Replicated Functional Evolution in Cichlid Adaptive Radiations

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ABSTRACT: Adaptive radiations highlight the mechanisms by which species and traits diversify and the extent to which these patterns are predictable. We used 1,110 high-speed videos of suction feeding to study functional and morphological diversification in 300 cichlid species from three African Great Lake radiations of varying ages (Victoria, Malawi, and Tanganyika) and an older, spatially dispersed continental radiation in the Neotropics. Among African radiations, standing diversity was reflective of time. Morphological and functional variance in Lake Victoria, the youngest radiation, was a subset of that within Lake Malawi, which itself was nested within the older Tanganyikan radiation. However, functional diversity in Neotropical cichlids was often lower than that in Lake Tanganyika, despite being much older. These two radiations broadly overlapped, but each diversified into novel trait spaces not found in the youngest lake radiations. Evolutionary rates across radiations were inversely related to age, suggesting extremely rapid trait evolution at early stages, particularly in lake radiations. Despite this support for early bursts, other patterns of trait diversity were inconsistent with expectations of adaptive radiations. This work suggests that cichlid functional evolution has played out in strikingly similar fashion in different radiations, with contingencies eventually resulting in lineage-specific novelties.

Keywords: geometric morphometrics, kinematics, morphology, adaptive radiation, cichlids.

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Introduction

Adaptive radiations provide glimpses into how traits diversify and evolve across related taxa in the presence of ecological opportunity (Simpson 1953; Gillespie et al. 2020). Studies of adaptive radiations have helped to explain how Caribbean anoles (Losos et al. 1998) and Hawaiian spiders (Gillespie 2004) have colonized new island habitats through repeated evolution of convergent ecomorphs. They have also highlighted mechanisms underlying trait divergence across adaptive peaks in Bahamian pupfishes (Patton et al. 2022), Galapagos finches, and Hawaiian honeycreepers (Tokita et al. 2016). A common theme in the literature on adaptive radiations is the degree to which trait evolution reflects predictable patterns of diversification versus the generation of novel phenotypic combinations, where replicated radiations provide insights into these patterns (e.g., Schluter 1996; Losos et al. 1998; Gillespie 2013).

Cichlid fishes are renowned for having multiple expansive radiations involving hundreds of species in each of three large African lakes—Victoria, Malawi, and Tanganyika (Fryer and Illes 1972; Stiassny and Meyer 1999; Seehausen 2006)—and a continental radiation in tropical South and Central America (López-Fernández et al. 2013; Arbour and López-Fernández 2014). The existence of these large radiations of related species provides an opportunity to capitalize on natural replication to address questions about the evolutionary repeatability of these systems at a scale beyond the first few niche expansions. The radiations also differ considerably in age, approximately 55 Ma for Neotropical cichlids, 10 Ma for Lake Tanganyika, 2 Ma for Lake Malawi, and 0.1 Ma for Lake Victoria (we discuss cichlid

ages further in the supplemental PDF). These differences present temporally spaced sample points that allow insight into the long-term unfolding of adaptive radiations and the relative importance of time and rate of diversification on current patterns of diversity.

Previous comparisons of phenotypic diversity in the three African lakes have drawn two main conclusions. First, diversity of body shape and trophic morphology differs between the radiations, with the oldest in Lake Tanganyika housing greater diversity of body shape and craniofacial morphology and the youngest in Lake Victoria having the lowest diversity (Young et al. 2009; Cooper et al. 2010). It is not known whether adaptive radiations of the African lakes have amassed greater morphological diversity and associated functional variation than the much older continental radiation in the Neotropics that includes roughly 500 species (López-Fernández et al. 2013). Second, convergent ecology and morphology are common both among and within radiations (Salzburger 2009), suggesting relatively predictable modes of diversification and broadly repeated patterns of evolution in feeding morphology (Hulsey et al. 2008; Cooper et al. 2010; Conith et al. 2019), body shape (Kocher et al. 1993; Young et al. 2009), or both (Rüber and Adams 2001; Muschick et al. 2012). Similar instances of convergence have been found among related groups of Neotropical cichlids (Burress et al. 2017; Arbour et al. 2020), as well as between Neotropical and African species (Winemiller et al. 1995). Thus, large cichlid adaptive radiations could generate similar, although not identical, sets of phenotypes where diversity accumulates over extended time periods.

The temporal sampling of cichlid radiations creates an opportunity to evaluate, at various stages of progression, patterns of functional diversification relative to our expectations of adaptive radiations. A classic prediction is an early burst in trait diversification, where evolutionary rates are initially rapid as open niches in newly colonized habitats are filled, followed by a nonlinear decay in rates after initial expansion (Simpson 1953). An assumption of the early-burst model is that the rapid increase in trait diversity is achieved by different lineages evolving toward separate adaptive zones where they subsequently undergo further diversification, resulting in comparatively greater variance among clades than within (Simpson 1953; Harmon et al. 2003).

In the present study, we describe the diversity of cichlid prey capture kinematics using high-speed video recordings of 300 species, sampled from the three African Great Lake radiations and the Neotropics. We contrast kinematic and morphological variation of the feeding mechanism in radiations of varying age to test the hypothesis that differences in standing functional diversity are due to time, as opposed to different rates of evolution. To assess the re-

peatability and predictability of cichlid adaptive radiation, we also quantify the extent to which each has produced similar ranges of feeding kinematics. Additionally, we test the key expectation that adaptive radiations exhibit an early burst in trait diversification that is achieved through the partitioning of traits among clades (Simpson 1953; Harmon et al. 2003).

A secondary objective of this work is to examine the relationship between morphological and functional diversity of the cichlid feeding mechanism. Preliminary estimates of body and craniofacial variation in the three large African lake radiations have typically been interpreted as reflecting functional diversity linked to locomotor and feeding biomechanics (Young et al. 2009; Cooper et al. 2010). In many cases, links between morphological variation and functional properties are well established (Hulsey and García de León 2005; Hulsey et al. 2006; Higham et al. 2007). Nevertheless, our current understanding of functional diversity in cichlids is largely inferred from morphological variation rather than direct measurements of functional traits. Comparisons of functional diversity allow us to test the reliability of morphological variation to reflect function and help to identify key axes of diversification that are cryptic when only morphology is considered.

Methods

Species Sampling and Feeding Videos

A total of 1,110 high-speed videos of feeding motions from 300 species of laboratory-filmed cichlids were studied (tables S1, S2; tables S1–S6 are available online). Species were broadly distributed phylogenetically from one of four focal radiations, three African Great Lakes—Victoria ($n = 40$), Malawi ($n = 86$), and Tanganyika ($n = 89$)—and the Neotropics ($n = 85$ species). We note that two study species belonging to the Lake Victoria region superflock, *Harpagochromis* sp. "golden duck" and *Pyxichromis orthostoma*, are endemic to Lake Kyoga, which retains a connection to Lake Victoria via the Victoria Nile River. All videos were filmed from a lateral perspective at 2,000 fps (McGee et al. 2016) and contained full-effort suction-feeding strikes on moderately evasive living midwater prey. Primary prey included mosquito larvae (*Culex pipiens*), black worms (*Lumbriculus* sp.), and *Daphnia magna*. Small fish were occasionally used as prey to elicit sufficiently full-effort feeding strikes from some species, which was important for reducing kinematic variation due to fish effort. Videos in which fishes displayed low-effort prey capture behaviors were not included in this study. We extracted 10 frames from each video, equally spaced in time from the initiation of the motion to peak expansion of the feeding apparatus, prior to mouth closing. For comparative analyses,

we matched filmed species to a recent cichlid phylogeny (McGee et al. 2020; supplemental PDF).

Morphological and Functional Traits

Cichlid functional diversity was determined using a variety of kinematic traits, all derived from a set of 10 landmarks and eight semilandmarks manually placed on cranial images for each of 10 video frames comprising a motion (figs. 1, S1; figs. S1–S4 are available online). Digitizing was done in tpsDIG2 (Rohlf 2015) and StereoMorph (Olsen and Westneat 2015). Landmark data for 53 species came from a previous study (Martinez et al. 2018), but the remaining 247 species comprised new data. First, subsets of landmarks were used to measure movements (i.e., maximum excursions) of key morphological features involved in prey capture. In total, we created six motion component traits (fig. 1B), three from rotational movements of bones (lower jaw rotation, cranial rotation, and maxillary rotation) and three from linear displacements (premaxillary protrusion, hyoid depression, and mouth gape). We analyzed these traits as a multivariate combination of all motion components, as well as individually. Because of the incommensurability of angles and linear displacements (Huttegger and Mitteroecker 2011), we converted the three rotational traits to distances by using the observed angle of rotation and the length of the rotating arm (measured on the fish at full gape) to determine the length of the arc transcribed by the structure in question (e.g., the Euclidean distance traveled by the distal end of the maxilla). All component traits were then scaled by dividing values by the centroid size of the fish's head in a closed-mouth state. Last, we averaged the components across repeated feeding trials within individuals and then across individuals to get a mean trait value for the species. All additional traits described below were similarly averaged to species for comparative analyses.

Additional kinematic traits were created using an approach that characterizes movements as trajectories of shape change (fig. 1), integrating the numerous moving parts involved in a complex motion into a single object that allows for comparisons at the whole-motion level (Adams and Cerney 2007; Adams and Collyer 2009; Collyer and Adams 2013; Martinez et al. 2018, 2022; Martinez and Wainwright 2019). Digitized cranial landmarks were aligned and scaled using generalized Procrustes analysis (GPA) with the gpagen function in the geomorph package (ver. 4.0.3) in the R statistical environment (ver. 4.1.3; Adams et al. 2021; R Core Team 2022), with alignment of sliding semilandmarks along the ventral margin of the head achieved by minimizing Procrustes distances. Once aligned with GPA, the progressive movements of landmark-tracked cranial features result in a trajectory of shape change (fig. 1A, 1E), the features of which can be used as traits that capture motion

variation (Martinez et al. 2018, 2022; Martinez and Wainwright 2019). In this study, for example, the length of each motion trajectory is a measure of cranial kinesis, or the amount of movement generated by the feeding apparatus during prey capture (fig. 1D). The total trajectory length was computed as the sum of Procrustes distances between consecutive motion shapes (Collyer and Adams 2013).

We also generated two composite traits designed to provide context about when and how kinesis is achieved. Kinesis skew was the ratio of kinesis across the final five motion shapes to the total kinesis for the motion, normalized by taking the natural logarithm for statistical analyses. This trait is a descriptor of the temporal distribution of kinesis within a movement, with smaller values indicating comparatively more movement toward the beginning of the feeding strike and larger values meaning that movement is concentrated near the end of the strike. Next, we measured a kinesis coefficient trait as an analog to kinematic transmission (Westneat 1994, 2004), which is commonly used with biomechanical linkage models to describe output movement of an anatomical feature given a degree of input motion from another. Here, we took the natural logarithm of total kinesis for a motion (output movement) divided by maximum cranial rotation (input movement) from the motion components described above. We used cranial rotation, which is powered by contraction of epaxial muscles posterior to the head, for the input value, as it facilitates expansion of the buccal cavity and drives movements of other features of the feeding apparatus (Camp et al. 2020).

The final functional trait we compared was motion pattern, briefly described here. For complex biomechanical systems composed of numerous mobile features, any change in relative timing and/or degree of movement across those features causes variation in the pattern of movement at the whole-motion level. We used anatomical landmarks to express feeding movements as an ordered series of changing shapes over time, or a trajectory through morphospace (fig. 1A). The paths forged by these trajectories each have their own shape and represent motion pattern—different trajectory shapes represent different patterns of movement that can be observed both within a single species feeding with different modes of prey capture (Martinez et al. 2022) and across species with divergently evolved feeding systems (Martinez et al. 2018). Motion components and motion pattern are both multivariate descriptors of feeding movements but capture contrasting aspects of their diversity. Motion components measure maximum excursions of key features of feeding motions, whereas motion pattern describes how and when those movements take place (Martinez et al. 2022). To compare motion patterns, we used modified code from the trajectory.analysis function in the R package RRPP (ver. 1.0.0) to align and scale trajectories
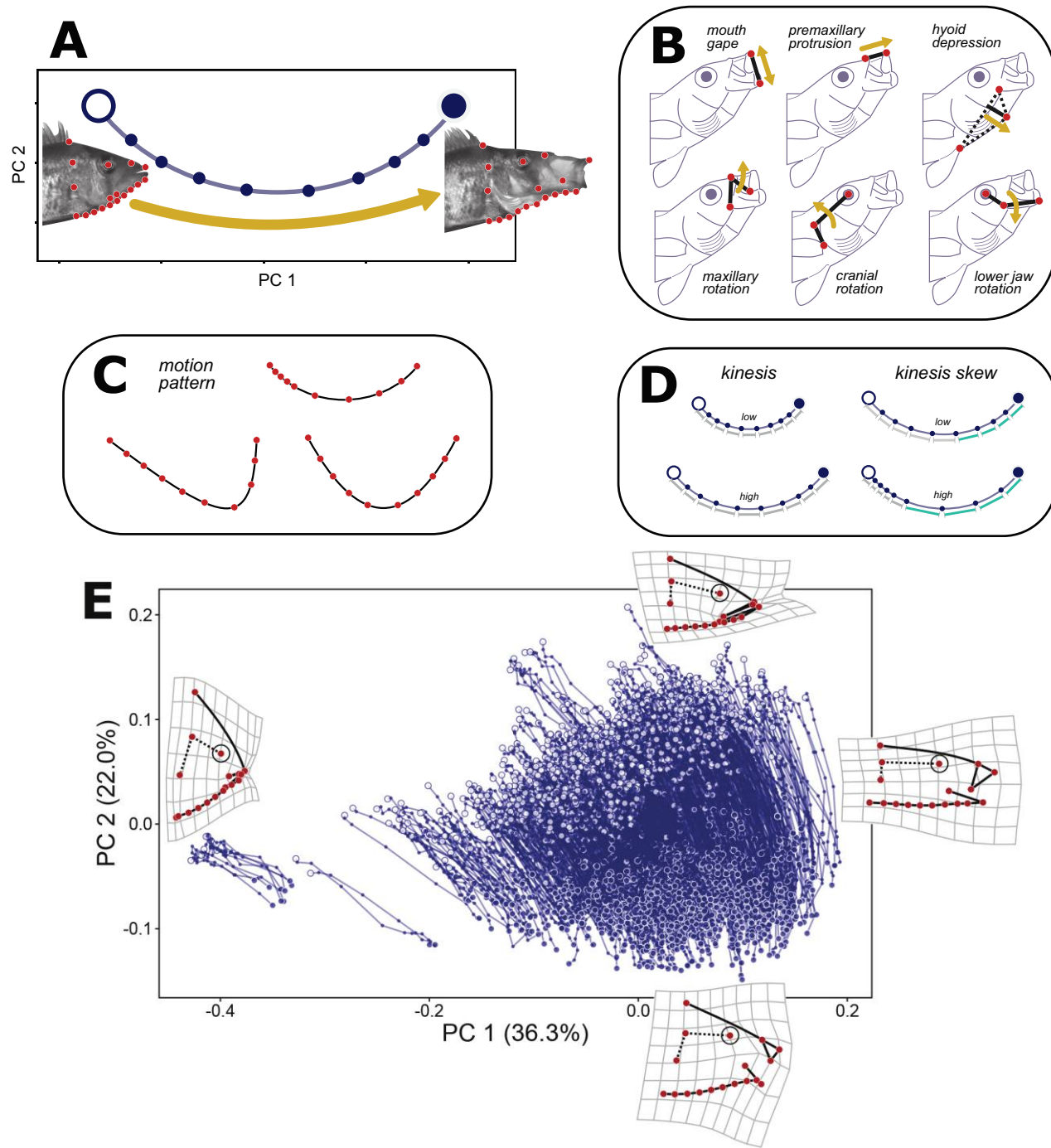
Figure 1: Functional traits examined across 300 species of cichlid in this study. *A*, Principal component axes (PC1 and PC2) displaying shape change for a single idealized trajectory composed of 10 hypothetical cranial shapes during a suction-feeding motion. *B*, Subsets of two or three landmarks were used to measure maximum excursions of six commonly measured motion components of suction feeding in percomorph fishes. *C*, Changes in the timing or extent of movements represent differences in motion pattern that are manifested as variation in trajectory shapes. Trajectories varied in spacing of cranial shape changes and the relative symmetry of trajectory paths. *D*, A series of trajectory-derived traits related to mobility included cranial kinesis (total trajectory length), kinesis coefficient (not pictured; total output kinesis divided by input movement from cranial rotation), and kinesis skew (kinesis over the last five motion shapes divided by total kinesis). *E*, PC1 and PC2 from 1,110 trajectories of suction-feeding motions, with deformation grids displaying shape change. All plots are shown in two dimensions for visualization, but data analysis was in the full dimensionality of the shape data unless otherwise noted.

(fig. S2; Collyer and Adams 2018, 2019). Here, the centroid size of the entire trajectory was the scaling factor.

To provide context to functional and kinematic patterns, we also examined interspecific cranial morphologies across cichlid species. We extracted head shape data from the starting positions of feeding motions, where the mouths were in a closed state. A separate shape alignment was done on head shape landmarks, which were then averaged to species prior to statistical analyses.

Trait Diversity and Overlap among Radiations

Variance of morphological and kinematic traits, both univariate and multivariate, were measured and statistically compared using the morphol.disparity function in geomorph with 10,000 permutations. In addition to variance, a measure of trait dispersion, we were interested in the degree of overlap (or lack thereof) of trait ranges between radiations as a means of describing the occupation of multivariate functional and morphological spaces. We created four-dimensional hypervolumes for motion components, motion pattern, and head shape data using the R package hypervolume (ver. 3.0.0; Blonder et al. 2014, 2018). We took the first four axes from a principal component analysis (PCA), as hypervolumes are best conducted on orthogonal variables (Blonder et al. 2014, 2018). Hypervolumes were made for each radiation (e.g., species from Lake Tanganyika) and for subsets of the data excluding each radiation (e.g., all species not from Lake Tanganyika). We then assessed hypervolume overlap and the fraction of unique space occupied by each radiation. Last, we estimated the likelihood of our observed results against a null distribution of hypervolumes generated by randomly permuting group assignments among species 10,000 times (e.g., Corn et al. 2022).

Rates of Evolution

For all traits, we estimated rates of evolution in each cichlid radiation with the compare.evol.rates function in geomorph. This function computes the Brownian rate parameter for both univariate and multivariate continuous trait data using distance matrices (Adams 2014). The approach has been shown to yield numerically equivalent rate estimates to covariance-based methods for univariate data, but it also accommodates high-dimensional data, like shapes (Adams 2014). Pairwise comparisons of rates were accomplished through permutations in which trait data were randomly assigned to tips. Statistical significance was then assessed by comparing observed rate ratios between pairs of radiations to the distribution of ratios obtained from 10,000 permutations.

Rates were estimated using a tree containing all four radiations examined in this study (McGee et al. 2020) to maintain a single, consistent phylogenetic hypothesis. This tree reconstructs the age of the Lake Tanganyika radiation at about 28 million years old. To ensure that the resulting rates did not bias comparisons across radiations or our interpretations of them, we additionally estimated rates for Lake Tanganyika using a time-calibrated tree based on whole genomes that dates the radiation to 10 Ma (Ronco et al. 2021). This tree was reconstructed with nearly all species of the in situ Tanganyikan radiation, so we trimmed it to our study species.

Modes of Trait Diversification

We examined both historical reconstructions of trait diversification and contemporary patterns of variation to explore the manner by which trait diversity was attained in cichlid radiations. We estimated the accumulation of trait disparity through time (DTT) in radiations using the dtt function in the R package geiger (ver. 2.0.7; Harmon et al. 2008; Pennell et al. 2014). DTT plots show relative disparities among subclades at each divergence event in the tree, estimating whether trait diversity is concentrated within or among subclades as an explicit test of the early-burst expectation (Simpson 1953). An output of this analysis is the morphological disparity index (MDI), a metric for comparing the difference between the estimated relative disparity of a clade and the disparity of the clade under simulated Brownian motion. MDI statistics were calculated for the first 75% of the tree's history, as missing species in the recent phylogeny may obscure patterns close to the present. We estimated DTT from subtrees of each radiation for motion components, motion pattern, and head shape using the first four axes from PCAs on each, consistent with our comparisons of hypervolumes. Tree topology is key to DTT analyses, so we note caution in interpreting results for young radiations, like Lakes Malawi and Victoria, in which a treelike model of lineage diversification is unlikely due to widespread hybridization (Joyce et al. 2011; Meier et al. 2017; Scherz et al. 2022). Consequently, we focus our discussion on the two older radiations in Lake Tanganyika and the Neotropics but provide results for all radiations in the supplemental PDF.

To further examine patterns of trait dispersion, we computed distances between extant species and radiation-specific ancestral states. For each cichlid radiation, we estimated ancestral states under Brownian motion with the gm.prcomp function in geomorph, extracting the value at the root of the tree as the most recent common ancestor (MRCA) for the radiation. Finally, we measured Euclidean distances (for motion components) and Procrustes distances (for motion pattern and head shape) between each species and its radiation's MRCA.

Results

Functional Diversity across Cichlid Radiations

Motion components, composed of six key features of fish cranial movement during feeding (fig. 1*B*), displayed 3.2 (Lake Tanganyika) and 2.9 (Neotropics) times greater variance in older radiations compared with the youngest radiation in Victoria (figs. 2, 3; table S3). Separate univariate analyses on the individual components did show some variation in rank orders of variances across traits (fig. 2; table S3). In all cases, Lake Victoria had the lowest variance, followed closely by Lake Malawi, but some traits displayed their highest variation in Lake Tanganyika (premaxillary protrusion, maxillary rotation, lower jaw rotation, and mouth gape), while others were most variable in the Neotropics (cranial rotation and hyoid depression).

Functional traits derived from trajectories of shape change also showed different levels of diversity across radiations. Cranial kinesis had significantly greater variance in Lake Tanganyika than all other cichlid radiations, having 1.9 times greater variance than the next closest (the Neotropics) and 2.9 times greater variance than the lowest (Lake Victoria). Kinesis skew (the proportion of kinesis in the latter half of the feeding motion) was again most variable in
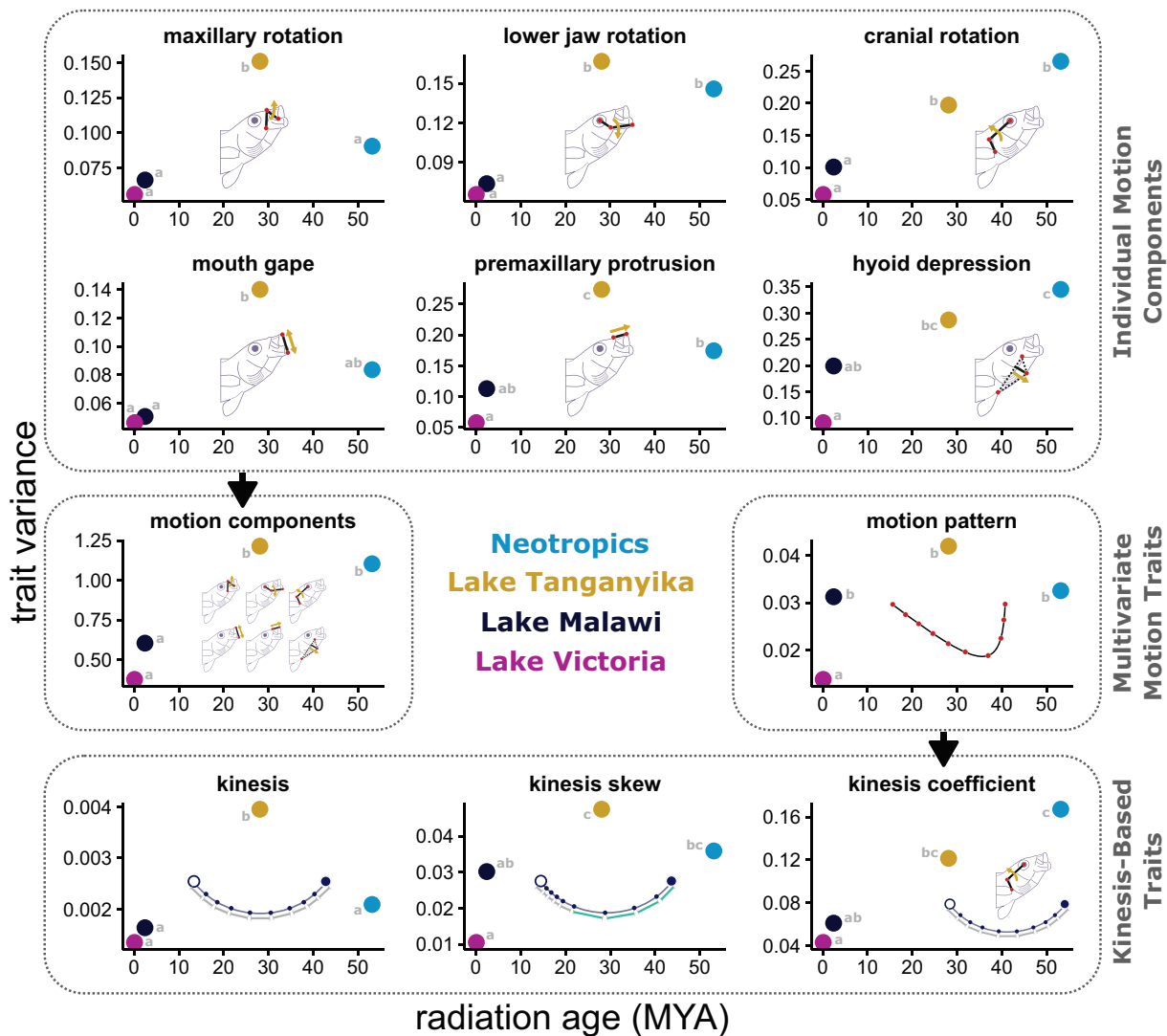

Figure 2: Functional trait variances in cichlid adaptive radiations plotted against maximum radiation age. Six individual kinematic traits are shown on top, with an arrow pointing to the multivariate motion components containing all of them. Motion pattern (i.e., the shape of a kinematic trajectory) is shown in the middle with an arrow pointing to composite kinesis-based traits measured from trajectories. Letters next to plot points denote significant *P* values from pairwise comparisons. Radiations sharing a letter do not have statistically different variances. MYA = million years ago.
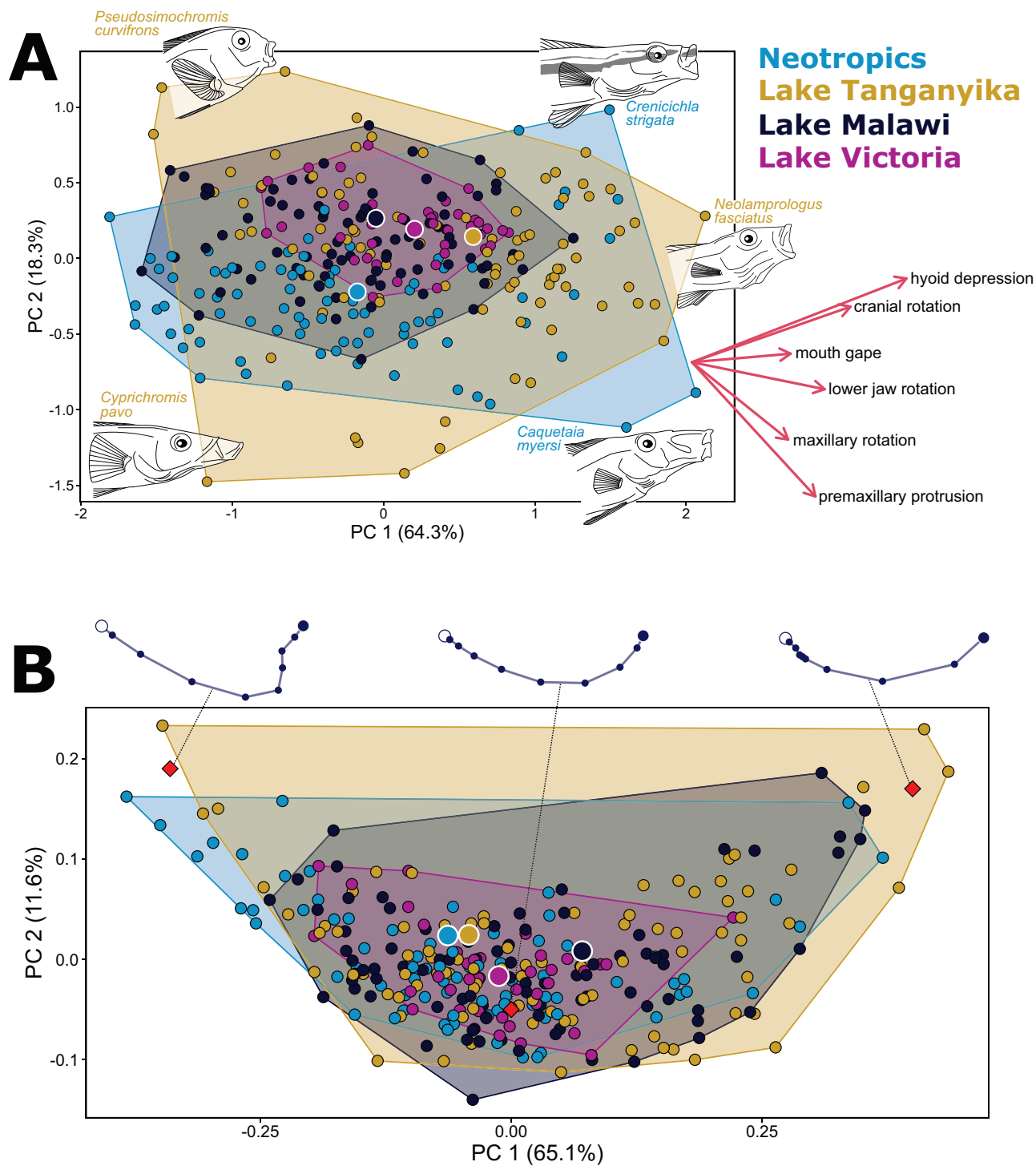
Figure 3: Primary dimensions of variation (PC1 and PC2) from separate principal component analyses for species-averaged multivariate functional traits. *A*, Motion components, a dataset comprising measurements of maximum excursions for six key kinematic features, are plotted by cichlid radiation. Representative drawings of cichlid heads at maximum gape are shown across the plot, and trait loadings are shown on the right. *B*, Variation in motion patterns by radiation, with two-dimensional representations of the shapes of kinematic trajectories provided at select locations of the plot (red diamonds). Low values on PC1 are motions with an abrupt shift in the manner of cranial movements, and large values are motions in which kinesis is disproportionately concentrated toward the end of the strike. For both multivariate traits, Lake Tanganyika and the Neotropics display unique occupation of this space, while Lakes Malawi and Victoria are almost entirely nested within them. Estimated values of radiation-specific most recent common ancestors are shown as large circles with white borders.

Lake Tanganyika, but there was greater parity among it, Lake Malawi, and the Neotropics (fig. 2). Kinesis coefficient (output kinesis relative to input movement from cranial rotation) showed the highest variance in the Neotropical radiation, which was significantly greater than that of the younger radiations in Lakes Malawi and Victoria (fig. 2). Finally, the rank order of variances for whole-motion pattern, a trait describing the timing and sequence of motion events, was the same as they were for kinesis skew—Lake Victoria was significantly lower than the other major radiations, followed by nearly identical values in Lake Malawi and the Neotropics, and the greatest variance again was in Lake Tanganyika (figs. 2, 3). Despite its young age, Lake Malawi showed surprisingly high diversity in motion pattern. This appears to be driven by a collection of species like *Tyrannochromis nigriventer*, *Copadichromis virginalis*, and *Caprichromis liemi* that have feeding motions in which kinesis is disproportionately concentrated toward the end of the strike (i.e., high kinesis skew, and motion patterns with large positive PC1 scores in fig. 3B).

Occupation of Novel Functional Spaces

Comparisons of hypervolumes identified whether individual radiations occupied unique regions of multivariate functional spaces. For the motion components, we found that 62% of Lake Tanganyika's and 49% of the Neotropics' functional space was unique to those radiations at the exclusion of all others, with both results occurring in the 99th percentile of randomized trials (fig. 3A; table S4). Cichlids occupying unique regions of the motion components functional space in Tanganyika included several specialized planktivores with high upper jaw protrusion in the genus *Cyprichromis* and elongate species with comparatively small gapes, like *Chalinochromis brichardi* and *Julidochromis dickfeldi*. In the Neotropics, multiple species of *Amatitlania* and a host of species in the tribe Geophagini (all with relatively small mouths, low cranial rotation, and low hyoid depression) occurred in one unique region, and species capable of extreme upper jaw protrusion, *Petenia splendida* and *Caquetaia myersi*, were found in another. In contrast to Tanganyika and the Neotropics, only 6% of this space in Lake Malawi and 3% in Lake Victoria were unique to those lakes, suggesting that most of their diversity is nested within the other cichlid radiations (fig. 3A; table S4).

Across all species, the diversity of motion patterns was largely restricted to a distinct concave or arched distribution (fig. 3B). This likely reflects a general constraint on motion diversity—despite differences in the relative magnitude of movements (motion components), patterns of feeding movements in cichlids are created by the same morphological features, moving in the same direction, and mostly in the same sequence. One extreme of the motion pattern

distribution was characterized by an abrupt shift in the direction of cranial shape change within morphospace toward the end of the motion, which manifested as an asymmetrical trajectory shape (left side of fig. 3B). During feeding sequences, this was caused by late-onset cranial rotation and hyoid depression after full protrusion of the upper jaw was achieved, presumably for continued buccal expansion posteriorly to prolong suction during prey acquisition. The other extreme contained symmetrically shaped trajectories in which the rate of cranial shape change was slow initially but fast toward the end of the strike, such that kinesis was disproportionately concentrated in later motion stages (right side of fig. 3B), a pattern reminiscent of high kinesis skew. Comparisons of hypervolumes for motion pattern revealed a mostly nested pattern in which Tanganyika (54%) occupied the greatest volume of unique space (upper 99th percentile of permutations), with much lower values for the Neotropics (26%), Malawi (14%), and Victoria (1%; table S4).

Rates of Functional Evolution

Across all functional traits, there was a strong inverse and nonlinear relationship between rates and radiation ages (fig. S3; table S5). In each case, Lake Victoria had much higher rates than other radiations, ranging anywhere from 42- to 95-fold faster diversification compared with the radiation with the lowest rates, the Neotropical cichlids. Similarly, the second youngest radiation, Lake Malawi, consistently had the second highest rates of trait evolution. Pairwise comparisons of rates between Lakes Victoria and Malawi were statistically significant at the $\alpha = .05$ level for functional traits except for hyoid depression and kinesis skew (table S5). One caveat is that these analyses assume a treelike pattern of lineage diversification, so we limit our interpretation primarily to emphasize the vastly different timescales over which functional trait diversity has accumulated in these radiations. Comparisons between the two older radiations revealed that rates in Lake Tanganyika (estimated from the McGee et al. [2020] tree) were always higher than in the Neotropics, ranging from 1.1-fold to 4.0-fold differences. However, unlike other pairwise comparisons among radiations, those between Tanganyika and the Neotropics failed to yield significant differences for more than half of the functional traits considered. Significant differences in rate of evolution in these two radiations were found for premaxillary protrusion, kinesis skew, kinesis, motion components, and motion pattern.

Rates for Lake Tanganyika computed with the phylogeny from Ronco et al. (2021) were between 1.4 and 2.7 times higher than those based on the McGee et al. (2020) tree (table S5), consistent with the younger reconstructed age of the radiation in the former. However, the rank order across

radiations remained the same regardless of tree, maintaining a clear trend of decreasing rate with radiation age (fig. S3). Given that rate estimates using the Ronco et al. (2021) tree did not impact interpretations of relative patterns across radiations, we focus our discussion on results from the McGee et al. (2020) tree that contained all radiations.

Modes of Functional Diversification

DTT analyses provided information about temporal patterns of diversification for two multivariate functional traits, motion components and motion pattern. In Lake Tanganyika and the Neotropics, neither trait displayed DTT trends that were statistically different from Brownian motion—the MDI, describing the deviation of observed DTT trends from the null expectation, was not statistically significant for either dataset (fig. 4; see table S6 for results from all radiations). However, while DTT trends for motion components largely stayed within the 95% range of simulated trait histories, those for motion pattern were above the Brownian expectation in all radiations, in some cases for prolonged durations, suggesting that trait variance was at times disproportionately concentrated within subclades. Notably, none of the functional DTT trends fell below the lower 95% range for Brownian motion, which would indicate a possible early burst of trait diversification (fig. 4).

Trait dispersion of extant species around radiation-specific MRCAs additionally captured patterns of trait space occupation across radiations at different stages of progression. Euclidean distances of motion components between species and MRCAs were continuously distributed for all radiations except Lake Victoria (fig. 4*B*), generally showing a single large cluster of species in functional space and not multiple clusters that might suggest divergence across discrete adaptive peaks. In Lake Victoria, a handful of species with comparably low upper jaw protrusion formed a small secondary peak that was more distantly situated from the radiation's MRCA (observations in the upper left of the Lake Victoria distribution in fig. 3*A*). These species consisted mostly of herbivorous cichlids from the genus *Neochromis* and omnivores in the genus *Pundamilia*, possibly representing a (weakly) isolated adaptive peak related to trophic ecology and jaw function. For motion pattern, Procrustes distances from radiation-specific MRCAs were right skewed, particularly in Lake Victoria and the Neotropics, seemingly reflecting the highly constrained distribution of the trait more than distinct adaptive zones (figs. 3*B*, 4*D*).

Cranial Morphology and Its Relation to Motion Diversity

Interspecific variance in head shape was highest in Neotropical cichlids but only marginally greater than in Lake Tanganyika (table S3). Still, the Neotropics boasted 2.3 times more head shape diversity than Lake Malawi and 2.7 times more than Lake Victoria. Pairwise comparisons of variances were statistically significant except between the two youngest radiations, Malawi-Victoria, and the two oldest, Neotropics-Tanganyika (table S3). Interestingly, the high head shape variance in the Neotropics did not directly translate to functional diversity, as Lake Tanganyika still had greater (but statistically similar) diversity in motion components and significantly higher variance in kinesis, motion pattern, premaxillary protrusion, and maxillary rotation (fig. 5*C*–5*E*).

Comparisons of hypervolumes for head shape revealed that 59% of cranial diversity in the Neotropics and 57% in Lake Tanganyika were unique to those regions, with both observations occurring in the upper 99th percentile of randomized permutations (table S4). One of the things that made the Neotropics stand out was expansion toward deep-headed taxa, like *Symphysodon discus* and *Pterophyllum scalare*, and several species in the genus *Amatitlania* that were not as extreme but still outside the space occupied by cichlids in other radiations (fig. 5*A*; lower scores on PC1). Some species from the African lake radiations occurred in a nonoverlapping region of morphospace with the Neotropics that contained many small-mouthed benthic biting and picking specialists, like *Tropheus brichardi*, *Melanochromis wochepa*, and *Chalinochromis popelini* (fig. 5*A*; lower scores on PC2). In addition, Lake Tanganyika possessed a fair degree of unique morphologies varying broadly in direction of mouth orientation (fig. 5*B*), from upturned (e.g., *Haplotaxodon microlepis*) to downward-deflecting profiles (e.g., *Xenotilapia ochrogenys*). Morphological diversity within Lakes Malawi and Victoria was almost entirely contained within the other regions, with only 5% and 3% unique morphospace occupation, respectively.

Rates of head shape evolution were again lowest in the older radiations (Neotropics and Lake Tanganyika), faster in Lake Malawi, and much faster in Lake Victoria (fig. S3; table S5). All pairwise comparisons of rates were statistically significant (table S5). Like functional analyses, DTT trends for head shape were statistically indistinguishable from a Brownian process (fig. S4; table S6). However, in the Neotropics the DTT trend dipped just below the 95% range for Brownian simulations briefly from 44 to 40 million years ago and again for an extended time from about 39 to 28 million years ago (fig. S4). During these periods, head shape disparity was concentrated among clades at a level beyond the null expectation, likely representing evolution of clades toward different adaptive peaks.

Discussion

In this study, we provide the first quantitative comparison of functional diversity across four major cichlid radiations, leveraging a large comparative kinematics dataset
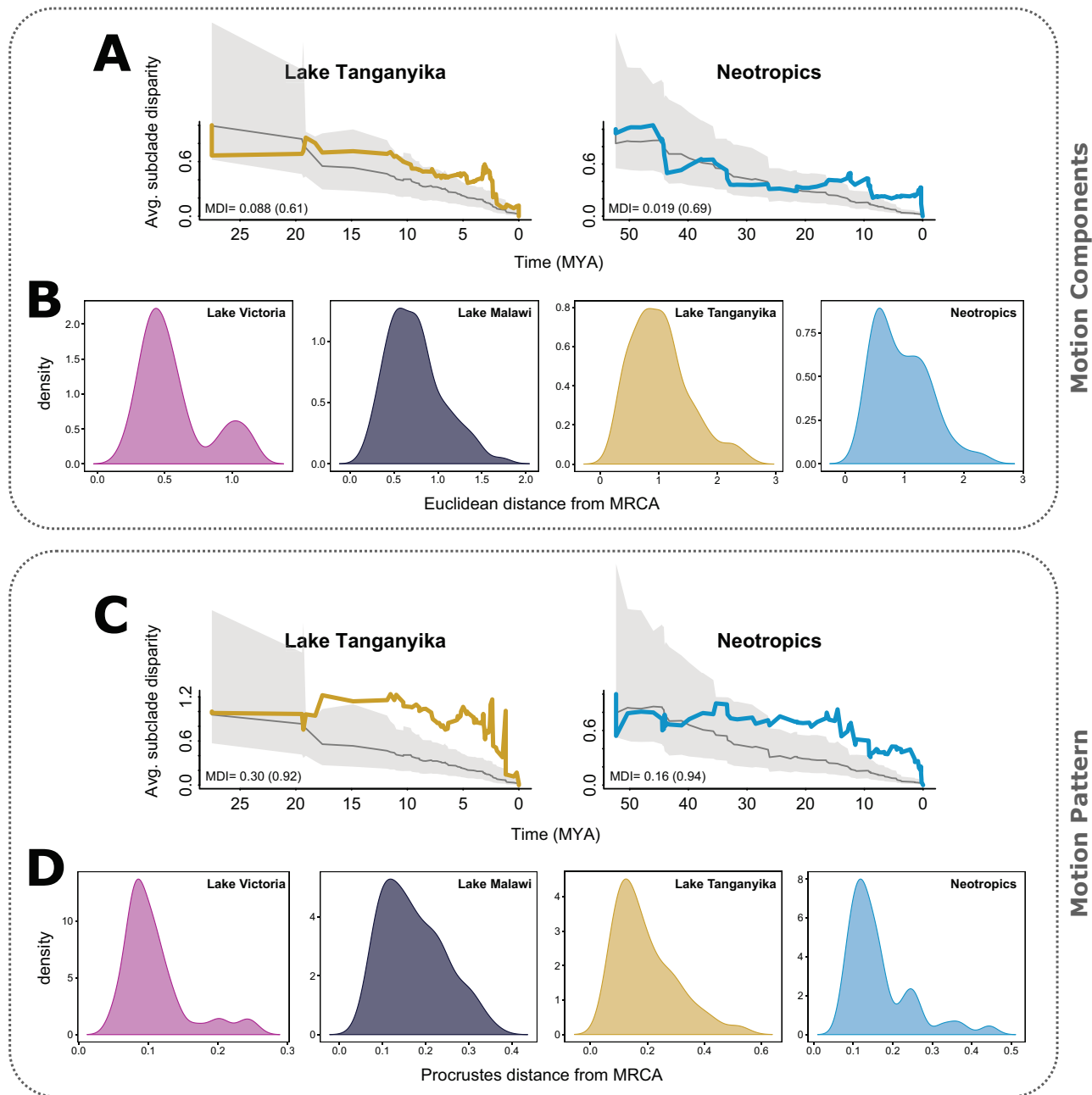
Figure 4: Disparity through time (DTT) plots in the two oldest cichlid radiations, Lake Tanganyika and the Neotropics, for motion components (*A*) and motion pattern (*C*). Also shown are distributions of Euclidean distances of motion components (*B*) and Procrustes distances of motion pattern (*D*) between extant species and their radiation-specific most recent common ancestor (MRCA). MDI = morphological disparity index; MYA = million years ago.

to contrast patterns of adaptive diversification across vastly different temporal scales (many thousands of years to over 50 million years) and spatial ranges (individual lakes vs. continental scale). We show that standing functional diversity in African cichlids is strongly related to radiation age and displays a striking nested pattern in

which trait spaces occupied in Lakes Victoria and Malawi were almost fully contained within that of Lake Tanganyika. Somewhat surprisingly, functional variance in the much older continental Neotropical radiation was lower than it was in Lake Tanganyika for many traits, making the high diversity in the latter all the more impressive. This suggests that
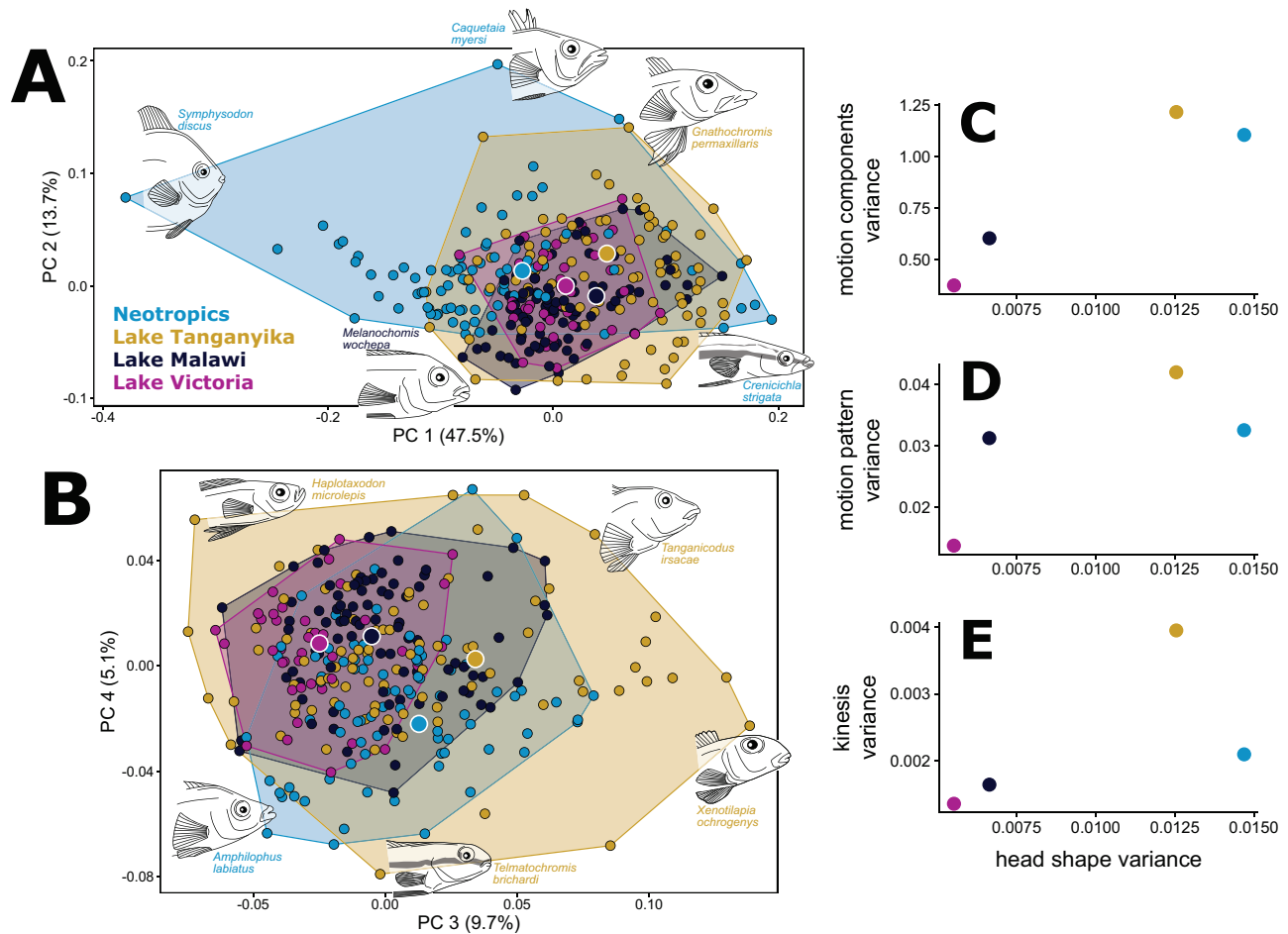
Figure 5: Species-averaged interspecific head shape diversity across cichlid radiations. *A*, Major axes of variation (PC1 and PC2) show the wide diversity of head shapes in Neotropical cichlids, most notably in head depth (left side of plot). Small-mouthed taxa in African lakes, often benthic biting feeders, are concentrated toward the bottom of the plot. *B*, PC3 and PC4 display axes on which Lake Tanganyika contains high morphological diversity, including variation in orientation of jaws. Most recent common ancestors for radiations are shown as large circles with white borders. *C–E*, Variance in head shape is shown in relation to select kinematic traits, displaying relationships between form and function. High morphological diversity in the Neotropics does not translate to commensurate kinematic diversity.

diversifying forces have operated more effectively in Lake Tanganyika, and likely across the African Great Lakes, compared with the largely riverine cichlids of Central and South America. Our results are consistent with recent work suggesting that many cichlid lake radiations beyond those examined in this study have experienced elevated rates of morphological evolution (Burress and Muñoz 2023).

Cichlid Feeding Systems and Adaptive Radiation

Cichlids have long served as a model system for understanding adaptive radiation (Stiassny and Meyer 1999; Seehausen 2006; Turner 2007), yet we recovered mixed evidence that functional diversification of their feeding systems adheres to traditional expectations of this process (Simpson 1953). A negative relationship was found be-

tween radiation age and rates of kinematic and morphological evolution (fig. S3), suggesting that phenotypic diversification proceeds fastest in early-stage radiations in a manner consistent with an early burst. Young cichlid radiations in Lakes Victoria and Malawi support modest levels of trait diversity, but they have acquired it at an incredibly fast pace due to high speciation rates (Seehausen 2006).

In addition to an early burst of diversification, an assumption of adaptive radiations remains that trait variance will be distributed disproportionately among clades versus within them (Simpson 1953; Harmon et al. 2003). Although cranial morphology showed some hints of elevated divergence among lineages in the Neotropics (fig. S4), none of the examined morphological or functional DTT trends were statistically different from Brownian motion (table S6). Furthermore, trait dispersion of extant species

around their MRCA was mostly continuous (fig. 4) with minimal evidence of clustering (i.e., discrete ecomorphs), a pattern largely consistent across radiations. One exception was found for motion components in Lake Victoria, which displayed a small secondary cluster of species with low values of jaw protrusion (fig. 4B), possibly representing divergence toward an adaptive peak associated with a substrate biting mode of feeding. That withstanding, comparably low trait variance in the two youngest radiations (fig. 2) are not suggestive of rapid divergence among lineages occupying distinct adaptive zones, where a significant portion of total potential diversity is achieved at initial stages of adaptive expansion.

Our study suggests that the diversification of feeding systems in cichlid adaptive radiations likely occurs by way of early burst, achieved not by adaptive divergence among clades but through extremely rapid within-clade dispersion in incipient radiations. Previous research has predicted such patterns in cichlids as a possible outcome of transgressive segregation during widespread introgression—a common theme of emerging African lake radiations—paired with ecological opportunity in newly colonized habitats (Seehausen 2004; Meier et al. 2017; Irisarri et al. 2018; Salzburger 2018; Meier et al. 2019; Selz and Seehausen 2019). Although we do not explicitly address the ecological dimensions across which diversification occurs, a large number of studies have linked morphological and functional evolution of the cichlid feeding apparatus with dietary diversity (e.g., Albertson et al. 2005; Hulsey and García De León 2005; López-Fernández et al. 2012; Soria-Barreto et al. 2019; Arbour et al. 2020). Additionally, previous work on Lake Malawi and Tanganyika cichlids suggests that feeding diversity is distributed continuously along an axis of prey evasiveness (Martinez et al. 2018), matching observed patterns in this study of time-dependent trait dispersion around radiation-specific MRCAs (fig. 4). If the landscape of ecological opportunity was discontinuously or sparsely distributed, for instance, it could pose challenges for a radiation diversifying via transgressive segregation since open adaptive zones are no longer adjacent to currently occupied zones, thereby reducing the probability that hybrid offspring happen upon a more distantly situated adaptive peak.

Clear evidence for early bursts appears to be the exception and not the rule in comparative trait data (Harmon et al. 2010). We found that no single radiation showed signs of an early burst for functional traits relating to prey capture but that a trend among radiations strongly supported such a pattern. Previous work on the Lake Tanganyika radiation also failed to recover an early burst for morphological traits of the jaw system (Ronco et al. 2021). A contributing factor in these findings could be the natural time dependency of macroevolutionary rate estimates (Harmon et al. 2021). Phylogenetic reconstructions of Lake Tanganyika cichlids

contain comparatively few long branches near the root (e.g., McGee et al. 2020; Ronco et al. 2021), but previous work has shown that the radiation experienced a burst in lineage diversification early in its history that was facilitated by hybridization (Salzburger and Sturmbauer 2002; Seehausen 2006; Irisarri et al. 2018), a pattern seen in Lakes Victoria and Malawi. In this context, we may think of young radiations as providing our best window into the mode of lineage (and trait) diversification that once characterized the early history of older radiations. In similar fashion, older radiations may be helpful for informing predictions of what is to come for young radiations.

Are We Watching the Same Film?

Stephen Jay Gould famously contemplated what the diversity of life on earth might look like if we had the ability to start over and replay the tape of life (Gould 1991). Would it be unprecedented and unrecognizable, or would we see familiar patterns as selection inevitably leads to diversification along predictable paths? Many others have since pondered this question (e.g., Lobkovsky and Koonin 2012; Orgogozo 2015; Blount et al. 2018). In one sense, evolution is constantly repeating its own version of this experiment at much smaller spatial and temporal scales—independent radiations in related groups of organisms provide replication and insight into evolutionary contingencies under conditions of varying similarity. A primary focus of this study was to examine whether four large cichlid radiations, each resulting in hundreds of species and celebrated levels of ecological and morphological variation, have generated similar patterns of functional diversity. Has diversification of feeding functional morphology played out following the same script in each radiation, or have they diversified along separate functional axes? The answer appears to be that both are true.

Considering, for a moment, only the three African lake radiations examined in this study, there is an argument to be made that both functional and morphological diversification have progressed in a similar fashion in each of the lakes. The high-dimensional spaces filled by motion components, motion pattern, and cranial shape data each show the younger radiations, Malawi and Victoria, occupying subspaces of the older and more diverse Tanganyikan radiation, with novelty only commonplace in the latter. This result is consistent with impressions of widespread convergence on trophic morphotypes in Lakes Malawi and Tanganyika (e.g., Stiassny 1981; Kocher et al. 1993; Stiassny and Meyer 1999; Conith et al. 2019; Ronco et al. 2021). Interestingly, the nested pattern indicates that convergence emerges as a consequence of time-dependent and continuously distributed trait expansion from a more or less common starting point—young lake radiations following in the footsteps of their older

counterparts. Still, it is unclear if we were to fast-forward the Lake Victoria tape millions of years to the current age of Lake Tanganyika whether we would find a carbon copy of that lake or whether selection would eventually lead the Victorian radiation into unfamiliar functional and morphological spaces.

When we expand beyond the large African lake radiations to contrast their phenotypic and functional diversity for the first time with the large Neotropical radiation, a different story emerges. The two older radiations are more diverse than the others and each has invaded novel, radiation-specific regions of functional and morphological space. Tanganyika boasts species with unique combinations of motion components, including highly specialized planktivores (*Cyprichomis*) and benthic foragers (e.g., *Pseudosimochromis curvifrons*, *Telamatochromis vittatus*). In the Neotropics, some geophagine cichlids feed with a strongly sequenced kinematic pattern, partitioned between distinct jaw protrusion and cranial rotation phases (asymmetrical motion patterns toward the left of fig. 3*B*). Additionally, an innovation in select piscivorous species from the tribe Heroini (e.g., *Petenia splendida* and *Caquetaia myersi*) results in extreme levels of premaxillary protrusion (Waltzek and Wainwright 2003; Hulsey and García de León 2005) that places them in a unique region of motion component space (fig. 3*A*). Both Lake Tanganyika and the Neotropics contain cichlids with functional profiles that do not occur anywhere else, showing that radiations can eventually diverge from each other in key areas of diversification. These observations amplify questions about the contrasting landscapes of ecological opportunity experienced by lake versus continental radiations.

Morphology Provides an Imperfect Index of Functional Diversity

The idea that morphological variation can be used as a proxy for functional diversity is commonly advanced, but the widespread presence of complex form-function relationships tests this assumption (e.g., Wainwright et al. 2005; Young et al. 2007, 2010; Lautenschlager et al. 2020). Neotropical cichlids, compared with the Lake Tanganyika radiation, illustrate that high variance in cranial morphologies does not always result in greater functional diversity. The primary axis of morphological variation in the Neotropics involved differences between elongate and slender (e.g., *Crenicichla*) versus deep heads with steep cranial profiles (e.g., *Symphysodon*, *Pterophyllum*, *Uaru*), which are adaptations typically found in species living in fast-flowing riverine environments and slow-moving water like lakes or floodplains, respectively (López-Fernández et al. 2013). It is therefore likely that an important source of variance in head shapes of Neotropical cichlids is due to selection on

habitat-specific body shape, perhaps involving adaptation of the locomotor system. Complex form-function relationships, particularly in biomechanical systems with many cooperating components, can make for challenging comparisons between morphologies and motions and impact how these traits accumulate during adaptive diversification (Alfaro et al. 2005). These findings suggest that caution is warranted in attributing observed morphological variation in fish feeding systems to functional diversity.

Conclusions

Cichlids have captivated the attention of biologists and aquarists alike with their remarkable diversity, boasting seemingly endless combinations of body shapes, sizes, coloration patterns, diets, and behaviors. Each radiation examined in this study has amassed an impressive variety of morphological and functional diversity. Adaptive radiation of cichlids has produced modest diversity of feeding kinematics in Lakes Victoria and Malawi, while Lake Tanganyika has surpassed even the much older Neotropical radiation, suggesting that the forces driving diversification in Tanganyika outstrip those in the Neotropics. However, these patterns of diversity have been established on very different timescales. Rates of functional evolution range from 40 to 95 times faster in Lake Victoria than in the Neotropics, supporting the notion that the African Great Lake radiations have experienced comparatively rapid evolutionary change. These observations suggest that while similarities exist, adaptive radiation of cichlid feeding kinematics has not always followed a common profile. Rather, evolutionary contingencies linked to time and biogeography explain varied patterns of morphological and functional diversification across this iconic group of fishes.

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Statement of Authorship

C.M.M. and P.C.W. conceptualized the project. C.M.M., K.A.C., and P.C.W. developed the methods. M.D.M. and

S.R.B. recorded most of the videos of cichlid feeding events, and C.M.M. contributed videos for the remaining species. C.M.M., K.A.C., S.W., D.S., A.S.R.-H., A.B., and P.C.W. digitized landmark data on video frames. C.M.M. prepared and curated data, and K.A.C. ran statistical analyses. C.M.M. and P.C.W. wrote the manuscript with edits from all authors.

Data and Code Availability

Data associated with this study are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.h18931zsh; Martinez et al. 2024*a*), and R scripts can be found on Zenodo (https://doi.org/10.5281/zenodo.8354124; Martinez et al. 2024*b*). The phylogenetic tree used for comparative methods was from a previously published article (McGee et al. 2020), also available in Dryad (https://doi.org/10.5061/dryad.fn2z34tr0).

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