecological characters were superimposed on the resulting tree using MacClade version 3.05 (Maddison and Maddison, 1992). The robustness of the phylogenetic estimates was tested by bootstrap analysis (Felsenstein 1985) using PAUP with 100 bootstrap replications and the support decay index (Bremer 1988).

Results

Ecology

Observations on the ecology and morphology of the *Pseudotropheus tropheops* species complex revealed differentiation among sympatric members of a community and similarities between communities at different localities (P. N. Reinthal, in prep.). From the surface to the depth at which rocky bottoms are replaced by sand (the entire depth range for the species in question), two of the four species at each locality were restricted to shallow water, and two were restricted to deeper water. At Otter Point, *P. t.* "broad mouth" and *P. t.* "orange chest" were shallow-water species, with individuals defending territories at mean depths of 2.0 ± 0.6 m (s.d.) and 2.9 ± 1.3 m, respectively. The deep-water species, *P. gracilior* and *P. microstoma,* showed mean territory depths of 5.2 ± 1.2 m and 4.5 ± 1.1 m. The maximum depth of utilizable habitat at this site is 7.6 m; no overlap in depth was observed between shallow- and deep-water species.

At Likoma Island, we also found two shallow-water and two deep-water species. The mean depth of territories for *P. t.* "yellow" and *P. t.* "red cheek" were 3.0 ± 2.3 m and 3.9 ± 2.7 m; *P. t.* "dark" and *P. t.* "membe" showed mean depths of 9.7 ± 2.3 m and 12.1 ± 0.9 m, respectively. Utilizable rocky habitat at Likoma extended from the surface to a depth of more than 30 m.

Sympatric species found at roughly the same depth differed by microhabitat utilization. In shallow water at Otter Point, *P. t.* "orange chest" defended territories centered over large boulders and rock surfaces. *P. t.* "broad mouth" was found over sand patches among rocks. In deeper water, *P. gracilior* defended large, sediment-covered rocks; *P. microstoma* occupied the rock/sand interface. Similar ecological differentiation by microhabitat occurred at Likoma Island. Shallow *P. t.* "red cheek" and deep *P. t.* "dark" were found over rock surfaces; shallow *P. t.* "yellow chin" and deep *P. t.* "membe" at the rock/sand interface.

The diets of each species reflect this habitat partitioning. The diets of species found over rocks in shallow water were dominated by attached algae (*Cladophora, Calothrix*), while the diets of species found over rocks in deep water were dominated by diatoms and other pelagic algae that settle on rock surfaces. Species found over rock/sand interfaces in shallow water were omnivores; similar species in deep water had the greatest dietary proportion of invertebrates.

Trophic morphological traits also showed differentiation among sympatric species. Characters such as neurocrania shape, mouth shape, tooth shape, and intestinal morphology can all be used as predictors of ecological strategy. There are many apparent convergences in morphology between ecologically similar species at the two different sites, but these are beyond the scope of the current paper (P. N. Reinthal, in prep.).

Molecular systematics

We found a remarkably high level of genetic similarity within the *Pseudotropheus tropheops* complex in Lake Malawi. In nine *P. tropheops* species examined, only 10 of 427 bases exhibited base substitutions. All of the observed substitutions are transitions

(Table 12.2), further supporting the notion that these species are very closely related (Brown et al. 1982). Only one transition involves a purine; pyrimindine transitions tend to be more frequent than purine transitions in fishes (Meyer 1993a).

Five substitutions were phylogenetically informative, and five were autapomorphic (sites 1, 2, 3, 6, and 7) (Table 12.2). Three of the five informative substitutions (sites 4, 8, and 9) supported the hypothesis that all species from a locality are more closely related to each other. A mutation at site 5 united three species from Likoma Island; another mutation at site 10 was shared by three species from Otter Point. No intraspecific variation was found in any species. It is interesting that retention of ancestral polymorphisms of mitochondrial DNA has been proposed for Malawi cichlids of the *Pseudotropheus zebra* species group (Moran and Kornfield 1993, 1995). However, if the results presented here were due to ancestral polymorphism, the polymorphism would be expected to be observed in at least one of the two localities. Given the number of individuals examined here (29), the probability of the data showing the biogeographic pattern observed here would be exceedingly low. This is not to say that the polymorphism did not become alternatively fixed at the two distinct localities or there was a possibilty of mtDNA capture. Until the presence of a polymorphism is detected in these taxa or variation in nuclear DNA is examined, the phylogenetic signal of the data presented here should not be ignored.

Conspecifics of *P. tropheops* "orange chest" from Zimbawe Island and Otter Point (Figure 12.2) differed only at site 10. The exceedingly small amount of genetic variation within and between communities supports the idea of an exceptionally recent origin of the Lake Malawi species flock, and agrees with other estimates of extremely limited genetic variation found in species of the Malawi and Victoria species flocks and in some Neotropical cichlids (Kornfield 1978; Owen et al. 1990; Meyer et al. 1990; Sturmbauer and Meyer 1992; Moran and Kornfield 1993, 1995; Sültmann et al. 1995; but see Klein et al. 1993 and Ono et al. 1993 for MHC variation in Lake Malawi cichlids). Because mtDNA evolves 5 to 10 times faster than a typical single-copy nuclear-protein-coding gene and the portion sequenced is the fastest evolving region of the entire mitochondrial genome (Meyer 1993a) the amount of DNA variation detected in this study would be unlikely to be increased by further sequencing of other portions of mtDNA.

Even though Otter Point and Likoma Island species differed from each other by only about 0.7% sequence divergence, we are able to use mtDNA sequences to assess the evolutionary relationships of these taxa. A parsimony analysis without outgroups results in a single most parsimonious tree (CI = 1.0, tree length = 10 steps) and reveals that all Otter Point species are genetically more closely related to each other than they are to any Likoma Island species and vice versa. This result supports the generalist-colonization model (Figure 12.3B) and documents the parallel evolution of extensive ecological and morphological similarity within a single species flock. A bootstrap analysis with no outgroup supports the Likoma-Otter Point distinction at levels over 95%. The central branch of an unrooted tree that differentiates the species from the two localities has a decay index of three. If the tree is constrained to support the specialist-colonization model, our data require a tree 23 steps in length. This would represent an additional 13 steps from the most parsimonious tree.

Genetic separation of Otter Point and Likoma species is supported by parsimony using representatives of two other mbuna genera (*Labeotropheus fuelleborni* or *Labidochromis* sp.) or another Malawi haplochromine cichlid species (*Chilotilapia rhoadesii*) as outgroup taxa. For example, with *Chilotilapia rhoadesii* as outgroup, we find three equally parsimonious trees (length = 13 steps; CI = 0.85; RI = 0.87). The strict consensus tree separates the Likoma species (with *Pseudotropheops tropheops* "red cheek," *P. t.* "dark" and *P. t.* "membe" as an unresolved trichotomy) from the Otter Point species (with *P. t.* "orange chest," *P. t.* "broad mouth," and *P. gracilior* as an unresolved trichotomy); it never supports the specialist-colonization model by placing ecologically equivalent species as sister taxa.

Including the Zimbawe *Pseudotropheops tropheops* "orange chest" population and *P. t. íchinyamwezií* in a parsimony analysis, using PAUP with *Chilotilapia rhoadesii* as an outgroup, results in nine most parsimonious trees (length = 14 steps; CI = 0.79; RI = 0.84). The strict consensus tree unites all four species from Likoma Island as sister taxa, but leaves those from Otter Point, Zimbawe, and Chinyamwezi as unresolved basal taxa. Three of the species – *P. t.* "red cheek," *P. t.* "dark," and *P. t.* "membe" – form an unresolved trichotomy sister to *P. t.* "yellow chin." These results also support the generalist-colonization model.

Discussion

Generalist-colonization model supported

The genetic data clearly support the model of *in situ* parallel evolution at separate geographic localities (Figure 12.3B) because we found that sympatric species are more closely related to each other than they are to their allopatric ecologically equivalent species. The most parsimonious explanation of these results is that colonization occurred, possibly by a generalist, at different parts of the lake and preceded ecological specialization and speciation events. These results make it unlikely that ecological specialization and speciation predated colonization, and instead support the hypothesis that speciation and morphological diversification occurred sympatrically on repeated, independent occasions at geographically separated sites in Lake Malawi. Interestingly, at localities where only a single *P. tropheops* species is found (e.g., Chinyamwezi, Figure 12.2), it expands its ecological range to encompass habitat used by all four species at Likoma Island or Otter Point (P. N. Reinthal, unpubl. data).

This finding tends to implicate ecological specialization as a driving force behind speciation and morphological differentiation at any one locality. However, divergence of trophic morphology need not necessarily precede or initiate speciation. If, for example, differences in coloration arose through sexual selection it could reproductively isolate populations in different habitats (Lande 1981; Dominey 1984; McKaye, Louda, and Stauffer 1990; McKaye 1991; Ribbink 1991; McElroy and Kornfield 1991), so that morphological and ecological divergence could then evolve secondarily (Reinthal 1990).

Parallel evolution of morphological traits within a single lake basin

Ecologically equivalent species from Likoma Island and Otter Point are very similar morphologically; there appears to be as much morphological variation within a species of *Pseudotropheops tropheops* at a given site as there is between it and the corresponding species from the other site. Morphometric analyses of neurocrania, body shapes, mouth shapes, and intestines revealed features that are more similar between ecologically equivalent species from different localities than between sympatrically occurring species (P. N. Reinthal, unpubl. data). Because ecologically equivalent species are not closely related genetically, it appears that morphological parallelism and convergence are important in cichlid evolution. Such homoplasy has previously been found to obstruct phylogenetic inference based on morphology at broader taxonomic scales (Meyer et al. 1990; Kocher et al. 1993). Convergence involving cichlids from different lakes has been identified using molecular techniques (Meyer et al. 1990; Meyer et al. 1991; Kocher et al. 1993; Meyer 1993b), including instances where it had been incorrectly interpreted as an indication of common recent ancestry (Greenwood 1983). This study presents the first clear example of within-lake parallel evolution in cichlid morphology and ecology, and suggests that speciation and adaptive radiation may have occurred sympatrically, at least in some instances.

Trophic morphology and phylogeny

Much of the morphological variation found in the family Cichlidae is related to trophic structures (particularly the oral jaw) which appear subject to strong selection pressures and convergence, so care must be taken in using trophic morphology as a basis for reconstructing cichlid phylogeny. There is a tendency for autapomorphies in trophic structure to mask synapomorphic variation in such traits as well. Previous work on phenotypic plasticity (e.g., Meyer 1987; Wimberger 1991) and trophic polymorphisms (Meyer 1990) demonstrated that trophic structures are highly labile and often easily influenced by environmental factors. Molecular phylogenetic analyses have also revealed that some cichlid species of Lake Tanganyika show large, discontinuous divergence in trophic structures even if they are closely related to each other (Sturmbauer and Meyer 1992, 1993; Verheyen et al. 1996).

Models of speciation

Our findings suggest that a significant amount of local differentiation takes place *in situ*. New species could potentially arise via a sympatric model in which differential habitat utilization and small genetic differences would promote reproductive isolation (Kondrashov and Mina 1986; Maynard Smith 1986). Sympatric speciation has been suggested as being responsible for the co-occurrence of a small number of cichlid species in two crater lakes in Cameroon (Schliewen et al. 1994). Alternatively, our results are also consistent with a micro-allopatric speciation model, in which a relatively small area of incompatible habitat (e.g., open water, or sand between rocks) forms an effective barrier to dispersal (Fryer 1977; Sturmbauer and Meyer 1992; Moran and Kornfield 1995). Species isolated over short distances could differentiate rapidly. Short-term fluctuations in lake level would re-unite differentiated taxa (i.e.,

sister species) and competition for shared, preferred resources would result in divergent selection for use of different microhabitats. Different patterns of microhabitat (e.g., depth) and resource utilization might lead to morphological differentiation between reproductively isolated taxa (as a result of differing selection pressures, phenotypic plasticity, or a combination of both) and reinforce reproductive isolation. The resulting distribution of genetic variation would have all members of a particular location more closely related to each other than to ecological equivalents from other more distant locations.

Several different speciation models have been suggested to account for the evolutionary origin of the adaptive radiation of cichlid fishes (Kosswig 1947; Fryer 1959; Fryer and Iles 1972; Fryer 1977; Kornfield 1978; Ribbink et al. 1983; Lewis et al. 1986; Meyer et al. 1990; Owen et al. 1990; Dominey 1991; Greenwood 1991; Sturmbauer and Meyer 1992; Meyer 1993b). Our data support the notion of true intralacustrine speciation. Speciation occurred (and is occurring) at many sites within Lake Malawi simultaneously and in parallel, probably through the mechanism of sympatric or micro-allopatric speciation. The availability of new habitat or open niches for these fishes is largely influenced by physical factors such as lake-level fluctuation (Ribbink et al. 1983; Scholz and Rosendahl 1988; Owen et al. 1990; Sturmbauer and Meyer 1992; Meyer et al. 1996; Verheyen et al. submitted; Sturmbauer et al. submitted). These abiotic changes contribute to intralacustrine speciation since gene flow seems to be extremely restricted and only newly available habitats allow for the expansion of species (Sturmbauer and Meyer 1992; Bowers et al. 1994; Moran and Kornfield 1995). We believe that these factors, in combination with tight ecological specialization and the combined synergistic effects of mating systems and sexual selection, are largely responsible for the evolutionary origin of the extraordinary species assemblages of cichlid fishes in Lake Malawi.

Conclusions

The remarkable adaptive radiations of cichlid fishes of the African Great Lakes are well known for their "explosive" rates of speciation and extensive ecological and morphological differentiation. The debate about which mechanisms of speciation underlie the formation of these adaptive radiations has not, however, been settled. The species flocks of cichlids in each lake provide model systems for testing different speciation scenarios for the origin of these adaptive radiations. Four pairs of cichlid species of the *Pseudotropheus tropheops* complex at two localities (separated by roughly 200 km) in Lake Malawi were found to have equivalent ecological strategies and morphological adaptations. To distinguish between two alternative models to account for this pattern, a 427-bp stretch of the fastest evolving portion of the mitochondrial genome was sequenced from 31 individuals of 12 species. Hypotheses based on an assumption of an initial colonization of each site by (i) several lineages of specialists or (ii) a generalist that then diversified *in situ* were tested by combining quantitative data on ecology and morphology with a molecular phylogeny based on DNA sequences. Cladistic analyses of the sequence data indicated that all species

from the same locality – irrespective of morphological or ecological specialization – share an immediate common ancestor, and thus evolved in a true intralacustrine manner. These results suggest that species evolved *in situ*, via sympatric or microallopatric speciation, and that convergent morphological diversification and ecological specialization evolved repeatedly and in parallel following colonizations of geographically separate areas within the basin of Lake Malawi.

Acknowledgments

We thank J. Coyne, J. Endler, D. Futuyma, E. Mayr, L. McDade, G. Orti, M. Stiassny, C. Sturmbauer, P. Wimberger, and G. Kling for insightful comments on earlier drafts and B. Curtsinger, J. Whitman, and R. Jennings for help during fieldwork in Malawi. We thank the Government of Malawi for permission to conduct the fieldwork and I. Kornfield and P. Moran for some purified mitochondrial DNAs. Part of this work was done while PNR was a Kalbfleisch Research Fellow at the American Museum of Natural History and AM an Alfred P. Sloan Postdoctoral Fellow in the laboratory of the late Allan C. Wilson at the University of California, Berkeley. Support came from the American Museum of Natural History and the National Geographic Society to PNR. Support came from the National Science Foundation (BSR-9107838) and the Alfred P. Sloan Foundation to AM and the National Science Foundation and the National Institutes of Health to the late Allan C. Wilson.

References

Bowers, N., Stauffer, J. R., and Kocher, T. D. 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.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803.

Brown, W. M, Prager E. M., and Wang, A. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. Journal of Molecular Evolution 18:225–239.

Coulter, G. W. 1991. Zoogeography, affinities and evolution with special regard to the fishes. Pp. 275–305 in *Lake Tanganyika and its life*, G.W. Coulter, ed. London, England: Oxford University Press.

Coyne, J. A. 1992. Genetics and speciation. Nature 355:511–515.

Dominey, W. J. 1984. Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiin *Drosophila*. Pp. 231–254 in *Evolution of fish species flocks*, A. A. Echelle and I. Kornfield, eds. Orono, ME: University of Maine Press.

Felsenstein, J. 1985. Confidence limits on phylogenies. Evolution 39:783–791.

Fryer, G. 1959. The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting Cichlidae. Proceedings of the Zoological Society of London 132:153–281.

Fryer, G. 1977. Evolution of species flocks of cichlid fishes in African lakes. Zeitschrift für Zoologische Systematik und Evolution-forschungen 15:141–165.

Fryer. G., and Iles, T. D. 1972. The cichlid fishes of the great lakes of Africa. Edinburgh, Scotland: Oliver and Boyd.

Greenwood, P. H. 1983. On *Macropleurodus, Chilotilapia* (Teleostei, Cichlidae) and the interrelationships of African cichlid species flocks. Bulletin of the British Museum of Natural History (Zoology) 45:209–231.

Greenwood, P. H. 1984. African cichlids and evolutionary theories. Pp. 141–154 in *Evolution of fish species flocks*, A. A. Echelle and I. Kornfield, eds. Orono, ME: University of Maine Press.

Greenwood, P. H. 1991. Speciation. Pp. 86–102 in *Cichlid fishes: behaviour, ecology and evolution,* M. H. Keenleyside, ed. London, England: Chapman and Hall.

Harrison, R. G. 1991. Molecular changes at speciation. Annual Review of Ecology and Systematics 22:281–308.

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.

Klein, D., Ono, H., O'Huigin, C., Vincek, V., Goldschmidt, T., and Klein, J. 1993. Extensive MHC variability in cichlid fishes of Lake Malawi. Nature 364:330–334.

Kocher, T. D., Conroy, J. A., McKaye, K. R., and Stauffer, J. R. 1993. Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. Molecular Phylogenetics and Evolution 2:158–165.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F. X., and Wilson, A. C. 1989. Dynamics of mitochondrial DNA evolution: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences, USA 86:6196–6200.

Kondrashov A. S., and Mina, M. V. 1986. Sympatric speciation: when is it possible? Biological Journal of the Linnean Society 27:201–223.

Kornfield, I. 1978. Evidence for rapid speciation in African cichlid fishes. Experientia 34:335–336.

Kosswig, C. 1947. Selective mating as a factor for speciation in cichlid fish of East African Lakes. Nature 159:604–605.

Lande, R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Proceedings of the National Academy of Sciences, USA 78:3721–3725.

Lewis, D. S. C., Reinthal, P. N., Trendall, J. 1986. *A guide to the fishes of Lake Malawi national park.* Gland, Switzerland: World Wildlife Fund.

Lynch, J.D. 1989. The gauge of speciation: on the frequencies of modes of speciation. Pp. 527–553 in *Speciation and its consequences,* D. Otte and J. A. Endler, eds. Sunderland, MA: Sinauer Associates.

Maddison, W. P,., and Maddison, D. R. 1992. *MacClade: Analysis of phylogeny and character evolution, version 3.05.* Sunderland, MA: Sinauer Associates.

Mayden, R. L. 1992. *Systematics, historical ecology and North American freshwater fishes.* Palo Alto, CA: Stanford University Press.

Maynard Smith, J. 1966. *Sympatric speciation.* American Naturalist 100:637–650.

Mayr, E. 1963. *Animal species and evolution.* Cambridge, MA: Harvard University Press

Mayr, E. 1984. Evolution of fish species flocks: a commentary. Pp. 3–11 in *Evolution of fish species flocks,* A. A. Echelle and I. Kornfield, eds. Orono, ME: University of Maine Press.

McElroy, D. M., and Kornfield, I. 1991. Coloration in African cichlids: diversity and constraints in Lake Malawi endemics. Netherlands Journal of Zoology 41:250–268.

McKaye, K.R. 1991. Sexual selection and the evolution of the cichlid fishes of Lake Malawi. Pp. 241–257 in *Cichlid fishes: behaviour, ecology and evolution,* M. H. Keenleyside, ed. London, England: Chapman and Hall.

McKaye, K. R., Louda, S., and Stauffer, J. 1990. Bower size and male reproductive success in a cichlid fish lek. American Naturalist 135:597–613.

McKaye, K. R., and Marsh, A. C. 1983. Food switching by two specialized algae-scraping cichlid fishes in Lake Malawi, Africa. Oecologia 56:245–248.

Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution 41:1357–1369.

Meyer, A. 1990. Ecological and evolutionary aspects of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces: Cichlidae). Biological Journal of the Linnean Society 39:279–299.

Meyer, A. 1993a. Evolution of mitochondrial DNA in fishes. Pp. 1–38 in *Biochemistry and molecular biology of fishes, vol. 2,* P.W. Hochachka and T. P. Mommsen, eds. Amsterdam, The Netherlands: Elsevier.

Meyer, A. 1993b. Phylogenetic relationships and evolutionary processes in East African cichlid fishes. Trends in Ecology and Evolution 8:279–284.

Meyer, A., Knowles, L., and Verheyen, E. 1997. Widespread geographic distribution of mitochondrial haplotypes in Lake Tanganyika rock-dwelling cichlid fishes. Molecular Ecology (in press).

Meyer, A., Kocher, T. D., Basasibwaki, P., and Wilson, A. C. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347:550–553.

Meyer, A., Kocher, T. D., and Wilson, A. C. 1991. African fishes. Nature 351:467–468.

Meyer, A., Montero, C., and Spreinat, A. 1994. Evolutionary history of the cichlid fish species flocks of the East African great lakes inferred from molecular phylogenetic data. Advances in Limnology 44:409–425.

Moran, P., and 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.

Moran, P., and Kornfield, I. 1995. Were population bottlenecks associated with the radiation of the mbuna species flock (Teleostei: Cichlidae) of Lake Malawi? Molecular Biology and Evolution 12:1085–1093.

Moran, P., Kornfield, I., and Reinthal, P. 1993. Molecular systematics and radiation of the haplochromine cichlids (Teleostei: Perciformes) of Lake Malawi. Copeia 1994:274–288.

Nishida, M. 1991. Lake Tanganyika as an evolutionary reservoir of old lineages of East African cichlid fishes: inferences from allozyme data. Experientia 47:974–979.

Ono, H., O'Huigin, C., Tichy, H., and Klein, J. 1993. Major-histocompatibility-complex variation in two species of cichlid fishes from Lake Malawi. Molecular Biology and Evolution 10:1060–1072.

Otte, D., and Endler, J. A. (eds.). 1989. *Speciation and its consequences.* Sunderland, MA: Sinauer Associates.

Owen, R. B., Crossley, R., Johnson, T. C., Tweddle, D., Kornfield, I., Davidson, S., Eccles, D. H., and Engstrom, D. E. 1990. Major low levels of Lake Malawi and their implications for speciation rates in cichlid fishes. Proceedings of the Royal Society of London, Series B 240:519–553.

Reinthal, P. N. 1990. Morphological analysis of the neurocranium of a group of rock-dwelling cichlid fishes (Cichlidae: Perciformes) from Lake Malawi, Africa. Zoological Journal of the Linnean Society 98:123–139.

Reinthal, P. N. 1993. Evaluating biodiversity and conserving Lake Malawi's fish fauna. Conservation Biology 7:211–219.

Ribbink, A. J. 1991. Distribution and ecology of the cichlids of the African Great Lakes. Pp. 36–59 in *Cichlid fishes: behaviour, ecology and evolution,* M. H. Keenleyside, ed. London, England: Chapman and Hall.

Ribbink, A. J., Marsh, B. A., Marsh, A. C., Ribbink, A. C., and Sharp, B. J. 1983. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. South African Journal of Zoology 18:149–310.

Schliewen, U. K., Tautz, D., and Pääbo, S. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368:629–632.

Scholz, C. A., and Rosendahl, B. R. 1988. Low lake stands in Lake Malawi and Tanganyika, East Africa, delineated with multifold seismic data. Science 240:1645–1648.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Stauffer, J. R., Jr., Bowers, N. J., Kocher, T. D., and McKaye, K. R. 1996. Evidence of hybridization between *Cynotilapia afra* and *Pseudotropheus zebra* (Teleostei: Cichlidae) following an intralacustrine translocation in Lake Malawi. Copeia 1996:203–207.

Stiassny, M. L. J. 1981. Phylogenetic versus convergent relationships between piscivorous cichlid fishes from Lake Malawi and Tanganyika. Bulletin of the British Museum of Natural History (Zoology) 40:67–101.

Stiassny, M. L. J. 1991. Phylogenetic intrarelationships of the family Cichlidae: an overview. Pp. 1–35 in *Cichlid fishes: behaviour, ecology and evolution,* M. H. Keenleyside, ed. London, England: Chapman and Hall.

Sturmbauer, C., and Meyer, A. 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. Nature 358:578–581.

Sturmbauer, C., and Meyer, A. 1993. Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes from Lake Tanganyika in Eastern Africa. Molecular Biology and Evolution 10:751–768.

Sturmbauer, C., Verheyen, E., and Meyer, A. 1994. Mitochondrial phylogeny of the Lamprologini, the major substrate spawning lineage of cichlid fishes from Lake Tanganyika in Eastern Africa. Molecular Biology and Evolution 11:691–703.

Sültmann, H., Mayer, W. E., Figueroa, F., Tichy, H., and Klein, J. 1995. Phylogenetic analysis of cichlid fishes using nuclear DNA markers. Molecular Biology and Evolution 12:1033–1047.

Swofford, D. 1991. *PAUP: phylogenetic analyses using parsimony, vers. 3.0s,* Champaign, IL: Illinois Natural History Survey.

Verheyen, E., Rüber, L., Snoeks, J., and Meyer, A. 1996. Mitochondrial phylogeography of rock-dwelling cichlid fishes reveals evolutionary influence of historical lake level fluctuations of Lake Tanganyika, Africa. Philosophical Transactions of the Royal Society of London, Series B 351:797–805.

Wimberger, P. 1991. Plasticity of jaw and skull morphology in the Neotropical cichlid *Geophagus brasiliensis* and *G. steindachneri.* Evolution 45:1545–1564.