Foraging behavior and ecology drive the evolution of humeral shape in hummingbirds

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Abstract

Skeletal morphology is central to avian flight performance and ecological adaptation. In hummingbirds, the humerus is critical for power generation and wing control, yet its evolutionary diversification remains poorly understood. We integrate geometric morphometrics, phylogenetic comparative methods, and ecological data to study humeral shape evolution across a diverse sample of 80 hummingbird species. Humeral shape variation was associated with ecological and geographical variables, including body mass, foraging strategy, range size and latitude. Larger body mass or smaller humeri corresponded to more robust, curved shapes, consistent with biomechanical demands of supporting greater forces. In contrast, broader geographic ranges are associated with more slender shapes, possibly reflecting aerodynamic efficiency across variable environments. Humerus shape also changed with foraging strategy: traplining species, which visit flowers along dispersed routes, exhibit elongated humeri favoring efficient, sustained flight, whereas territorial species showed shorter, robust shapes suited to rapid maneuvers. Morphological evolution rates differed among strategies, with trapliners showing highest rates, and increased significantly with range size. Strong phenotypic integration within the humerus indicated that evolutionary changes are tightly coordinated across the bone regions. These findings demonstrate how ecological pressures, scaling effects, and developmental constraints together shape flight-related morphology, underscoring the adaptive flexibility of the avian skeleton.

Keywords: Adaptive evolution, Allometry, Biomechanics, Bird flight, Geometric morphometrics, Phenotypic modularity and integration.

Teaser text

How do tiny hummingbirds balance agility, power, and efficiency in flight? By analyzing humeral shapes from 80 species, we reveal how body size, foraging strategy, and geography shape this key wing bone. Trapliners and territorial species show distinct morphologies linked to their flight demands, while broader geographic ranges correlate with faster evolutionary rates. Strong integration within the humerus suggests that changes in one region influence the entire bone. Our findings uncover the interplay of ecology, biomechanics, and evolutionary history in shaping the avian skeleton.

Introduction

Understanding the evolution of morphological diversity remains a central topic in evolutionary biology. Organisms diversify morphologically in response to ecological pressures, functional constraints, and historical contingencies, and disentangling these influences requires integrative approaches combining morphology, ecology, and phylogeny (Losos, 2011; Pigot *et al.*, 2020; Sayol *et al.*, 2025). Birds, among the most ecologically and morphologically varied vertebrates, offer an insightful system to explore these dynamics of trait diversification, especially in traits shaped by the demands of powered flight (Norberg, 1990; Dudley, 2002).

Among skeletal components, the avian humerus plays a pivotal role in flight mechanics and ecological adaptation. It connects the axial skeleton to the forelimb and anchors major flight muscles responsible for the downstroke and upstroke of the wingbeat (Tobalske & Biewener, 2008). In addition, humerus morphology affects lift, wing loading, and maneuverability (Agrawal *et al.*, 2022). Despite its biomechanical importance, the humerus remains underexplored in comparative studies of avian flight, which have often emphasized external features such as wing aspect ratio (Hieronymus, 2015). While such metrics effectively capture overall aerodynamic properties, they do not reflect underlying skeletal variation, potentially overlooking functionally significant musculoskeletal morphology.

In hummingbirds, the humerus is distinctively short, robust, and exhibits morphological adaptations that support their specialized hovering and maneuverable flight (Agrawal *et al.*, 2022). Unlike the long, slender humeri typical of birds adapted for gliding or forward-powered flight (Dial, 1992), the hummingbird humerus is shortened to perform exceptionally high wingbeat frequencies and rapid rotation of the wing on each upstroke (Warrick *et al.*, 2005). These changes allow hummingbirds to generate lift during both downstroke and

upstroke and exert fine control during rapid aerial maneuvers (Agrawal *et al.*, 2022). To meet these challenges, the humerus functions as a rigid, force-transmitting lever for enlarged flight muscles, and exhibits structural specializations such as increased cortical thickness, a relatively straight shaft, and pronounced muscle attachment sites at the deltopectoral crest. This morphology reflects a biomechanical compromise: a shorter, sturdier humerus reduces inertial resistance and facilitates maneuverability, but may constrain aspects of aerodynamic efficiency or range of motion, requiring precise tuning to maintain energetic economy during sustained flight. With over 300 species, the hummingbird clade presents a rich system for comparative study, offering insight into how skeletal structures evolve under varying ecological demands and locomotor constraints. Yet despite its biomechanical importance, the hummingbird humerus remains underexplored in evolutionary studies.

Hummingbirds exhibit diverse foraging strategies that reflect their ecological niches and influence their flight behavior. Some species defend floral territories aggressively, engaging in rapid, maneuver-demanding flights to repel intruders, while others adopt traplining behaviors, following predictable expansive foraging circuits that require sustained travel across larger areas and efficient navigation between patchy floral resources (Altshuler *et al.*, 2004b; Sargent *et al.*, 2021). These differing foraging strategies are expected to impose distinct aerodynamic and biomechanical demands, which may drive morphological divergence in the skeleton. Species engaging in frequent, high-intensity maneuvers—such as territorial defense—may benefit from shorter, more robust humeri that facilitate rapid changes in direction and powerful wing strokes (Agrawal *et al.*, 2022), In contrast, species relying on traplining may favor longer and more slender humeri that enhance flight efficiency and reduce energetic costs over longer distances, even if they still require precise control for floral approach and handling (Nudds *et al.*, 2007; Sievwright & Macleod, 2012).

Beyond foraging behavior, ecological factors such as those varying with elevation also shape hummingbird morphology. Species occupying high-altitude environments face hypoxia and reduced air density, which increase the biomechanical demands of flight (Altshuler, 2006; Scott & Milsom, 2006). Prior studies have documented phenotypic divergence along elevational gradients, particularly in wing shape, body size, and metabolic traits (Altshuler *et al.*, 2004a; Stiles, 2008). However, skeletal elements like the humerus remain understudied in this context, despite their critical role in flight mechanics.

Additional ecological and morphological factors may further influence humeral evolution. Species occurring at higher latitudes or occupying broader geographic ranges often encounter greater environmental heterogeneity and dispersal demands, potentially favoring either morphological flexibility or specialization (Claramunt *et al.*, 2012; Yang *et al.*, 2024). Body mass also imposes biomechanical constraints, as larger species must support greater gravitational and aerodynamic forces (Blackburn & Ruggiero, 2001). Finally, overall skeletal size may influence scaling relationships critical for wing loading and flight efficiency (Klingenberg, 2016).

Together, these patterns suggest that humeral morphology in hummingbirds is likely shaped by a complex interplay of biomechanical constraints, ecological demands, and evolutionary history. Yet, the extent to which ecological variables predict morphological divergence—and whether they are also associated with evolutionary rates—remains underexplored (Teplitsky *et al.*, 2014; Chira *et al.*, 2018). Evaluating evolutionary rates is important, as accelerated shape evolution may reflect ecological opportunity, functional innovation, or release from constraints (Harmon *et al.*, 2010; Cooney *et al.*, 2017). In hummingbirds, one may expect that body size, ecological and geographical factors could all influence not only humeral shape but also the tempo of its evolution. By testing for associations among such variables, one may

clarify how ecological and morphological factors jointly shape both the patterns and pace of morphological change in this key flight structure.

The hummingbird humerus, with its distinct proximal and distal functions, also provides an appropriate system to investigate phenotypic integration. The proximal region primarily serves as the attachment site for the pectoralis and supracoracoideus muscles, which are responsible for generating the downstroke and upstroke during flight, respectively (Poore *et al.*, 1997; Tobalske & Biewener, 2008). The distal portion of the humerus articulates with the radius and ulna, transmitting the forces generated by these muscles into wing motion and modulating the wing's orientation and amplitude. Understanding whether these regions evolve in a coordinated manner offers insights into developmental constraints and adaptive flexibility (Adams & Felice, 2014; Felice *et al.*, 2019; Hallgrímsson *et al.*, 2019). Morphological traits, especially those underpinning locomotion, rarely evolve independently (Orkney *et al.*, 2021). Instead, they exhibit integration and modularity due to shared developmental pathways and functional covariation (Klingenberg, 2008; Hallgrímsson *et al.*, 2009). While many studies of phenotypic integration focus on patterns across multiple bones or skeletal regions (e.g., skull and mandible, or entire limbs), exploring integration within a single element can reveal how distinct functional regions remain evolutionarily coordinated despite localized biomechanical specializations.

Here, we investigate how foraging strategy, ecological pressures, and evolutionary processes collectively shape humeral morphology across a broad sample hummingbird species. First, we hypothesized that species differing in foraging strategies would exhibit divergent humeral morphologies, reflecting adaptations to their specific flight demands and ecological contexts. We also explored whether these strategies are linked to variation in the evolutionary rates of humeral shape. Rather than assuming a single directional outcome, we framed this analysis as a hypothesis-generating approach informed by the contrasting demands of different foraging

behaviors. For example, traplining—characterized by repeated, sustained flight along spatially extensive routes—may select for morphologies that enhance energetic efficiency and maneuverability across heterogeneous environments. Territorial behavior, by contrast, involves frequent short-distance accelerations and complex aerial contests, potentially selecting for structural robustness and high maneuverability. Whether such behaviors promote morphological innovation or constrain it through stabilizing selection likely depends on evolutionary history, ecological variability, and biomechanical trade-offs. Given that evolutionary rates can reflect diverse processes, including directional selection, stabilizing selection, or release from constraint (Revell *et al.*, 2008), we interpret our rate comparisons within a framework that considers multiple evolutionary scenarios, rather than presupposing a single outcome.

Second, we predicted that humeral shape variation and evolutionary rates would be influenced by a combination of ecological and morphological factors. Specifically, species inhabiting higher elevations may exhibit distinctive humeral morphologies, as high-altitude environments impose greater aerodynamic and metabolic constraints that may drive adaptive changes. However, whether these conditions lead to accelerated evolutionary rates remains uncertain. In some cases, strong stabilizing selection or habitat filtering may constrain morphological evolution, resulting in low rates despite ecological pressure (Revell *et al.*, 2008). Similarly, while broad geographic ranges or occurrence at higher latitudes have been associated with increased environmental heterogeneity and dispersal challenges, the relationship with evolutionary rate is context-dependent and may vary across clades and traits. Regarding body mass, we predicted that larger-bodied species would exhibit corresponding shifts in humeral morphology, reflecting biomechanical scaling effects on flight performance and energetic costs. Although allometric relationships are expected, examining whether these scaling patterns vary across ecological contexts could provide insights into how size-related

constraints interact with ecological specialization. Finally, we expected overall skeletal size to correlate with humeral shape variation due to its impact on wing loading and flight efficiency.

Lastly, we hypothesized that the humerus exhibits strong internal phenotypic integration, with coordinated morphological evolution between its proximal and distal regions, given their shared role in force transmission and wing mechanics. Such integration is expected because modifications in one region (e.g., altering muscle attachment sites proximally) would necessitate compensatory changes distally to maintain flight performance. However, examples from other systems suggest that bones can evolve in a modular fashion when regions fulfill distinct functions or face divergent selective pressures (Felice & Goswami, 2018; Navalón *et al.*, 2020; Orkney *et al.*, 2021). Whether such modularity also occurs within the hummingbird humerus remains an open question but would imply that evolutionary changes could be targeted to specific regions without affecting the entire structure. By integrating ecological categories and morphometric analyses within a phylogenetic framework, our objective is to provide a comprehensive assessment of the factors driving morphological evolution in the hummingbird humerus.

Methods

Specimens and image acquisition

We analyzed 80 hummingbird species, representing approximately 22% of extant species diversity in the family Trochilidae (Gill *et al.*, 2023). To capture morphological variation of the humerus, we photographed the right humerus in cranial view from male specimens in the collection of the Louisiana State University Museum of Natural Science (LSUMNS). We focused on males to reduce potential shape variation due to sexual dimorphism and to ensure consistency across species, as male specimens are more frequently available and

taxonomically reliable in museum collections. Images were taken using a Canon EOS M50 camera equipped with a 28 mm macro lens. The camera was mounted on a copy stand at a fixed distance from the specimens to maintain consistent magnification and perspective. Each photograph included an embedded scale bar (10 mm with 0.1 mm divisions) for accurate scaling in subsequent analyses. This standardized view facilitated consistent comparative analysis of skeletal structure across species (Zelditch *et al.*, 2012).

A total of 25 anatomical landmarks were digitized on each humerus, focusing primarily on points along the contour to capture overall shape (Figure 1A-B). Landmarks were chosen based on biomechanical relevance and anatomical consistency across taxa (Bookstein, 1992; Klingenberg, 2016). No semilandmarks were employed, because we aimed to focus on homologous and discrete anatomical points rather than continuous curves. A detailed description of landmarks and specimens list is provided as supplementary material. Landmark digitization was conducted using TPSDig2 version 2.32 (Rohlf, 2015).

Given the rarity of skeletal material for many hummingbird species, sample sizes varied across taxa. For approximately 87% of species (70 of 80), only a single male specimen was available. For the remaining 10 species, where multiple specimens (two to four per species) were available, we calculated the species-specific mean shape by averaging landmark configurations across individuals (Cardini & Elton, 2007).

Morphometric data preparation

All data preparation and statistical analyses were conducted in R version 4.4.2 (R Core Team, 2024). Landmark data were aligned using a Generalized Procrustes Analysis (GPA) to remove non-shape variation related to position, orientation, and scale (Rohlf & Slice, 1990). GPA was performed using the gpagen() function in the *geomorph* package (Adams *et al.*, 2012). Following alignment, we calculated squared Euclidean distances from each specimen to the

mean shape configuration to identify and exclude outliers. We retained 97.5% of specimens closest to the mean shape, a typical threshold in morphometric studies. Euclidean distances were computed by converting the aligned 3D landmark array to a two-dimensional matrix, followed by row-wise distance calculations. Two specimens—*Calypte anna* and *Goethalsia bella*—were identified as outliers for their unusually high morphological deviation from the overall mean shape configuration, due to preservation or landmarking artifacts, and were thus removed from further investigation. Following this filtering, 78 species were retained for downstream analyses. A PCA analysis of the aligned coordinates was performed exclusively for graphical representation of the shape variation and morphospace visualization (Figure 1C).

Species traits

For each species, we recorded a suite of ecological and morphological traits. Foraging strategy was classified as territorial, trapliner, or opportunist, based on typical foraging behavior and resource use (Billerman *et al.*, 2022). We also recorded a species' maximum elevation as a proxy for elevational environment (Quintero & Jetz, 2018). Geographic range size (km^2) was obtained as we expect species occupying larger ranges to experience broader environmental heterogeneity. Body mass was included as a proxy for biomechanical constraints associated with size, given that larger species typically experience different mechanical stresses during flight (Altshuler *et al.*, 2004b). Range size, latitude at the centroid of the species' range, and body mass data were retrieved from Tobias et al. (2022). Additionally, as a structure-specific indicator of skeletal size, we measured humerus centroid size from the landmark data. While centroid size is mathematically derived from the distribution of landmark coordinates, it is widely recognized as a robust proxy for overall size in morphometric analyses (Klingenberg, 2016).

Phylogenetic signal

To assess whether humeral shape variation exhibited phylogenetic signal, we quantified Blomberg's multivariate K statistic (Blomberg *et al.*, 2003; Adams & Felice, 2014) using the physignal() function in *geomorph*, based on 1,000 permutations. We also computed a Z-transformed effect size to contextualize the observed K values relative to a null distribution. An ultrametric phylogeny was obtained from McGuire et al. (2014) for all comparative analyses.

Phylogenetic modeling of humeral shape variation

To evaluate how ecological and morphological predictors influence humeral shape, we implemented a phylogenetic generalized least squares (PGLS) approach using the procD.pgls() function in *geomorph* for high-dimensional shape data. We first optimized the lambda (λ) parameter for the phylogenetic correlation structure using likelihood profiling. The optimization converged on $\lambda = 0.99$, indicating strong phylogenetic structure consistent with Brownian motion. Permutation-based significance testing was conducted using 1,000 iterations, and effect sizes were calculated as Cohen's f^2.

The multivariate model included foraging strategy as a categorical predictor, alongside continuous ecological and morphological variables: maximum elevation, absolute latitude, range size, body mass, and humerus size. All predictors were incorporated simultaneously in the same phylogenetic generalized least squares (PGLS) regression framework. None of the variables exhibited strong collinearity (Pearson's $r < 0.36$), and all variance inflation factors were within acceptable limits (VIF < 1.5).

Discriminant analysis of humeral shape by foraging strategy

To further assess whether humeral shape is differentiated among the three major foraging strategies, we conducted a Canonical Variates Analysis (CVA). Prior to the CVA, we corrected allometric effects by regressing Procrustes-aligned shape coordinates against log-transformed

centroid size, following standard procedures (Klingenberg, 2016). The residuals of this regression represent shape variation independent of humerus size. We then analyzed these residuals using the CVA() function in the *Morpho* package (Schlager, 2017), specifying foraging strategy as the grouping variable. Statistical robustness of group discrimination was assessed with 1,000 permutations. We also projected phylogenetic relationships into the canonical morphospace using the phylomorphospace() function from *phytools* (Revell, 2012).

Evolutionary rates of humeral shape

To test whether the evolutionary rate of humeral shape varied among ecological categories, we compared rates among foraging strategies using the compare.evol.rates() function in *geomorph*, with 1,000 permutations. We further examined whether continuous predictors were associated with variation in evolutionary rates. For each species, we calculated Procrustes mean shape and reconstructed ancestral shapes using the fastAnc() function in *phytools*. Morphological change along terminal branches was computed as the Euclidean distance between ancestral and descendant shapes, standardized by branch length obtained from the ultrametric phylogeny. To minimize the influence of extreme values, we excluded the top 10% of evolutionary rates. We then modeled evolutionary rates as a function of five continuous predictors—maximum elevation, range size, absolute latitude, body mass, and humerus size—using a multivariate generalized additive model (GAM) implemented via the *mgcv* package in R. This approach allowed us to flexibly account for potential nonlinear relationships between predictors and evolutionary rates.

Phenotypic modularity and integration

We assessed phenotypic integration within the humerus by partitioning the landmark data into proximal and distal regions. The proximal region included the humeral head and articular surfaces, whereas the distal region encompassed the shaft and insertion points. We estimated

phylogenetic partial least squares (PLS) correlations between these modules using the phylo.integration() function (Adams & Felice, 2014), with statistical significance assessed through 1,000 permutations.

Results

Phylogenetic signal

Humeral shape exhibited a significant phylogenetic signal ($K = 0.309$, $p = 0.001$), indicating that closely related hummingbird species tend to share similar humeral morphologies. This result is further supported by a large effect size ($Z = 41.1$), underscoring the strength of phylogenetic structuring in shaping bone morphology. The geometric configuration used to quantify humeral shape reveals consistent variation in landmark positions across species, highlighting major axes of morphological differentiation (Figure 1A–C). The distribution of phylogenetic signal and its placement on the hummingbird phylogeny are shown in Figure 1D.

Predictors of humeral shape variation

The multivariate PGLS identified several significant predictors of humeral shape variation (Table 1; Figure 2). Humerus size had the strongest effect on shape ($R^2 = 0.062$, $Z = 3.29$, $p = 0.002$), consistent with expectations of allometric scaling influencing skeletal morphology. Body mass also emerged as a significant predictor ($R^2 = 0.032$, $Z = 2.25$, $p = 0.015$), suggesting that mass-associated biomechanical demands contribute to shape differences. Range size had a similarly significant effect ($R^2 = 0.031$, $Z = 2.51$, $p = 0.010$), indicating that species occupying larger geographic range distributions tend to exhibit distinct humeral configurations. Latitude, modeled as absolute distance from the equator, showed a marginal trend ($R^2 = 0.021$, $Z = 1.35$, $p = 0.073$), hinting at potential shape shifts across environmental

gradients, though this effect did not reach significance. Maximum elevation showed no statistically significant influence ($R^2 = 0.014$, $Z = 1.24$, $p = 0.103$). Foraging strategy was also marginally non-significant ($R^2 = 0.040$, $Z = 1.51$, $p = 0.068$), though its directional effects remained consistent with the results from group-wise comparisons. Overall, the model accounted for 27% of total humeral shape variation.

Morphospace differentiation by foraging strategy

The principal component analysis (PCA), used to visualize patterns of shape variation, revealed differences in humeral morphology associated with foraging strategies (Figure 3A). Notably, the morphospaces of traplining and territorial species were largely contained within the broader morphospace occupied by opportunistic species, as illustrated by the convex hulls outlining each group in Figure 3A. This suggests that opportunistic foragers may exhibit more generalist humeral shapes, while the other groups occupy more restricted morphological configurations. The projected phylogenetic relationships onto the morphospace showed some local clustering of related species, consistent with the significant phylogenetic signal detected in the shape data. However, the overall pattern lacked clear structure, with multiple lineages dispersed throughout the morphospace, indicating that a substantial proportion of shape variation is explained by ecological differentiation rather than shared ancestry. The Canonical Variate Analysis (CVA) further reinforced the role of foraging strategy in shaping humeral morphology (Figure 3C). The first canonical axis (CV1) primarily distinguished trapliners from both territorial and opportunistic species, while CV2 separated territorial from opportunistic species. Classification accuracy was 71.2%, significantly exceeding random expectations (permutation test, $p = 0.001$). The 90% confidence ellipses showed limited overlap, particularly for trapliners, which occupied a distinct region of morphospace. This group-level separation aligns with the differentiation detected in the multivariate regression models.

Evolutionary rates

Traplining species exhibited approximately 2.5 times faster rates of humeral shape evolution compared to territorial species (Figure 4), though this difference was not statistically significant ($p = 0.138$). Across all foraging strategies, evolutionary rates declined through time, consistent with an early burst followed by a deceleration of morphological change (Figure 4, upper left panel). When modeling the association between evolutionary rates and continuous predictors using a multivariate generalized additive model (GAM), only geographic range size showed a statistically significant effect. The relationship was nonlinear and smooth (edf = 2.69, $F = 6.85$, $p < 0.001$), suggesting that species with larger geographic ranges tended to evolve humeral morphology at faster rates. In contrast, maximum elevation ($p = 0.488$), body mass ($p = 0.774$), latitude ($p = 0.466$), and humerus size ($p = 0.662$) did not show significant associations with evolutionary rate estimates. These results indicate that among the ecological and morphological factors examined, geographic range size was the most consistent predictor of evolutionary tempo, while other variables exhibited no detectable effects under the current analytical framework.

Phenotypic integration

The modularity analysis revealed strong phenotypic integration between the proximal and distal regions of the humerus (r-PLS = 0.899, $Z = 4.22$, $p = 0.001$). This indicates that morphological changes in one region are closely mirrored by changes in the other, reflecting coordinated evolution across the bone (Figure 5).

Discussion

Phylogenetic signal

Our results reveal a clear phylogenetic signal in humeral morphology among hummingbirds (Figure 1). This finding indicates that evolutionary history continues to shape patterns of morphological similarity across species, consistent with general trends reported for other avian skeletal traits (Bright *et al.*, 2016; Shatkovska & Ghazali, 2020). The presence of a strong phylogenetic structure suggests that humeral shape has evolved in a way that is partly structured by shared ancestry throughout the radiation of hummingbirds. Rather than indicating strict evolutionary constraint, this pattern may reflect the retention of biomechanically effective configurations across lineages, as well as the influence of phylogenetic inertia during adaptive divergence (Poore *et al.*, 1997; Revell *et al.*, 2008; Holmes *et al.*, 2022).

Predictors of shape variation

Our multivariate phylogenetic regression identified several significant predictors of species differences in humeral shape, supporting the hypothesis that ecological and morphological factors contribute to skeletal morphology in hummingbirds. Humerus size showed the strongest effect in the model (Table 1), consistent with a marked allometric component in shape variation. As humerus size increased, species exhibited broader proximal epiphyses and straighter, shorter diaphyses. This pattern likely reflects biomechanical adaptations: larger humeri require expanded articular surfaces for muscle attachment (notably for the pectoralis and supracoracoideus) and reduced shaft curvature to minimize bending stress under greater loading (Poore *et al.*, 1997; Bleiweiss, 2002; Skandalis *et al.*, 2017). These shape changes are consistent with general scaling principles observed in avian flight bones, where increased size leads to disproportionate reinforcement of load-bearing regions (Biewener, 1982; Poore *et al.*, 1997).

Body mass also significantly predicted humeral shape. Larger-bodied species displayed more robust humeral shapes, particularly with increased shaft thickness and straighter diaphyses. Biomechanically, this likely compensates for the higher aerodynamic forces and inertial loads encountered during both flapping and hovering flight in heavier hummingbirds (Altshuler *et al.*, 2004a). These findings align with prior studies documenting allometric effects on limb bone robustness across volant birds (Nudds *et al.*, 2007).

Geographic range size was another significant ecological predictor. Species with broader geographic distributions tended to exhibit more streamlined humeral shapes, particularly with narrower shafts and reduced epiphyseal breadth (Figure 2). Wider ranges may be associated with longer average movement distances, whether through dispersal, exploratory behavior, or seasonal shifts, which in turn could favor morphologies that enhance aerodynamic efficiency and reduce the energetic costs of sustained flight (Claramunt *et al.*, 2012; Drury *et al.*, 2024). Alternatively, occupying a wide range of habitats may select for versatile skeletal configurations capable of performing effectively across diverse environmental conditions.

Latitude showed a marginal but notable trend, with species distributed farther from the equator exhibiting more robust humeri, characterized by expanded proximal epiphyses and straighter shafts. While this pattern could reflect biomechanical adaptations to greater environmental seasonality and structurally complex habitats, conditions that may require enhanced power generation and maneuverability during flight (Dakin *et al.*, 2018; Sonne *et al.*, 2020), it may also relate to broader-scale movement ecology. Specifically, species at higher latitudes often engage in longer-distance dispersal or seasonal movements, which could select for more mechanically robust skeletal traits that support sustained flight performance in variable conditions. In contrast, elevation did not show a significant effect, though the trend toward shorter diaphyses and deeper epiphyses in high-elevation species is biomechanically consistent with enhanced lift production under low air density and hypoxia

(Altshuler *et al.*, 2004a; Lim *et al.*, 2021). Similar morphological adjustments to elevation have been reported for wing shape and muscle architecture in other avian taxa (Altshuler, 2006).

Foraging strategy showed a marginal association with humeral shape ($p = 0.068$), yet the observed trends were biomechanically coherent. Traplining species tended to exhibit longer and more slender humeri, a morphology that likely supports sustained, energy-efficient flight over extended foraging circuits. A more elongated humerus can increase wing span and reduce wingbeat frequency, enhancing aerodynamic efficiency and decreasing energetic costs during long-distance movement between floral resources (Nudds *et al.*, 2007; Sievwright & Macleod, 2012; Zusi, 2013). In contrast, territorial species exhibited shorter, thicker, and more curved humeri, traits associated with enhanced maneuverability and force production. Shorter bones with greater curvature can reduce the moment of inertia around the shoulder joint by keeping mass closer to the body's center of rotation, enabling faster angular acceleration and tighter turning radii. These features are advantageous during rapid chases, evasive maneuvers, and sudden vertical ascents used in territorial defense (Altshuler *et al.*, 2004b; Dakin *et al.*, 2018; Sargent *et al.*, 2021). Additionally, a more robust humerus provides increased surface area for the attachment of flight muscles such as the pectoralis and supracoracoideus, which are critical for explosive bursts of power (Zusi, 2013). These divergent morphological configurations reflect adaptive tuning of the flight apparatus to meet the specific aerodynamic and behavioral demands imposed by alternative foraging strategies.

Together, these findings underscore that the humerus in hummingbirds is shaped by a complex combination of size-related biomechanics, ecological space use, and behavioral flight demands. The moderate explanatory power of the model ($R^2 = 0.27$) reflects the multifaceted nature of morphological evolution under interacting selective pressures and phylogenetic constraints

Morphospace differentiation by foraging strategy

The principal component analysis (PCA) showed that species using territorial and traplining strategies occupied partially distinct areas of morphospace. In contrast, opportunistic species encompassed much of the variation found in the other two groups (Figure 3A). This distribution suggests that opportunistic species tend to exhibit more generalized humeral morphologies, whereas territorial and traplining species display more specialized shapes adapted to their respective flight demands.

Building on these findings, the canonical variate analysis (CVA) demonstrated statistically significant morphological separation among the three foraging groups (Figure 3C). CV1 and CV2 effectively distinguished trapliners, territorial, and opportunistic species, confirming the shape differentiation patterns identified in the multivariate regression. The low overlap between groups, particularly for trapliners, reinforces that foraging strategies correspond to distinctive humeral morphologies, likely reflecting the biomechanical demands associated with each behavioral mode. Moreover, the distribution of species in the phylomorphospace (Figure 3A) revealed that some distantly related species converge on similar humeral configurations, suggesting that similar flight demands may drive morphological convergence across the hummingbird phylogeny despite evolutionary distance. This pattern highlights the role of ecological function in shaping skeletal form beyond phylogenetic constraints.

The reduced overlap of 90% confidence ellipses between groups in Figure 3B further underscores the functional and evolutionary relevance of humeral shape variation. While some convergence was observed between territorial and opportunistic species along CV2, the separable centroids indicate that their humeral morphologies remain distinct. This differentiation likely arises from the differing balance of maneuverability and energetic efficiency required by each strategy: territorial species must defend floral resources through

rapid, aggressive flights, while opportunistic species exploit transient feeding opportunities, potentially favoring more generalized morphologies (Tiebout, 1992; Sargent *et al.*, 2021). Notably, this group-level morphological differentiation persisted even after accounting for phylogenetic relatedness and allometric scaling, indicating that foraging strategy imposes a measurable and adaptive influence on humeral shape. These results corroborate the directional trends identified in the multivariate regression models and underscore the biomechanical relevance of humeral variation in relation to ecological demands.

Evolutionary rates

The analysis of evolutionary rates provided a nuanced picture of how ecological factors might shape the tempo of humeral evolution in hummingbirds. Traplining species exhibited evolutionary rates approximately 2.5 times higher than those of territorial and opportunistic species (Figure 4A), although this difference did not reach statistical significance ($p = 0.138$). While some aspects of traplining, such as sustained flight over long circuits, may impose biomechanical demands (Altshuler *et al.*, 2004b; Sargent *et al.*, 2021; Rombaut *et al.*, 2022), it remains unclear whether this strategy is consistently more selective than the rapid, acceleration-intensive flights involved in territorial defense. Given this uncertainty and the lack of statistical support, we suggest interpreting these rate differences with caution.

Among continuous predictors, geographic range size was the only factor significantly associated with evolutionary rate estimates, as revealed by generalized additive models (Figure 4B). This nonlinear association suggests that species distributed across larger geographic areas may experience greater morphological change in the humerus, potentially due to exposure to diverse environmental conditions, ecological heterogeneity and flight contexts (Webb & Gaston, 2000; Pie *et al.*, 2021). However, the underlying mechanisms remain speculative.

Although evolutionary rates appeared to decline over time across foraging groups (Figure 4A), consistent with an early burst model, species-level estimates mapped onto the phylogeny (Figure 1B) showed scattered pockets of elevated rates, especially near the tips. These patterns are not necessarily contradictory: temporal trends capture average changes over macroevolutionary time, while tip-specific estimates reflect recent shifts that may be driven by clade-specific adaptations or environmental changes. Such dual patterns have been observed in other studies of morphological evolution (Cooney *et al.*, 2017) and underscore the complexity of linking evolutionary rates to ecological scenarios.

Phenotypic integration

Our analysis revealed a very high degree of morphological integration between the proximal and distal regions of the humerus (Figure 5), supporting the prediction that these regions evolve in a coordinated manner. The strong integration observed suggests that evolutionary modifications in one region likely require compensatory changes in the other to preserve flight mechanics and biomechanical efficiency (Sievwright & Macleod, 2012).

Such integration is expected in highly loaded locomotor structures, where functional covariation and shared developmental pathways limit the potential for independent morphological change (Klingenberg, 2008; Goswami *et al.*, 2016). While this integration may limit the range of morphological trajectories available to hummingbirds, it likely facilitates the evolution of coordinated adaptations to ecological and biomechanical demands. This is consistent with broader patterns observed in vertebrate skeletal systems, where modularity and integration frequently emerge as key features of evolutionary flexibility and constraint (Randau & Goswami, 2017; Fabre *et al.*, 2020).

In hummingbirds, the persistence of a strongly integrated humeral structure across species suggests that even as humeral shape diverges in response to ecological demands, internal

morphological coordination remains evolutionarily conserved. This integration likely imposes constraints on the directions and combinations of shape changes that are viable, channeling morphological evolution along coordinated trajectories rather than permitting independent variation of individual regions. As a result, while ecological pressures may drive diversification in overall humeral form, such change is shaped by internal structural coherence, not unconstrained by it. Our findings thus highlight that phenotypic integration can both enable functional refinement and limit morphological disparity, shaping how form evolves under ecological and biomechanical selection.

Conclusion

Our results reveal that the evolution of humeral morphology in hummingbirds is driven by an interplay between ecological demands, biomechanical constraints, and evolutionary history. Despite strong phylogenetic signal, humeral shape is not purely conserved; instead, it exhibits clear associations with foraging behavior, spatial distribution, and body size. In particular, traplining species show faster evolutionary rates and more elongate humeri, suggesting that energetic efficiency under sustained locomotion can drive morphological divergence. Meanwhile, the influence of geographic range size on both shape and evolutionary tempo points to the role of spatial ecological heterogeneity as a driver of morphological diversification. At the same time, our finding of high phenotypic integration between proximal and distal humeral regions highlights the developmental and functional cohesion of this element, implying that adaptive evolution in one region must be coordinated with changes in the other. This internal integration likely acts as both a constraint and a scaffold, channeling morphological evolution along biomechanically viable pathways.

Taken together, this study underscores the value of integrating morphometrics, ecology, and phylogenetics to understand how complex structures evolve under multiple simultaneous pressures. It also highlights how even within a morphologically conservative lineage such as hummingbirds, fine-scale variation in ecology and behavior can leave clear imprints on skeletal design. Our results contribute to a broader understanding of how functionally integrated traits evolve in response to ecological and evolutionary forces, offering a model for investigating the dynamics of form and function across vertebrates.

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Tables

Table 1. Summary of the phylogenetic generalized least squares (PGLS) model evaluating humeral shape variation in relation to hummingbird species traits. The table presents the results of multivariate phylogenetic regression. Reported values include degrees of freedom (df), proportion of variance explained (R^2), F-statistic, effect size (Z), and empirical p-values based on 1,000 permutations.

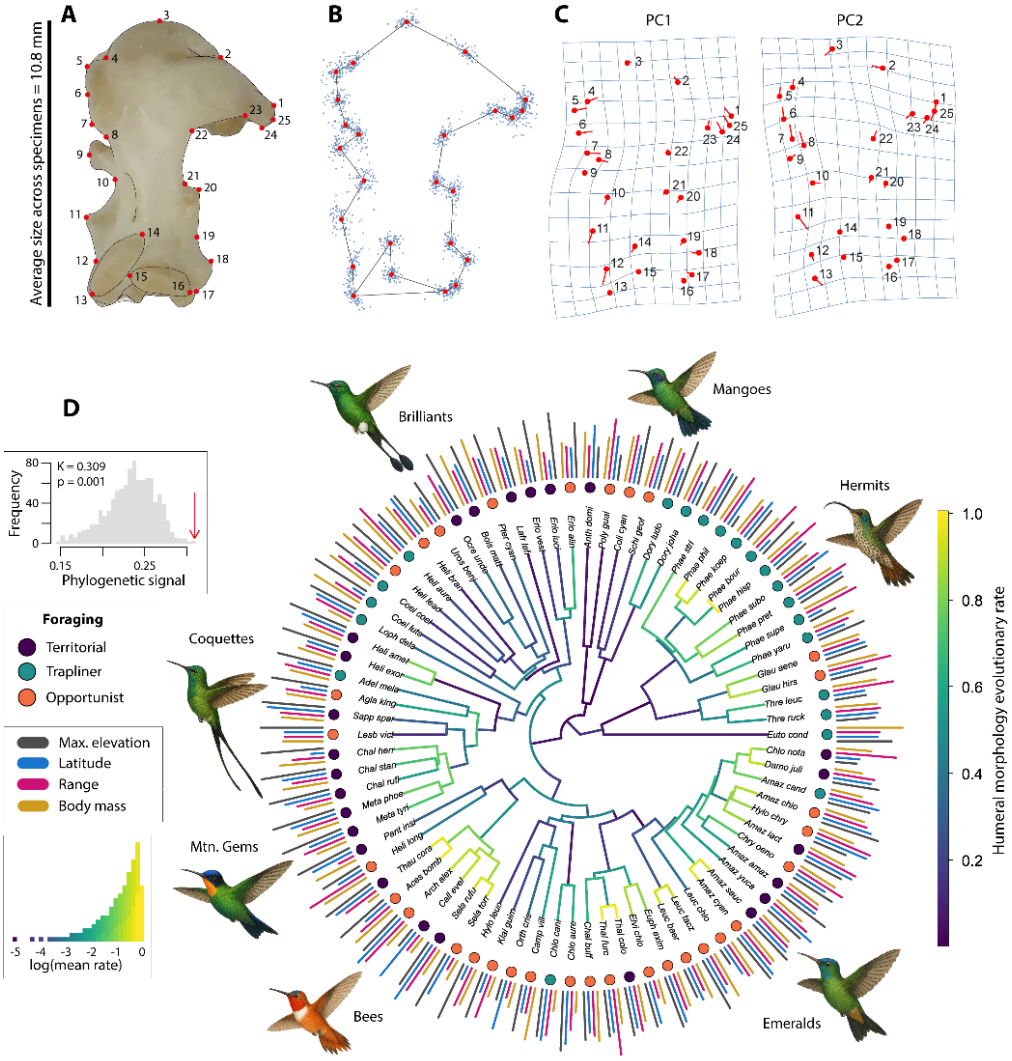
Predictor	df	R^2	F	Z	p-value	Significance
Foraging strategy	2	0.04	1.73	1.51	0.068	.
Latitude	1	0.021	1.85	1.35	0.073	.
Body mass	1	0.032	2.82	2.25	0.015	*
Humerus size	1	0.062	5.46	3.29	0.002	**
Range size	1	0.031	2.74	2.51	0.01	*
Elevation	1	0.014	1.21	1.24	0.103	

Table 2. Summary of generalized additive models relating evolutionary rate of humeral shape to species traits in hummingbirds. This table presents the results of univariate generalized additive models (GAMs) assessing the relationship between species-specific evolutionary rates of humeral shape and each continuous predictor. For each variable, the table reports the estimated degrees of freedom (edf), reference degrees of freedom (df), F-statistic, p-value, and level of statistical significance.

Predictor	edf	df	F	p-value	Significance
Range size	2.691	3.311	6.847	< 0.001	***
Elevation	1.430	1.745	0.977	0.488	
Body mass	1.365	1.638	0.159	0.774	
Latitude	1.000	1.000	0.541	0.466	
Humerus size	1.000	1.001	0.193	0.662	

Figures

Figure 1. Morphological evolution of the hummingbird humerus in a phylogenetic context. (A) Anatomical landmarks used to quantify humerus shape (n = 25), shown on a representative specimen. (B) Landmark variation across specimens illustrating the geometric morphometric dataset. (C) Shape deformation grids for the first two principal component axes (PC1 and PC2), showing the main axes of humeral shape variation and the anatomical regions most contributing to shape change. (D) Null distribution of phylogenetic signal (K) in humeral morphology across species with the observed value (red arrow) indicating significant phylogenetic structuring (K = 0.309, p = 0.001). The circular phylogenetic tree depicts evolutionary relationships among the 78 species analyzed. Branch colors represent species-specific evolutionary rates of humeral shape (from lower rates in dark purple to higher rates in bright yellow). Radial bars around the tree show normalized values of predictor variables and continuous traits. The lower left inset shows the frequency distribution of evolutionary rates across the tree (Log [mean rate]). Hummingbird illustrations were AI generated using Chat GPT-4o and corrected with Adobe Illustrator.


A
Average size across specimens = 10.8 mm
B
C
PC1
PC2
D
Frequency
K = 0.309
p = 0.001
Phylogenetic signal
Foraging
Territorial
Trapliner
Opportunist
Max. elevation
Latitude
Range
Body mass
log(mean rate)
Brilliants
Mangoes
Hermits
Coquettes
Mtn. Gems
Bees
Emeralds
Humeral morphology evolutionary rate

761

762

Figure 2. Humeral shape variation in relation to species traits. Each panel depicts the association between the first principal component (PC1) of humeral shape variation and one of the ecological and morphological predictors included in the PGLS model. PC1 represents a gradient from shorter, more robust humeri (lower PC1 values) to longer, more slender humeri (higher PC1 values), as illustrated along the y-axis. Continuous predictors are displayed as scatterplots with fitted phylogenetic regression lines and 95% confidence intervals (shaded areas). Foraging strategy, a categorical variable, is shown as boxplots with overlaid data points. While PC1 is shown here for illustrative purposes, the full multivariate PGLS model considers variation across the entire shape space. Note that latitude represents the absolute value at the centroid of the species' range.

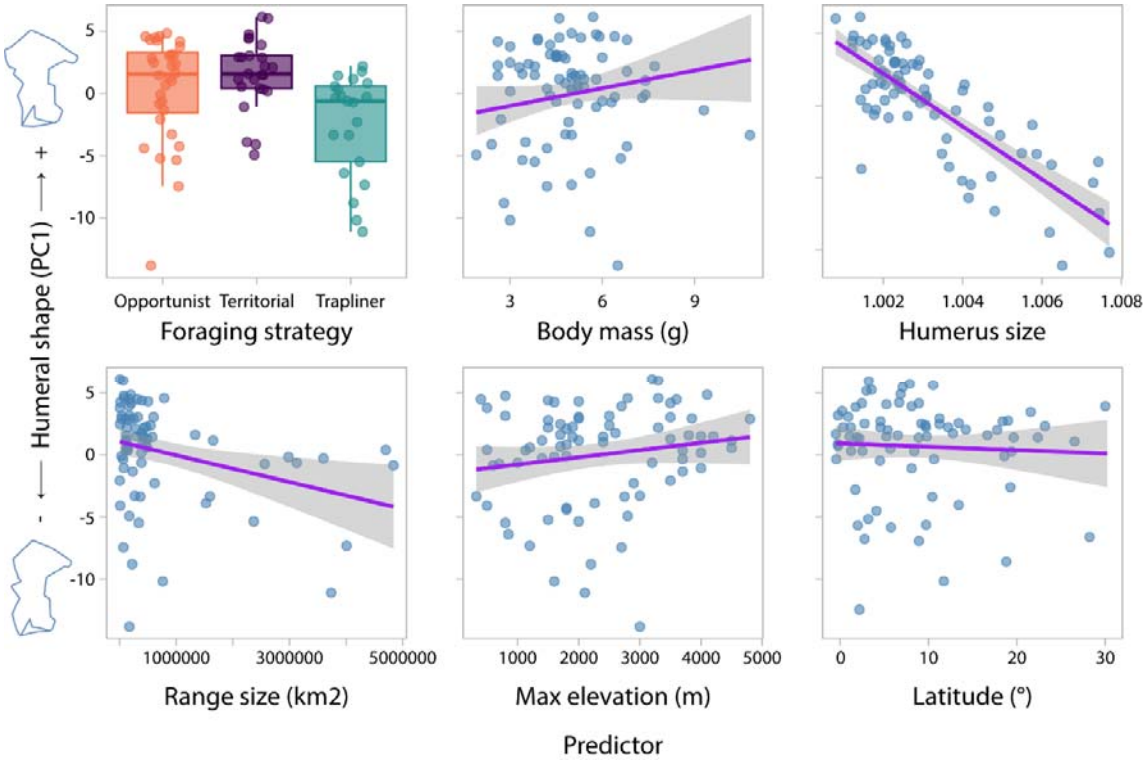
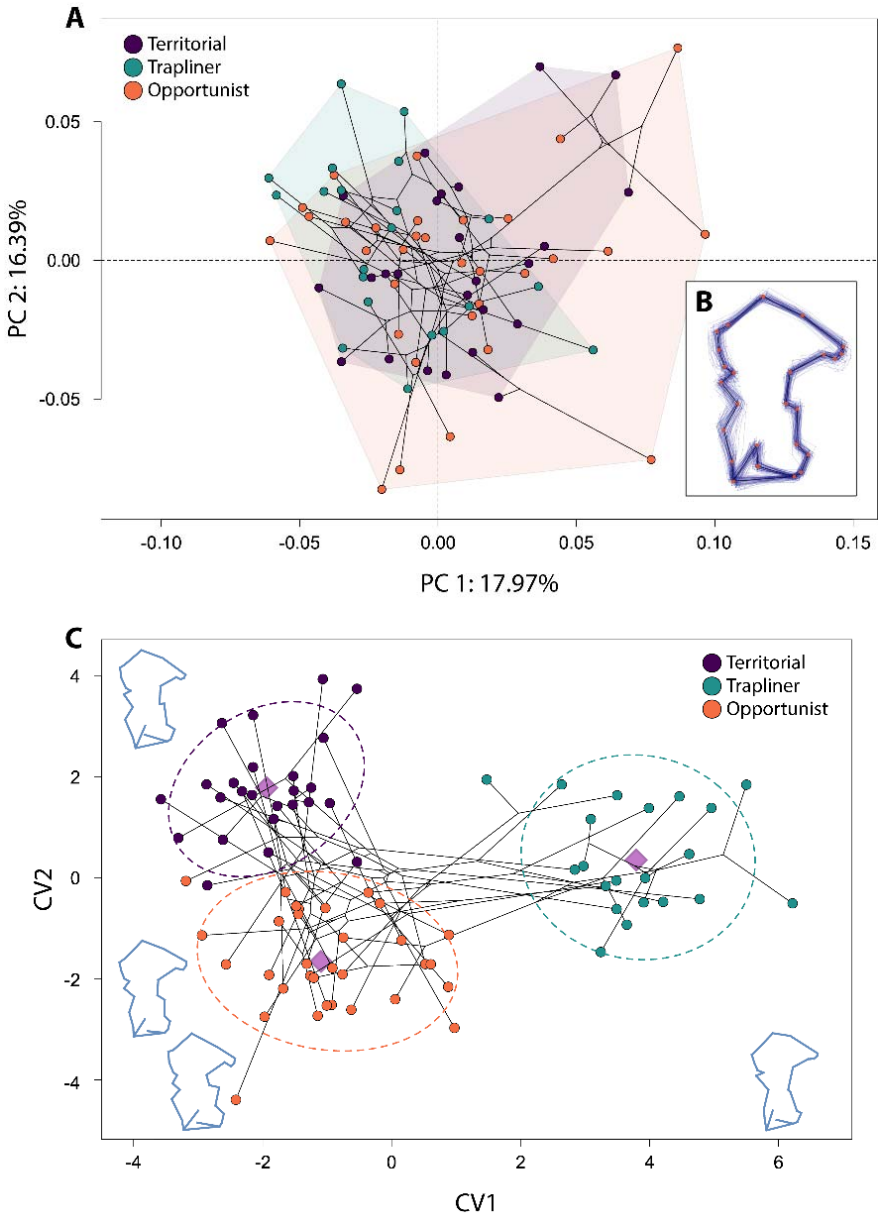


Figure 3. Morphospace of humeral shape variation in relation to foraging strategies. (A) Principal Component Analysis (PCA) of Procrustes-aligned humeral shapes across all specimens, colored by foraging strategy. PC1 and PC2 account for 17.97% and 16.39% of shape variation, respectively, capturing the major axes of morphological variation in the dataset. Shaded convex hulls delimit the morphospace occupied by each foraging group. Black lines represent phylogenetic relationships projected into the morphospace. (B) Mean humeral shape variation across all specimens, visualized as a thin-plate spline deformation grid relative to the consensus shape. Red dots represent each quantified landmark. (C) Canonical Variate Analysis (CVA) focusing on foraging strategies, which maximizes discrimination between groups. Dashed ellipses indicate 90% confidence intervals for each group, and purple diamonds mark the centroid of each group. Shape diagrams adjacent to the axes represent humeral shape changes associated with variation along CV1 and CV2.


A
Territorial
Trapliner
Opportunist
PC 2: 16.39%
PC 1: 17.97%
B
C
CV2
CV1

787

788

Figure 4. Ecological and geographic predictors of evolutionary rates in humeral morphology. (Top left) Temporal dynamics of evolutionary rates of humeral shape across foraging strategies. Each line represents a smoothed trajectory of rate variation through relative time (distance from the root), colored by foraging strategy. (Other panels) Relationships between the evolutionary rate of humeral shape and individual ecological and morphological predictor variables included in univariate regression models. Shaded areas represent 95% confidence intervals. Note that latitude represents the absolute value at the centroid of the species' range.

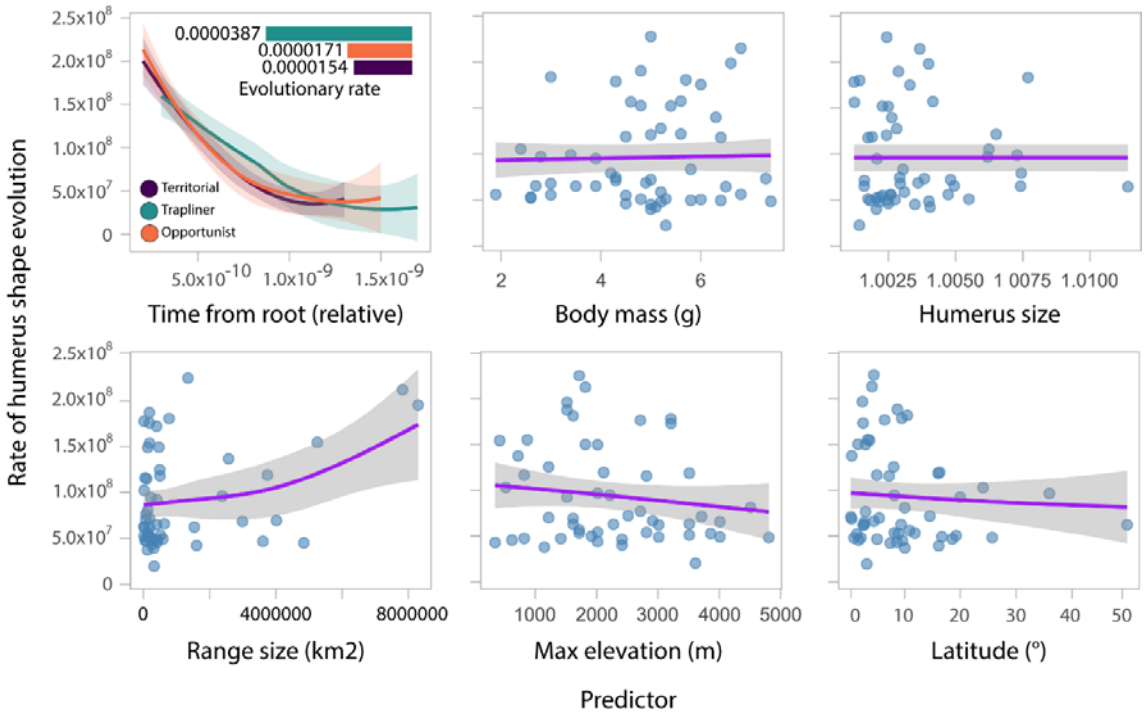
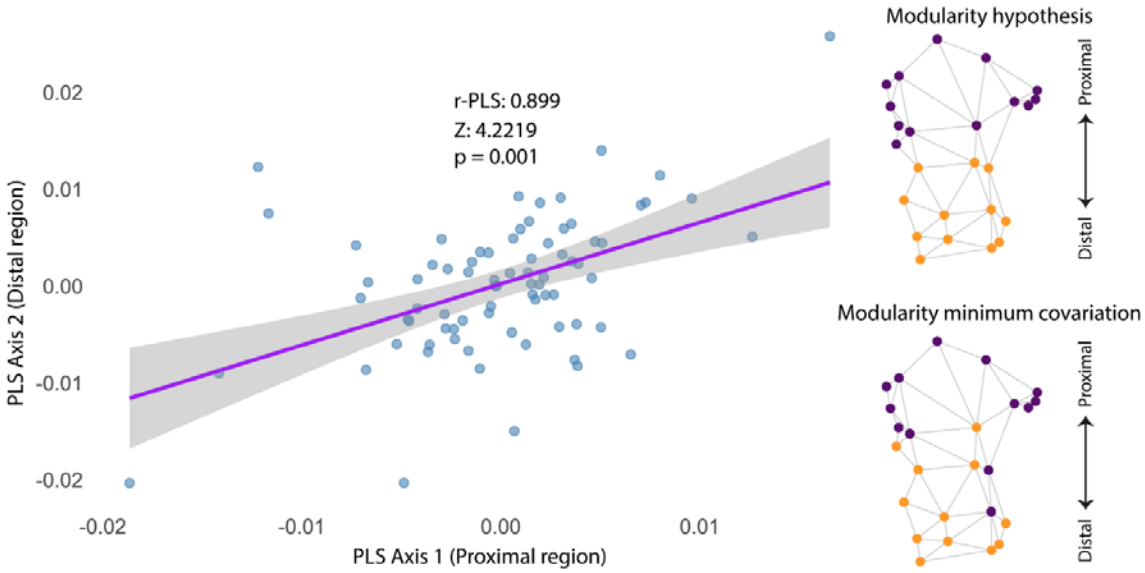



Figure 5. Phenotypic integration and modular organization of the hummingbird humerus. (Left) Partial Least Squares (PLS) analysis of shape covariation between the proximal and distal regions of the humerus. Each point represents a species' score on the first PLS axis for each region. The strong positive correlation (r-PLS = 0.899, p = 0.001) indicates high phenotypic integration, suggesting coordinated morphological evolution between regions. (Right) Hypothesized (top) and empirically derived (bottom) modular configurations of the humerus based on minimum covariation. Nodes represent landmarks, and colors denote module assignment. The empirical partition largely recovers the expected proximal–distal modular structure, reinforcing the developmental and functional differentiation of these anatomical regions.