Accelerated Brain Shape Evolution Is Associated with Rapid Diversification in an Avian Radiation

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Abstract: Niche expansion is a critical step in the speciation process. Large brains linked to improved cognitive ability may enable species to expand their niches and forage in new ways, thereby promoting speciation. Despite considerable work on ecological divergence in brain size and its importance in speciation, relatively little is known about how brain shape relates to behavioral, ecological, and taxonomic diversity at macroevolutionary scales. This is due in part to inherent challenges with quantifying brain shape across many species. Here we present a novel, semiautomated approach for rapidly phenotyping brain shape using semilandmarks derived from X-ray computed microtomography scans. We then test its utility by parsing evolutionary trends within a diverse radiation of birds: kingfishers (Aves: Alcedinidae). Multivariate comparative analyses reveal that rates of brain shape evolution (but not beak shape) are positively correlated with lineage diversification rates. Distinct brain shapes are further associated with changes in body size and foraging behavior, suggesting both allometric and ecological constraints on brain shape evolution. These results are in line with the idea of brains acting as a “master regulator” of critical processes governing speciation, such as dispersal, foraging behavior, and dietary niche.

Keywords: geometric morphometrics, computed tomography scanning, trait evolution, macroevolution.

Introduction

Expansion into new niche space is an important step during the speciation process. Novel behaviors or ecologies that allow populations to colonize new habitats or geographic regions are likely associated with concomitant changes in brain function and structure (Aristide et al. 2016; Montgomery and Merrill 2017). Larger brains have been shown to facilitate niche expansion and technical innovations in birds (Ducatez et al. 2015), suggesting that changes in brain size may spur speciation. If so, we would expect the brain itself to evolve as a response to the selective pressures exerted by different environments, either by concerted evolution or by mosaic evolution, with some brain regions expanding or contracting irrespective of others (Jerison et al. 1985; Finlay and Darlington 1995; Finlay et al. 2001).

Although several studies have investigated the ecological implications of interspecific variation in brain size (Corfield et al. 2008; Maspons et al. 2016; Sayol et al. 2018; Ksepka et al. 2020) and its potential role in speciation (Sol et al. 2005a; Carlson et al. 2011; Sayol et al. 2019), relatively little is known about how brain shape varies between closely related species and how brain evolution might relate to speciation (but see Ryan 1986; Carlson et al. 2011; Sayol et al. 2019). This shortcoming is due in part to challenges of quantifying brain shape as opposed to brain size (measured by total volume or weight). However, modern techniques, such as X-ray computed tomography (CT) scanning, are allowing biologists an ever more detailed view of internal traits that have been difficult to quantify, including brain shape (Aristide et al. 2016; Torres and Clarke 2018). Of the studies that have looked at brain shape to date, most have focused on primates and revealed significant variability between closely related species (Barton and Harvey 2000; Winter and Oxnard 2001; Aristide et al. 2016). For example, Aristide et al. (2016) showed a wide diversity of brain shapes both between and within subfamilies of New World monkeys and showed that shifts in brain shapes were also related to shifts in ecological niches. Therefore, changes in brain shape, as compared with brain size, might be an important factor in understanding macroevolutionary trends in brain evolution.
Changes in brain shape might relate to speciation in two ways. First, if distinct brain shapes (or sizes) directly influence behaviors that facilitate niche shifts and range expansions to drive speciation (Price 2008), then we would expect a pattern of trait-dependent speciation. This hypothesis predicts that certain brain shapes will be correlated with elevated speciation and lineage diversification rates (speciation minus extinction; Sayol et al. 2019). For example, if distinct brain shapes, such as larger optic lobes, are advantageous for dispersal, then lineages with these brain morphotypes may be more prone to colonization of new areas, such as islands, at higher rates than those with smaller optic lobes. Second, a nonexclusive alternative to trait-dependent speciation is a pattern in which brain morphology diverges as a by-product of— or in response to— ecological or nonecological processes that drive speciation. This hypothesis of speciation trait evolution predicts that elevated rates of trait evolution should be correlated with high speciation rates, which could occur either as species become geographically isolated (e.g., on different islands within an archipelago) or as a result of divergent natural or sexual selection during speciation (e.g., see Ingram et al. 2016). For example, functional traits involved in foraging and locomotion are expected to diverge in different environments (Nosil 2012). If changes in brain structure related to foraging behavior— such as shapes that limit brain damage during high-speed plunge-diving (as in our study system, kingfishers)— also influence reproductive isolation through modulating the production or viewing of mating displays, then changes in naturally selected brain shape will be linked to speciation events instead of changing gradually through time.

Here we develop a novel method of extracting rich 3D geometric-morphometric semilandmark data from CT scans of fluid-preserved specimens. We then test its utility in elucidating relationships between trait evolution, ecology, and lineage diversification rates in an ecologically and morphologically diverse clade of birds: kingfishers (Aves: Alcedinidae). Kingfishers exhibit a variety of dietary niches, ranging from generalist insectivory (e.g., Tanyt 英 tara paradise kingfishers of New Guinea) to obligate piscivory (e.g., belted kingfisher [Megaceryle alcyon] of North America). Even within genera (e.g., Cyxh), there is a wide range of diets associated with variable beak colors (from orange to black) and shapes, ranging from dagger-like in the little kingfisher (Cyxh pusillus) to spatula-like in the oriental dwarf-kingfisher (Cyxh erithaca; Moyle et al. 2007). The diverse polytypic species complexes of island kingfishers (Cyxh, Todiramphus) led Ernst Mayr, father of allopatriec speciation (Mayr 1942, 1963), to describe them as one of the Indo-Pacific’s “great speciators” (sensu Mayr and Diamond 2001). Body size across the family varies widely, from 9 g (African pygmy kingfisher [Ispidina picta]) to 490 g (laughing kookaburra [Dacelo novaegui neae]); even within the genus Todiramphus, there is a fourfold difference in body size (Fry 1992). Approximately half of all kingfisher species live in forested habitats, though they also occur in diverse habitats, from coral islands to arid deserts (Woodall 2001). Recent work in the group found considerable variation in diversification rates across the order, specifically finding cryptic diversity in these polytypic species complexes (Andersen et al. 2013, 2015) paired with elevated speciation rates (Andersen et al. 2018; McCullough et al. 2019). Kingfishers may therefore be an ideal clade to investigate evolutionary diversification in brain architecture and assess the consequences of these evolutionary changes in morphological and taxonomic diversification.

We test (1) whether brain shape evolution is related to lineage diversification (predicted under both speciation trait evolution and trait-dependent speciation models of evolution) and (2) whether there are ecological factors— such as foraging mode, habitat preferences, and geographic range size—that drive brain shape divergence. Additionally, we (3) compare macroevolutionary dynamics of brain evolution with that of beak shape, a commonly assessed trait in birds and proxy for species’ dietary and foraging niches (Grant 2006; Jonsson et al. 2012; Pigot et al. 2016; Cooney et al. 2017; Miller et al. 2017).

Material and Methods

Linear Trait Data Set

We took standard linear morphological measurements for all species in the avian order Coraciiformes, for which more than 60% of the species diversity is kingfishers (Gill and Donsker 2019). We measured at least four individuals of each species (two specimens for each sex), including subspecies shown to be distinct in previous molecular work (Andersen et al. 2018) that conflict with the International Ornithological Congress checklist (Gill and Donsker 2019). Because of limited numbers of specimens in museums, we could not measure four specimens for the Guadalcanal mustached kingfisher (Actenoides excelsus; N = 2) and the black-headed kingfisher (Actenoides capucinus; N = 3). To capture overall variation in beak shape, we used digital calipers (Mitutoyo) to measure three beak traits: beak length measured from the base to the tip, beak depth measured at the base, and beak width measured at the base. Also, we measured tarsus length using calipers and obtained mean values for body mass from Wilman et al. (2014). To account for measurement error, we took triplicate measurements of the three beak and tarsus traits and then used the averages for analyses. In total, we took 10,248 measurements on 854 coraciiform
Specimens representing all 188 recognized species in the order. We averaged species values and natural log transformed them before analyses.

**Specimen CT Scanning**

We sampled one individual for 65 out of 115 (56.5%) recognized kingfisher species, along with eight diverse outgroup taxa within Coraciiformes (fig. S1; figs. S1–S7 are available online). We scanned all specimens in the PaleoCT lab at the University of Chicago on a custom-built, dual-tube X-ray computed microtomography (microCT) scanner (General Electric, Boston) operating at 190 kV. Scan resolutions varied from 40 to 60 μm, depending on the size of the specimen. Importantly, our sampling within kingfishers spanned 13 out of 18 (72%) recognized genera and represented >68% of the phylogenetic diversity in the group, calculated with the pd function in picante (Kembel et al. 2010), and was not significantly overdispersed or clustered (mean pairwise distance = 57.3 My, $P_{\text{rand}} = 1$; mean nearest taxon distance = 10.9 My, $P_{\text{rand}} = .59$; see fig. S1). When possible, we chose to scan fluid-preserved specimens, as this ensured that the internal braincase was intact and not damaged during the skeletonizing or specimen-preparation process (see the supplemental PDF, available online, on fluid-preserved specimens). For three species, we could not find a fluid specimen in North American museums, so we chose to scan skeletons (see Dataset 2 in the Dryad Digital Repository [https://doi.org/10.5061/dryad.fbg79cs6; Eliason et al. 2021]).

**Semiautomated Method for Quantifying 3D Brain Shape**

Recent work shows that the internal surface of the braincase is a valid proxy for soft tissue shape in brains (Watanabe et al. 2019); thus, we hereafter use “brain” synonymously with the internal surface of the braincase. Fixed landmarks are useful in that they can be considered homologous among species, but they may underestimate shape variation compared with semilandmarks placed along curves or surfaces (Watanabe 2018; Bardua et al. 2019; Goswami et al. 2019). Traditional approaches to analyzing brain shape variation using semilandmarks involve time-consuming segmentation of 2D CT images to produce 3D representations of brains, or digital endocasts (Balanoff et al. 2015). This process can take from 8 h to weeks of computational time, depending on the level of detail required (C. Torres and C. Early, personal communication).

To address this issue of computational time and to quantify broad neuroanatomical variation across kingfishers, we developed a four-step, semiautomated procedure for placing semilandmarks on the internal skull (braincase) of birds. Briefly, we first generated a 3D “template” digital endocast in the endocast program (Michikawa et al. 2017) and subsequently placed a homologous set of fixed landmarks (table S1; tables S1–S7 are available online) on both this template endocast and the internal surface of 3D skull models of target specimens ($N = 65$). We then identified 500 approximately equidistant surface semilandmarks on the template endocast using k-means clustering with the digit.surface function of geomorph (ver. 3.1.2). Finally, we used buildtemplate to identify similar semilandmarks in the “target” skull models based on nearest neighbor distances to the template semilandmark set (Mitteroecker and Gunz 2009), after scaling and aligning models using the fixed landmark set. To benchmark our approach, we aligned semilandmarks using Procrustes distances, useful for describing global shape variation (Mitteroecker and Gunz 2009), and then compared Procrustes distances between endocast semilandmarks (i.e., the “gold standard”) and skull semilandmarks. Thus, assuming that our method places semilandmarks without error, the Procrustes distance between these two sets of semilandmarks should be zero. See figure 1 and the supplemental PDF for further details.

**Evolution of Brain Architecture**

**Principal Component Analysis (PCA).** To understand variability in brain shape, we used a PCA to reduce the dimensionality of our morphometric data set (i.e., 500 Procrustes-aligned, 3D semilandmark coordinates) and generate uncorrelated principal component (PC) axes relating to main trends in shape variation among species. We retained 44 PC axes for brain shape and two PC axes for beak shape, both explaining >95% of interspecific variation, for use in downstream multivariate comparative analyses. For comparative analyses and visualization of brain shapes, we opted for a standard PCA over a phylogenetic PCA (pPCA) because we wanted independent, easily interpretable shape axes that could be used directly in reconstructing 3D brain shapes at morphospace extremes (e.g., fig. 1A), and morphological disparities for the two approaches have been shown to be equivalent when considering all dimensions of PCA shape space as multivariate distances between species are preserved (Polly et al. 2013). Thus, for most analyses we analyze PC axes in a distance-based comparative framework; the use of pPCA would produce results identical to the PCA results presented here. However, for brain shape modularity, we used a pPCA implemented in the phyl.pca function of phytools (Revell 2011), with an input tree with branch lengths transformed according to the maximum likelihood (ML) estimate of multivariate phylogenetic signal (see below).
A. Fluid specimens were batch computed tomography scanned. B. One brain endocast (gray-headed kingfisher) was constructed automatically (step 1) using xendocast (Michikawa et al. 2017). Cutaway view of a skull shows manually placed fixed landmarks (yellow spheres, step 2) and 500 automatically placed semilandmarks (red spheres) on the endocast using the buildtemplate function in geomorph (step 3). C. Braincases of target specimens were semilandmarked (blue spheres) based on nearest distance to points on template endocast (step 4).
Visualizing Major Axes of Phenotypic Diversity. A strength of utilizing surface semilandmark data over brain size or fixed landmarks is in visualizing subtle shape variation and covariation among semilandmarks (Goswami et al. 2019). For example, semilandmarks that are coevolving should show PCA loadings of a similar magnitude and direction (Clavel et al. 2018). To visualize major axes of brain shape diversity (i.e., sets of semilandmark coordinates that are coevolving), we estimated loadings along phylogenetic axes pPC1 and pPC2 and plotted these values as a heat map on the surface of the brain. Similar colored patches indicate brain regions that are evolving independently from other regions.

Macroevolutionary Analyses

For all comparative phylogenetic analyses, we used a recent, time-calibrated, species-level phylogeny of Coraciiformes inferred with ultrasequences and fossils (McCullough et al. 2019). To estimate species-specific trait evolutionary rates, we used \( \text{RR}_{\text{phylo}} \) (Castiglione et al. 2018). This approach uses phylogenetic ridge regression to calculate evolutionary rates for each PC axis individually (along with an estimate of multivariate rate, i.e., the square root of the sum of the squared individual rates; for further discussion on using Euclidean distances as a multivariate rate estimate, see McPeek et al. 2008) and identify rate shifts from multivariate data (for further details, see Castiglione et al. 2018). We chose this approach to account for phylogenetic uncertainty by estimating multivariate evolutionary rates of brain shape evolution across all 44 PC axes (needed to capture 95% of overall variation) rapidly across a posterior set of trees from McCullough et al. (2019), available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.ffbg79cs6; Eliason et al. 2021). Alternatives for calculating multivariate rates (e.g., Venditti et al. 2011) are more computationally intensive and do not allow for multiple phylogenetic trees as input. Because we were interested in identifying broad, clade-wide shifts in rates of trait evolution, we used the single clade option in \( \text{RR}_{\text{phylo}} \). To further understand temporal trends in trait evolution and speciation rates, we sliced the tree into 1-My time bins and computed trait evolutionary rates and diversification rates for 100 draws from the posterior distribution, following Marki et al. (2019). We computed lineage diversification rates (i.e., species-specific rates of speciation minus extinction rates; hereafter, “diversification rates”) using the cladogenetic diversification rate shift test (Maliet et al. 2019). We chose this approach because it accounts for background extinction (assumed uniform across the phylogeny) that can be critical in investigating macroevolutionary trends (Beaulieu and O’Meara 2015). For analyses with alternative macroevolutionary rate metrics, see the supplemental PDF.

Speciation Trait Evolution. To test for a positive relationship between rates of trait evolution and diversification rates, as predicted under a model of speciation-trait evolution, we calculated multivariate rates of trait evolution (see above) for each of 100 posterior trees, for brain and beak shape. We then natural log transformed the rates and fitted phylogenetic generalized least squares (PGLS) models with natural log diversification rates as a predictor under a Brownian motion (BM) model of evolution for each simulated-rate data set. We verified that these data met two key assumptions of PGLS (homogeneity of variances and normality of residuals) by plotting residuals versus fitted values and with quantile-quantile plots, respectively. To account for different sources of uncertainty, we then estimated the variance in estimated slopes among trees (phylogenetic uncertainty) as well as the mean variance in parameter estimates (model uncertainty). The sum of these two variances was further used to statistically test for the effect of the different response variables in the models. That is, we determined \( \tau \) statistics as the mean slope divided by the square root of the summed variance and estimated \( P \) values from a Student’s \( t \)-distribution with degrees of freedom given as the number of tips in the tree, following Eliason et al. (2015).

Trait-Dependent Diversification. To assess whether specific trait values are associated with elevated diversification rates, as predicted under a model of trait-dependent speciation, we used a distance-based PGLS (d-PGLS) approach (Adams 2014a) implemented to link multivariate brain and beak shape to natural log diversification rates. Briefly, we first estimated multivariate phylogenetic signal for 44 brain and two beak PC axes using the mvgs function in mvMORPH (Clavel et al. 2015). These values were found to be highly uniform across posterior trees (fig. S2), and thus we used the maximum clade credibility (MCC) tree estimate to transform branch lengths of each posterior tree. We then fitted d-PGLS models for each posterior tree and determined support for trait-dependent diversification as the proportion of significant \( P \) values across trees.

Niche Divergence Tests

Predictors of Morphological Divergence. Morphological diversity at the clade level could be explained by switches in behavior or ecology that cause divergence in trait means among these adaptive regimes and/or stronger stabilizing selection (or relaxed selection) associated with a particular ecology that influences rates of trait evolution compared with lineages with different ecologies or behaviors (Collar et al. 2010). To test these two nonexclusive mechanisms of morphological change, we compared both the evolutionary means and the rates of brain shape among ecotypes.
(see below) using multivariate comparative methods. Specifically, we tested the following five predictors of morphological diversification (also see Cooper and Purvis 2009).

**Predictor 1: island-dwelling.** Species on islands have smaller, more fragmented ranges associated with reduced intraspecific competition (Stamps and Buechner 1985) and smaller population sizes that are more prone to drift and rapid selection of novel traits (Losos and Ricklefs 2009). Therefore, we predict faster rates of brain shape evolution in island lineages. Island living may further affect the specific kinds of brains that evolve, as islands are often associated with decreased predation that has been shown to mediate brain shape in fish (Kotrschal et al. 2017) and unpredictable environments that may select for larger brains (Sayol et al. 2018). Although brain shape was not studied by Sayol et al. (2018), if brains are evolving in a modular fashion (Balanoff et al. 2016), then expansion of overall brain size would result in uneven changes in different brain subregions associated with island colonization.

**Predictor 2: geographic range size.** Groups of species with larger ranges should evolve trait diversity more slowly because of the counteracting effects of gene flow and local adaptation (Stanley 1979). Furthermore, bird species that migrate have significantly smaller brains than sedentary species (Sol et al. 2005b), suggesting that brain morphology is related to dispersal behavior. Therefore, we predict that species with larger ranges will have distinct brain shapes relative to species with smaller ranges. To quantify range size, we downloaded species range maps from BirdLife International, converted them into presence-absence matrices using lets.presab in the letsR package (Vilela and Villalobos 2015) with a 2° grid resolution, and used the lets.rangesize function to calculate range size as the number of grid cells that a species occupies. This variable was then natural log transformed before analysis and used as a predictor in PGLS analyses (e.g., Chira et al. 2018).

**Predictor 3: body size.** Species with smaller body sizes should evolve faster because of their shorter generation times, shorter life spans, and higher mass-specific metabolic rates (Cooper and Purvis 2009). Changes in body size might also directly affect brain shape divergence, as was recently found for raptorial birds (Bright et al. 2016). Under this allometric hypothesis, we would predict that changes in body size will result in predictable changes in brain shape. We additionally tested for allometric changes in brain shape scaling with brain size (i.e., mean distance to the centroid). We obtained mean values for body mass from Wilman et al. (2014). For visualization purposes (see fig. 1), we estimated ancestral state of body size using an ML approach implemented in the fastAnc function of phytools (Revell 2011). Evolutionary shifts in rates of body size evolution were reconstructed using RRphylo, as described above.

**Predictor 4: plunge-diving behavior.** Traits should evolve more slowly in plunge-divers because of functional constraints (Chang et al. 2016). Switches to novel foraging behaviors could also spur trait divergence, with the prediction that plunge-divers will have larger relative optic lobes than species that do not plunge-dive, as similar changes have been linked to feeding behavior in diving ducks (Kalsinska 2005) and other species that primarily use beak-guided foraging behaviors (Sultan 2005). Because we were interested in the effects of air-water transitions on brain morphology, we used published data sets and life-history descriptions of kingfisher species (Woodall 1991, 2001) to characterize the feeding behaviors of kingfishers as either plunge-diving or non-plunge-diving.

**Predictor 5: forest-dwelling.** Species living in closed (e.g., forest) habitats should evolve trait diversity faster than those in open habitats because of greater structural heterogeneity (i.e., more potential niches; MacArthur and MacArthur 1961) and hence greater potential for reproductive isolation in closed habitats. More complex habitat types have also been linked to larger cerebella and telencephala in cichlids (Shumway 2008). We further predict enlarged olfactory bulbs in forest-dwelling species, as these changes have been proposed to be associated with selection for olfaction in forested environments where visual cues are less salient (Torres and Clarke 2018). We scored species as living in either closed (forested) habitats or open habitats using published species descriptions (Woodall 2001).

**Comparative Analyses.** To understand whether the above ecological predictors explain interspecific variation in rates of trait evolution, we used multivariate rate tests designed for discrete (Adams 2014b) and continuous (Revell 2011) predictors. We transformed branch lengths of each posterior tree according to the ML estimate of Pagel’s λ (0.477 and 0.90 for brain shape and beak shape, respectively) obtained from PC axes. Phylogenetic signal values estimated across posterior trees were highly similar (fig. S2), and therefore we used the MCC tree estimate of λ for all posterior trees. Models exist to test the effects of a discrete predictor trait (e.g., island-dwelling, habitat, foraging behavior) on the rate of evolution of a continuous multivariate phenotype (e.g., the first six PC scores derived from a PCA of brain landmarks). To do so, we used a distance-based approach (Denton and Adams 2015), implemented in the compare.evols.rates function of geomorph, to determine whether rates of evolution differed between ecotypes. To link continuous predictors to rates of multivariate evolution, we used the ratebystate approach (Revell 2011), with a modification to incorporate phylogenetic uncertainty and multivariate data. Briefly, we simulated multivariate BM evolution 100 times each across 100 posterior trees. For each
simulation and tree, we then calculated ancestral states of the predictor variable (log diversification rates) using ML implemented in the fastAnc function of phytools (Revell 2011) and the mean squared independent contrasts across all PC axes (i.e., multivariate rates) at each node (R code is available in the Dryad Digital Repository [https://doi.org/10.5061/dryad.ffbg79cs6; Eliason et al. 2021]). Finally, we determined the Pearson’s correlation between ancestral states and multivariate rates for the simulated traits to generate a null distribution of r values, as well as the observed correlation, and determined P values as the proportion of null values greater than or equal to the observed correlation (e.g., Burress et al. 2019).

To test whether ecological predictors explain differences in mean brain morphology, we used a distance-based regression framework (Adams 2014a). Briefly, for each posterior tree, we fitted PGLS models using 44 brain and two beak PC axes. We then used 1,000 simulations to assess significance and determined significant divergence along distinct PC axes by comparing the observed PGLS estimates with these null distributions. Finally, we determined support for a significant relationship between a given predictor and morphological divergence as the number of posterior trees showing a significant (P < .05) relationship, following Burress et al. (2019).

Results

Evolution of Brain Architecture

Size-corrected brain shape varied in a modular fashion, with pPC1 showing coordinated changes in the optic lobe and medial edges of the telencephalon (fig. 2C) and pPC2 showing correlated changes mainly in the dorsal surface of the telencephalon and the cerebellum (fig. 2D). Rates of brain shape evolution are heterogeneous across kingfishers (fig. 3C). Specifically, we identified two significant (P < .05) rate shifts in the Todiramphus and Ceyx subclades (fig. 3C).

Accelerated Brain Shape Evolution Is Correlated with Rapid Diversification Rates

The relationship between lineage diversification rates and brain shape evolution rates was highly significant (β = .63 ± .18, P = .0012; fig. 3A), with most of the variance (~88%) coming from model uncertainty rather than phylogenetic uncertainty (see table 1 for details). Rates of beak shape evolution were not significantly associated with diversification rates (P = .54; see fig. 3B; table 1). Results were qualitatively similar in a multiple regression framework using PC axes derived from fixed braincase landmarks (β = .63 ± .20, P = .027), suggesting that the described link between diversification rates and brain shape evolution rates is not an artifact of methodological error in semilandmark placement. Furthermore, our results were robust to different metrics for diversification (table S3) and inclusion of body mass as a potential covariate (βbrain_rate = .66 ± .16, P < .01; βmass = .04 ± .08, P = .64), shown to be linked to brain PC1 (fig. 2A). Diversification rates were not significantly related to multivariate brain (P = .149) or beak shape (P = .18), as predicted under a model of trait-dependent diversification (for alternative rate metrics, see tables 2, S4).

Brain Phenotypes and Rates of Evolution Differ among Niches

Brain shape was significantly related to body size (table 3), with larger species having smaller optic lobes and a “taller” telencephalon or forebrain (fig. 4A). Brain shape was also significantly related to foraging behavior, with plunge-divers showing relatively broader cerebella (fig. 4B). These results were qualitatively similar when analyzed in a multiple regression framework using PC axes derived from fixed landmarks (table S5) and semilandmarks (table S6). Rates of brain shape evolution were significantly explained by habitat openness, with species living in closed, forest habitats exhibiting faster rates of brain shape evolution than species in more open habitats (table 4). However, contrary to our prediction, variation in mean brain shape was not related to habitat openness (table 3).

Discussion

We created and implemented a novel method for quantifying brain shape diversity across a clade of birds (fig. 1) to study the evolutionary rates and patterns of a complex anatomical trait. We find a link between accelerated speciation rates and rates of brain (but not beak) shape evolution (fig. 3). In addition, we found that rate increases in brain shape evolution occur in two species-rich clades of kingfishers (Ceyx, Todiramphus) that predominately live on isolated oceanic islands (fig. 3C). We found significant relationships between ecology and brain shape evolution, with kingfishers that live in closed, forested habitats displaying elevated rates of brain shape evolution (table 4) and general trends of distinct brain shapes related to body size and plunge-diving behavior (table 3; fig. 4). These results suggest that phenotypic lability of brain shape over macroevolutionary timescales may have enabled kingfisher species to take advantage of spatial opportunity often associated with rapid speciation in island radiations (Losos and Ricklefs 2009).
Brain shape divergence has been explored recently among disparate clades of birds (Balanoff et al. 2013), but our current understanding of how brain shape evolves within major avian lineages remains limited (Bright et al. 2016). With growing access to CT scan data in online repositories (e.g., MorphoSource; Boyer et al. 2016), novel analytical approaches are needed to process these data to meet the demands of modern comparative biology. A strength of our semiautomated approach is that it will enable researchers to investigate brain shape across many taxa more efficiently. Creating 3D model digital endocasts of brains—the current state of the art (Balanoff et al. 2015)—is often time-consuming. Recent methods for generating endocasts automatically from CT scan data (Michikawa et al. 2017) may provide a way forward, but this approach still involves time-consuming preliminary steps (e.g., image cropping, file conversion, isosurface determination) before endocast landmarking. By contrast, our hybrid approach of placing fixed landmarks directly on skulls and utilizing a single template endocast took only ~15 min of manual work per specimen. Subsequently placing surface semilandmarks on the braincase using computer algorithms was surprisingly accurate (fig. S3) and robust to human error in the original placement of fixed landmarks (fig. S4). Our approach is similar to the semiautomated procedure of Felice and Goswami (2018), in which they place semilandmarks on the external surface of skulls. Key differences in our approach are that we use a brain endocast as a template for semiautomated landmarking (rather than a
hemispherical shape) and we seek to place semilandmarks on internal (rather than external) skull surfaces. Internal measurements—made possible by these methods of obtaining dense surface semilandmarks from CT data—may uncover evolutionary trends that are not apparent with traditional linear measurements or fixed landmark analyses (e.g., resource use traits, including body size; Goswami et al. 2019). Indeed, in contrast to the evolutionary patterns seen for brain shape, rates of beak shape evolution showed no significant relationship with diversification rates (fig. 3B; table 1). Kingfishers also showed more brain shape variation than previously reported (Brusaferro and Insom 2009). Aristide et al. (2016) similarly showed extensive brain shape variation across New World monkeys linked to distinct foraging and dietary niches, and Carril et al. (2016) found two distinct brain morphotypes inferred from 3D digital
endocasts in Neotropical parrots. Qualitative analysis of
digital endocasts in shorebirds suggests high levels of
morphological variation (Smith and Clarke 2012) and
highlights the opportunity for further work with quanti-
tative, morphometrically based analyses. For example, geo-
metric morphometrics enable researchers to study mod-
ularity and phenotypic integration (Goswami and Polly
2010) and may improve morphological systematics in
rapid radiations (Parins-Fukuchi 2018).

**Accelerated Brain Shape Evolution and Rapid Diversification Rates**

Given a link between trait evolution and diversification
rates (fig. 3A; table 1), does allopatric speciation directly
promote trait diversity, or are kingfishers more speciose
because they have more evolutionarily labile brains? These
alternative hypotheses are difficult to separate with current
comparative methods. However, additional empirical evi-

dence for high levels of plasticity (Bentley et al. 1999) and
modularity (Balonoff et al. 2016) in avian brain shape, along
with habitat-driven changes in brain shape (fig. 4) and links
between behavioral flexibility and larger brains (Sol et al.
2005a), together suggest that brain shape lability may trig-
ger diversification in birds. Lineages with higher rates of
brain shape evolution might be able to occupy a wider
range of ecological niches and colonize harsher environ-
ments than those with less labile brain morphologies. This
could promote diversification if niche divergence results in
reproductive isolation among populations (Nosil 2012).

Alternatively, brain morphologies in kingfishers, if linked
to frequent behavioral innovations (Sol et al. 2005a) or pat-
terns that govern dispersal (Sol et al. 2010), could drive
rapid speciation on islands (Andersen et al. 2018) through
enabling clades to take advantage of ecological or “spatial”
opportunity (sensu Lambert et al. 2019) available in island
archipelagos that causes genetic divergence between popu-
lations (Price 2008). Indeed, the two kingfisher subclades
with the fastest diversification rates, *Todiramphus*
and *Ceyx* (Andersen et al. 2018), also have the highest rates
of brain shape evolution (fig. 3C) and are found primarily
on oceanic islands. These two island kingfisher clades are
marked by significant phenotypic disparity (i.e., plumage)
between closely related taxa (Andersen et al. 2013, 2015).

Alternatively, brain shape lability could be tied to lower ex-
tinction rates, for example, if the ability to evolve novel
brain shapes is linked to colonization success. We did not
consider this hypothesis because current approaches
for estimating species-specific diversification rates either
focus only on speciation (Harvey and Rabosky 2018) or
take extinction as a constant across the tree (Maliet et al.
2019), but future work could explore this possible link with
extinction rates. Nonetheless, our results suggest that, in
the same way that certain genes regulate key processes
(e.g., sex determination in animals), brain shape in king-
fishers may be acting as a phenotypic “master regulator”
(Ohno 1979) of key processes involved in speciation and/or
extinction (e.g., dispersal, migration, foraging behavior,
and sexual selection). In this study, we sampled a fraction
of the species-level diversity within the *Ceyx* (12 of 24) and
*Todiramphus* (10 of 28) clades (Gill and Donsker 2019).
Future research into brain shape evolution of these insular
species complexes should exhaustively sample species within
these genera to elucidate the relationship between brain

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**Table 1:** Testing a model of speciation trait evolution
in kingfishers

| Response               | Slope  | P     | % errorphylo | N  |
|------------------------|--------|-------|--------------|----|
| Brain shape rates      | .63 ± .18 | <.01  | 11.76        | 57 |
| (PC1–PC44)             |        |       |              |    |
| Beak shape rates       | .53 ± .87 | .54   | 12.32        | 57 |
| (PC1–PC2)              |        |       |              |    |

Note: Models were fitted using phylomol, with multivariate rate estimates for principal component (PC) axes describing >95% of overall interspecific variation as response variables and in diversification rate as the predictor variable. Error was decomposed into model uncertainty and phylogenetic uncertainty (shown above) estimated over 100 posterior trees. *P* values account for both sources of error (for details, see “Material and Methods”). For results with alternative macroevolutionary rate metrics, see table S3 (available online).

**Table 3:** Predictors of mean brain shape divergence
in kingfishers

| Predictor                  | $R^2$ | Z    | P     | Support |
|---------------------------|-------|------|-------|---------|
| In body mass              | .106  | 6.07 | <.01  | 1.00    |
| In range size             | .029  | 1.75 | .04   | .15     |
| Plunge-diving behavior    | .032  | 2.15 | .03   | 1.00    |
| Island-dwelling           | .025  | 1.28 | .10   | .00     |
| Forest-dwelling           | .019  | .36  | .35   | .00     |

Note: Models were fitted using distance-based phylogenetic generalized least squares regression implemented in the procD.pgl function of geomorph, with principal component (PC) axes PC1–PC44 as a response variable. *P* values were assessed with 1,000 randomizations of tip values. “Support” indicates the proportion of posterior trees showing a significant relationship ($P < .05$).
shape evolution, species diversification, and ecological divergence during rapid island radiations.

**Ecomorphology of Brain Shape and Size**

Brain regions are thought to change shape and size independently (i.e., modular evolution) rather than simply scaling together to produce overall larger or smaller brains (i.e., concerted evolution; Iwaniuk et al. 2004). Across kingfishers, after accounting for evolutionary covariation by treating brain shape as a single evolving phenotype, we find several significant trends with both brain shape and brain size. First, significant changes primarily in cerebellar shape are linked to plunge-diving behavior in kingfishers (fig. 4B). This relationship could be related to motor control needed for precise, beak-guided foraging, as was found for diving ducks (Kalisińska 2005), but this would need to be verified with neural and behavioral data. Indeed, cerebellar changes have been hypothesized to be related to cognition (Day et al. 2005). Second, in addition to these cerebellar changes, we found significant effects of behavior and ecology on the shape of the olfactory bulbs and telencephalon (fig. 4), which is the most variable region of the avian brain (Cate and Healy 2017). The flattened forebrains of plunge-diving species (fig. 4A) could be the result of skull streamlining to reduce drag during dives, as this would presumably result in compression of the available braincase volume. Our results also point to

![Figure 4](image-url)

**Figure 4**: Ecological predictors of brain shape divergence in kingfishers. Brain shape divergence is significantly explained by variation in body mass (A) and foraging behavior (B; with 0 and 1 indicating absence or presence of plunge-diving behavior). Although our analyses account for multivariate divergence along 44 principal component (PC) axes, only the most divergent PC axes between ecotypes are shown here for visualization purposes (for full results, see table 3). Digital endocasts depict 3D brain shapes at each extreme predictor value. Heat map colors correspond to regions of the brain that are larger (yellow) or smaller (dark blue) relative to the other ecotype.

| Predictor         | Parameter | Test                | $P$  | Support |
|-------------------|-----------|---------------------|------|---------|
| In body mass      | −.013     | ratebystate         | .93  | .00     |
| In range size     | −.014     | ratebystate         | .88  | .00     |
| Plunge-diving behavior | .927 | compare.evol.rates  | .31  | .00     |
| Island-dwelling   | 1.146     | compare.evol.rates  | .07  | .24     |
| Forest-dwelling   | 1.174     | compare.evol.rates  | .03  | .96     |

Note: Support indicates the proportion of posterior trees exhibiting a significant relationship ($P < .05$) between multivariate rates of evolution and a given predictor. The parameter for ratebystate tests is the correlation coefficient ($r$) between diversification rates and trait evolution rates, and for compare.evol.rates tests the parameter is the rate ratio between regimes (e.g., plunge-diving to non-plunge-diving species).
habitat openness as a predictor of brain shape evolution, with faster rates of brain shape evolution among species living in forests (table 4). Given that no single brain shape was associated with forest habitats (table 3), brain shape in these lineages may be diverging stochastically, perhaps through genetic drift in fragmented landscapes or in response to microhabitat characteristics not captured by our broad habitat categories (i.e., “open” and “closed”). Nonetheless, if populations become fragmented and reproductively isolated upon entering forested habitats, this should result in speciation, and therefore habitat could be mediating the observed correlation between rates of lineage diversification and brain shape evolution (fig. 3A).

Higher proportions of forest-dwelling species in Todiramphus (20 of 28, 71%) and Ceyx (22 of 24, 92%) relative to other “background” kingfisher lineages (36 of 81, 44%; Woodall 1991) provide some support for this mechanism. However, these clades are also found primarily on oceanic islands (Andersen et al. 2018), and thus geographic isolation among populations on different islands might explain both brain shape divergence and speciation rates. Neuroanatomical innovations might also be driving the differentiation of signals that cause reproductive isolation between populations and thereby promote speciation (Carlson et al. 2011). We recommend that future researchers study diverse signal traits of kingfishers, such as color patterns (Eliason et al. 2019) and vocal behaviors, to understand whether and how signal diversity relates to brain shape diversity. We hypothesize that high levels of neural diversity across a rapid radiation, such as island-dwelling kingfishers (Andersen et al. 2018; McCullough et al. 2019), will correlate with rapid evolution of acoustic and visual signals (Eliason et al. 2019) among closely related taxa.

Allometry of Brain Shape Evolution

We uncovered expected patterns of allometry, as well as some unexpected and novel results. For example, larger-bodied species have significantly larger brains (table S7). Interestingly, kingfishers with large ranges have significantly smaller brains than species with smaller ranges (table S7). This agrees with the pattern found by Vincze et al. (2015) and might relate to the energetic costs of migration (e.g., in Todiramphus sanctus and Megaceryle alcyon) and long-distance dispersal. The main driver of brain shape evolution is body mass, explaining >10% of the interspecific variation in brain shape (fig. 4A; table 3). Part of this trend stems from the major shift in body size between pygmy kingfishers (subfamily Alcedininae) and other subclades (fig. 2A).

Brain shape allometry (figs. 2A, 4A) could occur if some brain regions scale allometrically while others do not. One possible explanation for this pattern is that species with proportionally large jaw muscles needed to support a larger head would have predictably deeper temporal fossa, a groove in the posterior region of the skull that is particularly conspicuous in kingfishers (Burton 1984). This would constrain the available space on the interior of the skull, resulting in the observed allometric scaling of brain shape (figs. 1A, 4). Despite this significant relationship between body mass and brain shape, these two traits have decoupled evolutionary rates. For example, a slowdown in the rate of body mass evolution in Ceyx kingfishers is coincident with elevated rates of brain shape evolution (fig. S7). The fact that rates of body size evolution are accelerated in the Todiramphus genus but decelerated in Ceyx (fig. S7) suggests different modes of evolution in these nested radiations. Todiramphus kingfishers are evolving primarily along a body size axis (fig. 2A), with little variation in foraging niche as most species are generalists (Woodall 1991). Instead, body size variation might be linked to prey size (Remsen 1991). By contrast, Ceyx kingfishers show greater partitioning of foraging niche space (e.g., plunge-diving for fish and sallying for insects) associated with distinct brain morphologies (fig. 4B) and are found primarily in forest habitats shown to be linked to elevated rates of brain shape evolution (table 4). Thus, our results suggest that both nonadaptive (e.g., allometric constraints) and adaptive processes may have contributed to the considerable brain shape diversity of kingfishers (fig. 2A).

Conclusion

In this study, we investigate diversity of brain shapes in kingfishers with a new template-based landmarking approach. We find that beak shape, inferred from a linear data set that is typical of avian evolutionary studies, did not drastically change in areas of the tree associated with elevated diversification rates (e.g., Todiramphus). Conversely, we show brain shape—a relatively understudied trait in birds—to have elevated rates of evolution that align with the timing of these rapid radiations, particularly in island-dwelling kingfishers. Our results hint that evolutionary lability in brain shape allows for species to take advantage of spatial opportunities during a rapid radiation. Novel findings such as these highlight the value of natural history collections and having CT data generally accessible for researchers through sites such as MorphoSource (https://morphosource.org) and augmented by general scanning of fluid specimens.

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Statement of Authorship

C.M.E. conceptualized the research, C.M.E. and J.M.M. collected and analyzed data, and all authors wrote the manuscript.

Data and Code Availability

All code and data sets required to replicate these analyses are available in the Dryad Digital Repository (Elaison et al. 2021; https://doi.org/10.5061/dryad.fb79cs6). CT scans are available on MorphoSource (https://www.morphosource.org/Detail/ProjectDetail/Show/project_id/1134).

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A river kingfisher (Ceyx azureus) from Australia with a freshwater crayfish. Photo credit: Tyrone Lavery.