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Gliding towards an understanding of the origin of flight in bats

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Abstract

Bats are the only mammals capable of powered flight and have correspondingly specialized body plans, particularly in their limb morphology. The evolution of bat flight is still not fully understood due to an uninformative fossil record, but it is widely hypothesized that flying evolved from gliding. Here, we test the gliding to flying hypothesis of the origin of bat flight by using phylogenetic comparative methods to model the evolution of forelimb and hind limb traits on a dataset spanning four extinct bats and 231 extant mammals with diverse locomotor regimes. Our results reveal gliders' elongate forelimb adaptive optima to be intermediate between those of bats and arborealists and that gliders and bats share a single adaptive zone characterized by elongate and narrow hind limb traits. Together, our results inform how glider-like postcranial morphology may have gradually evolved into bat-like morphology prior to powered-flight behavior. Finally, we propose a hypothetical adaptive landscape based on

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the length and width optima trends that we interpret considering the macroevolutionary transition from arborealism to gliding to flying in mammals.

Keywords

adaptive landscape; ecomorphology; limb morphology; macroevolution; phylogenetic comparative methods

Introduction

Powered flight has evolved at least four times over the course of organismal evolution, consistently serving as a gateway to ecomorphological and species diversification. As the only mammals capable of powered flight, bats have correspondingly specialized body plans that include highly derived limbs. These specialized morphologies are understood to be the result of adaptations for the demands of flight; the skeletal elements of bat forelimbs are elongated to support flight membranes and increase aerodynamic efficiency, whereas bat hind limbs are specialized for hanging and catching prey in flight [1-3].

The evolutionary origin of flight in bats remains one of the biggest mysteries in mammalian evolution due to an uninformative fossil record linking bats to their close relatives. Darwin [4] posited the origin of bat flight as one of the biggest challenges to the theory of evolution. Nevertheless, bat flight is widely hypothesized to be the result of an ancestral “trees-down” transition from arborealist (tree-dweller) to glider to flyer species [5-7], albeit this idea has not been tested quantitatively. The alternative “ground up” hypothesis [8,9] posits that flight evolved from running and jumping vertebrates without a gliding intermediate step; bipedal running is a key evolutionary step in how birds are thought to have evolved flight [10,11].

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Following the arborealist-to-glider-to-flier hypothesis, the ancestors of bats, which originated about 64 million years ago [12], are hypothesized to have evolved more elongated limbs, followed by patagia (wing membranes) on the armwing that facilitated gliding, and finally membranes in the handwing that enabled powered flight. The armwing consists of stylopodal (i.e., humerus) and zeugopodal (i.e., ulna and radius) bones, while the handwing consists of autopodal bones (i.e., the metacarpals and phalanges). The evolution of the handwing is thought to be the key innovation that catalyzed bats' adaptive radiation [13-15], which has resulted in the evolution of over 1,500 extant species [16].

Previous work has examined many biological aspects associated with the evolution of bat flight, such as sensory adaptations (e.g., echolocation or vision), aerodynamics (e.g., aerofoil and flight performance), ecology (e.g., diet, habitat, roosting), and development (e.g., patagia development and digit elongation) [1,5,16]. Morphological approaches to understanding the origin of bat flight further include analyses of skeletal dimensions in the context of development [17,18], biomechanics [20], and functional morphology [15]. These studies often focus on aspects of the bat forelimb (e.g., [13]) or hind limb (e.g., [21]), but seldom both in tandem in a phylogenetic context (but see [20]). Here, we test the gliding to flying hypothesis of the origin of bat flight by modeling the evolution of forelimb and hind limb traits on a dataset spanning four extinct bats and 231 extant mammals with diverse locomotor regimes including ground-dwellers, arborealists, gliders, and flyers.

Based on the arborealist-to-glider-to-bat hypothesis, we test two predictions regarding the evolution of limb morphologies. First, we predict that the evolutionary optima of glider forelimb traits will be intermediate in value to those of arborealists and bats. If supported, this suggests that gliders are on the same evolutionary trajectory as bats or on an intermediate adaptive peak

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between bats and arborealists. Specifically, we predict wider and longer skeletal element optima progressing from arborealists to gliders to bats. Longer bones (especially in the handwing) and larger-diameter bones (especially in the armwing), may enable bats to secure enough muscle attachment area for powered flight [15] while still increasing their patagia (wing membrane) area. Gliders with intermediate forelimb length and width optima between arborealists and bats may represent an intermediate adaptive zone between the anatomical adaptations required for tree-dwelling [22] and those required for powered flight [3]. Support for this prediction would suggest selective pressures for flight influenced the evolution of bat forelimb skeletal elements from glider-like forelimbs.

Second, we predict that bat hind limb morphologies will be most similar to gliders, suggesting that bat and glider hind limb traits share a single trait optimum (i.e., adaptive peak) separate from ground-dwelling and arboreal mammals. Previous work has considered bat hind limbs to be “reduced” in size (e.g., [7,23-24]), but it is unclear whether that is relative to arborealists or gliders. Here, we aim to identify where bat hind limb adaptive optima lie relative to other mammals. Specifically, we predict narrower and longer skeletal element optima progressing from arborealists to gliders and bats. Longer and lower-diameter hind limb bones may enable bats to decrease weight while still generating lift by increasing their plagiopatagium surface area, as their hind limbs are incorporated into this membrane [1]. Support for this prediction would suggest that shared selective pressures for less-quadrupedal motion (i.e., as gliding and flying both incorporate hind limbs into the wing membrane) influenced the evolution of bat hind limb skeletal elements from ancestors with glider-like hind limbs.

We include fossils of extinct bats in our morphological dataset; we predict that the extinct flyers’ limbs are more similar to extant bats’ than extant gliders’ due to the degree of

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morphological similarity previously described [5–7,13]. Even if morphologically similar, we predict the inclusion of these fossil bats will alter estimations of the adaptive zone between bats and gliders (i.e., the inclusion of fossils may make the bat-glider trait optima more similar or more disparate) and thus inform the understanding of the adaptive landscape underlying the evolution of mammalian flight.

Methods

Morphological Data

We compiled linear measurements of 14 forelimb and 15 hind limb traits (electronic supplementary material, figure S1) from 231 extant mammals across 21 orders (78% of mammalian orders) and 73 families (47% of mammalian families). We collected limb data from specimens at the Burke Museum of Natural History and Culture and the literature [25-28]. All specimens measured for this study were fully mature, determined by the closure of exoccipital–basioccipital and basisphenoid–basioccipital sutures on the cranium and ossification of all vertebrae and limb bones. Specimens were a mixture of male, female, and unknown sexes. We size-corrected all linear measurements by calculating log-shape ratios of each trait measurement [29] by $\log_{10}$-transforming the mean trait value divided by the cube root of body mass prior to running all statistical analyses. We compiled body mass data from the PanTHERIA database [30] and the *Handbook of the Mammals of the World* book series [31].

We also collected the 29 measurements for four extinct bats (*Onychonycteris finneyi, Icaronycteris index, Hassianycteris messelensis, and Tachypteron franzeni*) using ImageJ [32] and published full-body images that include scale bars [2,7,33].

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To visualize the distribution of locomotor groups in the forelimb and hind limb morphospaces, we performed principal component analyses (PCA) of the morphometric data using the *princomp* function in the *R* package *stats* [34].

Ecological Data

We categorized species into four locomotor groups: ground dwellers (n = 117), arborealists (n = 71), gliders (n = 16), and flyers (n = 27) following [25-28]. The ground-dwelling mammals include diverse ambulatory, cursorial, saltatorial, and fossorial taxa. This 'catch-all' category was intentional as we are interested in investigating the arboreal to gliding to flying specialization trends only. Our arborealist category was also broad in that it includes both fully arboreal (tree-dwelling) and scansorial taxa. Gliders include tree-dwelling mammals with derived morphologies (i.e., patagia and elongated limbs) for gliding locomotion [26]. Flyers included just bats.

Phylogenetic Comparative Analyses

We used generalized evolutionary models [35,36] to examine the influence of locomotor modes on the evolution of forelimb and hind limb morphologies in our extant-only and extant+extinct datasets. For each of the 29 traits, we fit a Brownian motion model (BM), a single-optimum Ornstein-Uhlenbeck model (OU1), and two multiple-peak OU (OUM) models, an OUM_{loc4} model that allows a separate adaptive optimum for each of the four locomotor modes (i.e., ground-dwellers, arborealists, gliders, flyers) to test our forelimb-specific predictions, and an OUM_{loc3} model that allows separate optima for three groups (i.e., ground-dwellers, arborealists, and gliders+flyers) to test our hind limb-specific predictions. All models were fit

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using the *R* package *OUwie* [37]. All four models were fit across 250 stochastically mapped trees (see electronic supplementary materials, supplementary methods) to account for uncertainty in phylogenetic topology and the ancestral character states. We assessed relative model support using small sample corrected Akaike weights (AICcW). Models with ΔAICc scores less than two were considered supported and interpreted as evidence of locomotor groups influencing species' adaptive zones. We computed 95% confidence intervals for all model parameters of the best-fit model using parametric bootstrapping. See the electronic supplementary materials for full methods.

We additionally fit univariate models to PC1 scores of the extant-only forelimb dataset. We acknowledge that it can be problematic to fit models to a single PC axis [38,39], however, the first PC axis served to capture a majority (80.2%) of the forelimb dataset's variation. We chose not to fit models to other PC axes for the fore- or hind limb, as none explain the majority of trait variation (i.e., 8.5% for forelimb PC2; 48.2% for hind limb PC1; 17.5% for hind limb PC2).

We repeated the above model fitting approaches on a merged dataset that includes extant species and the four fossil species. All four models were fit across 50 stochastically mapped trees to account for uncertainty in the ancestral character states. We manually placed the four extinct bats on the phylogeny as a polytomy with extant bats at 69.8 MYA.

Results

Forelimb model-fitting

The OUM_{loc4} model was the best-fitting model for 11 of the 14 forelimb traits (electronic supplementary material, tables S2a and S4a), whereas OUM_{loc3} was the best-fitting model for

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humerus distal width and ulna olecranon length (electronic supplementary material, tables S3a and S4a). The OU1 model was the best fit for the proximal phalanx width of the third manual digit, but this may be due to little variation between ground-dwellers, arborealists, and gliding species, as previous research found proximal phalanx diameter to be relatively lower in bats compared to non-volant mammals [20].

OUM_{loc4} optima for the armwing (i.e., humerus and radius) lengths gradually increase from ground-dwellers to arborealists to gliders to flyers, and parametric bootstrapping revealed little to no overlap in the 95% confidence intervals of optima between most locomotor groups (figure 1b-c). Optima of armwing widths indicated that bats exhibit relatively wider humeri at the mid-shaft and at the distal and proximal ends compared to all other locomotor groups (figure 1g-i; electronic supplementary material, table S2a).

Bats exhibit relatively higher OUM_{loc4} optima for handwing traits (i.e., longer third manual metacarpal, proximal phalanx, and intermediate phalanx) than all other locomotor groups (figure 1d-f; electronic supplementary materials, table S2a). Ground-dwellers, arborealists, and gliders show little difference between some handwing optima (metacarpal length) and slightly more separation in others (proximal and intermediate phalanx relative length) (figure 1d-f). In handwing width traits, bats exhibit relatively higher optima for metacarpal widths compared to all other locomotor groups (figure 1j), but no differences in proximal phalanx width as evidenced by OU1 support for this trait.

An adaptive optimum for a bat-glider combined group in the OUM_{loc3} models was supported for humerus distal width and ulna olecranon length (figure 1i; electronic supplementary material, table S3a). Finally, bats exhibited higher optima for scapulae relative width and length than all other locomotor groups, while the glider scapula height optimum was

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relatively lower than those of all other groups (figure 1a; electronic supplementary material, table S2a).

Adding fossil bats into forelimb model-fitting analyses yielded overall slightly more support for OUM_{loc3} models compared to extant-only modeling (electronic supplementary material, table S5a). OUM_{loc4} models were still widely supported as the best-fitting model for eight traits and comparable fits for four traits; OU1 was still the best-fitting model for proximal phalanx width (electronic supplementary material, table S5a). Overall, the length and width optima for the forelimb were very similar between extant+extinct and extant only-modeling; however, we were unable to examine the trends in finer detail because forelimb bootstrapping failed with the addition of fossil species (electronic supplementary material, table S7).

Forelimb morphospace

PC1 (figure 2a) explains 81.5% of the forelimb variation and separates flyers (both extant and extinct) from the remaining three locomotor modes. PC1 also slightly separates gliders from arborealists and ground-dwellers. This axis describes the relative lengths of the armwing (humerus, radius, ulna) and the handwing (metacarpals and phalanges) (electronic supplementary material, table S1a). PC1 varies across shortened (-PC1) to elongate (+PC1) bones, revealing a trend within the dataset in the elongation of multiple forelimb bone elements from arborealists to gliders to flyers. PC2 explains 7.8% of the morphological variation and primarily describes the relative widths of the long bones and hands (electronic supplementary material, table S1a). This variation of wide to narrow bones mostly occurs within locomotor groups, especially within the diverse ground-dweller group. Fossil bats overlap with extant bats in morphospace, which is consistent with the lack of transitional forms in the bat fossil record (figure 2a).

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PC1 of the extant forelimb dataset was best fit with an OUM_{loc4} model (electronic supplementary material, table S6a). The bootstrapping reveals the optima gradually increases from ground-dwellers to arborealists to gliders to flyers (electronic supplementary material, table S6b), very similar to the univariate arm- and handwing-length optima trends (i.e., the traits dominating PC1).

Hind limb model-fitting

The OUM_{loc3} model was the best-fitting model ($\Delta AIC = 0$) for six of 15 traits (electronic supplementary material, table S4b), and the second best-fitting model ($\Delta AIC < 2$) for an additional five traits. The OUM_{loc4} model was the best-fitting model for seven of 15 traits (electronic supplementary material, table S4b), and the second best-fitting model for an additional five traits. OUM_{loc4} alone was supported ($\Delta AIC < 2$) for four traits: pelvis length, ilium length, ischium length, and fibula length. The OU1 model was the best fitting model for the remaining two traits (tibia midshaft width and pedal proximal phalanx length), but OUM_{loc3} was additionally supported for these two traits, indicating that adaptive evolution may play some role in the evolution of these traits (electronic supplementary material, table S4b).

In total, the OUM_{loc3} model was supported ($\Delta AIC < 2$) for 11 of the 15 traits. Parametric bootstrapping of these OUM_{loc3}-supported models revealed that the glider-bat group had relatively longer femur, tibia, and intermediate phalanx length optima and relatively shorter metatarsal length optima than the other locomotor groups (figure 3f; electronic supplementary material, table S3b). The glider-bat group widths are even more disparate from the other groups, with relatively smaller femur distal width, femur shaft width, tibia proximal width, metatarsal width, and proximal pedal phalanx width optima (figure 3g, i, j-k; electronic supplementary

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material, table S3b). OUM_{loc3} hind limb optima for the glider-flyer group showed less separation from arborealist optima only for tibia midshaft width and proximal pedal phalanx length (figure 3j, e; electronic supplementary material, table S3b), but these two traits were best supported by an OU1 model (ΔAIC = 0 for both) and had lower OUM_{loc3} weights (AICcW = 0.31 and 0.22, respectively).

In total, OUM_{loc4} was supported (ΔAIC < 2) for 12 of 15 traits. Gliders have relatively longer tibia and femoral optima compared to bats and the other locomotor groups (electronic supplementary material, table S2b). However, parametric bootstrapping of the OUM_{loc4} models reveals overlapping 95% confidence intervals for gliders and bats for others of these traits (e.g., figure 3b-c; electronic supplementary material, table S2b). Additionally, width optima for long bones and digits overlap for bats and gliders for all but femur distal width (figure 3h), where bats have significantly smaller width optima than all other groups (electronic supplementary material, table S2b).

The pelvis, ilium, and ischia optima appear unique among hind limb traits by being relatively smaller for bats than for the other locomotor groups, which showed little variation (i.e., figure 3a; electronic supplementary material, table S2b). Additionally, pelvis-associated lengths and fibula length (rudimentary in bats) were the only hind limb traits supported solely by OUM_{loc4} (table S4b).

Adding fossil bats into hind limb model-fitting analyses yielded overall slightly more support for OUM_{loc4} models (best-fit, ΔAIC = 0, for nine traits, and comparable fits, ΔAIC < 2, for four) compared to extant-only modeling (electronic supplementary material, table S6b). The OUM_{loc3} model was the best for five traits and a comparable fit model for three. OU1 was still the best-fitting model for pedal proximal phalanx length. The length and width optima for the

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hind limb were very similar between extant+extinct and extant only-modeling (electronic supplementary material, table S8). However, we were unable to examine the extent of this consistency because hind limb bootstrapping failed with the addition of fossil species (electronic supplementary material, table S8).

Hind limb morphospace

PC1 explains 47.5% of hind limb variation and describes the relative shortening (-PC1) to elongation (+PC1) of the fibula, metatarsal, and pedal proximal and intermediate phalanx (figure 2b; electronic supplementary material, table S1b). PC1 slightly separates gliders and bats, with gliders falling towards +PC1 and bats falling towards -PC1, indicating glider hind limb autopodia are overall more elongated than bats'. Arborealists and ground-dwellers are spread across PC1 (figure 2b). PC2 explains 17.7% of the variation, where +PC2 is associated with relatively lengthened pedal proximal, intermediate phalanx, and ischium and -PC2 with relatively narrower pedal proximal and intermediate phalanges (electronic supplementary material, table S1b). PC2 loosely separated ground-dwellers, arborealists, and the glider-flyer group (figure 2b). Based on these loadings (electronic supplementary material, table S1b), variation between the four locomotor groups mainly occurs throughout the hind limb autopodia region.

Discussion

Forelimb trait evolution

Our forelimb results provide some support for the hypothesis that selective pressures for flight influenced the evolution of bat forelimbs from glider-like forelimbs. We found that glider

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forelimb optima are intermediate between bat and arborealist optima for length (figure 1a-f), but not width (figure 1g-j) traits across both the armwing and handwing. The decoupled patterns of length results from width results represents an interesting breakdown between adaptive zones in limb bone length and width among gliding and flying mammals and across both the armwing and handwing. Consistent with the rapid evolution of a handwing being a necessary adaptation for bats [13-15], bat handwing length optima are farther from glider handwing length optima than armwing length optima are between these two groups. Model results from fitting the forelimb PC1 axis (dominated by relative lengths) similarly find gliders to be intermediate between arborealists and bats (electronic supplementary material, table S6). Bats display relatively wider skeletal element optima across the armwing compared to all other locomotor groups, consistent with findings that bat humeri and radii have significantly larger diameters than nonvolant mammals' [20]. Bats' relatively robust humeral width optima may reflect the higher muscle mass closer to their center of gravity required for powered flight [15]. If the scapula is considered part of the armwing, gliders are intermediate between arborealists and bats for scapula length (figure 1a) but not width optima likely because the bat scapula serves as the attachment point for many of the necessarily large muscles controlling their wing-beat cycle [15].

The handwing width patterns are more complex. The dorsoventral diameters of bat metacarpals are relatively larger than other mammals' [20], congruent with our findings that bats have relatively thicker third metacarpal optima (figure 1j). Proximal phalanx diameters are also smaller in bats compared to nonvolant mammals [20]. Here, an OU1 model was supported for proximal phalanx width (electronic supplementary material, table S4a), suggesting that selection does not strongly influence this trait's evolution. This finding may conflict with the

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biomechanical necessity for distal bat phalanx elements to be relatively narrower to enable bending and thus reduce torsional forces during flight [20,40].

For gliders, the width optima are very similar to those of mammals of other locomotor modes, suggesting gliders maintain widths while continuing to elongate long bones [26]. This is unsurprising because gliders may remain gracile to increase patagial area while decreasing wing loading [41], and may not need to concentrate muscle mass proximally because they do not rely on muscle use for gliding as opposed to flapping flight. Overall, gliders appear to be evolving more elongate, gracile forelimb bones while bats are evolving more elongate but relatively robust forelimb bones.

Overall, our results largely support our prediction that the four locomotor groups (ground-dwellers, arborealists, gliders, and flyers) exhibit separate adaptive optima in forelimb traits and place gliders on the same evolutionary trajectory as bats. Furthermore, the lack of support for the OUM_{loc3} model indicates different selective pressures between gliders and bats. The only traits for which we have OUM_{loc3} support, humerus distal width and ulna olecranon length (electronic supplementary material, table S4a), may both correspond to traits most pronounced for digging locomotion and thus be functionally irrelevant for either gliding or aerial locomotion [42]. Therefore, our use of univariate models parsed out fine-scale trait differences (i.e., long bone diameters and scapula dimensions) and revealed more of the functional complexity associated with gliding versus flying adaptive zones.

Hind limb trait evolution

Our results generally support the hypothesis that bat hind limb morphology is most similar to that of gliders to facilitate aerial locomotion. We found that bat and glider hind limb

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traits exhibit a single optimum separate from ground-dwelling and arboreal mammals for many length and width traits. For traits best fit with two separate optima for gliders and bats, we see broad overlap in confidence intervals of optima. This shared bat-glider adaptive zone leads to shared narrower and longer hind limb optima for gliders and bats, especially in the autopod region.

The bat-glider combined group has relatively long femur, tibia, and third intermediate phalanx length optima (figure f; electronic supplementary materials, table S3b) from other locomotor groups. This trend contrasts with the bat-glider group having a relatively shorter metatarsal length optimum (figure 3d) from other locomotor groups. The relatively long femur and tibia optima, in addition to variably long/short foot elements, may be due to the autopod tending to accumulate more variation because it is the last limb element to develop [43]. Regardless, we see bats and gliders following the same length optima trends throughout the hind limb, distinct from arborealists.

The bat-glider group has relatively narrow hind limb optima across the stylopod and autopod skeletal elements, with more variability in the zeugopod (confidence intervals for tibia midshaft width overlap for all three locomotor groups) (figure 3g-i). These narrow femur, metacarpal, and phalanx width optima for both bats and gliders support our prediction that bat-glider hind limb length and width optima will reflect the elongated and narrow bones necessary for both bat and glider weight requirements, bat roosting behaviors [1], and glider patagial surface area requirements [41,44].

Although the OUM_{loc4} model was supported for 12 of 15 traits, there is weak evidence for the trait optima of bats and gliders being different, as indicated by their overlapping confidence intervals (electronic supplementary material, table S2b); this finding provides some support for

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our initial prediction. In particular, though bats display relatively shorter femoral and tibia optima than gliders, the groups' 95% confidence intervals overlap. The only traits for which OUM_{loc4} and not OUM_{loc3} were supported were the pelvic lengths, which are generally laterally compressed in bats though vary considerably between families [15], and fibula lengths, which are vestigial in bats (figure 3a; electronic supplementary material, table S4b). Therefore, four-locomotor group model support is unsurprising for these skeletal traits that reflect bat-specific hind limb specializations as distinct from glider hind limbs.

Our results show that bat hind limb bone optima shorten somewhat as their forelimbs' optima elongate, and bat hind limb bone optima narrow as their forelimbs' optima widen. These results add to the findings of Swartz and Middleton [20], who did not examine hind limb distal elements, by extending bats' thinner diameter hind limb trend distally to the third metatarsals and third proximal pedal phalanges. This trend reflects a unique decoupling of both bone length and width in bats relative to other mammals where both fore- and hind limbs undergo lengthening or narrowing. We find agreement with Hallgrímsson et al.'s [45] model of limb covariance, which predicts that between-limb covariation decreases as forelimb and hind limb size and functions decouple. Here, bats' forelimb and hind limb sizes and functions have decoupled and led to wider forelimb but narrower hind limb bones. Both the differential expression and signaling of growth factors like the bone morphogenic proteins between the bat forelimb and hind limb autopod [14] and the postnatal divergence in chondrocyte activity between the bat forelimb and hind limb autopod [23] may help explain how bats can achieve this decoupling where other mammal species cannot.

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Incorporating fossil bats

The morphospace positions of fossil bats in the forelimb PCA confirm extinct bats' morphological similarity to extant bats in the degree of forelimb specialization (figure 2a). Amador et al. [13] modeled aerodynamic traits instead of skeletal traits and found that the Eocene bat *Onchonycteris finneyi* occupies an area of aerofoil morphospace between extant gliders and bats. In our morphospace, *O. finneyi* is the closest fossil bat to gliders across PC1 (electronic supplementary material, figure S2a), congruent with Amador et al.'s [13] findings.

Fossil bats occupy the same region of hind limb morphospace as extant bats, but three of four species approach the morphospace of non-bats (figure 1b). *Tachypteron franzeni* most clearly overlaps with extant bats, and is also the only fossil we included from an extant bat family, Emballonuridae [33]. Simmons et al. [7] suggested that, based on its intermediate limb traits, *O. finneyi* was likely more proficient at climbing than extant bats and may have employed quadrupedal locomotion and under-branch hanging. Interestingly, *O. finneyi* and *Desmodus rotundus* (the common vampire bat) are almost indistinguishable in the hind limb morphospace (electronic supplementary material, figure S2b). Vampire bats are known for their adept quadrupedal locomotion and climbing (e.g., [21]), so *O. finneyi*'s similar morphology may lend support to more walking-capable early bats. However, the linear measurements we used here may not reflect the complex adaptations required for these behaviors (e.g., ninety-degree joint rotations for under-branch hanging, [21]), so the placements in morphospace leave open many possibilities for ancestral locomotor behaviors.

Including fossil bats in our model-fitting analyses did alter estimations of the adaptive zone between bats and gliders; the inclusion of fossils appeared to make the bat-glider trait

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optima more similar for the forelimbs and more disparate for the hind limbs. Fossil addition to forelimb model-fitting analyses yielded overall slightly more support for OUM_{loc3} models compared to extant-only modeling (electronic supplementary material, table S5a). In particular, the OUM_{loc3} support in the metacarpals suggests that there is some selective pressure on a bat-glider group for this defining handwing trait. Fossil addition to hind limb model-fitting analyses yielded overall slightly more support for OUM_{loc4} models compared to extant-only modeling (electronic supplementary material, table S5b). Interestingly, these OUM_{loc4} models include autopod optima, which had OUM_{loc3} support in extant-only modeling. However, overall the length and width trends are the same as above between locomotor groups across the fore- and hind limb, though we lack bootstrap support to gain more insight into whether any adaptive optima confidence intervals overlap (electronic supplementary material, table S7). Considering the differential model support, selection appears to be acting on fore- and hind limb optima towards three- and four-locomotor group selective regimes, respectively, when fossils are included as the closest morphologies we have to bat-glider intermediaries.

Evolutionary transitions and the adaptive landscape: Are gliders evolving towards a bat optimum?

Our results provide insight on the adaptive landscape from arboreal to gliding to flying locomotions. We found that, overall, glider forelimb optima fall intermediate between arborealists and bats in either their lengths or widths and glider hind limb optima overlap with bats' in their lengths and widths to some degree, emphasizing that selection on key length/width traits may be "pulling" gliders towards a flying adaptive zone. Bishop [5] proposed that the high

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degree of convergence among gliders suggests functional constraints on the evolution of powered flight from gliding; however, more recent work found that gliding lineages display incomplete convergence, with little evidence that gliding is under strong selective pressures toward a single-optimum gliding morphology [26]. The clustering of bat species in forelimb morphospace (figure 2a) may represent a steep adaptive peak within which forelimb morphologies are highly conserved. In contrast, the broad glider morphospace may represent a gradually inclined selection plane, with gliders still far from a peak [26]. This broad adaptive slope could represent the foothill of a steep bat peak such that gliders are very slowly evolving towards a bat peak (figure 4a). The evolutionarily oldest glider lineages, dermopterans (colugos) and anomalurids (scaly-tailed squirrels), have evolved farthest from ancestral arborealists [26]. Here, the dermopteran species *Cynocephalus volans* and *Galeopteras variegatus* are the gliders closest to the bat forelimb morphospace (figure 1a; electronic supplementary material, figure S2a), suggesting a trajectory in morphospace from gliding to flying. Further, colugos are known as the “mitten gliders” because of their interdigital membranes, which, along with their common carpal morphology with bats [46], illustrates a possible early mechanism for this transition [47,48].

In contrast, our results suggest that both bats and gliders exhibit broad adaptive optima in the hind limb with overlapping relative widths and somewhat overlapping relative lengths (figure 4b). These broad adaptive optima could represent neighboring peaks for gliders and bats separated by only a shallow fitness valley (figure 4b). This hypothetical adaptive landscape is valuable here because the transition from quadrupedalism to hind limb suspension has been hypothesized to enable forelimbs to evolve more freely as they would no longer need to be weight-bearing structures [5]. There is both quadrupedal suspension and hind limb suspension in

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dermopterans, the gliders potentially closest to a bat optimum, resulting in a tradeoff in the ability to walk quadrupedally upright [49,50]. Indeed, *G. variegatus* is the only glider to fall directly within the bat hind limb PC1 and PC2 morphospace (figure 2b; electronic supplementary material, figure S2b), reflecting a hind limb adaptive optimum potentially more easily attained than that of bats' specialized forelimbs (figure 4).

Our results support the hypothesis that selection is "pulling" specific limb traits towards a bat optimum, offering support for the arborealist-to-glider-to-flyer hypothesis of the evolution of bat flight. In particular, gliders' intermediate forelimb length and overlapping hind limb length/width optima between that of arborealists and gliders could be the traits pulling lineages across fitness valleys due to their greater effect in, for example, the functionality of the gliding/flying apparatus.

Conclusions

Our findings complement earlier work investigating the evolution of bats' powered flight by testing the selective regimes acting on length and width traits across the forelimb and hind limb. Aerodynamically, gliders appear to be plausible precursors to bats [6,7,13]. Here, we contribute to the understanding of how such a transition from gliding to flying may have occurred by highlighting how selection on key morphological traits may "pull" gliders towards a flying adaptive optimum. Our results reveal gliders' elongate forelimb optima to be intermediate to those of bats and arborealists and suggest gliders' elongate and narrow hind limb optima relative to arborealists is yet somewhat overlapping with bats. Thus, the traditional "reduced" view of bat hind limbs may represent a more malleable adaptive zone than previously thought. Together, our results inform how glider-like postcranial morphology may gradually evolve into

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bat-like morphology prior to powered-flight behavior. Though not in question, our results also refute the alternative running-to-flying hypothesis [8,9] of bat flight because we do not find ground-dwelling optima approaching bat optima or even glider optima for the vast majority of traits. This study therefore has significance to evolutionary biology by testing models of adaptive evolution on a longstanding question, the evolutionary origins of bats' powered flight.

Authors' contributions. A.E.B. and C.J.L. conceived the study, measured the specimens, and performed model-fitting and statistical analyses with input from D.M.G. and S.E.S. A.E.B. estimated the fossil measurements from images and collected literature data. All authors interpreted the data, drafted and revised the manuscript, and gave final approval for publication.

Competing interests. We have no competing interests.

Funding. A.E.B. was supported by the Mary Gates Endowment and the American Society of Mammalogists Grant-in-Aid of Research Award; D.M.G. and S.E.S. were supported by National Science Foundation IOS-2017738; and C.J.L. was supported by National Science Foundation DBI-1906248 and DBI–2128146, the Gerstner Family Foundation and the Richard Gilder Graduate School at the American Museum of Natural History, and a University of Texas Early Career Provost Fellowship.

Acknowledgements. We thank Jeff Bradley (UWBM) for extensive access to specimens and Lucas N. Weaver for giving permission for our use of his original skeletal illustrations of *Rattus norvegicus*.

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Figures

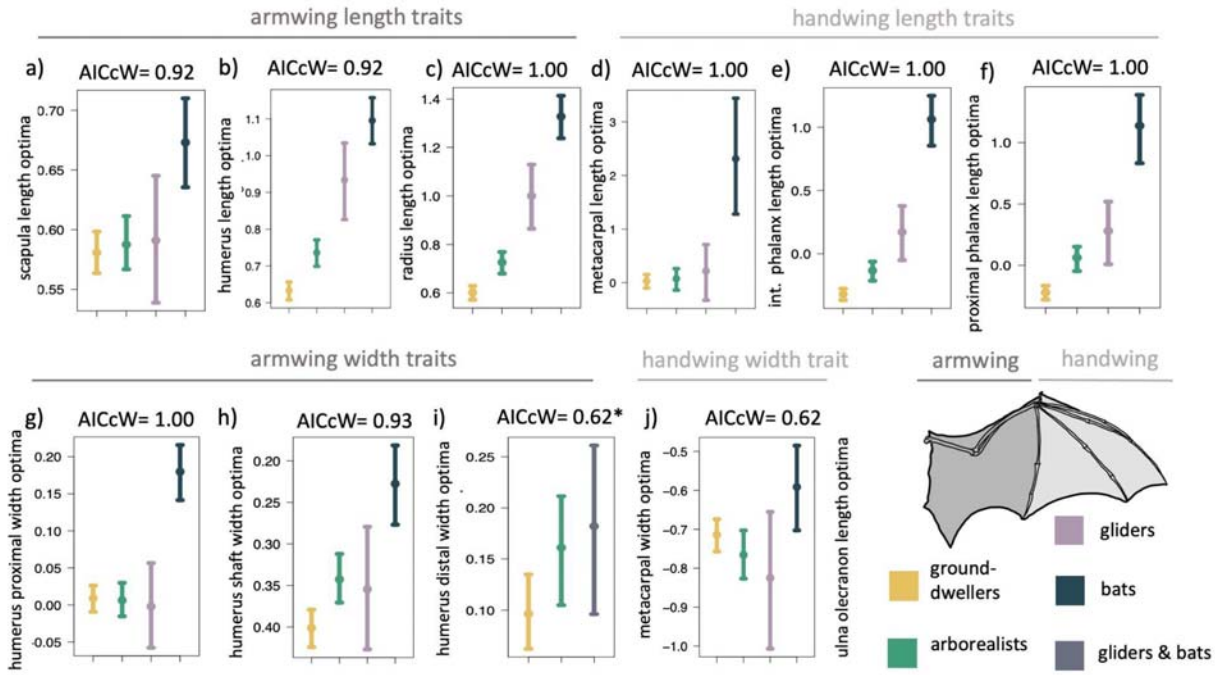

Figure 1. Forelimb trait optima (θ) with 95% confidence intervals estimated from the best-fitting OU models (electronic supplementary material, tables S2a,3a,4a). Only traits that were best fit by multiple-peak OU models are shown. The AICcW of the model is on top of each panel with * indicating OUM_{loc4} was supported (ΔAIC < 2) in addition to OUM_{loc3} (electronic supplementary material, table S4a).

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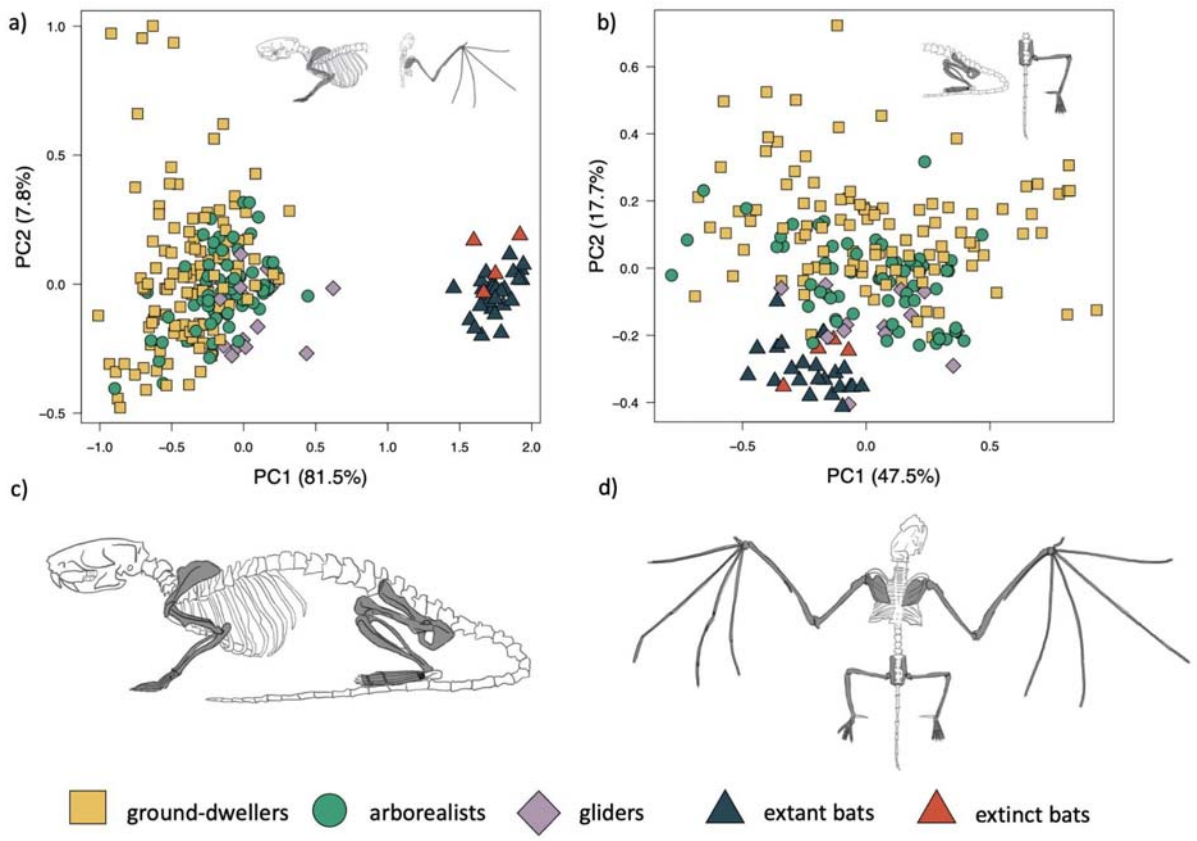

Figure 2. Forelimb and hind limb PCAs. Morphospaces consist of PC1 and PC2 of a) 14 forelimb traits and b) 15 hind limb traits with cartoons of a c) quadrupedal mammal skeleton (*Rattus norvegicus*) versus a d) bat skeleton (*Pipistrellus abramus*). Dark gray shaded bones on the cartoon skeletons correspond to those measured to generate the morphospace (the specific traits are listed in the electronic supplementary material, figure S1).

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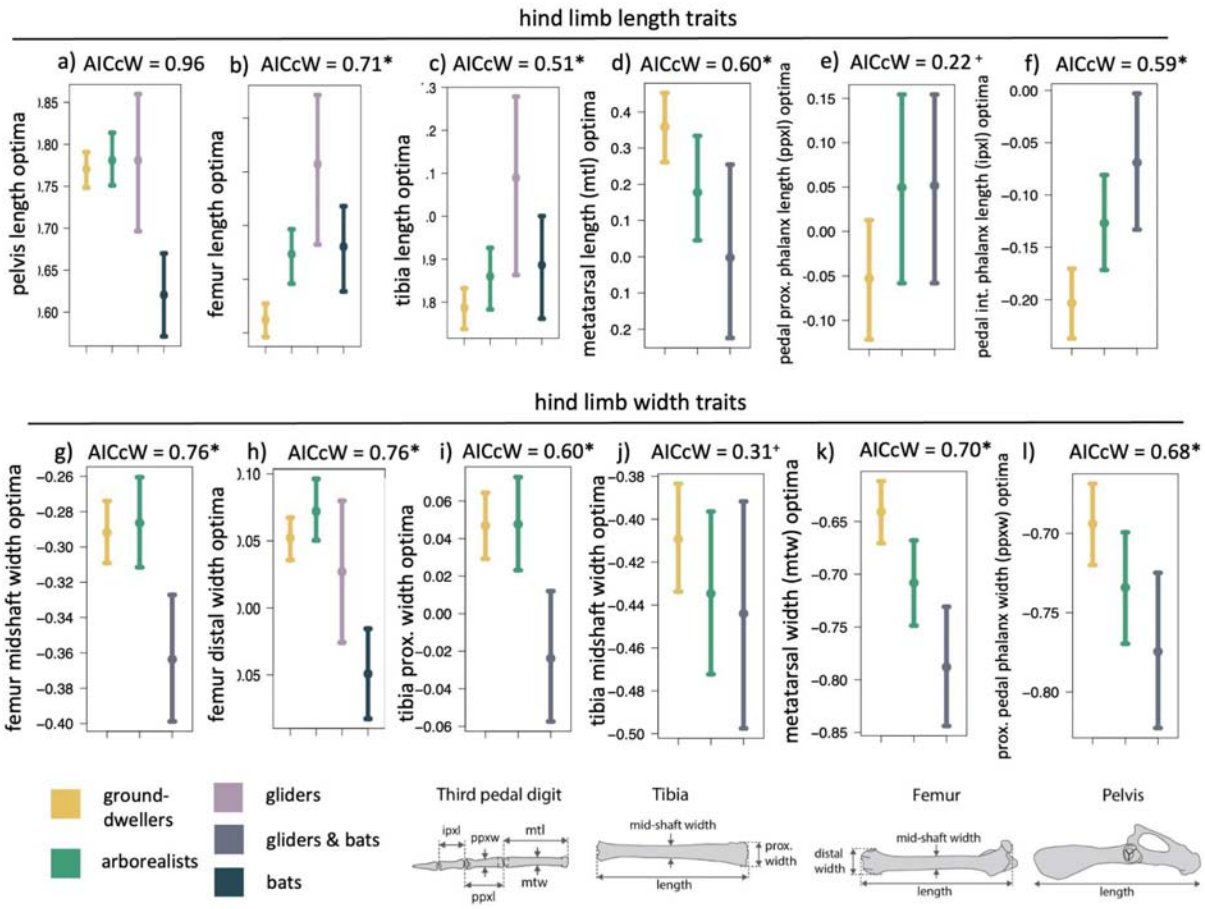

Figure 3. Hind limb length trait optima from model-fitting of univariate traits. OUM optima (θ) as a size-corrected trait value (log-shape ratio) with 95% confidence intervals (electronic supplementary material, tables S2b,3b,4b) for the best-fitting models (ΔAIC = 0) for 12 traits. The AICcW of the model is on top of each panel with * indicating another OUM model was also supported (ΔAIC < 2) and $^{+}$ indicating the model shown was the second-best supported after OU1 (electronic supplementary material, table S4b).

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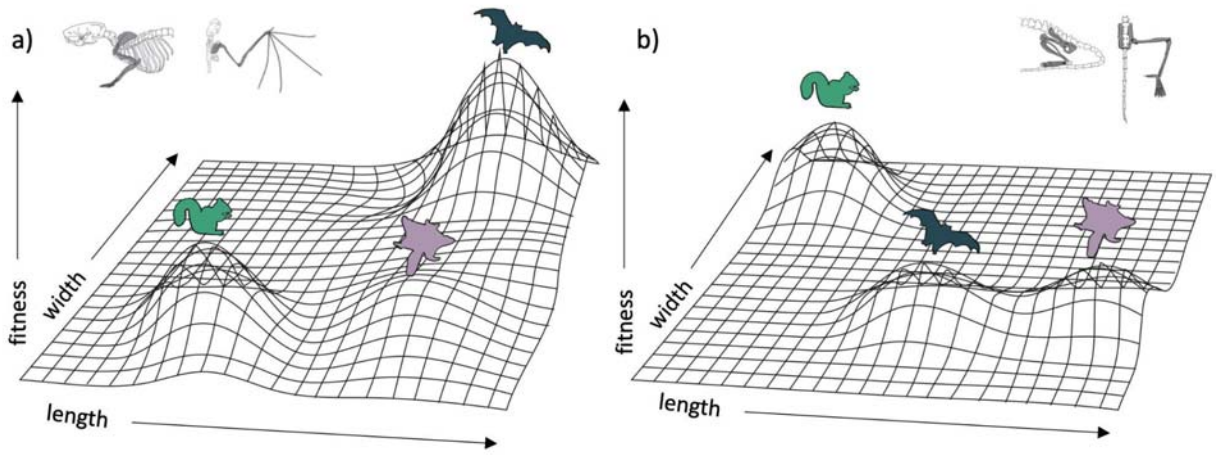

Figure 4. Hypothetical adaptive landscapes of forelimb and hind limb skeletal relative length and width evolution. Hypothetical fitness peaks are shown for arborealists, gliders, and bats for a broad summary of our conclusions from the skeletal trait optima results discussed. The "length" and "width" in the figure refer to the lengths and widths of individual limb elements. For the a) forelimb, we depict arborealists on a relatively steep adaptive peak at relatively lower length/width optima, gliders on a broad, shallow peak/incline at relatively higher length but not width optima, and bats on a very steep peak at relatively higher length and width optima. For the b) hind limb, we depict arborealists again on a fairly steep adaptive peak at relatively higher width but not length optima, bats on a shallow steep peak at relatively higher length and lower width optima, and gliders on a peak similar to and overlapping with bats' at relatively higher length but similar width optima.