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

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Abstract

Skeletal morphology underlies avian flight performance and ecological diversification. In hummingbirds, the humerus is central to power generation and maneuverability, yet its macroevolutionary dynamics remain poorly understood. Here, we integrate geometric morphometrics, ecological data, and phylogenetic comparative methods to examine humeral shape evolution across 80 species. Humeral shape varied significantly with body mass, centroid size, foraging strategy, geographic range size, and latitude, reflecting the joint influence of scaling, spatial ecology, and behavioral demands. Traplining, territorial, and opportunistic species occupied partially distinct regions of morphospace, and foraging strategy exerted a significant effect on evolutionary tempo, with trapliners evolving humeral morphology at consistently faster rates. Continuous ecological predictors, however, did not explain variation in evolutionary rates. Phylogenetic partial least squares revealed strong evolutionary integration between proximal and distal humeral regions, indicating that morphological changes are tightly coordinated across the bone. Together, these results show that humeral evolution in hummingbirds is shaped by the interaction of ecological differentiation, biomechanical constraints, and strong internal integration, illustrating how functionally complex structures diversify under multiple selective pressures.

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 shape across 80 species, we uncover how body size, foraging behavior, and geographic distribution shape this key wing bone. Traplining and territorial species exhibit partially distinct humeral morphologies consistent with their contrasting flight demands, and trapliners evolve humeral form at significantly faster rates. Strong evolutionary integration between proximal and distal regions shows that changes in one part of the bone propagate through the entire structure. Our findings reveal how ecology, biomechanics, and evolutionary history interact to shape the hummingbird 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 [1–3]. 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 [4,5].

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 [6]. In addition, humerus morphology is understood to influence wing kinematics, which in turn likely affect the magnitude and direction of aerodynamic forces and aspects of maneuverability [7]. 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 [8]. 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 [7]. Unlike the long, slender humeri typical of birds adapted for gliding or forward-powered flight [9], the hummingbird humerus is shortened to perform exceptionally high wingbeat frequencies and rapid rotation of the wing on each upstroke [10]. These changes allow hummingbirds to generate lift during both downstroke and upstroke and exert fine control during rapid aerial

maneuvers [7]. 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 (the lift-to-drag (L/D) ratio) 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 [11,12]. 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 [7], 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 [13,14].

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 [15,16]. Prior studies have documented phenotypic divergence along elevational gradients, particularly in wing shape, body size, and metabolic traits [17,18]. 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 [19,20]. Body mass also imposes biomechanical constraints, as larger species must support greater gravitational and aerodynamic forces [21]. Finally, overall skeletal size may influence scaling relationships critical for wing loading and flight efficiency [22].

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 [23,24]. Evaluating evolutionary rates is important, as accelerated shape evolution may reflect ecological opportunity, functional innovation, or release from constraints [25,26]. 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 [6,27]. 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 [28–30]. Morphological traits, especially those underpinning locomotion, rarely evolve independently [31]. Instead, they exhibit integration and modularity due to shared developmental pathways and functional covariation [32,33]. 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 [34], 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 [34]. 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 [31,35,36]. 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 [37]. 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 with a Canon EOS M50 (6000 × 4000 px) using a 28 mm macro lens. Because specimens were photographed in strict orthogonal orientation near the center of the frame, geometric distortion at this focal length is minimal, and no additional distortion correction was applied. 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 [38].

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 [22,39]. 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 in Table S1. Landmark digitization was conducted using TPSDig2 version 2.32 [40].

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 [41].

To assess the potential influence of geometric uncertainty on the morphometric dataset, we considered several non-statistical sources of measurement error. Pixel resolution of the Canon EOS M50 (6000 × 4000 px) introduces at most 1–2 pixels of uncertainty in landmark position, corresponding to ~0.1% of humeral length in our images. Intra-observer variation in landmark placement in similar studies is typically 0.5–1.5%, and because all specimens were digitized by the same observer, inter-observer error is likely below 1%. Anatomical ambiguity in a subset of landmarks may contribute an additional 1–3% variability in local point placement. Together, these factors suggest an overall geometric uncertainty on the order of 2–4% of inter-landmark distances, well below the magnitude of interspecific shape differences captured by the Procrustes analysis. Consequently, these sources of error are unlikely to influence the main axes of humeral shape variation or the phylogenetic patterns recovered in our analyses.

Morphometric data preparation

All data preparation and statistical analyses were conducted in R version 4.4.2 [42]. Landmark data were aligned using a Generalized Procrustes Analysis (GPA) to remove non-shape variation related to position, orientation, and scale [43]. GPA was performed using the gpagen() function in the *geomorph* package [44]. 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 [45]. We also recorded a species' maximum elevation as a proxy for elevational environment [46]. 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 [11]. 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 [22].

Phylogenetic signal

To assess whether humeral shape variation exhibited phylogenetic signal, we quantified Blomberg's multivariate K statistic [30,48] 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. Because distance-based methods do not estimate Pagel's λ by maximum likelihood [50], we fixed λ at 0.99, a value consistent with the strong phylogenetic signal detected in humeral shape ($K = 0.309$, $p = 0.001$). Significance testing was conducted using 1,000 permutations of residuals under a Brownian correlation structure.

We evaluated a set of *a priori* candidate ecological models (Table S2) and then fitted a full multivariate model including foraging strategy (categorical), body mass, humerus centroid size, maximum elevation, geographic range size, and absolute centroid latitude. None of the predictors showed problematic collinearity (Pearson's $|r| < 0.36$), and all variance inflation factors remained low ($VIF < 1.5$), indicating that the model structure was appropriate for multivariate estimation.

Because procD.pgls uses Type-I sequential sums of squares, we implemented a principled ordering of predictors to maximize model stability and ensure biological interpretability. First, we performed an exhaustive permutation analysis of all 720 possible predictor sequences. This analysis consistently ranked centroid size as the strongest predictor, followed by body mass, with environmental predictors emerging as the weakest contributors. Based on this result, and following best practices for sequential sum-of-squares models, we ordered predictors as follows: (1) centroid size, (2) body mass, (3) foraging strategy, (4) range size, (5) absolute latitude, and (6) maximum elevation. This ordering minimizes instability due to predictor order and reflects both biological expectations and empirical model performance.

The final PGLS model was then used to generate multivariate effect estimates, partial residuals, and projected PC1 relationships shown for visualization. Although PC1 is used for illustrative purposes, all hypothesis tests were based on the full multivariate shape data.

Discriminant analysis of humeral shape by foraging strategy

To evaluate whether humeral shape differs among foraging strategies, we performed a Canonical Variates Analysis (CVA) using size-corrected Procrustes coordinates. Allometric effects were removed by regressing aligned shape coordinates on log-transformed centroid size, and the resulting residuals were used as inputs for all subsequent analyses [22]. CVA was conducted using the CVA() function in the *Morpho* package [51], with foraging strategy specified as the grouping factor. Statistical support for group separation was assessed via 1,000 permutation tests, which evaluate whether between-group differences exceed those expected under random assignment of specimens to groups. To visualize phylogenetic structure in the discriminant space, we projected species relationships onto the CVA axes by connecting species scores according to the tree topology using *phytools* [52]. This allows assessment of whether phylogenetic relatedness aligns with group clustering in canonical

morphospace. All analyses were conducted on the same dataset and grouping scheme used in the main phylogenetic regression models to ensure consistency across analytical frameworks.

Evolutionary rates of humeral shape

To test whether foraging strategies differed in their evolutionary tempo, we used the compare.evol.rates() function, specifying foraging strategy as the grouping factor and performing 999 permutations to assess statistical significance under a null model of equal rates. This analysis evaluates whether the mean evolutionary rate of each group departs from expectations given the phylogeny and the distribution of shape change across species.

For continuous predictors, we tested associations between evolutionary rates and ecological or morphological variables using the search.trend() function in RRphylo [53], which fits linear regressions between branch-specific rate estimates and predictor values while accounting for phylogeny. Because branch-specific rate estimates can be influenced by extreme values in individual species, we applied standard RRphylo diagnostics to identify potential outliers or leverage points using the built-in functions RRphylo::outliers() and RRphylo::search.trend(). No species were removed, but diagnostics are reported in Table S3. To evaluate time-dependent patterns in evolutionary tempo, we extracted ancestral rate estimates from RRphylo and regressed them against node ages, allowing us to assess whether rate magnitudes varied systematically across the temporal depth of the phylogeny.

We additionally modeled species-specific evolutionary rates as a univariate response using phylogenetic generalized least squares (PGLS). These models were fitted using the gls() function in the *nlme* package, with a Brownian-motion correlation structure (corBrownian) derived from the same ultrametric tree used in the RRphylo analyses. Each ecological or morphological predictor (body mass, centroid size, geographic range size, absolute latitude,

and maximum elevation) was evaluated in a separate univariate PGLS model. Predictor variables were log-transformed when appropriate to improve linearity and homoscedasticity.

This complementary framework allowed us to test whether continuous predictors showed phylogenetically structured associations with evolutionary tempo that were consistent with the patterns suggested by RRphylo's rate estimates.

Phenotypic modularity and integration

To evaluate the degree of phenotypic integration within the humerus, we partitioned the Procrustes-aligned landmark data into two functional modules defined a priori based on avian flight anatomy. The proximal module included the humeral head, tubercles, and associated articular surfaces involved in shoulder rotation and load transfer during both hovering and flapping. The distal–shaft module comprised the diaphysis, trochlea, capitulum, and distal muscle insertion sites, which primarily mediate force transmission, wingbeat mechanics, and resistance to bending loads during forward flight. This subdivision reflects the well-established biomechanical differentiation between the articulation region of the humerus and its load-bearing shaft [27,54] and was selected to test a specific functional hypothesis rather than to infer empirical modular structure.

We quantified evolutionary covariation between these two modules using phylogenetic partial least squares (pPLS) implemented in the phylo.integration() function of *geomorph*. This method estimates the correlation between paired blocks of shape variables while explicitly accounting for phylogenetic non-independence, thereby capturing integration patterns that reflect coordinated evolutionary changes between modules. Statistical significance was assessed using 1,000 phylogenetically informed permutations. We did not perform alternative modularity searches (e.g., minimum covariation partitions or EMMLi-based module optimization), because our goal was to test a focused, anatomically motivated prediction

regarding integration between proximal articulation structures and the distal shaft, rather than to explore the full modular landscape of the humerus.

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 showed the strongest effect ($R^2 = 0.081$, $Z = 3.08$, $p = 0.002$), consistent with strong allometric contributions to humeral morphology. Range size also emerged as a significant predictor ($R^2 = 0.031$, $Z = 2.52$, $p = 0.008$), indicating that geographically widespread species tend to occupy distinct regions of humeral morphospace. Body mass showed a moderate but significant association with shape ($R^2 = 0.022$, $Z = 1.89$, $p = 0.031$), suggesting that mass-related biomechanical demands influence humeral configuration. Importantly, foraging strategy also had a significant effect on humeral shape ($R^2 = 0.035$, $Z = 1.87$, $p = 0.035$), reflecting detectable morphological differentiation among territorial, traplining, and opportunistic species. Absolute latitude exhibited only a marginal

trend ($R^2 = 0.016$, $Z = 1.46$, $p = 0.070$), whereas maximum elevation showed no significant influence on variation in shape ($R^2 = 0.013$, $Z = 1.25$, $p = 0.102$). Overall, the final model explained approximately 20% of the total multivariate variation in humeral shape.

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 occupy a broader and more variable humeral morphospace, although the overall overlap among groups was substantial. 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. Cross-validated classification accuracy was low (43.6%), only modestly exceeding random expectations, and the canonical morphospace showed broad overlap among strategies. Traplining species displayed slightly higher correct assignment (47.6%) than territorial or opportunistic species (40.6–44.0%), but all groups exhibited extensive intermingling. These modest patterns of group separation are consistent with the significant but relatively small effect of foraging strategy detected in the multivariate regression models.

Evolutionary rates

Estimates of evolutionary rates derived from RRphylo revealed a strongly right-skewed distribution, with most hummingbird species exhibiting very low rates of humeral shape change. Across the 78 species analyzed, median rate was 0.0063, with the 25th and 75th percentiles at 0.0030 and 0.0120, respectively. Only a small subset of species displayed markedly elevated rates (Table S3). These high-rate branches represent isolated episodes of accelerated morphological divergence, whereas most species retained relatively conserved humeral configurations. Phylogenetic signal in evolutionary rates was weak ($\lambda = 0.027$) and not significantly different from zero (LR = 0.041, $p = 0.840$), indicating that closely related species did not evolve at more similar rates than expected under phylogenetic independence. This demonstrates that rate variation is not phylogenetically structured and arises idiosyncratically across the tree.

Group-wise comparisons using compare.evol.rates() detected a statistically strong effect of foraging strategy on evolutionary tempo ($R^2 = 0.062$, $Z = 3.465$, $p = 0.001$). Traplining species exhibited the highest mean evolutionary rates (approximately 2.5 times faster than territorial species) followed by opportunistic taxa (Figure 4). When examining temporal patterns, rates across all strategies declined through time, consistent with an early burst followed by a deceleration of humeral shape evolution (Figure 4, upper left panel).

To assess whether continuous ecological or morphological traits predicted evolutionary rates, we fitted univariate PGLS models using a Brownian correlation structure. None of the predictors showed statistically significant associations with rate estimates: latitude ($p = 0.132$), body mass ($p = 0.476$), humerus centroid size ($p = 0.925$), geographic range size ($p = 0.678$), and maximum elevation ($p = 0.890$) all produced weak and non-significant effects (Table 2). Although the predictors themselves exhibited moderate phylogenetic signal ($\lambda =$

0.57–0.63), this structure did not translate into variation in evolutionary tempo. These results indicate that foraging strategy is the only ecological factor with a detectable association with evolutionary rates in the humerus, while other variables show no consistent influence under a phylogenetic regression framework.

Phenotypic integration

The phylogenetic PLS 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). This high level of integration is consistent with the functional coupling between articulation surfaces and the load-bearing shaft, where evolutionary changes in joint morphology necessarily co-vary with adjustments in shaft curvature, thickness, or distal anatomy.

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 [55,56]. 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 [27,34,57].

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 [27,58,59]. 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 [27,54].

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 [17]. These findings align with prior studies documenting allometric effects on limb bone robustness across volant birds [13].

Geographic range size was another significant ecological predictor. Species with larger geographic ranges tended to exhibit humeri with a more slender mid-shaft, characterized by reduced mediolateral breadth relative to anteroposterior depth (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 flight efficiency and reduce the energetic costs of sustained flight (i.e. longer overall wing (via

elongation of the ulna, manus, or primary feathers), increased wing area, and reduced wing loading; 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 [61,62], it may also relate to broader-scale movement ecology. This pattern is biomechanically consistent with the demands of lift production in hummingbirds: a shorter diaphysis reduces the moment of inertia of the wing, enabling faster angular accelerations, while deeper epiphyses provide larger areas for the shoulder musculature to generate torque. 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 [17,63]. Similar morphological adjustments to elevation have been reported for wing shape and muscle architecture in other avian taxa [15].

Foraging strategy showed a significant but modest effect on humeral shape ($p = 0.035$), and the differences among strategies exhibited overlap in morphospace. Nonetheless, the observed trends were biomechanically coherent. Traplining species tended to exhibit longer and more slender humeri, a morphology likely supporting sustained, energy-efficient flight over extended foraging circuits. A more elongated humerus can increase wingspan and reduce

wingbeat frequency, enhancing aerodynamic efficiency (approximated here as a higher lift-to-drag ratio) and decreasing energetic costs during long-distance movement between floral resources. Insofar as a longer proximal wing segment increases the span and reduces induced drag during forward flight. [13,14,64]. 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 [11,12,62]. 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 [64]. These divergent morphological configurations reflect adaptive tuning of the flight apparatus to meet the specific aerodynamic and behavioral demands imposed by alternative foraging strategies.

Importantly, elongation of the humerus is not the only anatomical pathway for increasing wing span or improving L/D ratio; elongation of primary feathers could theoretically achieve similar aerodynamic benefits with lower inertial costs. Our interpretation therefore does not assume a one-to-one link between humeral length and wing length. Rather, we propose that changes in the bony proximal wing segment may interact with distal elements (ulna, manus, feathers) to modify overall wing planform in ways relevant to forward-flight efficiency. Humeral elongation should therefore be viewed as a plausible, but not exclusive, morphological route contributing to differences in flight performance among 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 (~20% of total variation) 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 occupy a broader and more variable region of morphospace, although all three strategies show partial overlap.

Building on these findings, the CVA revealed modest differentiation among groups, with overlap in canonical morphospace despite statistically detectable effects. (Figure 3C). CV1 and CV2 effectively distinguished trapliners, territorial, and opportunistic species, which is consistent with the significant but modest effect of foraging strategy detected in the multivariate regression. 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 90% confidence ellipses showed partial overlap, particularly between territorial and opportunistic species, while trapliners occupied a more distinct region of morphospace. These patterns likely arise from the differing balance of maneuverability and energetic efficiency required by each strategy, producing partially convergent solutions rather than discrete morphotypes: territorial species must defend floral resources through rapid, aggressive flights, while opportunistic species exploit transient feeding opportunities, potentially favoring more

generalized morphologies [12,65]. 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) and this difference was statistically significant ($R^2 = 0.062$, $Z = 3.465$, $p = 0.001$). Traits associated with traplining, such as sustained flight along extended foraging circuits, may impose distinct biomechanical and energetic demands that contribute to the observed rate acceleration [11,12,66], although the precise functional mechanisms warrant further investigation. These results indicate that foraging behavior is a consistent predictor of evolutionary tempo in the humerus, with traplining lineages exhibiting reliably faster rates of morphological change than territorial and opportunistic species.

Continuous ecological and morphological predictors showed no significant association with evolutionary rate in univariate PGLS models, indicating that variables such as body mass, elevation, latitude, centroid size, and geographic range size do not systematically predict evolutionary tempo under a phylogenetic regression framework.

Although evolutionary rates showed a temporal decline consistent with an early burst model (Figure 4A), species-level estimates mapped onto the phylogeny showed scattered pockets of elevated rates (Figure 1B), 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 [26] and underscore the complexity of interpreting rate heterogeneity in macroevolutionary datasets, where isolated pulses of accelerated divergence coexist with long periods of relative stasis.

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 indicates that the humerus behaves as a single functional unit, with evolutionary changes in the proximal region tightly associated with coordinated adjustments along the shaft and distal end, reflecting the mechanical continuity required during hovering and forward flight [14].

Such integration is expected in highly loaded locomotor structures, where functional covariation and shared developmental pathways limit the potential for independent morphological change [33,67]. 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 [68,69]. The strength of this integration also suggests that evolutionary covariation dominates over any individual-level plasticity associated with adult bone remodeling.

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 high integration likely constrains the directions along which humeral morphology can evolve, channeling change along coordinated trajectories rather than allowing independent diversification of proximal and distal regions. As a result, while ecological pressures may drive diversification in overall humeral form, such change is shaped by internal structural coherence, constrained 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. Thus, even when ecological pressures promote divergence in overall humeral morphology, internal structural coherence remains evolutionarily conserved.

Conclusion

Our results show that humeral evolution in hummingbirds reflects an interplay between ecological demands, biomechanical constraints, and phylogenetic history. Although humeral shape displays moderate phylogenetic structuring, evolutionary rates exhibit little phylogenetic signal, indicating that tempo and mode are decoupled. Shape variation is associated with foraging behavior, spatial distribution, and body size, and traplining species evolve humeral morphology at significantly faster rates, consistent with the demands of sustained circuit-foraging. Geographic range size further predicts differences in humeral shape, although it does not influence evolutionary tempo.

Strong evolutionary integration between proximal and distal humeral regions highlights the cohesive functional design of the bone. This integration implies that evolutionary modifications are channeled along coordinated, biomechanically viable trajectories rather than occurring independently across regions.

Together, these findings demonstrate that even within a lineage constrained by flight performance, ecological and behavioral variation leaves detectable signatures on skeletal design. The combined analysis of morphometrics, ecology, and phylogenetics provides a clearer understanding of how integrated locomotor structures evolve under multiple selective pressures.

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Tables

Table 1. Ecological and morphological predictors explain variation in hummingbird humeral shape, with size, range, and body mass showing the strongest effects. Humerus shape variation is significantly associated with humerus size, geographic range size, body mass, and foraging strategy. The table reports effect sizes and significance values from the final multivariate PGLS model, including degrees of freedom (df), coefficient of determination (R^2), F-statistics, Z-scores, and p-values for each predictor. Asterisks indicate significance levels (* $p < 0.05$, ** $p < 0.01$).

Predictor	df	R^2	F	Z	p-value	Significance
Foraging strategy	2	0.035	1.536	1.872	0.035	*
Latitude	1	0.016	1.445	1.458	0.070	.
Body mass	1	0.022	1.927	1.894	0.031	*
Humerus size	1	0.081	7.120	3.078	0.002	**
Range size	1	0.031	2.774	2.520	0.008	**
Elevation	1	0.013	1.209	1.251	0.102	

Table 2. Foraging strategy shows significantly different evolutionary rates of humeral shape across regimes, whereas continuous predictors show no detectable univariate effects. Foraging strategy explains significant differences in evolutionary rates of humeral morphology when tested using compare.evol.rates, whereas RRphylo-based univariate regressions show no meaningful effects for latitude, body mass, humerus size, range size, or elevation. The permutation ANOVA (1000 residual randomizations) reports Df, R^2, F, Z, and p-values for the categorical test. The Kappa statistic summarizes overall model fit. The bottom section reports univariate PGLS results based on RRphylo tip rates, including parameter estimates, standard errors, maximum-likelihood λ, and p-values. Significance codes: *** $p < 0.001$.

Analysis of variance using residual randomization (1000 permutations)

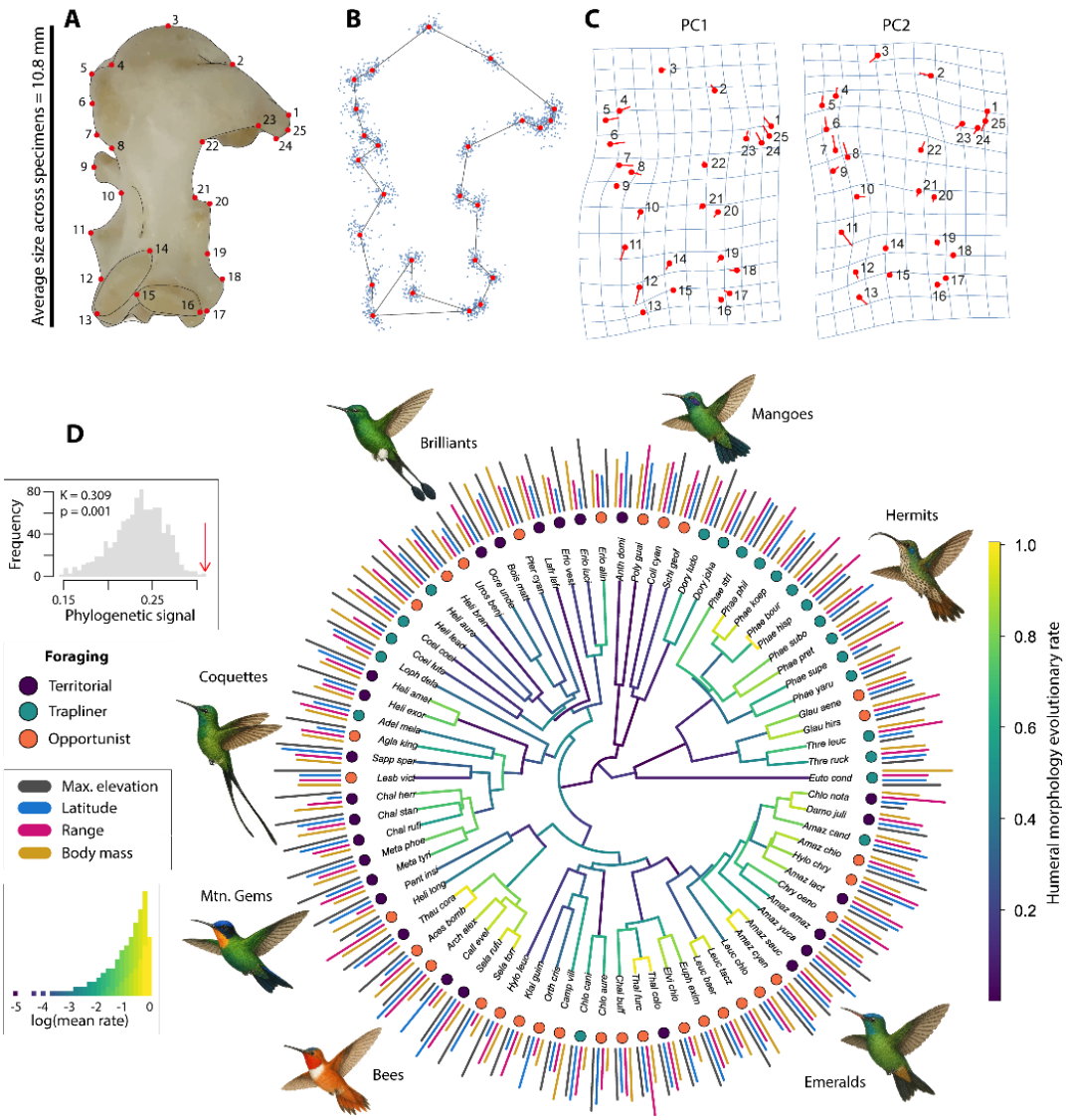
Predictor	Df	R^2	F	Z	Pr(>F)	Significance
Foraging strategy	2	0.062	2.517	3.465	0.001	***
Residuals	75	0.937				
Kappa statistic	0.143					

Univariate PGLS values

Predictor	Estimate	Std. Error	Lambda (ML)	p-value
Latitude	-0.0001	8.92E-05	0.630	0.132
Body mass	0.000	0.00048	0.571	0.476
Humerus size	-0.038	0.406	0.572	0.925
Range size	1.49E-10	3.56E-10	0.568	0.678
Elevation	-8.95E-08	6.47E-07	0.570	0.890

Figures

Figure 1. Hummingbird humeral shape shows strong phylogenetic structure and substantial evolutionary rate heterogeneity across major clades. Phylogenetic structure in humeral morphology is high (K = 0.309), and evolutionary rates vary markedly across hummingbird clades, with several lineages showing accelerated morphological evolution. (A) Twenty-five anatomical landmarks used to quantify humerus shape. (B) Landmark dispersion across specimens illustrating within-family morphological diversity. (C) Morphological deformation grids for PC1 and PC2 showing major axes of shape variation. (D) Null distribution of phylogenetic signal with the observed value (red arrow). The circular tree shows the phylogeny of the 78 species analyzed; branch colors depict species-specific evolutionary rates, and radial bars reflect normalized ecological and morphological predictors. The lower-left inset shows the distribution of log-transformed mean evolutionary rates. Hummingbird illustrations were AI-generated (ChatGPT-4o) and refined in 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

862

863

Figure 2. Humeral shape (PC1) is strongly associated with humerus size, range size, body mass, and foraging strategies. Humeral shape (PC1) shows significant associations with foraging strategy, body mass, humerus size, and geographic range size, consistent with the multivariate PGLS results. Each panel shows the relationship between PC1 and a predictor included in the model. Continuous predictors are displayed as scatterplots with fitted regression lines and 95% confidence intervals (shaded areas), and foraging strategy is shown as boxplots with point distributions. PC1 reflects a gradient from shorter, more robust humeri (higher values) to longer, more slender humeri (lower values). Although PC1 is shown here for visualization, the full multivariate analysis evaluates shape variation across the entire morphometric space.

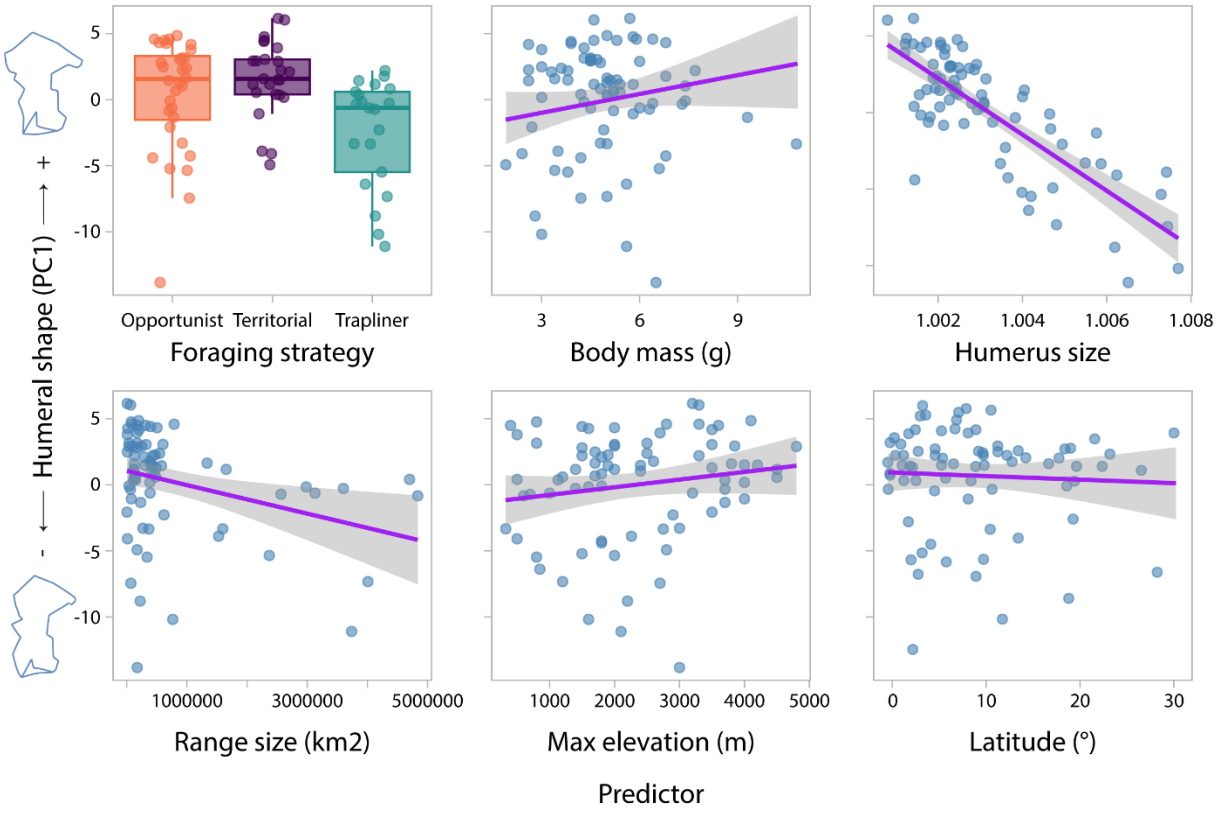
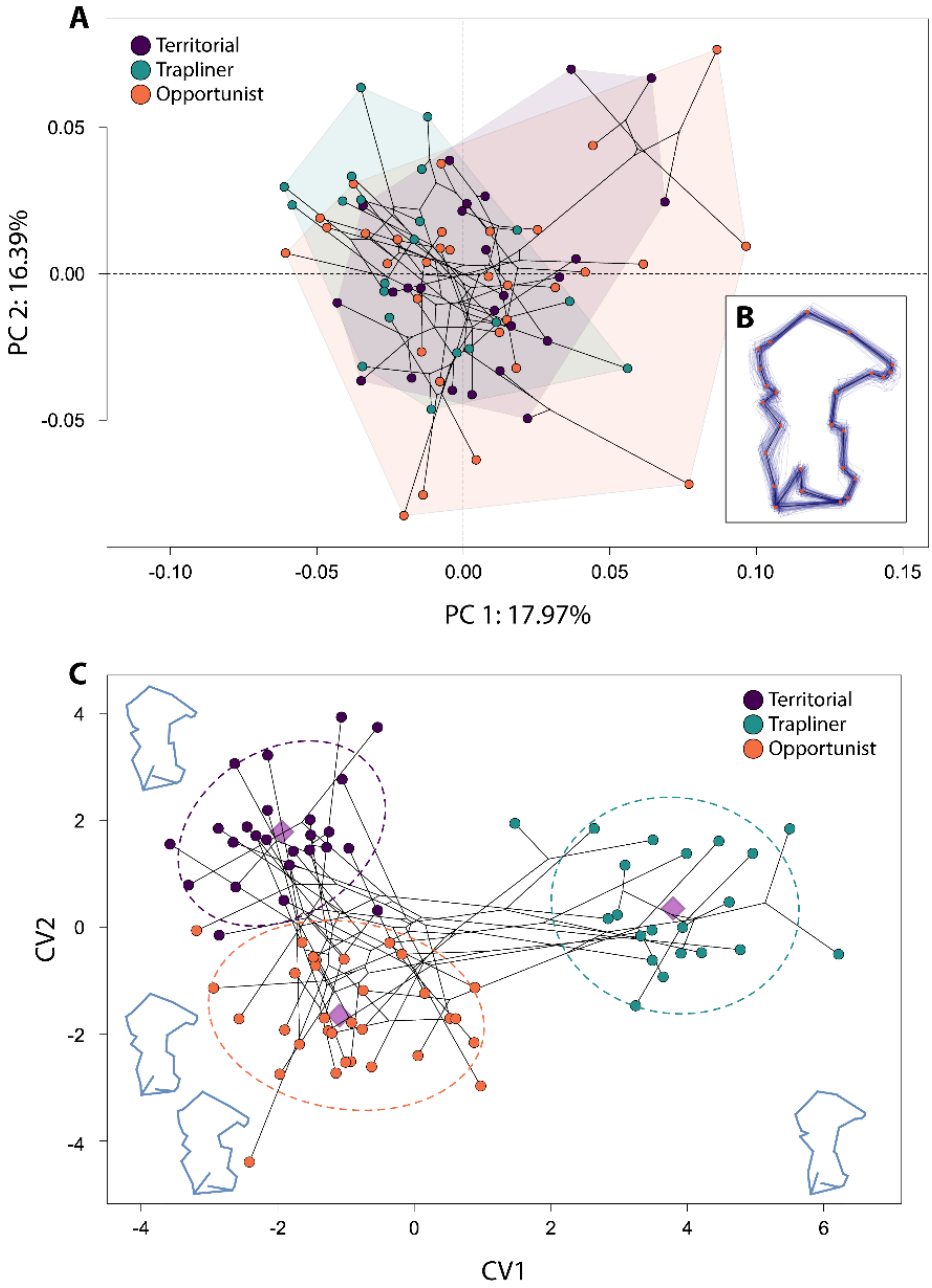


Figure 3. Hummingbird foraging strategies occupy distinct regions of humeral morphospace, with clear group separation in both PCA and CVA analyses. Foraging strategies differ in their occupation of humeral morphospace, with territorial, traplining, and opportunistic species forming partially distinct clusters in both unsupervised (PCA) and supervised (CVA) shape spaces. (A) PCA of Procrustes-aligned humeral shapes, with PC1 and PC2 explaining 17.97% and 16.39% of variation, respectively. Points are colored by foraging strategy; shaded convex hulls denote group morphospaces, and thin black lines represent phylogenetic relationships projected into shape space. (B) Mean humeral shape variation across specimens visualized as a thin-plate spline deformation of the consensus configuration; red points denote landmarks. (C) Canonical Variate Analysis maximizing group separation based on foraging strategy. Dashed ellipses show 90% confidence regions, and purple diamonds mark group centroids. Shape outlines adjacent to the axes illustrate humeral changes associated with CV1 and CV2.


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

889

890

Figure 4. Evolutionary rates of humeral shape vary among foraging strategies and show no detectable associations with continuous ecological predictors. Foraging strategies differ in their average evolutionary rates of humeral shape, while RRphylo-derived rates show no significant relationships with body mass, humerus size, range size, elevation, or latitude. The top-left panel displays group-level evolutionary rates estimated using compare.evol.rates, illustrating differences among territorial, traplining, and opportunistic species. All remaining panels show RRphylo tip rates plotted against continuous predictors; points represent species-level $\log_{10}$-transformed evolutionary rates, and purple lines indicate fitted PGLS regressions with 95% confidence intervals (shaded areas). "Time from root" corresponds to the relative phylogenetic distance of each tip and is shown for visualization only.

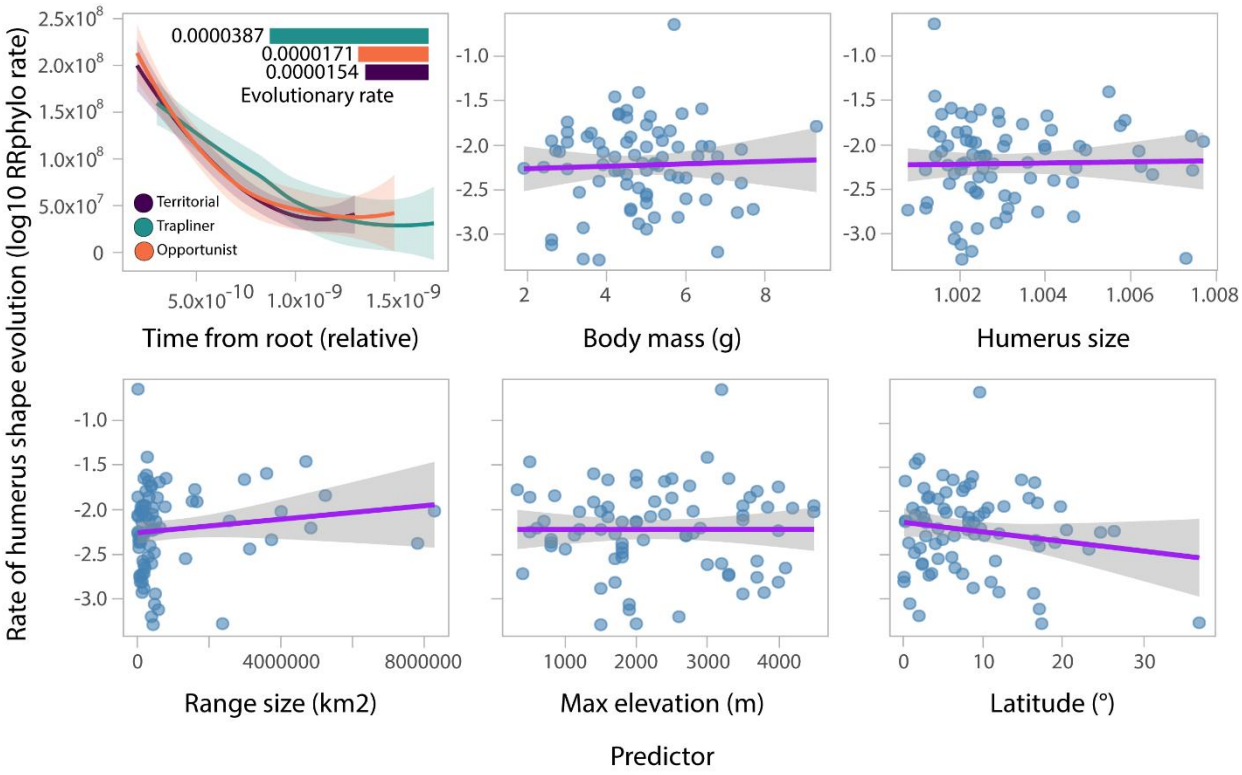
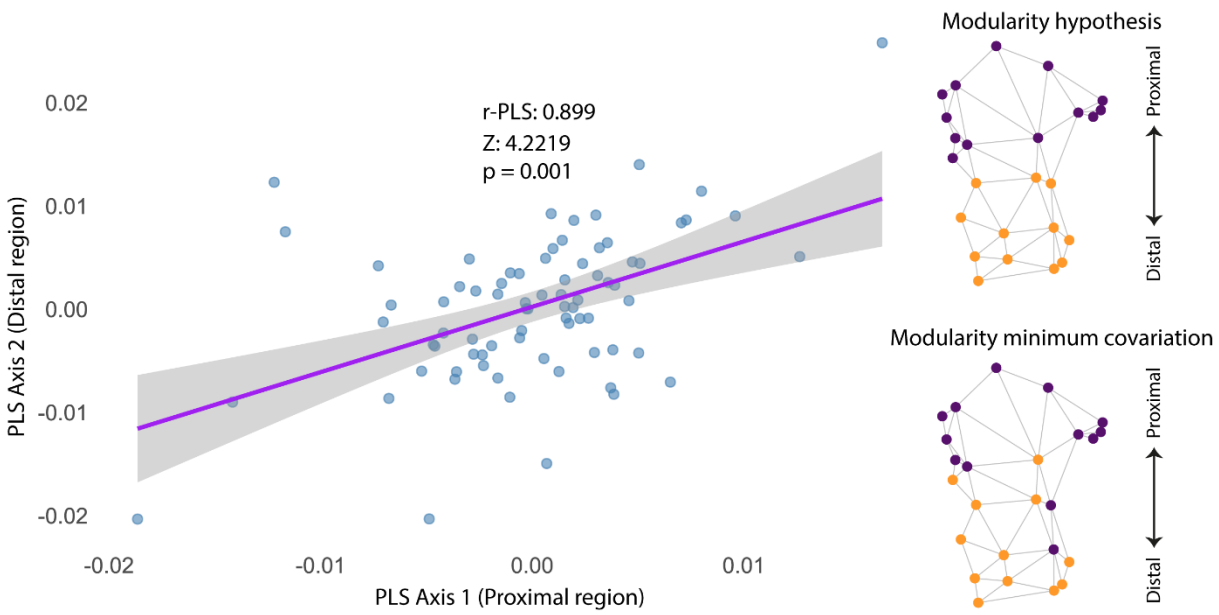



Figure 5. Proximal and distal regions of the hummingbird humerus are strongly integrated, supporting a functional linkage rather than modular independence. The proximal and distal regions of the humerus exhibit strong morphological integration, indicating coordinated evolutionary changes rather than independent modular evolution. The scatterplot shows the relationship between PLS1 scores of the proximal region and PLS2 scores of the distal region, with a high correlation (r-PLS = 0.899) and strong statistical support (Z = 4.2219, p = 0.001). The fitted regression line and 95% confidence interval illustrate the magnitude and direction of covariation across species. The diagrams on the right depict the *a priori* modularity hypothesis (upper panel), representing the expected division of the humerus into proximal and distal functional units, contrasted with the data-driven minimum-covariation modular structure (lower panel). Nodes represent landmarks and edges represent strength of covariation. The strong positive PLS correlation demonstrates that evolutionary changes in the proximal humerus (e.g., deltopectoral crest and articular surface geometry) are tightly linked to changes in the distal region (e.g., condyles and shaft curvature), consistent with shared biomechanical demands during hovering and forward flight in hummingbirds.