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The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics

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Abstract With more than 3,000 species, the fish family Cichlidae is one of the most species-rich families of vertebrates. Cichlids occur in southern and central America, Africa, Madagascar, and India. The hotspot of their biodiversity is East Africa, where they form adaptive radiations composed of hundreds of endemic species in several lakes of various sizes and ages. The unparalleled species richness of East African cichlids has been something of a conundrum for evolutionary biologists and ecologists, since it has been in doubt whether these hundreds of species arose by allopatric speciation or whether it is necessary to invoke somewhat less traditional models of speciation, such as micro-allopatric, peripatric, or even sympatric speciation or evolution through sexual selection mediated by female choice. Ernst Mayr's analyses of these evolutionary uniquely diverse species assemblages have contributed to a more direct approach to this problem and have led to a deeper understanding of the patterns and processes that caused the formation of these huge groups of species. We review here recent molecular data on population differentiation and phylogenetics, which have helped to unravel, to some extent, the patterns and processes that led to the formation and ecological maintenance of cichlid species flocks. It is becoming apparent that sexually selected traits do play an important role in speciation in micro-allopatric or even sympatric settings. Species richness seems to be roughly correlated with the surface area, but not the age, of the lakes. We observe that the oldest lineages of a species flock of cichlids are often less species-rich and live in the open water or deepwater habitats. While the species flocks of the Lake Malawai and the Lake Victoria areas were shown to be mono-

phyletic, the cichlid assemblage of Lake Tanganyika seems to consist of several independent species flocks. Cichlids emerge as an evolutionary model system in which many fundamental questions in evolution and ecology can be tested successfully, yet for other fish species flocks the relative importance of alternative mechanisms of speciation is likely to differ from that in cichlid fish.

Introduction

Cichlid fishes are tropical freshwater fish that are unsurpassed by any other group of vertebrate in terms of their sheer number of species (~3,000), variety of body shapes, assortment of coloration, behavioral diversity, and degree of trophic and ecological specialization (see Fryer and Iles 1972; Coulter; Meyer 1993; Stiassny and Meyer 1999; Kornfield and Smith 2000; Schluter 2000; Kocher 2004). Since the publication of the first reports on the East African fish fauna at the end of the nineteenth century (Boulenger 1898), the exceptional diversity of fishes in the family Cichlidae has attracted the attention of evolutionary biologists. Cichlids were viewed by some as an enigmatic, hard-to-explain, aberrantly species-rich group. Yet, their exuberant variation made them important models and textbook examples for rapid speciation and diversification (Fryer and Iles 1972; Stiassny and Meyer 1999; Kornfield and Smith 2000; Schluter 2000; Kocher 2004).

Molecular phylogenetic analyses (Zardoya et al. 1996; Streebman et al. 1998; Farias et al. 2000, 2001; Sparks 2004) have revealed that the phylogenetic relationships among the major lineages of cichlids are consistent with an initially Gondwanaland distribution, with the Indian and Malagasy representatives forming the most basal lineages and the reciprocally monophyletic African and American lineages being sister groups—a distributional pattern that is congruent with models of vicariance biogeography rather than overseas dispersal (Fig. 1). Only about a dozen extant species represent the most basal

Dedicated to Prof. Ernst Mayr

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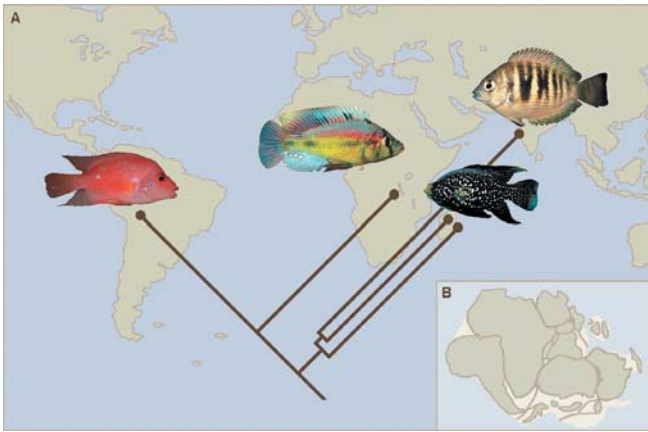


Fig. 1A, B Distribution of the Cichlidae. **A** The distributional pattern of the cichlids, with the representatives from India, Sri Lanka, and Madagascar forming the most basal lineages and the reciprocally monophyletic African and American lineages as sister-groups, is consistent with an initially Gondwanaland distribution (Zardoya et al. 1996; Streebman et al. 1998; Farias et al. 2000, 2001; Sparks 2004). **B** The supercontinent of Gondwanaland some 200 million years ago (MYA)

paraphyletic lineages of cichlids from India/Sri Lanka and Madagascar, which are the two landmasses that split off first from the supercontinent of Gondwanaland between 165 and 130 million years ago (MYA). The Americas are inhabited by an estimated 400–500 species. Their center of biodiversity is East Africa, where the cichlids inhabit several small and some exceptionally large lakes (as well as rivers) and have formed so-called “species flocks” with sometimes hundreds of endemic species in each of these lakes. By far the greatest diversity of cichlids occurs in East Africa in Lakes Victoria, Malawi and Tanganyika (Fig. 2), where a total of probably more than 1,800 species may occur.

With an age of 9–12 million years, Lake Tanganyika is the oldest of East Africa’s Great Lakes (Cohen et al. 1993, 1997), followed by Lake Malawi with a probable age of 2–5 million years (Johnson and Ng’ang’a 1990; Delvaux 1995). Both Lake Tanganyika and Lake Malawi have the shape and characteristics of a typical rift lake, as they are located in the rift between the east African and central African tectonic plate. They are relatively narrow and extremely deep (up to ~1,450 m in the case of Lake Tanganyika). Lake Victoria, however, the youngest of East Africa’s Great Lakes, is a rather shallow lake (~80 m maximum depth) that formed between 250,000 and 750,000 years ago (Johnson et al. 1996). Around the size of Ireland (about 68,800 km²), Lake Victoria is the world’s largest tropical lake and contains 500 or more endemic species of cichlids (Seehausen 1996). Molecular phylogenetic studies suggest that the Lake Victoria “superflock” (Greenwood 1979, 1980, 1984a) includes not only the endemic cichlids of this inland water, but also those of some of its neighboring lakes such as Lakes Albert, Edward, George, Kyoga, and Kivu, and that it is about 100,000 years old (Meyer et al. 1990; Nagl et al. 2000; Seehausen et al. 2003; Verheyen et al. 2003). Lake

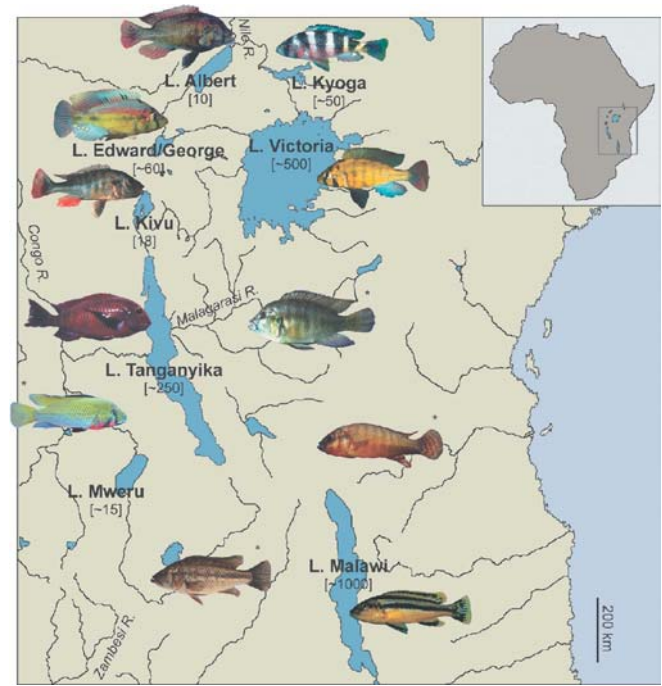


Fig. 2 The cichlids’ center of biodiversity is East Africa, where they inhabit the rivers and lakes and have formed species flocks of unparalleled species richness and morphological diversity. More than 2,000 cichlid species are recognized, 80% of which belong to a single tribe, the Haplochromini. The most species-rich assemblages are found in the East African Great Lakes: Tanganyika, Malawi, and Victoria. More than 200 species live in rivers (four representatives marked with an asterisk are depicted). Estimated species numbers are given in square brackets

Malawi contains 500–700 (some estimates even reach 1,000) cichlid species (Turner et al. 2001), while for Lake Tanganyika “only” about 200–250 species of cichlids are estimated (Snoeks et al. 1994). It should be noted that this number is likely to be an underestimate of the actual number of biological cichlid species in this lake.

Modes of speciation

Historically, the enormous diversity of the assemblages of cichlid species has been considered a challenge, “the cichlid problem”, which was seen, at least by several investigators to question or even contradict traditional models of speciation. Several researchers, including some of the earliest workers on speciation theory (Woltereck 1931; Rensch 1933; Kosswig 1947; Brooks 1950), invoked sympatric speciation models to explain how so many species could arise within one body of water, without obvious barriers to gene flow and thereby genetic population differentiation. Clearly, there is something special about the evolutionary success of cichlid fishes, which might—at least it was seen this way by some researchers—require special explanations. Two of the major issues in the cichlid problem are to explain (1) how such large numbers of species of cichlids, and not of the other

families of fishes that also inhabit these lakes, could have arisen, and (2) how the species are ecologically maintained without outcompeting each other and driving each other to extinction.

Several factors are believed to be responsible for the diversity of cichlids. One has to do with their specialized anatomy. Cichlids possess two sets of jaws: one shapes the “normal” mouth, to suck, scrape, or bite off bits of food; another “internal” one, which is derived from the 5th gill arch, is in the throat to crush, macerate, slice, or pierce the morsel before it is ingested. The particular organization of the pharyngeal jaw apparatus is a synapomorphy shared by all cichlids (Liem 1973, 1980). The jaws are exceedingly versatile and adaptable; for example, they can change in form even within the lifetime of a single individual (see discussion in Meyer 1990a, 1990b). A second factor has to do with the cichlid’s sophisticated breeding behavior and in particular with the various modes of broodcare behavior that are found in the East African lake cichlids (Fryer and Iles 1972; Goodwin et al. 1998; Stiassny and Meyer 1999; Barlow 2000; Kornfield and Smith 2000; Klett and Meyer 2002). More recent studies suggested that sexual selection through female choice of male (nuptial) coloration might also play an important role in the evolution of East African cichlids (Dominey 1984; Meyer 1993; Turner and Burrows 1995; Deutsch 1997; Seehausen and van Alphen 1999; Barlow 2000; Danley and Kocher 2001). Speciation on the basis of sexual selection in East African cichlids has been proposed on the basis of field observations (van Oppen et al. 1998) as well as mate choice experiments in the laboratory (Knight et al. 1998; Seehausen et al. 1999). Further evidence for sexual selection came from the observable breakdown of visual reproductive barriers under monochromatic light conditions or in turbid waters (Seehausen et al. 1997).

Ernst Mayr and speciation in cichlids

The “cichlid problem” has been, for more than 60 years, a topic that Ernst Mayr has returned to periodically in his writings since he first mentioned it in his landmark book *Systematics and the Origin of Species* (Mayr 1942). He discussed various aspects of the cichlid problem and suggested that the size and age of a lake might be much more important determinants of (i.e., might be positively correlated with) species number than predation, the prevailing hypothesis of that time (Worthington 1937, 1940). Mayr suggested that predators might have an a posteriori effect, in that “they can exterminate aberrant types” (Mayr 1942, p 272) and would be a “conservative factor by preventing super-specializations, but it is not apparent how they could possibly affect the establishment of discontinuities.”

Twenty years ago Mayr (1984) succinctly reviewed the work on fish species flocks in a commentary to a compendium of largely empirical studies (Echelle and Kornfield 1984) and summarized the major open questions about the formation of the species flocks of cichlids thus:

1. Is the presence of hundreds of closely related species within a single lake inconsistent with traditional allopatric speciation modes?
2. Do the species flocks refute the principle of competitive exclusion?

Mayr (1984) went on to outline the deficiencies in the empirical data and the problems that this lack of information presented in terms of answering some of the fundamental problems that the cichlid species flocks pose to evolutionary theory. Reviewing the available literature and analyzing the then state of knowledge, or rather the relative lack of it at that time, Mayr (1984) summarized the three major areas of uncertainty, as follows.

1. *Uncertainty regarding the age of the species flocks and their habitats*

Since the ages of the flocks were still unknown 20 years ago, it was unclear as to whether the flocks really arose at unfeasibly fast rates of speciation that might throw doubt on traditional models of speciation.

2. *Uncertainties regarding taxonomy in systematics*

Taxonomically, the species flocks were insufficiently known, and in many cases sister group relationships were unclear and entirely based on morphological traits. Many of those were later found to be susceptible to high levels of homoplasy (see below). That most (or all) of these putative species are genuine biological species was not questioned. It was asserted that they are genetically well separated, even if they often are extremely similar morphologically.

3. *Uncertainties regarding cladistic analyses*

Mayr (1984) laments that the—as he, probably rightly, sees it—limitations of cladistic analysis have been reached, given the extreme genetic and morphological similarity of such a large number of species (see also Mayr and Brock 2002). Interestingly, Mayr recognizes that homoplasy and parallelism which are likely observed (but not proven then to be homoplasy rather than synapomorphies) among large sets of closely related species pose a challenge to the phylogenetic reconstruction of evolutionary relationships and, possibly more important, to the investigation of the processes of lineage formation.

The imperfections of analytical techniques as well as the paucity of crucial data were summarized by Mayr (1984), and three major potential causes for the large number of cichlid species were considered:

1. multiple colonization of lake habitats from several lineages of riverine ancestors (questioning the monophyly of these species flocks)
2. amalgamation of smaller lakes and their subflocks into larger species flocks due to rising lake levels
3. intralacustrine speciation by one of several possible mechanisms of speciation.

Mayr (1942) as well as Rensch (1933), but not Kossig (1947), initially favored explanations such as multiple colonization and fusions of smaller flocks into larger ones as likely partial explanations for the emergence of the extraordinarily large species flocks of cichlids. However, Mayr later felt that intralacustrine speciation was responsible for the formation of these large species assemblage (Mayr 1984).

Intralacustrine speciation, according to Mayr (1984), can be envisioned to work in several ways. Partial separation—through major lake level fluctuations—of large and deep lake basins such as those of Lakes Tanganyika or Malawi into several sub-basins may promote allopatric speciation. Allopatric speciation might also work if a large lake basin is not divided into smaller ones. Cichlid fishes are known for their pronounced philopatry and their, in general, low dispersal abilities, which, according to Mayr (1984), promotes allopatric intralacustrine speciation.

Moreover, most cichlids are very tightly linked to particular substrate types, e.g., rocky substrate or sandy habitats, due to extreme food specialization. The mosaic distribution of different types of habitats along the shores of the large African lakes provides ample opportunity for population subdivision and are effective barriers to gene flow.

Finally, the spatial distribution of spawning sites is discontinuous, which, in conjunction with, and as a byproduct of, small effective population sizes would promote the rapid evolution of premating isolating mechanisms. Mayr (1984) suggested that spawning site fidelity might be an important contributing factor to speciation in the species flocks of East African cichlids. This suggestion has not really been followed up so far. Mayr's contention is that speciation is largely a non-adaptive byproduct of isolation. Dobzhansky (1937), on the other hand, argued that during the process of speciation pre-isolating mechanisms are formed only incompletely in periods of isolation and are completed through reinforcement, through natural selection against intermediate phenotypes (inferiority of hybrids), after contact with neighboring incipient species. Accordingly, morphological as well as ecological differences between these diverging populations would be exacerbated through character displacement.

Mayr (1984) postulated that two sets of factors need to be shown to act in the ecological setting of the African lakes to explain the evolution of their cichlid species flocks, the existence of isolating mechanisms (ecological or behavioral) that would prevent the nascent species from merging with other species, and factors that might be responsible for the facilitation of the evolution of isolating mechanisms in the first place. The great evolutionist always argued against ecological factors and for geographic aspects as major initial driving forces for isolating mechanisms (Mayr 1947, 1963) because he felt that speciation as a byproduct of ecological divergence for incipient species would not be likely, since the niche differences of many nascent species would be too slight

for complete reproductive isolation. Based on Dominey (1984) he instead argued that sexual selection might be a stronger force for speciation than ecological factors. Mayr (1984) went on to argue that sexual selection should be regarded as “micro-allopatric” speciation, since rapid evolution of premating isolating mechanisms would have resulted in “mating clusters” of individuals that are spatially somewhat separated.

In the following section we attempt to summarize the major advances of the last 20 years, mostly due to the application of molecular techniques, since the publication of Mayr's (1984) assessment of the “cichlid problem”. We review the current knowledge on the phylogenetic relationships and population structure of the cichlid species flocks of the Great Lakes in East Africa and discuss the species flock concept in the light of these hypotheses. We then focus on intralacustrine speciation and the mechanisms by which the unique diversity of cichlids in East Africa might have emerged. We conclude that future evolutionary genomic approaches would greatly advance our understanding of the patterns and processes involved in explosive speciation and adaptive radiation in cichlids, and also in other groups of organisms.

Molecular data and the East African cichlid species flocks

Lake Victoria

The cichlid species assemblage of Lake Victoria (see Fig. 2) was among the first endemic faunas to be studied by means of molecular phylogenetic tools (Sage et al. 1984; Meyer et al. 1990). The study of Meyer et al. (1990) was based on a segment of the mitochondrial cytochrome *b* gene, where no variation at all was found, and the mitochondrial DNA control region, the fastest evolving section of the mitochondrial genome. Among other findings, this paper established the monophyly of the Lake Victoria region cichlid species flock. However, Meyer et al. (1990) did not include specimens of all the neighboring lakes of Lake Victoria, which have been grouped into a “superflock” of closely related species based on morphological grounds (Greenwood 1973, 1979, 1980). Our more recent phylogeographic study proposed an origin of the “superflock” (now including the cichlid faunas from Lakes Victoria, Albert, Edward, George, Kyoga, and Kivu) from Lake Kivu endemics and revealed that the Lake Victoria cichlid assemblage is technically diphyletic (Verheyen et al. 2003). In addition, the age of the “superflock” has been discussed in detail (Meyer et al. 1990; Fryer 1997, 2001, 2004; Booton et al. 1999; Nagl et al. 2000; Sturmhuber et al. 2001; Seehausen 2002; Seehausen et al. 2003; Verheyen et al. 2003), particularly after geological data suggested that the entire lake basin dried out completely between 15,600 and 14,700 years ago (Johnson et al. 1996). Molecular phylogenetic studies agree that the age of Lake Victoria's cichlid fauna is less than 200,000 years (Meyer et al. 1990; Nagl et al. 2000), and is

probably about 100,000 years (Verheyen et al. 2003; Salzburger et al. 2004). Due to the extremely young age of the assemblage, the phylogenetic inference among the cichlid species in the superflock is rather difficult, and species often share mitochondrial haplotypes (Verheyen et al. 2003; R.O. Abila et al., unpublished). Also in non-coding regions of the nuclear DNA the persistence of polymorphisms was detected among Lake Victoria cichlids (Nagl et al. 1998).

Lake Malawi

From the almost 1,000 species of Lake Malawi only a relatively small proportion have been subjected to phylogenetic analyses (Meyer et al. 1990, 1996b; Kocher et al. 1993; Moran et al. 1994; Reinthal and Meyer 1997; Albertson et al. 1999; Shaw et al. 2000; Takahashi et al. 2001a; Allender et al. 2003). In a study based on two mitochondrial gene segments and including specimens from all main lineages identified so far, representatives of the pelagic genera *Rhamphochromis* and *Diplotaxodon* were resolved as sister group to a clade formed by the rock-dwelling “*mbuna*” cichlids, the “non-*mbuna*” cichlids, *Copadichromis virginalis*, and the riverine representative *Astatotilapia calliptera* (Meyer et al. 1996b; Shaw et al. 2000). The reconstruction of the relationship between Lake Malawi cichlids is made more complicated by the persistence of ancestral polymorphisms across species (Moran and Kornfield 1993), which reflects the young age of the species flock. Recent studies using the AFLP method to overcome this problem have provided phylogenetic hypotheses for larger subgroups of the *mbuna* cichlids (Albertson et al. 1999; Allender et al. 2003). Population-genetic analyses based on microsatellite markers have also been successfully applied in Lake Malawi *mbuna* cichlids and it has been shown that deep waters and sandy stretches may act as strong barriers to gene flow (van Oppen et al. 1997; Arnegard et al. 1999; Markert et al. 1999). Sometimes a habitat discontinuity of only about 35 m seems sufficient to isolate two populations (Rico and Turner 2002).

Lake Tanganyika

The phylogeny of the Lake Tanganyika cichlid assemblage is the best understood, which is—at least to some extent—facilitated by the greater age of the lake and its species assemblage. This leads to more pronounced differences in genetic diversity (Sturmbauer and Meyer 1992; Salzburger et al. 2002a) as well as in morphology, ecology, and behavior of its species (Fryer and Iles 1972; Greenwood 1984b; Poll 1986), making phylogenetic inference feasible. The cichlids of Lake Tanganyika were grouped into 12 eco-morphologically distinct tribes (Poll 1986), whereas the monophyletic species flocks of Lakes Victoria and Malawi consist of representatives of one of these tribes only, the Haplochromini. The first attempts to

reconstruct the evolution of the species flock using allozyme data (Nishida 1991) identified several ancient lineages that are basal to the substrate-spawning Lamprologini plus the mouthbrooding “H-lineage,” which led to the notion that Lake Tanganyika is an evolutionary reservoir for old African cichlid lineages (Nishida 1991). Subsequent work based on mitochondrial DNA sequences (see, e.g., Sturmbauer and Meyer 1992; Sturmbauer et al. 1994, 2003; Kocher et al. 1995; Salzburger et al. 2002a; Baric et al. 2003; Koblmüller et al. 2004) and on SINE insertion patterns (see, e.g., Takahashi et al. 1998) were in astonishing agreement—with the exception of only a handful of species that were suggested to be in need of reassignment to other tribes—with the reciprocal monophyly of the eco-morphologically defined tribes of Poll (1986). Unlike the monophyletic haplochromine species flocks of Lake Malawi and the Lake Victoria region, the species assemblage of Lake Tanganyika turned out to be oligophyletic, i.e., several (riverine) lineages are likely to have entered the lake habitat after its formation and radiated in parallel (Salzburger et al. 2002a). While more ancestral lineages combine a handful of species only, the substrate-spawning Lamprologini (~100 species) and the Tanganyikan mouthbrooders (~80 species) [the “H-lineage” of Nishida (1991) but excluding the goby-like Eretmodini] are relatively species-rich (see Fig. 3). However, no comprehensive nuclear DNA phylogeny exists so far for the Lake Tanganyika cichlid assemblage.

The species flock concept in light of molecular phylogenetic analyses

A species flock (or species swarm) is generally referred to as a monophyletic assemblage of closely related species that coexist in the same area (Greenwood 1984a; Mayr 1984), and it was Mayr (1942) who closely associated the two concepts of explosive speciation and species flock. Three criteria for a species flock and its members were recognized in an ichthyologic context:

1. a geographical circumscription
2. a high level of endemism
3. a close phyletic relationship (Greenwood 1984a).

For most lacustrine species flocks, the lake habitat itself resembles the **geographical circumscription**. That the confines of a single lake circumscribe the species flock is not the case for the Lake Victoria region superflock, which combines the species assemblages of Lakes Victoria, Albert, Edward, George, Kyoga, Kivu and several smaller lakes in the region (Greenwood 1973, 1979, 1980; Verheyen et al. 2003). However, for the Lake Victoria superflock a clear geographic boundary is also evident that, in this case, includes more than one body of water and rivers as well as lakes (Nagl et al. 2000; Seehausen et al. 2003; Verheyen et al. 2003). We note that the reverse conclusion, that all cichlid species of these lakes strictly belong to the superflock, is not valid; there are several

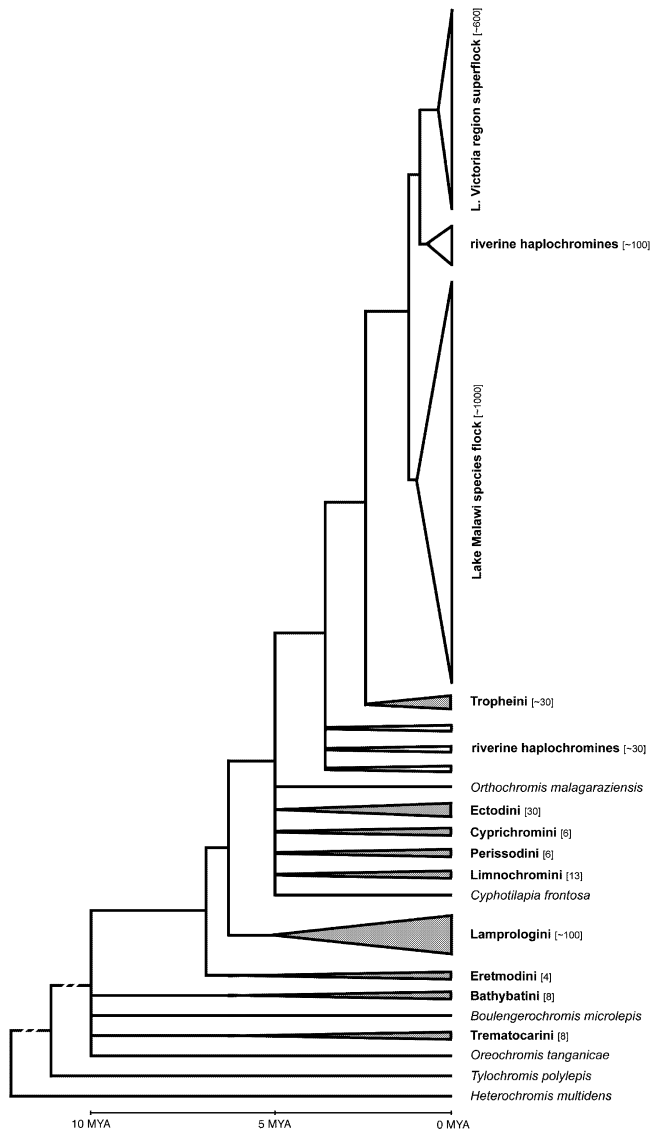


Fig. 3 Molecular phylogeny of the East African cichlids (combined after Klett and Meyer 2002; Salzburger et al. 2002a; Verheyen et al. 2003). Within the Lake Tanganyika assemblage, several sub-radiations can be delineated (depicted in gray), with the substrate-spawning Lamprologini being the most species-rich tribe (Poll 1986). Several cichlid lineages have secondarily left Lake Tanganyika (Sturmbauer et al. 1994; Salzburger et al. 2002a), and also the common ancestor of the East African riverine haplochromines, the Tanganyikan Tropheini as well as the species flocks of Lake Malawi and the Lake Victoria region seem to be of Lake Tanganyika origin (Nishida 1991; Kocher et al. 1995; Salzburger et al. 2002a). *MYA* million years ago

more ancestral haplochromine species in these lakes, such as *Astatoreochromis alluaudi* and *Pseudocrenilabrus multicolor* in Lake Victoria, *Haplochromis gracilior* in Lake Kivu and *Haplochromis* spp. in Lake Edward/George that do not belong to the superflock (Nagl et al. 2000; Salzburger et al. 2002a; Verheyen et al. 2003). Also, several species of tilapia are found in the water-bodies of this area (Fryer and Iles 1972), which are rather distantly

related to the haplochromines and are ultimately of West-African origin (Klett and Meyer 2002).

For the Lake Tanganyika assemblage, or at least for some Tanganyikan tribes, the criterion of a geographical circumscription is problematic, as several lineages of Tanganyikan origin left the lake habitat secondarily and their descendants are now distributed over large parts of the African continent (Sturmbauer et al. 1994; Salzburger et al. 2002a, 2002b). Also the ancestor of the species flocks of Lake Malawi and the Lake Victoria region is likely to be of Tanganyikan origin (Kocher et al. 1993; Salzburger et al. 2002a) (see also Nishida 1991; Takahashi et al. 2001b; Takahashi and Okada 2002 in support of the sister-group relationship between the Tropheini and haplochromine representatives of lakes Victoria and Malawi). Likewise, a strict geographical circumscription of the Lake Malawi species flock fails, technically, if—as has been suggested based on mitochondrial DNA markers (Moran et al. 1994; Meyer et al. 1996b; Shaw et al. 2000) and AFLP analyses (Seehausen et al. 2003)—the riverine species *Astatotilapia calliptera* is not the most ancestral taxon of the species flock but nested therein. Molecular phylogenetic and phylogeographic studies have thus revealed that at least some East African lacustrine cichlid adaptive radiations were not restricted to the respective lake habitat.

The second fish species flock criterion of *high levels of endemism* (Greenwood 1984a; Mayr 1984) is certainly fulfilled by all species flocks of cichlid fishes in East Africa, as the amount of endemism in the different lakes' cichlid assemblages reaches almost 100%. Remarkably, the species flocks/assemblages of Lakes Tanganyika, Malawi, and Victoria do not have a single cichlid species in common (Fryer and Iles 1972).

Similarly, the third criterion of *close phyletic relationships* (Greenwood 1984a; Mayr 1984) is true for the East African cichlid species flocks. Moreover, the two most species-rich species flocks, those of the Lake Malawi region and the Lake Victoria region, have been confirmed to be (almost) monophyletic (see, e.g., Meyer et al. 1990; Shaw et al. 2000; Verheyen et al. 2003). Lake Tanganyika, on the other hand, represents an assemblage of oligophyletic origin (Nishida 1991; Kocher et al. 1995; Salzburger et al. 2002a) and part of its eco-morphological diversity is actually explained by the fact that several more ancient lineages are likely to have radiated in parallel after the formation of the lakes. For example, the substrate-spawning Lamprologini and the Tanganyikan mouthbrooders (Salzburger et al. 2002a), which account for about 90% of the lakes' cichlid species, have radiated in parallel. As a consequence of its oligophyly, the Lake Tanganyika cichlids should not be considered as a single species flock but rather as several species flocks within a single lake. According to the molecular phylogenies (Nishida 1991; Kocher et al. 1995; Salzburger et al. 2002a), one should consider the Trematocarini (8 species), the Bathybatini (8 species), the Eretrmodini (4 species), the Lamprologini (~100 species), and the Tanganyikan mouthbrooders or even subgroups thereof (~80

species) as independent or parallel intralacustrine species flocks (see Fig. 3). All these lineages are likely to have seeded the lake independently and to have subsequently radiated in the lacustrine environment (Salzburger et al. 2002a), so that the criterion of a close phyletic relationship (Greenwood 1984a), which we, and others previously (Kosswig 1963; Greenwood 1984a), consider a synonym for monophyly, is not fulfilled. That the Lake Tanganyika species assemblage consists of several species flocks had already been suggested by (Kosswig 1963), who saw in the Lamprologini an independent radiation compared with other cichlids of the lake. The entire cichlid community in Lake Tanganyika should then be referred to as “assemblage” rather than “species flock.” Also, for the haplochromines that inhabit Lake Victoria, which are part of the Lake Victoria region superflock (Verheyen et al. 2003), the term “assemblage” would reflect better the fact that the lake’s cichlids flock into a higher-level species amalgamation which includes the species of neighboring lakes and rivers as well.

Three areas of uncertainty

In his 1984 review, Ernst Mayr highlighted three major areas of uncertainty that, by that time, prevented a definitive answer to the question whether sympatric speciation occurs in species flocks of freshwater lakes, and, furthermore, what the relative importance of different allopatric processes in the formation of these flocks was. He noticed uncertainties concerning the age of the species flocks, the taxonomic status of the species, and the cladistic techniques by which the members of the species flocks were analyzed (Mayr 1984).

Advances in the fields of paleogeology and paleolimnology have in the meantime advanced our understanding of the evolution of the East African Rift System and the formation of the lakes (see e.g., Cohen et al. 1993, 1997; Delvaux 1995; Lezzar et al. 1996). Even more important types of data on the “cichlid problem” were recent phylogenetic, phylogeographic, and population-genetic outcomes on the age, the phyletic status (mono- vs. oligophyletic), and the origin of the seeding lineages of the assemblages of the three large lakes as well as other bodies of water in East Africa (Meyer et al. 1990; Kocher et al. 1993, 1995; Salzburger et al. 2002a; Seehausen et al. 2003; Verheyen et al. 2003).

While the timing of the evolutionary processes in East African cichlids is fairly well documented (see above), there are ongoing debates on the taxonomic status of many cichlid species, lineages, and even species flocks (see e.g., Snoeks et al. 1994; Turner et al. 2001; Genner et al. 2004). The taxonomy of the Tanganyikan cichlids is relatively well established (Poll 1986), which is mostly due to the existence of morphological characteristics that distinguish the different tribes and species. Molecular phylogenetic studies largely confirmed the tribal assignments and well-supported phylogenetic hypotheses exist that interrelate the tribes and their respective members

(see e.g., Kocher et al. 1995; Takahashi et al. 1998; Salzburger et al. 2002a); and, moreover, it is now possible to infer sister-species relationships by standard molecular techniques (see e.g., Kocher et al. 1995; Salzburger et al. 2002a; Sturmbauer et al. 2003; Koblmüller et al. 2004). Also, unlike in the species flocks of Lake Malawi and the Lake Victoria region (Moran and Kornfield 1993; Verheyen et al. 2003; Salzburger et al. 2004; R.O. Abila et al., unpublished), shared haplotypes between species have not been observed in Tanganyikan lineages (Sturmbauer et al. 2003) except as a result of hybridization (Rüber et al. 2001). Taxonomic uncertainties still remain among closely related taxa in Lake Malawi and, in particular, in the hundreds of closely related species of the Lake Victoria cichlid assemblage. In the latter case, due to the extreme pace of species formation, neither morphological nor molecular data has so far been able to provide sufficient phylogenetic resolution to infer statistically reliable sister-species relationships or hypotheses about the relationships between different genera. It has, however, also been pointed out that the population structure within Lake Victoria cichlids has not been studied extensively (Rico et al. 2003).

That the inference of the phylogeny based on molecular data among closely related cichlid species in Lake Malawi, and particularly in Lake Victoria, remains a challenge has to do to some extent with the type of data and the analyses that have been used so far to address the problem. Additionally, it is still an elusive goal to establish a well-supported phylogeny of the Lake Victoria superflock, since these species are so young and the genetic differences are so slight. Mitochondrial DNA sequences have proved to be reliable markers for the reconstruction of the phylogeny of the major cichlid lineages and older species assemblages (Meyer et al. 1990; Kocher et al. 1995; Shaw et al. 2000; Klett and Meyer 2002; Salzburger et al. 2002a) and the inference of phylogeography in all East African cichlids (here, shared haplotypes between species do not pose a problem) (Sturmbauer and Meyer 1992; Meyer et al. 1996a; Verheyen et al. 1996, 2003; Rüber et al. 2001; Baric et al. 2003; R.O. Abila et al., unpublished). In the cases where the insertion of short interspersed nuclear elements (SINEs) in the nuclear genome has been studied, the prior mitochondrial phylogenies were confirmed (Takahashi et al. 1998, 2001a, 2001b; Takahashi and Okada 2002). But, since SINE insertions are evolutionarily rare events, they may never provide enough phylogenetic resolution to resolve relationships among the more closely related species (flocks). Mitochondrial DNA sequence data also proved useful to investigate the parallel evolution of certain morphological traits between the species assemblages of Lakes Tanganyika and Malawi as well as within the Lake Tanganyika cichlid assemblage (see e.g., Kocher et al. 1993; Rüber et al. 1999; Sturmbauer et al. 2003).

For phylogenetic inference among closely related taxa in the younger assemblages, mitochondrial DNA markers, although evolving five to ten times faster compared with the nuclear genome, are still too slowly evolving, which is

reflected by shared haplotypes between species (Moran and Kornfield 1993; Verheyen et al. 2003; R.O. Abila et al., unpublished). Recently, Hey et al. (2004) developed a method which uses the information provided by microsatellite repeats and the nuclear haplotypic information provided by flanking region sequences in closely related Lake Malawi *mbuna* species. Albertson et al. (1999) used a novel approach to overcome the problem of the persistence of ancestral polymorphism in closely related *mbuna* cichlids of Lake Malawi by analyzing more than a thousand polymorphic markers derived from a DNA fingerprinting method (AFLP). This method has meanwhile been adopted by others (Allender et al. 2003; Seehausen et al. 2003). Allender et al. (2003) was able to show, for example, that in the Lake Malawi *mbuna* cichlids, similar types of nuptial coloration have evolved repeatedly in several lineages. However, only a few taxa have been studied so far using the AFLP method; also the comparison of results between studies from different laboratories is problematic when using this technique. Nonetheless, approaches using thousands of polymorphic markers that are distributed over the whole genome, such as AFLPs, or the analysis of hundreds of single nucleotide polymorphisms (SNPs), have the potential to considerably advance our understanding of the phyletic relationships within arrays of closely related cichlid species. This type of data might be necessary for the analysis/determination of patterns of relationships as well as studies of the mechanisms of speciation.

Multiple colonization, species flock amalgamation, or intralacustrine speciation?

Molecular phylogenetic and phylogeographic studies clearly show that multiple colonization from riverine stocks (Mayr 1984) is a negligible factor for species richness, as the most species-rich flocks—those of the Lake Malawi region and the Lake Victoria region—have each been founded by a single lineage of haplochromine ancestors only (Meyer et al. 1990; Kocher et al. 1993; Moran et al. 1994; Nagl et al. 2000; Shaw et al. 2000; Seehausen et al. 2003; Verheyen et al. 2003). For the Lake Tanganyika cichlids (Nishida 1991; Kocher et al. 1995; Salzburger et al. 2002a), several independent species flocks derived from a small number of seeding lineages make up the assemblage (see above), and several lineages have left the lake secondarily (Sturmbauer et al. 1994; Salzburger et al. 2002a). However, the ages of the parallel intralacustrine species flocks of Lake Tanganyika, where all radiations have occurred after the formation of the lakes (Sturmbauer et al. 1994; Salzburger et al. 2002a), suggest that the diversity within Lake Tanganyika's parallel species flocks was almost entirely shaped via intralacustrine speciation.

The Lake Victoria superflock might be viewed as an amalgamation of independent species flocks in Lakes Victoria, Albert, Edward, George, Kyoga, and Kivu—all were founded by the same lineage of haplochromine an-

cestors. Moreover, the 500 or so species of the main lake (Lake Victoria) were recently suggested to be diphyletic (Verheyen et al. 2003), which seems to implicate that the amalgamation of several independent species flocks could explain the unique species richness in that lake or in the whole superflock. However, the second seeding lineage of Lake Victoria cichlids is represented by one rock-dwelling species so far, so that the second lineage combines some 500 species that most likely have arisen in the lake basin within the last ~100,000 years (Fryer 1997, 2001, 2004; Nagl et al. 2000; Verheyen et al. 2003). Molecular data thus agree in affirming that intralacustrine speciation is the dominant mode of species multiplication in all East African lake cichlid species flocks (Meyer et al. 1990; Kocher et al. 1993; Meyer 1993; Stiassny and Meyer 1999; Salzburger et al. 2002a).

Allopatric intralacustrine speciation

The term “intralacustrine speciation” connotes not much more than that the speciation events took place within the confines of the respective lakes, and that neither multiple colonization nor amalgamation of independent species flocks is responsible for the observed species richness. Importantly, intralacustrine speciation does not specify which mechanism(s) of speciation was/were involved. Mayr (1984) mentioned three possibilities through which allopatric intralacustrine speciation might take place.

1. *Major lake level fluctuations* may be factors that “would permit some extent of allopatric speciation within the lake” (Mayr 1984). Large lake level changes caused by variations in rainfall, evaporation, and/or tectonic activities are known in all East African lakes (Beadle 1974; Scholz and Rosendahl 1988; Cohen et al. 1993, 1997; Delvaux 1995; Johnson et al. 1996; Lezzar et al. 1996; Nicholson 1999). Such fluctuations have been interpreted as important external modulators promoting speciation within arrays of closely related species in all the African lakes (Rossiter 1995; Rüber et al. 1998; Sturmbauer 1998; Kornfield and Smith 2000; Sturmbauer et al. 2001), and several studies support the view that lake level fluctuations may influence the genetic divergence in East African lake cichlids and promote allopatric speciation (Owen et al. 1990; Rüber et al. 1998; Danley et al. 2000; Sturmbauer et al. 2001, 2003; Baric et al. 2003).

Allopatric speciation would, according to Mayr (1984), also be possible if a lake basin is not divided into sub-basins. He argued that the spotty distribution of many cichlid species would favor intralacustrine allopatric speciation and pointed out that almost none of the cichlid species in East Africa's Great Lakes have a lake-wide distribution and that they occur typically only in an, often small, portion of a lake. Here, phylogeography, i.e., the combination of biogeography and phylogenetics/population-genetic data (Avice et al. 1987), recently offered the opportunity to

not only map the geographic distribution of morphologically recognized taxa, but also to investigate the biogeography of genetic lineages along the shoreline of a lake. In Tanganyikan rock-dwelling cichlids, for example, mitochondrial lineages tend to have restricted distribution patterns and only a few lineages have a lake wide-distribution (Meyer et al. 1996a; Verheyen et al. 1996; Sturmbauer et al. 1997; Rüber et al. 1998, 2001; Taylor et al. 2001; Baric et al. 2003), which is in agreement with Mayr's (1984) notion. Species of the Lake Malawi *mbuna* group seem to have even more fine-grained ranges compared with Tanganyikan rock-dwellers (Konings 1990); both assemblages are known for their sometimes extreme philopatry (see, e.g., van Oppen et al. 1997; Arnegard et al. 1999; Rüber et al. 2001; Rico and Turner 2002).

2. Mayr (1984) emphasized the importance of the *patchy habitat distribution*, due to the alternation of rocky, sandy, and muddy habitats in the Great Lakes, for allopatric intralacustrine speciation. That the island-like properties of these habitats combined with the cichlid's specialization to particular habitat types are an important factor for intralacustrine speciation had already been suggested (Brooks 1950; Mayr 1963; Fryer and Iles 1972); the patchy distribution of many littoral cichlid species, and in particular rock-dwelling ones, in the East African Great Lakes lends obvious support to this hypothesis. Also, the observation that the size—but not the age, as has also been suggested by Mayr (1942)—of a lake is positively correlated with the species number of a lake's cichlid assemblage (Fig. 4), indicates that the many more ecological opportunities and/or the higher potential for spatial separation a larger lake provides might be an important promoter for micro-allopatric intralacustrine speciation (see also Barluenga and Meyer 2004).

Molecular ecological work has indeed confirmed the importance of substrate discontinuities or deep-water habitats as barriers to gene-flow. Studies on Lake Malawi *mbuna* cichlids (Reinthal and Meyer 1997; van Oppen et al. 1997; Arnegard et al. 1999; Markert et al. 1999; Danley et al. 2000; Rico and Turner 2002) showed that populations are sometimes extremely small and that sister-species are separated by often only few hundred meters of shoreline. On the other hand, population structuring has not been observed in pelagic cichlids from Lake Malawi, and it has been suggested that these might form lake-wide panmictic populations (Shaw et al. 2000). Demersal cichlid species in Lake Malawi, which are probably as species-rich as the rock-dwelling *mbuna*, also seem to display low levels of population structuring (Taylor and Verheyen 2001).

3. Mayr (1984) also argues that the *isolation of spawning sites* would be an important promoter for allopatric intralacustrine speciation. In Mayr's view, "the isolating mechanisms are acquired during spatial isolation as a byproduct of the genetic changes of the isolated population" (Mayr 1984). He disagrees with Dob-

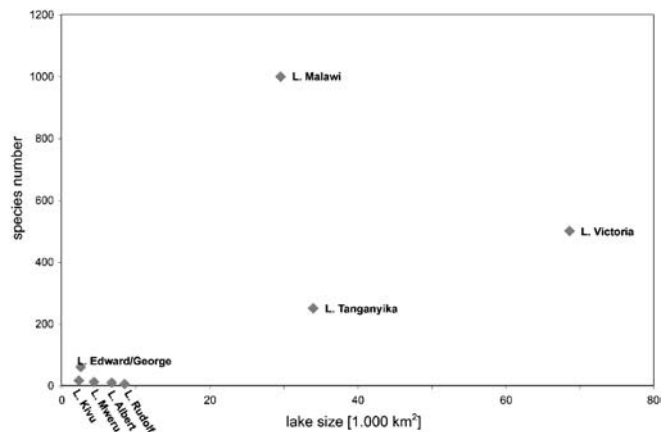


Fig. 4 The size, but not the age, of a lake roughly correlates with its species number of endemic cichlids (Mayr 1942), suggesting that the much higher number of ecological niches and/or the higher potential of spatial separation due to large discontinuous shoreline that are provided by large lake habitats facilitate intralacustrine allopatric speciation (see also Barluenga and Meyer 2004). Lake Malawi is somewhat of an exception, since it has about four times more species than the similarly sized Lake Tanganyika. This can—to some extent—be explained by a different taxonomic treatment of its taxa. However, it is likely that the adaptive radiation in Lake Malawi is at a different stage than that of Lake Tanganyika, and that the Lake Tanganyika species assemblage had been more species-rich at some point in the past

zhansky's (1937) concept of hybrid inferiority causing the perfection of isolating mechanisms in secondary sympatry and attests that the almost complete absence of hybrids in the cichlid assemblages of the East African lakes would support his view.

Mayr's and Dobzhansky's views have both been used to explain the evolution of isolating mechanisms in East African lake cichlids (see, e.g., Fryer and Iles 1972; Deutsch 1997; Seehausen and van Alphen 1999; Kornfield and Smith 2000). However, the extreme pace of species formation in the East African cichlids poses difficulties in determining which mode-isolating mechanisms were acquired and possibly perfected secondarily. In addition, the role that hybridization might play in the evolution of cichlid species flocks is disputed [the supposed absence of hybridization was used as argument by Mayr (1984)]. Hybridization has been observed in conditions of reduced water clarity (Seehausen et al. 1997) or after translocation of fishes (Stauffer and Bowers 1996). Recent molecular studies have also revealed that hybridization events did occur between distinct species (Rüber et al. 2001; Salzburger et al. 2002a; Smith et al. 2003). However, the frequency of the occurrence of hybridization events in cichlids has not been studied in sufficient detail to allow statements as to whether hybridization is rare and exceptional or rather common in the cichlid species assemblages.

Ecological factors vs. sexual selection

Ernst Mayr (1984) stated that “the probable causal factors in the origin of isolating mechanisms has recently been discovered in sexual selection” and refers to Dominey (1984) in the same compendium, who was the first to discuss the Fisherian runaway sexual selection model with respect to cichlid fishes. Sexual selection via female choice of male (nuptial) coloration is now regarded as important factor in cichlid evolution (see, e.g., Meyer 1993; Turner and Burrows 1995; Deutsch 1997; Seehausen and van Alphen 1999; Kornfield and Smith 2000; Allender et al. 2003). Assortative mating was also seen as a possible mechanism by which sympatric speciation might occur (Turner and Burrows 1995; Seehausen and van Alphen 1999; Wilson et al. 2000; Schliewen et al. 2001; Allender et al. 2003; Barluenga and Meyer 2004), particularly after simulations predicted that sympatric speciation is the theoretical outcome of such set-ups (Dieckmann and Doebeli 1999; Higashi et al. 1999; Kondrashov and Kondrashov 1999; Gavrilets and Waxman 2002).

Mayr (1984), on the other hand, argued that sexual selection would act in small populations, i.e., spatially isolated “mating clusters,” and he designated the process as “micro-allopatric.” In his view, the first characters of species to diverge would be related to courtship, e.g., behavioral and/or colorational traits. Trophic traits related to ecological differences in the food uptake would be diverging secondarily, in particular, once sympatry was re-established as a form of character displacement after secondary contact (Mayr 1984). Empirical observations show that sibling cichlid species tend to differ in coloration rather than in trophic differences, lending support to this hypothesis (Deutsch 1997; Reinthal and Meyer 1997; Seehausen and van Alphen 1999). Recent molecular studies on sympatric speciation in a trophically and colorationally polymorphic Nicaraguan crater lake cichlid species (Wilson et al. 2000; Barluenga and Meyer 2004) also revealed more pronounced differences between color but not pharyngeal jaw morphologies (a trait related to trophic differences; see Meyer 1989, 1990a, 1990b). Mayr himself considers cichlid examples (e.g. Mayr 2001; p 100), as the most convincing examples for sympatric speciation (E. Mayr, personal communication)

If, however, cichlid assemblages of entire lake habitats are considered, it seems more likely that, initially, ecological factors lead to a spatial separation into more benthic and more limnetic lineages (Danley and Kocher 2001). Danley and Kocher (2001) presented a three-stage model for the evolution of the cichlid species in Lake Malawi, in which the first stage is characterized by the creation of the *macrohabitat clades* (see also Streebman et al. 2003b). The second stage would be the *trophic specialization* within lineages associated with changes in the trophic morphology (Liem 1973, 1980), whereas the third stage, that would lead to the most substantial cycles of species multiplication, would be related to *reproductive*

characters, and particularly to coloration (Danley and Kocher 2001; Streebman and Danley 2003).

The situation in Lake Tanganyika is somewhat similar, in that more ancient lineages tend to occupy marginal or deep-water habitats (Salzburger et al. 2002a); these lineages are also relatively species-poor (Poll 1986). Also, the tribes that are combined in the Tanganyikan mouth-brooders seem to first of all display a specialization to particular habitat types in the lake (Salzburger et al. 2002a), which may be secondarily reversed (Koblmüller et al. 2004). Within these tribes, the more recent radiation and speciation processes seem to be as well related to trophic morphologies (see, e.g., Sturmbauer et al. 2003), whereas colorational differences are found within arrays of closely related species, for instance of the Cyprichromini and the Tropheini. Unlike in Lake Malawi, however, in Lake Tanganyika the different colored morphs are usually not considered as separate species (Poll 1986; Snoeks et al. 1994; Genner et al. 2004).

Clearly, despite the relatively great age of the family Cichlidae, it is not the old lineages that are now represented with large species numbers, but the astonishingly diverse lineages are typically the relatively young ones (see Meyer et al. 1990, 1994; Verheyen et al. 2003). The old lineages also tend to live in open-water and deep-water areas of the lakes. It is therefore possible that the older species flocks of cichlids (in particular that of Lake Tanganyika) had been more species-rich initially, and were “pruned” of species later during their evolution (Meyer 1993; Schluter 2000; Salzburger et al. 2002a; Streebman and Danley 2003). To test this assumption one would need to find out whether older lineages, compared with younger ones, are characterized by being species-poor due to extinction events. There are comparative method approaches (Nee et al. 1994; Harvey and Rambaut 2000; Pybus and Harvey 2000) that use molecular phylogenies with which these might be testable. However, without a fossil record, it may remain difficult to know whether those ancient lineages used to be more species-rich (and possibly colorful) and how many species became extinct (possibly due to competitive extinction by more competitive younger lineages) or whether those rather ancient lineages were never particularly successful in terms of the formation of new species.

Conclusions and outlook

Twenty years ago, Ernst Mayr stated that the extraordinary evolutionary success of cichlids might require special explanations. “It is thus evident that there must be some aspect in the life history and perhaps the genome of certain taxa which permit them to speciate rapidly inside of lakes” (Mayr 1984). In this age of genomics, the types of molecular data that are being collected on the “cichlid problem” are becoming increasingly sophisticated and promise to yield entirely new insights that might one day, sooner rather than later, answer the question as to whether there are some special molecular evolutionary mecha-

nisms acting in the genome of cichlids that might be at least partly responsible for their evolutionary success compared with that of other families of fishes that also live in East African lakes. Several types of genomic resources such as genetic maps, cDNA and BAC libraries, and cDNA-microarrays have been established and there is currently an effort underway to have a genome of a cichlid fish determined (Kocher et al. 2004; Kocher 2004). In addition, approaches such as QTL (quantitative trait loci) studies (Albertson et al. 2003; Streelman et al. 2003a) have been employed and more work is in progress using this novel method. This entirely new approach, which was still quite inconceivable 20 years ago, promises to unveil some of the secrets of the cichlids that they still hold as a model system for exuberant biological diversification and explosive speciation.

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