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 origin of bat flight is still not fully understood due to an uninformative fossil record, but it is widely hypothesized that bats evolved from gliding ancestors. Here, we test predictions of 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 non-gliding arborealists. Our results also show that gliders and bats share an adaptive zone characterized by elongate and narrow hind limb bones. We propose a hypothetical adaptive landscape based on skeletal length and width optima trends, which we interpret within our robust mammal limb dataset. Our results support the potential for glider-like postcranial morphology to evolve into bat-like morphology prior to powered-flight behavior, setting a foundation for future developmental, biomechanical, and evolutionary research to test this idea.

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 (Heard and Hauser 1995; Hunter 1998; Jetz et al. 2012; Nicholson et al. 2014). 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 (Norberg 1990; Gunnell and Simmons 2005; Giannini 2012).

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 (1859) 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 non-gliding tree-dweller (hereafter, 'arborealist') to glider to flyer species (Norberg 1985; Bishop 2008; Simmons et al. 2008), albeit this idea has not been tested using phylogenetic comparative methods. The alternative "ground up" hypothesis (Padian 1982; Caple et al. 1983; Adams and Shaw 2013) holds 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 (Long et al. 2003; Kurochkin and Bogdanovich 2010).

Following the arborealist-to-glider-to-flier hypothesis, the ancestors of bats, which originated about 70-60 million years ago (Teeling et al. 2000; Upham et al. 2019; Álvarez-Carretero et al. 2022), are hypothesized to have evolved more elongated limbs, followed by

patagia (wing membranes) on the armwing to facilitate gliding, and finally membranes in the handwing to enable 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 (Vaughan 1970; Sears et al. 2006; Amador et al. 2019), resulting in the evolution of over 1,500 extant species (Simmons and Cirranello 2022B).

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) (Bishop 2008; Cooper and Tabin 2008; Giannini 2012). Morphological approaches to understanding the origin of bat flight further include analyses of skeletal dimensions in the context of development (Adams 1992, 2008; Sears et al. 2006; Nojiri et al. 2023), biomechanics (Swartz and Middleton 2008), and functional morphology (Vaughan 1970). These studies often focus on aspects of the bat forelimb (e.g., Amador et al. 2019) or hind limb (e.g., Riskin et al. 2005), but seldom both in tandem in a phylogenetic context (but see Swartz and Middleton 2008 and Nojiri et al. 2023). 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 selection will lead forelimb traits of arborealists, gliders, and bats towards three separate trait optima (commonly interpreted as the

location of an adaptive peak), where the glider forelimb optimum will be intermediate in value to those of arborealists and bats. If supported, this suggests that glider morphology may fall on an intermediate adaptive peak between bat and arborealist morphologies. 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 (Vaughan 1970) while still increasing their patagia (wing membrane) area. Glider optima with intermediate forelimb length and width optima between arborealist and bat optima may represent an intermediate adaptive zone between the anatomical adaptations required for tree-dwelling (Cartmill 1985) and those required for powered flight (Norberg 1990). 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 optima will be most similar to those of gliders', suggesting that bat and glider hind limb traits share a 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., Farnum et al. 2008; Simmons et al. 2008; Louzada et al. 2019), 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 (Giannini 2012). 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 morphological similarity previously described (Norberg 1985; Bishop 2008; Simmons et al. 2008). 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 (Fig. 1) from 231 extant mammals across 21 orders (78% of mammalian orders) and 73 families (47% of mammalian families). We collected limb data from 26 species of bats (48 specimens) at the Burke Museum of Natural History and Culture and obtained published data (Chen and Wilson 2015; Grossnickle et al. 2020; Weaver and Grossnickle 2020; Pevsner et al. 2022). We collected additional measurements from 42 species that were missing hind limb traits. The datasets sampled one or two specimens per species. 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 (Mosimann 1970) 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 (Jones et al. 2009) and the *Handbook of the Mammals of the World* book series (Wilson and Mittermeier 2009).

We also collected the 29 measurements for four extinct bats (*Onychonycteris finneyi, Icaronycteris index, Hassianycteris messelensis, and Tachypteron franzeni*) using ImageJ (Schneider et al. 2012) and published full-body images that include scale bars (Storch et al. 2002; Gunnell and Simmons 2005; Simmons et al. 2008).

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* (R Core Team 2021).

Ecological Data

We categorized species into four locomotor groups: ground dwellers (n = 117), arborealists (n = 71), gliders (n = 16), and flyers (n = 27) following (Chen and Wilson 2015; Grossnickle et al. 2020; Weaver and Grossnickle 2020; Pevsner et al. 2022). 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 (Grossnickle et al. 2020). Flyers included bats only.

Phylogenetic Comparative Analyses

We used generalized evolutionary models (Hansen 1997; Butler and King 2004) 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. The OUM models included 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 using the *R* package *OUwie* (Beaulieu et al. 2012). We applied a single selection parameter (α) and rate parameter (σ^2) across all selective regimes while allowing θ to vary between regimes (Hansen 1997; Butler and King 2004). All four models were fit across 250 stochastically mapped trees to account for uncertainty in phylogenetic topology and the ancestral character states (Fig. 1). We used the *make.simmaps* function in the *R* package *phytools* (Revell 2012) to simulate 10 stochastic character maps across 1,000 tree topologies randomly drawn from the posterior distribution of "completed" trees from Upham et al. (2019), resulting in 10,000 character maps of locomotory regimes. We randomly sampled 250 trees for subsequent analyses. 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. For traits in which the OUM model was the best-supported model, we computed 95% confidence intervals using parametric bootstrapping of the model parameters with the *OUwie.boot* function in the *R* package *OUwie* (Beaulieu et al. 2012). We randomly sampled 100

of our character maps and performed 10 bootstrap replicates per stochastic character map sampled.

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 (Uyeda et al. 2015; Adams and Collyer 2018), 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 (Table S2a, S4a), whereas OUM_{loc3} was the best-fitting model for humerus distal width and ulna olecranon length (Tables S3a, S4a). The OU1 model was the best fit for the proximal phalanx width of the third manual digit.

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 (Fig. 2b-c). Optima of armwing widths indicated that bats exhibit relatively wider humeri at the

distal end, proximal end, and mid-shaft compared to all other locomotor groups (Fig. 2g-i; 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 (Fig. 2d-f; Table S2a). For handwing width traits, bats exhibit relatively higher optima for metacarpal widths compared to all other locomotor groups (Fig. 2j), 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 (Fig. 2i; 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 relatively lower than those of all other groups (Fig. 2a; Table S2a).

Adding fossil bats into forelimb model-fitting analyses yielded overall slightly more support for OUM_{loc3} models compared to extant-only modeling (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 (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 (Table S7).

Forelimb morphospace

PC1 explains 81.5% of the forelimb variation and separates flyers (both extant and extinct) from the remaining three locomotor modes (Fig. 3a; Table S1a). 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). 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. 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.

PC1 of the extant forelimb dataset was best fit with an OUM_{loc4} model (Table S6a). The bootstrapping reveals the optima gradually increase from ground-dwellers to arborealists to gliders to flyers (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 supported for 11 of the 15 traits (Table S4b), serving as the best-fitting model ($\Delta AIC = 0$) for six traits and as the second best-fitting model ($\Delta AIC < 2$) for the other five 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 (Fig. 4f; 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 (Fig. 4g, i, j-k). 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 (Fig. 4j, e), but these two traits were both best supported by an

OU1 model (ΔAIC = 0 for both) and had lower OUM_{loc3} weights (AICcW = 0.31 and 0.22, respectively).

The OUM_{loc4} model was supported for 12 of 15 traits (Table S4b), serving as the best-fitting model for seven traits and as the second-best-fitting model for five traits. Gliders have relatively longer tibia and femoral optima compared to bats and the other locomotor groups (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., Fig. 4b-c; Table S2b). Additionally, width optima for long bones and digits overlap for bats and gliders for all but femur distal width, where bats have significantly smaller width optima than all other groups (Fig. 4h; Table S2b).

The pelvis, ilium, and ischium optima appear unique among hind limb traits by being relatively smaller for bats than for the other locomotor groups, which showed little variation (e.g., Fig. 4a; 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 slightly more support for OUM_{loc4} models (best-fit for nine traits and comparable fits for four traits) compared to extant-only modeling (Table S6b). The OUM_{loc3} model was the best for five traits and a comparable fit model for three traits. OU1 was still the best-fitting model for pedal proximal phalanx length. The length and width optima for the hind limb were very similar between extant+extinct and extant-only modeling (Table S8). However, we were unable to examine the extent of this consistency because hind limb bootstrapping failed with the addition of fossil species (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 (Fig. 3b; 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. 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. PC2 loosely separated ground-dwellers, arborealists, and the glider-flyer group. These loadings show that 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 forelimb optima are intermediate between bat and arborealist optima for length (Fig. 2a-f), but not width (Fig. 2g-j) traits across both the armwing and handwing. Consistent with the rapid evolution of a handwing being a necessary adaptation for bats (Vaughan 1970; Sears et al. 2006; Amador et al. 2019), 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 (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' (Swartz and Middleton 2008). Bats' relatively robust humeral width optima may reflect the higher muscle mass closer to their center of gravity required for powered flight (Vaughan 1970). If the scapula is considered part of the armwing, gliders are intermediate between arborealists and bats for scapula length (Fig. 2a) but not width optima. This is likely because the bat scapula serves as the attachment point for many of the necessarily large muscles controlling their wing-beat cycle (Vaughan 1970). In the handwing, the diameters of bat metacarpals are relatively larger than other mammals' (Swartz and Middleton 2008), congruent with our findings that bats have relatively thicker third metacarpal optima (Fig. 2j). While the proximal phalanx width of the third manual digit was best fit with an OU1 model, this is consistent with Swartz and Middleton's (2008) finding that the proximal phalanx diameter is not significantly smaller in bats compared to nonvolant mammals.

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 (Grossnickle et al. 2020). This is unsurprising because gliders may remain gracile to increase patagial area while decreasing wing loading (Thorington and Santana 2007), 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, the decoupled patterns of length versus width optima represent an interesting breakdown between adaptive zones in limb bone length and width among gliding and flying mammals and across both the armwing and handwing. 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 (Table S4a), may both correspond to traits most pronounced for digging locomotion and thus be functionally irrelevant for either gliding or aerial locomotion (Hildebrand 1985). Therefore, our use of univariate models parsed out finer-scale morphometric 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 traits exhibit a single optimum (i.e., OUM_{loc3}) 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 (i.e., OUM_{loc4}), there is 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 longer femur, tibia, and third intermediate phalanx length optima (Table S3b) compared to other locomotor groups. This trend contrasts with the bat-glider group having a relatively shorter metatarsal length optimum (Fig. 4d) 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 (Young and Hallgrímsson 2005). Regardless, bats

and gliders follow 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) (Fig. 4g-i). These common narrower elements support our prediction that bat-glider hind limb length and width optima will reflect the demands of bat and glider weight requirements, bat roosting behaviors (Giannini 2012), and glider patagial surface area requirements (Thorington and Heaney 1981; Thorington and Santana 2007).

Although the OUM_{loc4} model was supported for 12 of 15 traits, there is weak evidence for the trait optima of bat and glider hind limbs being different, as indicated by their overlapping confidence intervals (Table S2b); this finding provides some support for 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 (Vaughan 1970), and fibula lengths, which are vestigial in bats (Fig. 4a; 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 (2008), 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 (2002) 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 (Sears et al. 2006) and the postnatal divergence in chondrocyte activity between the bat forelimb and hind limb autopod (Farnum et al. 2008) may help explain how bats can achieve this decoupling where other mammal species cannot.

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 (Fig. 3a). Amador et al. (2019) 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 (Fig. S1a), congruent with Amador et al.'s (2019) 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 (Fig. 2b). *Tachypteron franzeni* most clearly overlaps with extant bats, and is also the only fossil we included from an extant bat family, Emballonuridae (Storch et al. 2002). Simmons et al. (2008) 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 (Fig. S1b). Vampire bats are known for their adept quadrupedal locomotion and climbing (e.g., Riskin et al. 2005), 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, Riskin et al. 2005), 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 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 (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 (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 (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.

Limb trait optima and the adaptive landscape

Our results provide insight on the potential adaptive landscape connecting arboreal, gliding, and 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" glider traits towards a flying adaptive zone. Bishop (2008) proposed that the high 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 (Grossnickle et al. 2020). The clustering of bat species in forelimb morphospace (Fig. 3a) 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 (Grossnickle et al. 2020). This broad adaptive slope could represent the foothill of a steep bat peak such that gliders are very slowly evolving towards a bat peak (Fig. 5a). The evolutionarily oldest glider lineages, dermopterans (colugos) and anomalurids (scaly-tailed squirrels), have evolved farthest from ancestral arborealists (Grossnickle et al. 2020). Here, the dermopteran species *Cynocephalus volans* and *Galeopteras variegatus* are the gliders closest to the bat forelimb morphospace (Fig. 2a; Fig 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 (Simmons and

Geisler 1998), illustrates a possible early mechanism for this transition (Smith 1977; Anderson and Ruxton 2020 but see Gardner and Dececchi 2022 for an alternate view).

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 (Fig. 5b). These broad adaptive optima could represent neighboring peaks for gliders and bats separated by only a shallow fitness valley (Fig. 5b). 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 (Bishop 2008). There is both quadrupedal suspension and hind limb suspension in dermopterans, resulting in a tradeoff in the ability to walk quadrupedally upright (Fujiwara et al. 2011; Granatosky 2018). Indeed, *G. variegatus* is the only glider to fall directly within the bat hind limb PC1 and PC2 morphospace (Fig. 3b; Fig. S1b), reflecting a hind limb adaptive optimum potentially more easily attained than that of bats' specialized forelimbs (Fig. 5).

Our modeling approach offers a first step towards support for the hypothesis that selection may more strongly "pull" certain limb traits towards a bat adaptive zone. In particular, gliders' intermediate forelimb length and overlapping hind limb length/width optima between that of arborealists and bats could be the traits driving lineages across fitness valleys due to their greater effect in, for example, the functionality of the gliding/flying apparatus. However, these results could be strengthened by more comprehensive coverage of the lengths/widths and biomechanical studies tying these traits to specific selective pressures to complete the tentative adaptive landscape presented here (Fig. 5).

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 mammalian forelimb and hind limb. 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, solely based on morphometrics, the traditional highly specialized view of glider/bat forelimbs and the "reduced" view of bat hind limbs may represent more malleable adaptive zones than previously thought. There is a need for future studies to test the biomechanical consequences of such trait optima alongside more synergistic studies taking into account bat and gliders' complex physiology and development to test these hypotheses regarding the evolution of mammalian 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.

Data availability. Data and R scripts will be available from the Dryad Digital Repository at XXXXXX (upon publication).

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References

Adams, D. C., and M. L. Collyer. 2018. Multivariate Phylogenetic Comparative Methods: Evaluations, Comparisons, and Recommendations. Syst. Biol. 67:14–31.

Adams, R. A. 1992. Comparative skeletogenesis of the forearm of the little brown bat (Myotis lucifugus) and the norway rat (Rattus norvegicus). J. Morphol. 214:251–260.

Adams, R. A. 2008. Morphogenesis in Bat Wings: Linking Development, Evolution and Ecology. Cells Tissues Organs 187:13–23.

Adams, R. A., and J. B. Shaw. 2013. Time's Arrow in the Evolutionary Development of Bat Flight. Pp. 21–46 *in* R. A. Adams and S. C. Pedersen, eds. Bat Evolution, Ecology, and Conservation. Springer New York, New York, NY.

Álvarez-Carretero, S., A. U. Tamuri, M. Battini, F. F. Nascimento, E. Carlisle, R. J. Asher, Z. Yang, P. C. J. Donoghue, and M. dos Reis. 2022. A species-level timeline of mammal evolution integrating phylogenomic data. Nature 602:263–267. Nature Publishing Group.

Amador, L. I., N. B. Simmons, and N. P. Giannini. 2019. Aerodynamic reconstruction of the primitive fossil bat *Onychonycteris finneyi* (Mammalia: Chiroptera). Biol. Lett. 15:20180857.

Anderson, S. C., and G. D. Ruxton. 2020. The evolution of flight in bats: a novel hypothesis. Mammal Rev. 50:426–439.

Beaulieu, J. M., D.-C. Jhwueng, C. Boettiger, and B. C. O'Meara. 2012. Modeling stabilizing selection: Expanding the Ornstein-Uhlenbeck model of adaptive evolution. Evolution 66:2369–2383.

Bishop, K. L. 2008. The Evolution of Flight in Bats: Narrowing the Field of Plausible Hypotheses. Q. Rev. Biol. 83:153–169.

Butler, M. A., and A. A. King. 2004. Phylogenetic Comparative Analysis: A Modeling Approach for Adaptive Evolution. American Naturalist 164:683–695.

Caple, G., R. P. Balda, and W. R. Willis. 1983. The Physics of Leaping Animals and the Evolution of Preflight. Am. Nat. 121:455–476.

Cartmill, M. 1985. Chapter 5. Climbing. Pp. 73–88 *in* M. Hildebrand, D. M. Bramble, K. F. Liem, and D. B. Wake, eds. Functional Vertebrate Morphology. Harvard University Press.

Chen, M., and G. P. Wilson. 2015. A multivariate approach to infer locomotor modes in Mesozoic mammals. Paleobiology 41:280–312.

Cooper, K. L., and C. J. Tabin. 2008. Understanding of bat wing evolution takes flight. Genes Dev. 22:121–124.

Darwin, C. 1859. On the origin of species. 1st ed. John Murray, London.

Farnum, C. E., M. Tinsley, and J. W. Hermanson. 2008. Forelimb versus Hindlimb Skeletal

Development in the Big Brown Bat, *Eptesicus fuscus:* Functional Divergence Is Reflected in Chondrocytic Performance in Autopodial Growth Plates. Cells Tissues Organs 187:35–47.

Fujiwara, S., H. Endo, and J. R. Hutchinson. 2011. Topsy-turvy locomotion: biomechanical specializations of the elbow in suspended quadrupeds reflect inverted gravitational constraints: Elbow mechanics in suspended quadrupeds. J. Anat. 219:176–191.

Gardner, N. M., and T. A. Dececchi. 2022. Flight and echolocation evolved once in Chiroptera: comments on 'The evolution of flight in bats: a novel hypothesis.' Mammal Rev. 52:284–290.

Giannini, N. P. 2012. Toward an integrative theory on the origin of bat flight. Pp. 353–384 *in* G. F. Gunnell and N. B. Simmons, eds. Evolutionary History of Bats. Cambridge University Press, Cambridge.

Granatosky, M. C. 2018. Forelimb and hindlimb loading patterns during quadrupedal locomotion in the large flying fox (*Pteropus vampyrus*) and common vampire bat (*Desmodus rotundus*). J. Zool. 305:63–72.

Grossnickle, D. M., M. Chen, J. G. A. Wauer, S. K. Pevsner, L. N. Weaver, Q.-J. Meng, D. Liu, Y.-G. Zhang, and Z.-X. Luo. 2020. Incomplete convergence of gliding mammal skeletons*. Evolution 74:2662–2680.

Gunnell, G. F., and N. B. Simmons. 2005. Fossil Evidence and the Origin of Bats. J. Mamm. Evol. 12:209–246.

Hallgrímsson, B., K. Willmore, and B. K. Hall. 2002. Canalization, developmental stability, and morphological integration in primate limbs. Am. J. Phys. Anthropol. Suppl 35:131–158.

Hansen, T. F. 1997. Stabilizing Selection and the Comparative Analysis of Adaptation.

Evolution 51:1341–1351.

Heard, S. B., and D. L. Hauser. 1995. Key evolutionary innovations and their ecological mechanisms. Hist. Biol. 10:151–173.

Hildebrand, M. 1985. Chapter 6. Digging of Quadrupeds. Pp. 89–109 *in* M. Hildebrand, D. M. Bramble, K. F. Liem, and D. B. Wake, eds. Functional Vertebrate Morphology. Harvard University Press.

Hunter, J. P. 1998. Key innovations and the ecology of macroevolution. Trends Ecol. Evol. 13:31–36.

Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. Nature 491:444–448. Nature Publishing Group.

Jones, K. E., J. Bielby, M. Cardillo, S. A. Fritz, J. O'Dell, C. D. L. Orme, K. Safi, W. Sechrest, E. H. Boakes, C. Carbone, C. Connolly, M. J. Cutts, J. K. Foster, R. Grenyer, M. Habib, C. A. Plaster, S. A. Price, E. A. Rigby, J. Rist, A. Teacher, O. R. P. Bininda-Emonds, J. L. Gittleman, G. M. Mace, and A. Purvis. 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology 90:2648–2648.

Kurochkin, E. N., and I. A. Bogdanovich. 2010. Origin of feathered flight. Paleontol. J. 44:1570–1588.

Long, C. A., G. P. Zhang, T. F. George, and C. F. Long. 2003. Physical theory, origin of flight, and a synthesis proposed for birds. J. Theor. Biol. 224:9–26.

Louzada, N. S. V., M. R. Nogueira, and L. M. Pessôa. 2019. Comparative morphology and scaling of the femur in yangochiropteran bats. J. Anat. joa.12996.

Mosimann, J. E. 1970. Size Allometry: Size and Shape Variables with Characterizations of the

Lognormal and Generalized Gamma Distributions. J. Am. Stat. Assoc. 65:930–945.

Nicholson, D. B., A. J. Ross, and P. J. Mayhew. 2014. Fossil evidence for key innovations in the evolution of insect diversity. Proc. R. Soc. B Biol. Sci. 281:20141823. Royal Society.

Nojiri, T., I. Werneburg, V. T. Tu, D. Fukui, M. Takechi, S. Iseki, T. Furutera, and D. Koyabu. 2023. Timing of organogenesis underscores the evolution of neonatal life histories and powered flight in bats. Proc. R. Soc. B Biol. Sci. 290:20221928. Royal Society.

Norberg, U. M. 1985. Evolution of Vertebrate Flight: An Aerodynamic Model for the Transition from Gliding to Active Flight. Am. Nat. 126:303–327. [University of Chicago Press, American Society of Naturalists].

Norberg, U. M. 1990. Vertebrate Flight. Springer Berlin Heidelberg, Berlin, Heidelberg.

Padian, K. 1982. Running, Leaping, Lifting Off. The Sciences 22:10–15.

Pevsner, S. K., D. M. Grossnickle, and Z.-X. Luo. 2022. The functional diversity of marsupial limbs is influenced by both ecology and developmental constraint. Biol. J. Linn. Soc. 135:569–585.

R Core Team. 2021. R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Riskin, D. K., J. E. A. Bertram, and J. W. Hermanson. 2005. Testing the hindlimb-strength hypothesis: non-aerial locomotion by Chiroptera is not constrained by the dimensions of the femur or tibia. J. Exp. Biol. 208:1309–1319.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9:671–675.

Sears, K. E., R. R. Behringer, J. J. Rasweiler, and L. A. Niswander. 2006. Development of bat flight: Morphologic and molecular evolution of bat wing digits. Proc. Natl. Acad. Sci. 103:6581–6586.

Simmons, N. B., and A. L. Cirranello. 2022B. Bat Species of the World: A taxonomic and geographic database.

Simmons, N. B., and J. H. Geisler. 1998. Phylogenetic relationships of Icaronycteris, Archaeonycteris, Hassianycteris, and Palaeochiropteryx to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. Bulletin of the AMNH ; no. 235. Relationships of Eocene bats. [New York] : American Museum of Natural History.

Simmons, N. B., K. L. Seymour, J. Habersetzer, and G. F. Gunnell. 2008. Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. Nature 451:818–821.

Smith, J. D. 1977. Comments on Flight and the Evolution of Bats. Pp. 427–437 *in* M. K. Hecht, P. C. Goody, and B. M. Hecht, eds. Major Patterns in Vertebrate Evolution. Springer US, Boston, MA.

Storch, G., B. Sigé, and J. Habersetzer. 2002. Tachypteron franzeni n. gen., n. sp., earliest emballonurid bat from the Middle Eocene of Messel (Mammalia, Chiroptera). Paläontol. Z. 76:189–199.

Swartz, S. M., M. B. Bennett, and D. R. Carrier. 1992. Wing bone stresses in free flying bats and the evolution of skeletal design for flight. Nature 359:726–729. Nature Publishing Group.

Swartz, S. M., and K. M. Middleton. 2008. Biomechanics of the Bat Limb Skeleton: Scaling, Material Properties and Mechanics. Cells Tissues Organs 187:59–84.

Teeling, E. C., M. Scally, D. J. Kao, M. L. Romagnoli, M. S. Springer, and M. J. Stanhope. 2000. Molecular evidence regarding the origin of echolocation and ¯ight in bats. 403:5.

Thorington, R. W., and L. R. Heaney. 1981. Body Proportions and Gliding Adaptations of Flying Squirrels (Petauristinae). J. Mammal. 62:101–114.

Thorington, R. W., and E. M. Santana. 2007. How to Make a Flying Squirrel: *Glaucomys* Anatomy in Phylogenetic Perspective. J. Mammal. 88:882–896.

Upham, N. S., J. A. Esselstyn, and W. Jetz. 2019. Inferring the mammal tree: Species-level sets of phylogenies for questions in ecology, evolution, and conservation. PLoS Biol. 17:e3000494.

Uyeda, J. C., D. S. Caetano, and M. W. Pennell. 2015. Comparative Analysis of Principal Components Can be Misleading. Syst. Biol. 64:677–689.

Vaughan, T. A. 1970. Vaughan, T. A. (1970). The skeletal system. In Biology of Bats 1, ed. W. A. Wimsatt. New York: Academic Press. pp. 98–139. Pp. 98–139 *in* Biology of Bats. New York: Academic Press.

Weaver, L. N., and D. M. Grossnickle. 2020. Functional diversity of small-mammal postcrania is linked to both substrate preference and body size. Curr. Zool. 66:539–553.

Wilson, and R. A. Mittermeier. 2009. Handbook of the Mammals of the World. Wilson&R. A. Mittermeier, Eds. Lynx Edicions.

Young, N. M., and B. Hallgrímsson. 2005. Serial Homology and the Evolution of Mammalian Limb Covariation Structure. Evolution 59:2691–2704.

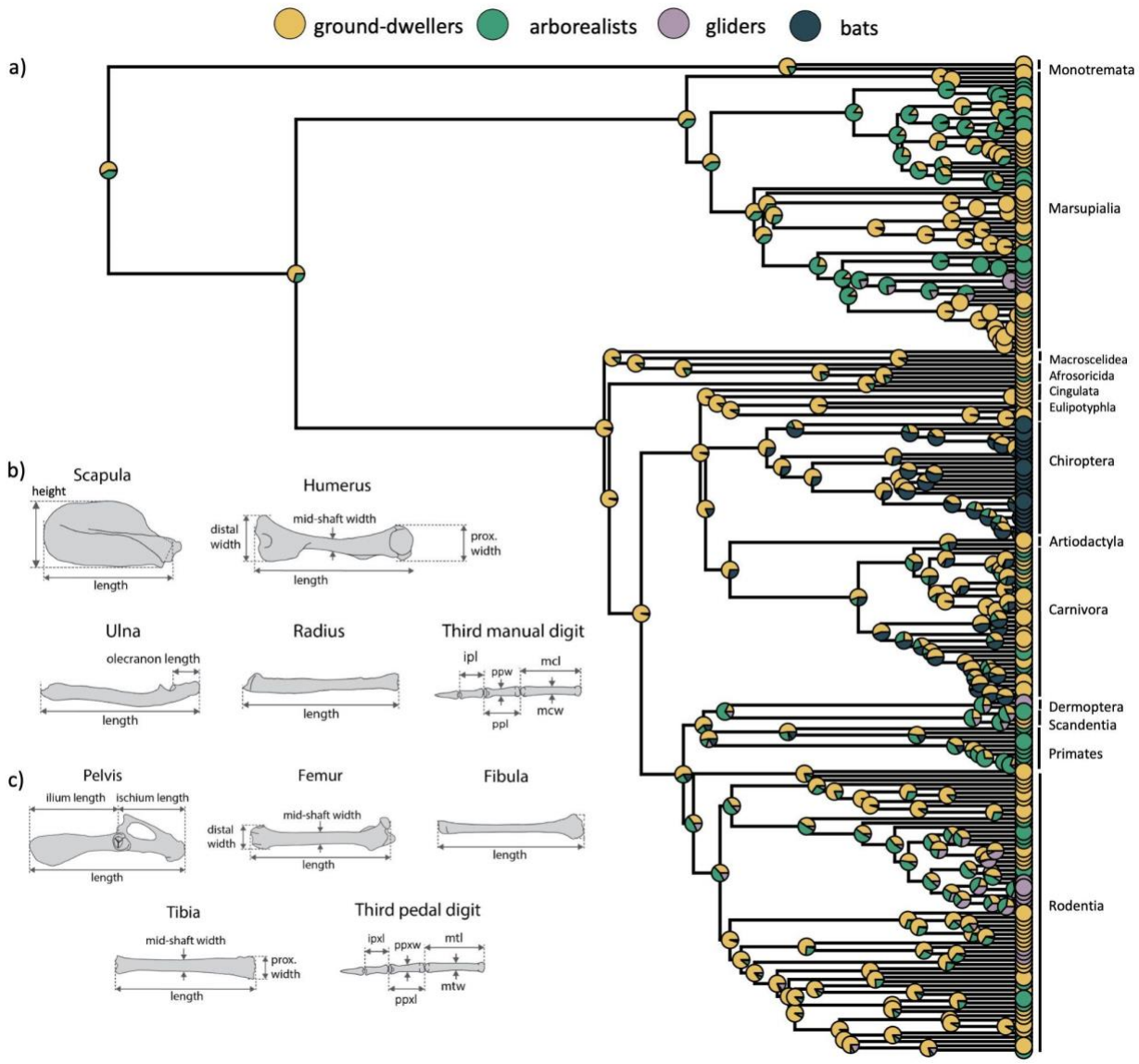

Figure 1. Morphological measurements and phylogeny. We conducted analyses based on this a) phylogeny (Upham et al. 2019) across b) 14 forelimb linear measurements for each specimen of scapula height and length (sh, sl), humerus length (hl), distal width (hdw), proximal width (hpw), and mid-shaft width (hsw), radius length (rl), ulna length and olecranon length (ul, uol), and third manual digit metacarpal length and width (mcl, mcw), proximal phalanx length and width (ppl, ppw), and intermediate phalanx length (ipl) and we collected c) 15 hind limb measurements consisted of pelvis, ilium, and ischium length (pel, il, isl); femur length (fl), distal width (fdw), and midshaft width (fsw); tibia length (tl), proximal length (tpw), and midshaft width (tmw); fibula length (fbl), and third pedal digit metatarsal length and width (mtl, mtw), proximal phalanx length and width (ppxl, ppxw), and intermediate phalanx length (ipxl). We measured only the third digit in this dataset (and not, for example, the fifth even though that might be of biological interest) in order to build on previous datasets in the literature to generate a large enough dataset for evolutionary modeling.

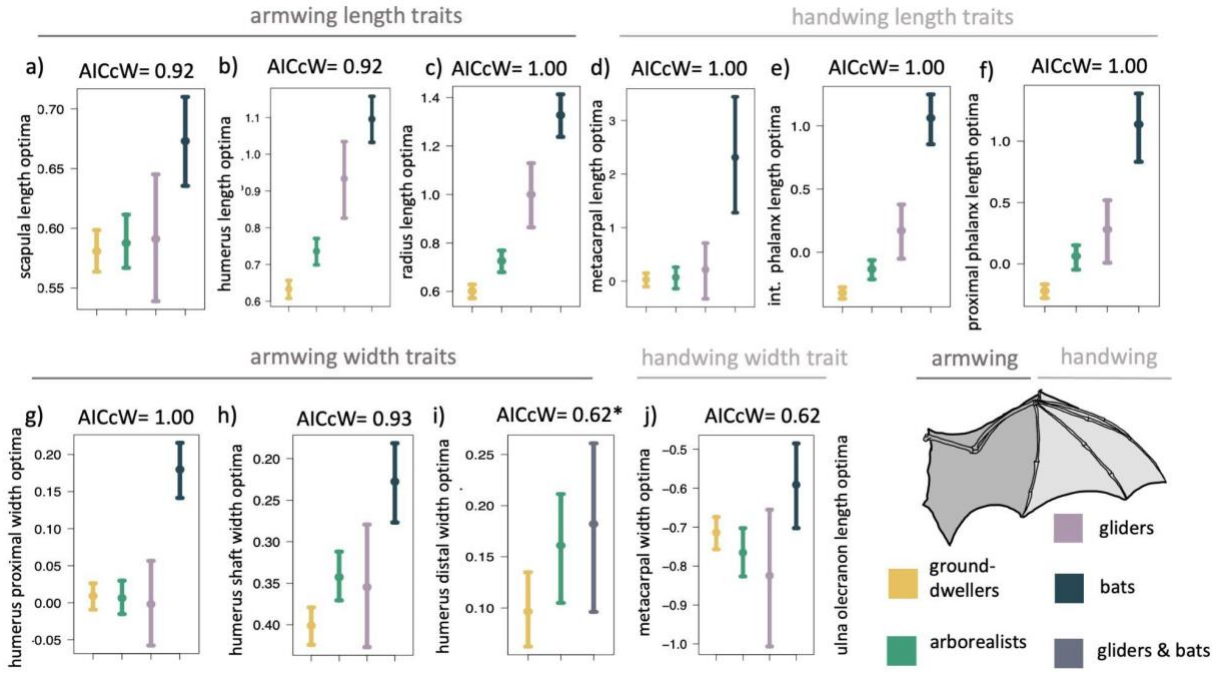

Figure 2. Forelimb trait optima from model-fitting of univariate traits. Forelimb trait optima (θ) with 95% confidence intervals estimated from the best-fitting OU models (Tables S2a, 3a, 4a). Only traits that were best fit by multiple-peak OU models are shown. The AICcW of the model is on top of each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

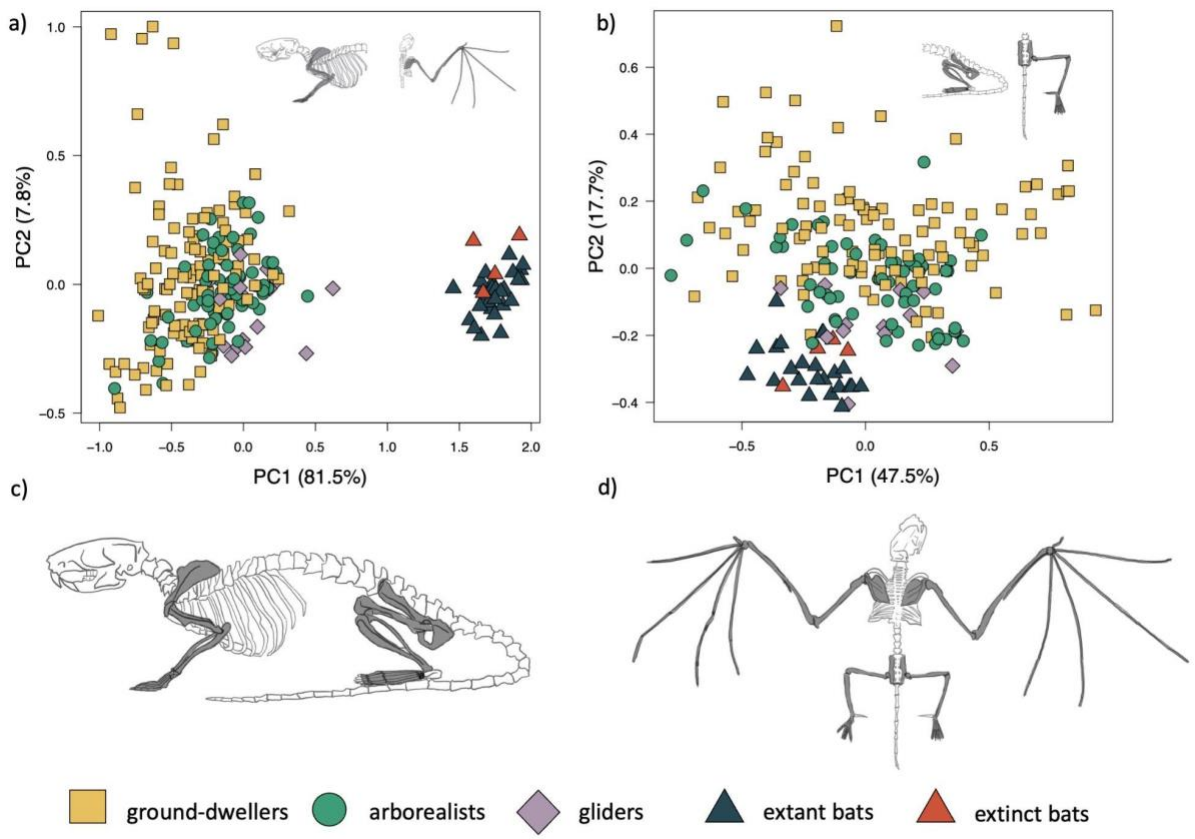

Figure 3. Forelimb and hind limb PCAs. Morphospaces consist of PC1 and PC2 of a) 14 forelimb traits and b) 15 hind limb traits with cartoons of a c) quadrupedal mammal skeleton (*Rattus norvegicus*) versus a d) bat skeleton (*Pipistrellus abramus*). Dark gray shaded bones on the cartoon skeletons correspond to those measured to generate the morphospace.

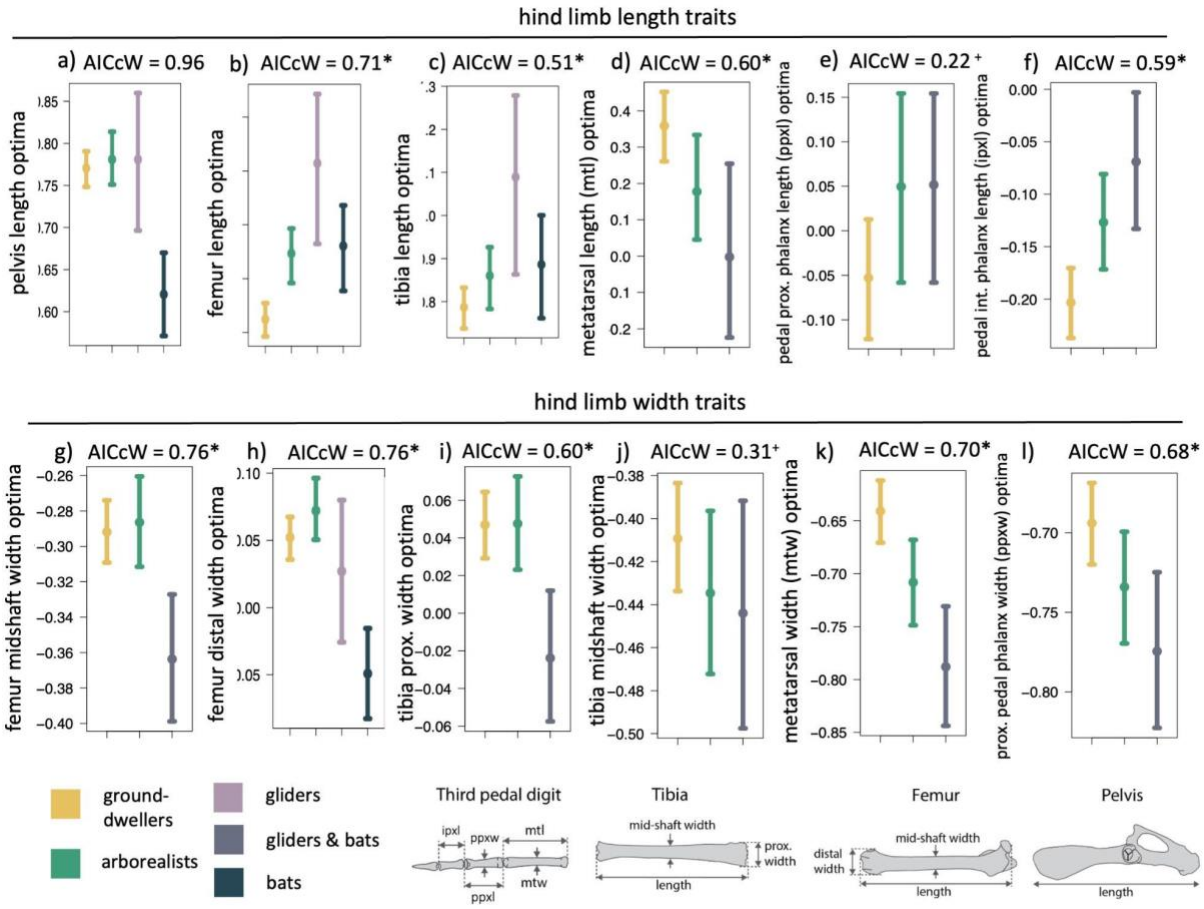

Figure 4. Hind limb trait optima from model-fitting of univariate traits. OUM optima (θ) as a size-corrected trait value (log-shape ratio) with 95% confidence intervals (Tables S2b, 3b, 4b) for the best-fitting models (ΔAIC = 0) for 12 traits. The AICcW of the model is on top of each panel with * indicating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

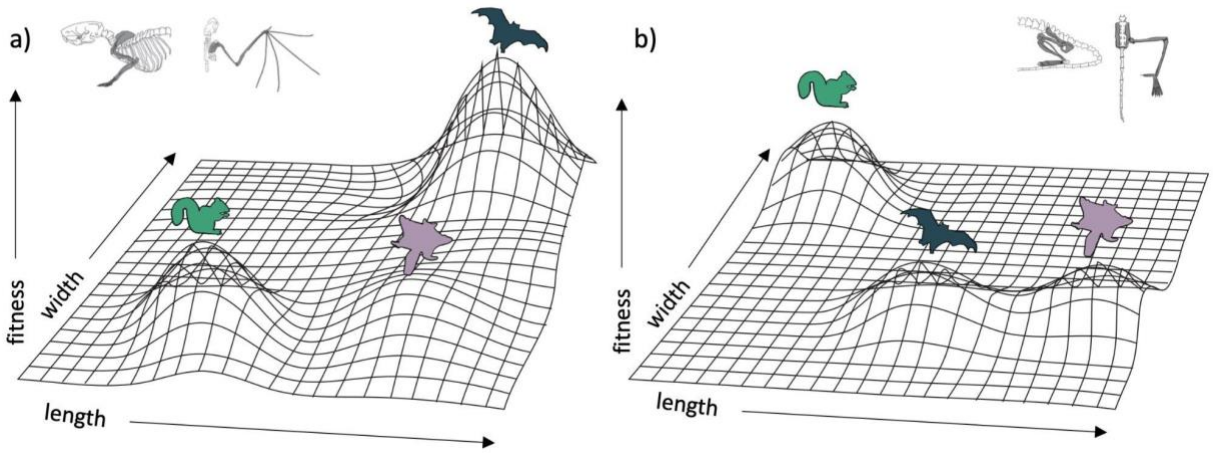

Figure 5. Hypothetical adaptive landscapes of forelimb and hind limb skeletal relative length and width evolution. Hypothetical fitness peaks are shown for arborealists, gliders, and bats for a broad summary of our conclusions from the skeletal trait optima results discussed. The "length" and "width" 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.