

Parallel life history evolution in mouthbrooding cichlids from the African Great Lakes

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The existence of ancient deep-water lakes provides an opportunity to study the independent adaptation of aquatic organisms to pelagic, benthic, and rocky shore habitats. With improving resolution of their phylogenetic relationships, the many cichlid fish species endemic to the African Great Lakes Malawi, Tanganyika, and Victoria provide a significant resource for the comparative study of such evolutionary processes. Here, we show that cichlid lineages colonizing rocky shores and pelagic habitats in the different lakes have independently evolved larger eggs and lower fecundities than benthic lineages, suggesting parallel adaptive life-history evolution. By contrast, other pelagic teleost fishes in both marine and freshwater habitats, including African lakes, typically produce large numbers of very small eggs. Our results also suggest that decreased fecundity and increased egg size not only occurred independently in each lake but occurred independently in the colonization of rocky and pelagic habitats.

parallelism | phylogenetically controlled comparative analysis | phylogeny

Elucidating whether similar ecological specializations in closely related lineages are the result of unique or repeated evolutionary events is of particular interest in the understanding of adaptive evolution (1, 2). The demonstration of parallel evolution of closely related yet phylogenetically distinct lineages in similar environments provides strong evidence of evolution driven by natural selection because genetic drift is unlikely to produce repeated evolution in the same direction (3, 4). Instances of parallelism abound in animal evolution (5), but most cases refer to morphological traits such as in cave amphipods (6–8), Anolis lizards (9), or lake whitefish (10). Examples of repeated evolution of the same life-history traits are scarcer, although a convincing case has been made for independent adaptations to predator-mediated mortality in several populations of Trinidadian guppies (11). To build such a case, one needs to demonstrate that multiple populations or species exposed to similar environments have evolved similar traits independently in different localities and evidence that the similarities among populations are the product of the same sort of natural selection (11).

The cichlid fishes of the East African Great Lakes represent an ideal model for the study of parallel evolution. An estimated 660–1,319 species of cichlid fishes have been recorded from Lakes Malawi, Tanganyika, and Victoria, most endemic to a single lake catchment (12). These species flocks are so rich that, collectively, they provide the best example of rapid adaptive radiation in vertebrates (13–16). The independent radiations (14, 15) in the three lakes have produced very similar communities. Many of these species are habitat specialists, with most of them confined either to rocky shores or to sand/mud bottoms (benthic), whereas a few are found in open water (pelagic) habitats (17). There are several well documented examples of parallel evolution of morphology associated with independent colonization of similar habitats (18–20) as well as parallel evolution of coloration (21, 22). Here, we show that parallel evolution of habitat specialization in East African Great Lake cichlids is further accompanied by hitherto undocumented parallel evolution of life-history traits and by a reproductive strategy

exceptional for pelagic teleost fish. Unusually, all known cichlid species provide parental care of eggs and young. Many species are biparental substrate-spawners, whose eggs and young are guarded by both parents. Many other species are maternal mouthbrooders, in which females pick up and brood eggs and larvae for a few weeks, with some species even allowing independently feeding young to return to the mouth when they are threatened with predation (17, 23). All cichlid species from Lake Victoria and all except one from Lake Malawi are maternal mouthbrooders. Although nearly every type of parental care is represented in Lake Tanganyika, all pelagic species and many of the rocky shore and benthic species are mouthbrooders [supporting information (SI) Table S1 and *SI Text*]. Mouthbrooders typically produce fewer, larger eggs than substrate spawners (17), and therefore, to avoid the confounding effect of the type of parental care on the ecological correlates of life-history strategies, all species included in this study are mouthbrooders. We compared batch fecundities and egg sizes of cichlid fishes from pelagic, benthic, and rocky-shore habitats in all three lakes using new and published data. We adopted a two-step approach for data analysis: first, testing for significant effects on the life-history traits with linear models and then assessing whether significant effects were confounded by phylogenetic nonindependence among species (24). The second step required the estimation of a phylogeny for the species included in our dataset. The phylogeny of the Victorian cichlids is still poorly resolved (25), and DNA sequences were available for few species for which we had life-history data. For Lakes Malawi and Tanganyika, we used comparative methods to account for the effect of phylogenetic relationships on life-history correlations (26, 27) and Markovian models to reconstruct evolutionary transitions among habitats (28).

Results

Fecundities differed significantly among habitats and lakes (Fig. 1A and Table 1), but the lack of a significant interaction indicated similar contrasts among habitats in all three lakes. Controlling for phylogeny (Fig. 2A), the difference among lakes was no longer significant, which was expected because the haplochromine species flocks of lakes Malawi and Victoria represent independent colonizations (14), but the habitat effect remained significant. In the analysis without phylogenetic correction, egg diameter varied significantly among both habitats and lakes (Fig. 1B and Table 1), but again, the difference among lakes was confounded with phylogeny (Fig. 2B). A significant interaction term, even after phylogenetic correction (Fig. 2B), indicated that the contrast among habitats was

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and reduction of clutch size was observed as benthic species adapted to either rocky or pelagic habitats.

Life-history theory is dominated by the concept of tradeoffs (32). Our data do not permit us to consider directly the overall level of lifetime or per-clutch reproductive investment nor to estimate postzygotic investment but only to consider, for a given level of total prezygotic investment in oocyte production, the within-clutch tradeoff between offspring number and per-offspring investment (33). Such a tradeoff is well known among fishes, from comparisons between species (34), populations (35), and even siblings (36).

Physical factors may favor the production of few, large eggs by pelagic mouthbrooding cichlids, at least some of which spawn off the substrate in Lakes Malawi (37), Tanganyika (38, 39), and Victoria (40). Midwater-spawning females spin round to catch their eggs, and the laying of fewer eggs at a time may increase retrieval rates and reduce predation risk. However, this advantage does not apply to rocky-shore species that lay their eggs on the bottom.

All known cichlid fish provide parental care to their offspring (23). All of the species in our study are maternal mouthbrooders, but there may be differences between species in the nature and duration of parental care. Riverine maternal mouthbrooding cichlids, like their biparental substrate-spawning relatives, typically care for free-swimming independently feeding fry for periods lasting weeks or months. Although information is patchy and remains to be thoroughly reviewed, many benthic cichlid species in both lakes (and a few rocky-shore species in Malawi) guard free-swimming fry, as apparently do all of the Lake Victoria species irrespective of habitat. Fry guarding may favor the production of larger numbers of smaller offspring, because parental care may increase the survival rates of smaller fry. On rocky shores in Malawi and Tanganyika, fry of many maternal mouthbrooding species are immediately independent on first release, after absorption of the yolk sac, and attempt to establish territories in refuges among rocks (41). As far as we know, there has been little recognition or discussion of the scarcity of parental care of free-swimming fry on rocky shores: The high density of possible predators, the abundance of cover for their approach, and the difficulty in moving a free-swimming brood among the high density of territorial fish may all reduce the benefits of parental care. Notably, the majority of species in Lake Malawi that do provide care for free-swimming fry over rocky habitats are large, often predatory, species that might be expected to be particularly effective guarders (42). Thus, it may be that the principal advantage to the production of large eggs for rocky-shore cichlids lies in the larger size at hatching of the offspring. Larger offspring are likely to be less vulnerable to a certain component of the predator community or vulnerable for a shorter period and be better competitors over food and space (43, 44). It has been suggested that there is high predation pressure on young fish in the crowded rocky shores of the African Great Lakes (17). Less is known about parental strategies of pelagic cichlid fish. Females of the Malawian *Rhamphochromis longiceps* migrate inshore to release fry in lagoons (45), and mouthbrooding female *Rhamphochromis esox* are often found over rocks (46), but it is thought that most other species remain offshore throughout their breeding cycle. It is not known whether pelagic cichlids guard free-swimming fry, but juveniles of some species are retained in their mothers' mouths until they are >30 mm and have started exogenous feeding (47, 48). Prolonged mouthbrooding in pelagic cichlids is probably an adaptation to increase juvenile survival, because increased mouthbrooding duration was positively related to predation risk in other mouthbrooding cichlids (49). Perhaps high-predation risks in the open pelagic zone select against fry guarding and favor large offspring size, but this remains speculative.

Most pelagic marine and freshwater teleost fishes produce many small eggs, whereas pelagic cichlids produce few large eggs. The existence within the same lake of divergent reproductive strategies among fishes living in the same habitat suggests that the direction a particular lineage evolves toward may depend on its starting point

(50). Although we lack sufficient information to say which direction they have evolved in since colonizing the lakes, the endemic noncichlid pelagic fishes retain the high fecundity–small eggs pattern typical of the families centrropomidae, clupeidae, and cyprinidae (51). Clearly, the pelagic cichlids have not evolved toward this kind of life-history strategy. These cichlids have likely arisen from ancestors that, like all known cichlids, were batch spawners with low fecundities and large eggs. Mouthbrooding cichlids (as in this study) lay even fewer, larger eggs than others (17, 23). Phylogenetic analysis suggests that there have been few, if any, reversals from mouthbrooding to substrate spawning in the evolution of African cichlid parental care (52) and none at all within the Great Lakes. Nevertheless, pelagic cichlids might be expected to have relatively high fecundities and small eggs among mouthbrooding cichlids, rather than the fewer, larger eggs indicated by our analysis.

Low fecundity and large egg size are indications of high levels of parental investment per offspring. Large-scale patchiness of spatial and temporal variability in resource availability is believed to favor production of large numbers of small offspring as a bet-hedging strategy for pelagic teleosts (29, 31). However, reduction of the risk of starvation in prey-poor environments could also select for small broods of large offspring (29). This might apply to cichlids because the pelagic zone of the African Great Lakes is relatively oligotrophic compared with the productive inshore habitats (53, 54). If so, this would suggest that the evolution of large offspring size had different causes among rocky-shore and pelagic cichlids. The small fry of pelagic cyprinids and clupeids initially feed on unicellular algae (55–57), whereas the much larger fry of cichlid fish in all habitats feed mainly on crustacean zooplankton (55, 58, 59). Differential spatiotemporal patchiness of zooplankton and phytoplankton could lead to differing selection pressures among the fish families in the pelagic zones of the African lakes, simply from the initial divergence in offspring size and trophic level.

A comparable approach to offspring size and fecundity in marine species may be illuminating. Although marine pelagic teleosts produce many small offspring, it has been suggested that, among elasmobranchs, transition to a pelagic lifestyle is associated with the evolution of viviparity (60), although this relationship has yet to be quantitatively tested with phylogenetically controlled methods. Elasmobranchs, even more than cichlids, typically produce few, large offspring, and viviparous species have larger offspring (61), although the evidence for reduced fecundity is equivocal (61, 62). Thus, there are intriguing hints that phylogenetically constrained multiple adaptive life-history peaks also exist in the marine pelagic zone.

Our results indicate that an increase in per-egg investment occurred not only in pelagic cichlids but also to a lesser extent in the evolution of specialized rocky-shore cichlids. There are few specialized rocky-shore fishes among the other fish families in the African Great Lakes, but there is a high incidence of parental care among fishes of marine rocky shores (63). This may indicate a common selection pressure with rocky-shore cichlids favoring high per-offspring investment. There are two main life-histories groups among coral reef fishes, each of approximately the same diversity and abundance. The first group mainly consists of species with large body size, long life, and late maturation, which release huge numbers of tiny pelagic eggs (64), often in communal spawning aggregations (65). The other group is mainly made up of small cryptic species that provide parental care for small numbers of benthic eggs (64). So, again, there are interesting hints of a double adaptive peak.

The independent and parallel evolution of similar reproductive traits within the same mouthbrooding mode of parental care in the pelagic, rock-dwelling, and benthic cichlid fishes inhabiting the three lakes strongly suggests that these reproductive strategies are adaptive to the colonization of these habitats. Our results also suggest that decreased fecundity and increased egg size not only occurred independently in each of the three African Great Lakes

but occurred independently in the colonization of rocky and pelagic habitats. With finer phylogenetic resolution and more life-history data, future analyses may show further parallel transitions in egg size, fecundity, and perhaps other life-history traits associated with independent transitions between the same habitats by different lineages within the same lake. Additional phylogenetically controlled analysis of life-history traits may reveal parallel evolution and multiple adaptive peaks within other aquatic habitats.

Materials and Methods

Categorization of Taxa. We analyzed the species most strongly associated with the pelagic zone in each lake and compared them with more rocky-shore- or bottom-living (benthic) cichlids from the same lake. This classification was based on knowledge of the distribution, diet, breeding habits, morphology, and phylogenetic affinities of species. For example, in Lake Malawi, the genera *Diplotaxodon* and *Rhamphochromis* comprise at least 20 species of silvery, countershaded, streamlined, midwater-feeding zooplanktivores and piscivores, of which at least 5 species are abundant throughout all open water habitats, even where the bottom depth exceeds 250 m and is deoxygenated. *Pallidochromis tokolosh* is a deep-water species that feeds on benthic fish (66) but is morphologically similar to *Rhamphochromis* and has recently been shown to be a member of the *Diplotaxodon* clade (67, 68). *Copadichromis quadrimaculatus* is a laterally compressed zooplanktivore, morphologically and genetically similar to other inshore-living species presently classified in *Copadichromis*. It breeds in shallow rocky and sandy areas, but large adult specimens are an important part of the open-water catches (47, 69). Therefore, *C. quadrimaculatus* and *P. tokolosh* were classified as pelagic. Shallow-water, rocky-shore species were distinguished from benthic species occupying sandy or muddy bottoms. For lakes Tanganyika and Victoria, categorization of taxa followed the sources (see [Tables S2 and S3](#)).

Sources of Data. Species identification features for Lake Malawi fish were described elsewhere (70), and nomenclature follows Turner (69) for benthic and offshore species and Ribbink *et al.* (71) for rock-dwelling species. Benthic and pelagic fish species were collected by monthly trawl catches in the north of the Southwest arm of Lake Malawi. Rock-dwelling species were caught by gill nets in the same area (70). Fish were measured (standard length) to the nearest millimeter and weighed to the nearest 0.1 g. Ripe gonads were fixed in 5% formalin for later determination of gonadosomatic index (GSI = gonad weight/total body weight \times 100), fecundity, and mean oocyte weight. Based on gonads in the final preovulation maturation stage (stage 4), fecundity was estimated as the number of oocytes belonging to the size class of the greatest diameter. This group was clearly distinguished by eye and essentially corresponds to the oocytes that would be released in the next spawning bout. Thus, fecundity was measured as the number of oocytes to be released at the next spawn (i.e., batch fecundity). Oocyte weight measurements were carried out on samples preserved for 3 weeks in 5% formalin. The average oocyte weight per female was determined by weighing 50 oocytes belonging to the pool of oocytes considered for fecundity estimates. To allow comparison of oocyte weights of different species, measurements were made at the same vitellogenic stage, when growth has been completed. To determine the GSI threshold above which oocyte weights no longer increase significantly, the mean oocyte weights of females of each species were plotted against their GSI. The GSI corresponding to the beginning of the asymptotic part of the curve (GSI_a) was visually determined, and any fish whose GSI was lower than the GSI_a were removed. The final GSI threshold was reached when no correlation remained between the mean oocyte weight and the GSI (72).

Data from Lake Malawi species were obtained mainly from our own surveys as well as from published sources (12 pelagic, 34 benthic, and 23 rock-dwelling species, [Table S1](#)). Data for species from Lake Tanganyika (4 pelagic, 19 benthic, and 17 rock-dwelling species, [Table S2](#)) and Lake Victoria (6 pelagic, 10 benthic, and 3 rock-dwelling species, [Table S3](#)) were obtained from the literature. For comparison, we also compiled published data for pelagic species of families other than Cichlidae from East African Great Lakes (5 species) and from several marine pelagic taxa (33 species, [Table S4](#) in SI).

Most of the reproductive data collected in the bibliography refer only to body length not body weight. As body shapes of cichlid fishes within a given habitat are similar in the three lakes (17, 18), the weights corresponding to the published lengths of Lake Tanganyika and Victoria species were estimated by using Lake Malawi per-habitat length–weight (L/Wt) relationships: Benthic: $Wt (g) = 0.000155 SL (mm)^{3.13}$, $n = 11\ 222$, $r^2 = 0.957$.

Rock: $Wt (g) = 0.000616 SL (mm)^{2.85}$, $n = 3\ 667$, $r^2 = 0.865$.

Pelagic: $Wt (g) = 0.0000813 SL (mm)^{2.73}$, $n = 3\ 172$, $r^2 = 0.880$.

For the Malawi species, we used actual weights, except for *R. macrophtalmus*, *R. grayi*, and *R. esox*, where weight was estimated by using the Malawian L/Wt. For the pelagic L/Wt relationships, we added individual L/Wt raw

data for *Rhamphochromis* spp. (provided by A. B. Thompson) to augment the sample sizes and get a better accuracy for the large fish.

For Lakes Tanganyika and Victoria, “egg size” refers to oocyte maximum diameter. For Lake Malawi, we had egg weights for every species, but only a few egg diameters were measured. For the sake of data homogeneity among the three lakes, oocyte maximum diameter was then used for comparison of reproductive investment. For Lake Malawi, oocyte diameter was estimated from a regression of mean oocyte weight versus mean length of the largest oocyte diameter of 13 Lake Malawi cichlid species (73), from <http://cichlidresearch.com/eggtab3.html#Malawi> and from data collected in the present study, giving oocyte diameter (mm) = 0.055 weight (mg) + 2.886, $r^2 = 0.7542$. The trend of between-habitat differences in egg length was similar to that observed using egg weight.

Fecundity and egg size in cichlids are generally positively correlated with body mass (74, 75). Such correlations were observed at the intra- and interspecific level for fecundity but only at the interspecific level for egg diameter. Details on the relationships between fecundity or egg diameter and body mass for all combinations of habitat and lakes are presented in [Figs. S1 and S2](#), respectively). Batch fecundity and oocyte diameter were compared by using standard analyses of covariance (ANCOVA, with lake, habitat, and body mass as covariates) performed with R for cichlid fishes occupying pelagic, benthic, and rocky habitats in the three lakes. For a clearer graphic representation of the fecundity and egg diameter contrasts between lakes and habitats among Great Lakes cichlids and between pelagic cichlids and other pelagic teleost fishes ([Fig. 1](#)), relative fecundity (number of eggs per kilogram of female) was used.

Phylogenetic and Comparative Analyses. Given that no published phylogenies of African cichlids include a representative number of species in our dataset, we built a phylogeny using DNA sequences from GenBank. We initially considered five genes (mitochondrial control region, cytochrome *b*, NADH-2, and nuclear RAG1-exon 3, and RAG1-intron 2), but after alignment, only the control region (581 sequences of 126 species) and the NADH-2 (134 of 79) datasets were further considered.

Alignment was performed with Clustal X with default parameters and then visually inspected. The accession numbers of the 581 sequences of control region and of the 134 sequences of the NADH-2 are listed in [SI Text](#). An examination of the alignment of the control region revealed that the number of sequences per species was highly biased, and many sequences were very short (53.3% of the data were alignment gaps on 1,008 sites). We removed some sequences to homogenize this dataset. This led us to keep 252 sequences representing 125 species (43.3% of alignment gaps). All 134 aligned NADH-2 sequences were used in subsequent analyses (79 species, 1,048 sites, 1.9% of alignment gaps).

The estimation of the species phylogeny was difficult because many species did not appear as monophyletic with these mitochondrial sequences (usually owing to introgression through past hybridization (76)). To take all available information for a given species into account, we used the distance-based neighbor-joining method [NJ (77)] where, in a first step, we computed the distances among all aligned sequences, and, in a second step, we computed the distances between each pair of species as the mean of the distances between the sequences belonging to these two species. The NJ tree was estimated from this interspecies distance matrix. To assess which substitution model best fitted the data, we first fitted different models by maximum likelihood using PHYML. We found that the GTR and Tamura-Nei models were very close as best models. We selected the latter to compute the distances between pairs of sequences because there is no closed formula for the former. The estimated trees by NJ are displayed in [SI Text](#) ([Fig. S3](#) and [Fig. S4](#) for the control region and the NADH-2 dataset, respectively). We also estimated trees by NJ and by maximum likelihood (ML) with PHYML from the aligned sequences (78). From the between-sequences distance matrix, we computed the between-species distances by averaging all distances from sequences belonging to a pair of species. This was done independently for the control region and NADH-2 data as a way to assess the reliability of our results with respect to a particular molecular phylogeny. The trees were rooted with *Boulengerochromis microlepis* (14). Before doing the comparative analyses, the trees were trimmed to keep only the species for which we had life-history data (55 and 45 species for the control region and the NADH-2 trees, respectively).

To assess phylogenetic uncertainty, we performed a bootstrap analysis on both datasets by resampling 100 times the aligned sequences and repeating the procedure described above: We ended up with 200 trees. We repeated the comparative analyses with these 200 trees. The comparative analyses were done with a generalized estimating equations (GEE)-based method (24), which allowed us to fit a generalized linear model (that is similar to the ANCOVA performed previously) taking phylogenetic correlation into account. We plotted histograms of the 200 *P* values (for each tree) for each GEE-based test to assess whether the comparative analyses were influenced by a particular tree.

Evolutionary transitions among habitats were analyzed with Markovian models fitted by maximum likelihood (26). We built several models with different

parameterizations of the transition rates among habitats. For instance, the most general model assumes that all rates of habitat change are different and so has six parameters, whereas the simplest model has one parameter (all rates are equal). These different models were compared by likelihood ratio tests and the Akaike information criteria (AIC) when they were not nested. All comparative analyses were done with APE (27).

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