Gliding towards an understanding of the origin of flight in bats

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, from the perspective of a functional transition, 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 modes. Our results reveal that gliders exhibit adaptive trait optima (1) toward relatively elongate forelimbs that are intermediate between those of bats and non-gliding arborealists, and (2) toward relatively narrower but not longer hind limbs that are intermediate between those of non-gliders and bats. We propose a hypothetical adaptive landscape based on limb length and width optimal trends derived from our modeling analyses. Our results support the potential for glider-like postcranial morphology to precede a bat-like morphology adapted to powered-flight, 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 not only specialized for hanging and catching prey in flight (Norberg 1990; Gunnell and Simmons 2005; Giannini 2012), but also contribute to flight itself (Cheney et al. 2014).

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 non-flying ancestors. 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 (Norberg 1985B; Bishop 2008; Simmons et al. 2008), although this idea has not been tested using phylogenetic comparative methods. It should be noted that all phylogenomic evidence shows that bats are not closely related to gliders (Upham et al., 2019; Álvarez-Carretero et al., 2022); bats are nested within Laurasiatheria where neighboring clades are predominantly adapted to terrestrial locomotion. Laurasiatheria is far from the placental gliders, including dermopterans and rodents, located in the Eucarchontoglires superorder. 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 features 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; Anthwal et al., 2023; Feigin et al., 2023). 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 et al. 1992; Swartz 1997; 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 modes including ground-dwellers, arborealists, gliders, and flyers.

Following the arborealist-to-glider-to-bat hypothesis, we test two predictions regarding the evolution of limb morphologies using an Ornstein-Uhlenbeck evolutionary model-fitting

approach. First, we predict that selection will lead the forelimb skeletal traits of arborealists, gliders, and bats towards three separate adaptive trait optima/attractors (i.e., θ parameters of fitted OU models, which are commonly interpreted as the locations of adaptive peaks in morphospace; hereafter "optima"), where the glider forelimb optimum will be intermediate in value to those of arborealists and bats. If supported, this demonstrates that glider morphology may fall on an intermediate adaptive peak between bat and arborealist morphologies. Specifically, we predict wider and longer skeletal element optima values progressing from arborealists to gliders to bats. Relatively longer bones (especially in the handwing) and larger-diameter bones (especially in combination with thinner, highly mineralized walls), may give bats enough resistance to torsion during powered flight (Swartz et al. 1992) while still increasing their patagia 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 exhibit a distinct trait optimum (i.e., adaptive peak) separate from ground-dwelling, arboreal, and gliding 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), whereas gliders exhibit relatively lengthened hindlimbs (Grossnickle et al. 2020; Linden et al. 2023). Therefore, the relative lengths and widths of the hindlimb bones are unclear between these aerial groups as well as in relation to arborealists. 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 values progressing from arborealists to bats

and gliders. We predict similar but distinct hindlimb optima between gliders and bats because relatively longer and lower-diameter hind limb bones in bats may decrease weight while still generating lift by increasing their plagiopatagium surface area, as their hind limbs are incorporated into this membrane (Giannini 2012). Similarly, relatively longer and wider hindlimb bones in gliders would increase the surface area for lift during gliding. 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.

Methods

Morphological Data

We compiled linear measurements of 14 forelimb and 15 hind limb traits (Fig. 1) from 231 extant mammals (1–2 specimens per species) across 21 orders (78% of mammalian orders) and 73 families (47% of mammalian families). Of these, 26 species were bats. Linear measurements were obtained from specimens held at the Burke Museum of Natural History and Culture or from previously published work (Chen and Wilson 2015; Grossnickle et al. 2020; Weaver and Grossnickle 2020; Pevsner et al. 2022). 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. Unfortunately, most specimens lacked accompanying body mass data;

therefore we compiled species-level 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) on published full-body images that include scale bars (Storch et al. 2002; Gunnell and Simmons 2005; Simmons et al. 2008). We estimated the body masses of extinct bats using a linear regression between the body mass and geometric mean of all linear measurements from the extant bats of our dataset and used those body mass estimations to size-correct our fossil data again using log-shape ratios. We included fossil bats in our morphological dataset because, although extinct bats have a high degree of morphological similarity to extant bats (Norberg 1985B; Bishop 2008; Simmons et al. 2008), the fossil bat data may alter model estimations of the adaptive zone between bats and gliders in an informative way. For example, the inclusion of fossils may make the bat-glider trait optima more similar or more disparate by adding more phylogenetic history information and thus inform the understanding of the adaptive landscape underlying the evolution of mammalian flight.

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 previous work (Chen and

Wilson 2015; Grossnickle et al. 2020; Weaver and Grossnickle 2020; Pevsner et al. 2022). The ground-dwelling mammals included 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 included 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.

We first tested the hypothesis that locomotor modes influenced the evolution of the forelimb and hind limb using multivariate generalized evolutionary models on our extant datasets (Butler & King, 2004; Hansen, 1997) in the R package mvMORPH (Clavel et al., 2015). To reduce the dimensions of our data, we conducted a principal component analysis (PCA) on our 14-trait forelimb dataset and 15-trait hind limb dataset. We retained the first three principal components (PCs) of our forelimb dataset (91.8% of the forelimb variation) and hind limb dataset (85.3% of hind limb variation). We then fit six multivariate models to the scores of these three PCs. We fit a multivariate Brownian motion model (mvBM), which models trait variance in a random walk over time; a single-regime multivariate Ornstein-Uhlenbeck model (mvOU1), in which scores of each PC evolve towards a single trait optimum (θ parameter of OU models), giving a single mean optimum for all locomotor groups; and multivariate OU models (mvOUM)

with a given number of optima corresponding to selective regimes (based on locomotor modes). To test our hypotheses on overall limb evolution, we fit a four-peak OU model ($mvOUM_{loc4}$) for distinct optima among ground-dweller, arborealist, glider, and flyer regimes. To test alternative hypotheses, we fit three three-peak models ($mvOUM_{loc3a\text{-}c}$) with distinct combinations of the four locomotor groups. The $mvOUM_{loc3a}$ model tests for distinct optima among ground-dweller, arborealist, and a combined glider-flyer regimes. The $mvOUM_{loc3b}$ model tests for distinct optima among ground-dweller, combined arborealist-glider, and flyer regimes. The $mvOUM_{loc3c}$ model tests for distinct optima among a combined ground-dweller-arborealist, glider, and flyer regimes.

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 and fit all six models to these trees. 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 acknowledge that using a subset of PCs instead of the full dataset could bias our results (D. C. Adams & Collyer, 2018; Uyeda et al., 2015). However, our results remained consistent when we increased the number of PCs in our model-fitting analyses to the first four or five PCs. Further, we used simulations to assess whether we had adequate power to accurately distinguish between complex mvOU models from Brownian motion (Boettiger et al., 2012).

If the $mvOUM_{loc4}$ model is best-supported, we interpreted this as each locomotor regime having its own distinct adaptive optimum. This result alone does not provide evidence for the evolutionary origin of bats. However, if gliders are located at an optimum between arborealists and bats (i.e., if arborealist < glider < bat optima or arborealist > glider > bat optima), there is support for an evolutionary "pathway" between the two locomotor modes relative to an ancestral arborealist or ground-dwelling state (i.e., one could theoretically bridge adaptive peaks via these pathways). Therefore, if the optima gradually increase/decrease from arborealists to gliders to bats, our hypothesis that bats evolved from gliding ancestors is supported.

If the $mvOUM_{loc3a}$ model is best-supported, we interpret this as gliders+flyers having a shared adaptive optimum and arborealists and ground-dwellers having their own distinct evolutionary optima. This model provides the clearest support for flyers evolving from gliding ancestors because lineages of both groups are evolving towards the same shared adaptive optima. Therefore, if this model best fit the data, our hypothesis is supported.

If the $mvOUM_{loc3b}$ model is best-supported, we interpret this as arborealists+gliders having a shared adaptive optimum and flyers and ground-dwellers having their own distinct evolutionary optima. There is phylogenetic justification for this grouping because all gliders evolved from ancestrally arboreal groups (e.g., gliding squirrels evolved from tree squirrels; see tree in Fig. 1). These groups are similar to the 4-group model in that if arborealists+gliders have an optimum between those of ground-dwellers and bats, there is support for an evolutionary "pathway" between the two locomotor modes relative to an ancestral ground-dwelling state. Therefore, if the optima gradually increase/decrease from ground-dwellers to arborealists+gliders to bats, our hypothesis that bats evolved from gliding ancestors is supported.

Finally, if $mvOUM_{loc3c}$ model is best-supported, we interpret this as ground-dwellers+arborealists having a shared adaptive optimum and flyers and gliders having their own distinct evolutionary optima. As with the $mvOUM_{loc4}$ and $mvOUM_{loc3b}$ models, if gliders have an optimum between those of ground-dwellers+arborealists and bats, there is support for an adaptive "pathway" between the two locomotor modes relative to an ancestral arborealist or ground-dwelling state, providing evidence for our hypothesis that bats evolved from gliding ancestors.

We also examined the influence of locomotor modes on the evolution of each individual forelimb and hind limb trait in our extant-only and extant+extinct datasets using univariate evolutionary models. For each of the 29 traits, we fit a Brownian motion model (BM), a single-optimum Ornstein-Uhlenbeck model (OU1), and four multiple-peak OU (OUM) models. The OUM models included OUM_{loc4}, OUM_{loc3a}, OUM_{loc3b}, and OUM_{loc3c}, featuring the same locomotor regimes as the multivariate mvMORPH models. 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 optima (θ) to vary between regimes (Hansen 1997; Butler and King 2004). For traits in which an 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 repeated the above multivariate and univariate 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 morphospace and multivariate model-fitting

PC1 explains 81.5% of the forelimb variation and separates flyers (both extant and extinct) from the remaining three locomotor modes (Fig. 2a; 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 relatively shorter (-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. PC3 explains 2.9% of the morphological variation with +PC3 dominated by metacarpal length, ulna olecranon length, and scapula height and -PC3 dominated by intermediate phalanx length and humerus distal width. Fossil bats overlap with extant bats in morphospace, which is consistent with the lack of transitional forms in the bat fossil record.

The $mvOUM_{loc3b}$ model, which assigns arborealists and gliders to the same regime, was the best-fitting model for the extant multivariate forelimb dataset (PCs 1-3) (AICcW = 0.99, Table 1a). Distinct optima were supported for these three locomotor groups, with PC1 and PC2 optima scores increasing in value from ground-dweller to arborealist+glider to flyer hind limbs (Fig. 2c). Repeating the multivariate model-fitting with the addition of the four extinct bat

species yielded a $mvOUM_{loc4}$ model as the best fit (mAICcW = 0.99; Table S6a). However, the optima exhibited unrealistic magnitudes (Table S6b).

Forelimb univariate model-fitting

The OUM_{loc4} model was the best-fitting model for six of the 14 forelimb traits (AICcW = 0.51-1.00), OUM_{loc3b} was the best-fitting model for five traits (AICcW = 0.36-0.65), OUM_{loc3c} was the best fitting model for metacarpal length (AICcW = 0.63), and OUM_{loc3a} was the best-fitting model for ulna olecranon length (AICcW = 0.58; Table S2a). The OU1 model was the best fit for the proximal phalanx width of the third manual digit (AICcW = 0.51).

Armwing (i.e., humerus and radius) length traits have optima (from the best-fitting OUM_{loc4} models) that gradually increase from ground-dwellers to arborealists to gliders to flyers; parametric bootstrapping revealed little to no overlap in the 95% confidence intervals of optima between most locomotor groups (Fig. 3b-c). Optima of armwing widths (from the best-fitting OUM_{loc3b} models) indicated that bats exhibit relatively wider humeri at the distal end, proximal end, and mid-shaft compared to all other locomotor groups (Fig. 3g-i; Table S3a).

Bats exhibit relatively higher optima for handwing length traits (i.e., longer third manual metacarpal, proximal phalanx, and intermediate phalanx) than all other locomotor groups (Fig. 3d-f; Table S3a). For each, glider optima were intermediate between ground-dweller and arborealist optima. For handwing width traits, bats exhibit relatively higher OUM_{loc3b} optima for metacarpal widths compared to all other locomotor groups (Fig. 3j), 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_{loc3a} models was supported for ulna olecranon length only (Table S2a). In total, however, glider optima (in

combination with arborealists) were between the 95% confidence intervals of ground-dweller and bat optima for all traits except scapula length/width, humerus proximal width, and metacarpal width (Fig. 3a, 3g, 3j; Table S3a). For these last trait optima, glider+arborealist optima were statistically the same as that of ground-dwellers while bats exhibited higher optima than all other locomotor groups (Fig. 3a, 3g, 3j; Table S3a).

Adding fossil bats into forelimb model-fitting analyses yielded overall slightly more support for OUM_{loc3a} models compared to extant-only modeling; scapula height and humerus proximal width demonstrate support for a bat-glider combined group (Table S4a). OUM_{loc4} and OUM_{loc3b} models were still widely supported as the best-fitting model for six traits and five traits, respectively; OU1 was still the best-fitting model for proximal phalanx width (Table S4a). Overall, the length and width optima values for the forelimb were very similar between extant+extinct and extant only-modeling (Table S5a); however, we were unable to examine the trends in finer detail because forelimb bootstrapping failed with the addition of fossil species.

Hind limb morphospace and multivariate model-fitting

PC1 explains 47.5% of hind limb variation and more positive PC1 scores describe the relative elongation (+PC1) of the fibula, metatarsal, and pedal proximal and intermediate phalanges (Fig. 2b; 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 17.8% of the variation, where +PC2 is associated with relatively lengthened pedal proximal phalanges, intermediate phalanges, and ischium and -PC2 with relatively narrower pedal proximal and intermediate phalanges. PC2 loosely separated ground-dwellers, arborealists,

and the glider-flyer group. PC3 explains 11.3% of the morphological variation with +PC3 dominated by ilium length, femur length, and fibula length and -PC3 dominated by metatarsal width, intermediate phalanx width, and proximal phalanx width. These loadings show that variation between the four locomotor groups mainly occurs throughout the hind limb autopodia region.

The $mvOUM_{loc4}$ model was the best-fitting model for the extant multivariate hind limb dataset (PCs 1-3) (AICcW = 0.92, Table 1b). Distinct optima were supported for the four locomotor regimes, with PC1 and PC2 optima scores decreasing in value from ground-dweller to arborealist to glider to flyer hind limbs, albeit with overlap (Fig. 2d). Adding in the four extinct bat species yielded again the $mvOUM_{loc4}$ model as the best fit, and very little change in the optima values (Table S6).

Hind limb univariate model-fitting

The OUM_{loc4} model was the best-fitting model for three of 15 traits, all of which were hind limb long bone lengths (AICcW = 0.33-0.60; Table S2b). Specifically, gliders have relatively longer tibia and femoral optima compared to bats and the other locomotor groups (Fig. 4b-c, Table S3b). Fibula length was the third hind limb trait supported by OUM_{loc4}, but fibulae are rudimentary in bats. The OUM_{loc3a} model best fit three traits (AICcW = 0.36-0.52), all of which were widths: femur shaft width, metatarsal width, and pedal proximal phalanx width (Table S3b). Parametric bootstrapping of these OUM_{loc3a}-supported models revealed that the glider-bat group had relatively narrower femur shafts, metatarsals, and pedal proximal phalanges optima than the other locomotor groups (Fig. 4f, i, j; Table S3b). The OUM_{loc3b} model was the best-fitting model for four of the 15 traits (AICcW = 0.44-0.71): ilium, ischium, metatarsal, and

intermediate proximal phalanx length (Table S2b). Bats had significantly smaller relative ilium and ischium length optima than other locomotor groups. Gliders+arborealists had metatarsal and intermediate proximal phalanx optima intermediate between bats and ground-dwellers (Fig. 4d-e; Table S3b). The OUM_{loc3c} model best fit three traits (AICcW = 0.42-0.48): pelvis length, femur distal width, and tibia proximal width (Table S2b). In the latter two traits, gliders exhibited intermediate width optima between bats (which were smaller) and ground-dwellers+arborealists (where were larger). For pelvis length, like ilium and ischium length, there was no clear trend in the direction of the optima values across locomotor groups (Table S3b).

Adding fossil bats into hind limb model-fitting analyses yielded only one difference compared to extant-only modeling: pelvis length was now best fit with a OUM_{loc3b} instead of OUM_{loc3c} model (Table S4b). The length and width optima for the hind limb were very similar between extant+extinct and extant-only modeling (Table S5b). However, we were unable to examine the extent of this consistency because hind limb bootstrapping failed with the addition of fossil species.

Discussion

Forelimb trait evolution

We found evidence that mammalian glider forelimb morphologies are evolving toward adaptive peaks that are intermediate between those of arborealists and bats. Our multivariate forelimb results and univariate length trait results provide support for the hypothesis that selective pressures for flight influenced the evolution of bat forelimbs from glider-like forelimbs. Using multivariate modeling, we found a three optima model with distinct selective regimes for ground-dwellers, arborealists+gliders, and bats as the best fit to the first three PCs of our data

(Table 1a). The arborealist+glider optima are intermediate between those of bats and ground-dwellers (Fig. 2c). PC1 is mostly explained by the variation in the forelimb bone lengths and PC2 by their widths, creating a coarse picture that forelimb bones likely become relatively longer and possibly wider from ground-dwellers to arborealists+gliders to bats. Our univariate model results are consistent with these findings, revealing that glider forelimb optima are intermediate between bat and arborealist optima for length traits (Fig. 3a-f), but only partially for width traits (Fig. 3g-j) across both the armwing and handwing (See Table S7 for a breakdown of whether each univariate model supports our hypothesis).

Bats exhibit trait optima indicating evolution of relatively longer forelimb bones compared to all other locomotor groups (Fig. 2a, Fig. 3a-f) particularly in the handwing, which exhibited rapid evolution as a necessary adaptation for bats (Vaughan 1970; Sears et al. 2006; Amador et al. 2019). Increased bone lengths may enhance the surface area of the patagium for greater lift (Norberg 1985b). Bats also displayed evidence of evolution toward relatively wider bones in the armwing compared to all other locomotor groups (Fig. 3g-j) consistent with findings that bat humeri and radii exhibit significantly larger diameters compared to nonvolant mammals (Swartz and Middleton 2008). These relatively wider proximal forelimb bones combined with thinner yet highly mineralized walls provide resistance to bending and torsion during flapping (Swartz et al. 1992; Papadimitrious et al. 1996). Furthermore, the transition from relatively more robust proximal ends of the humerus to relatively less robust distal ends may also reflect greater muscle mass closer to the center of rotation of the wing joints, which would greatly decrease flight inertial power and facilitate economical flight (Norberg 1985A; Swartz 1997). In the handwing, we found bats exhibit relatively thicker third metacarpals (Fig. 3j) that corresponded with its relatively increased length (Fig. 3d) compared to nonvolant mammals. In contrast, the

proximal phalanx length of the third manual digit was relatively longer compared to nonvolant mammals whereas its width was best supported with an OU1 model, consistent with previous work showing that the proximal phalanx diameter is not significantly smaller in bats compared to nonvolant mammals (Swartz and Middleton 2008). As a result, bats exhibit relatively thick bone cortices to combat greater bending moments associated with this relatively increased elongation (Swartz et al. 1992; Papadimitriou et al. 1996; Swartz 1997). Increased cortical thickness along with the reduction of mineralization of the handwing work together to resist bending failure while simultaneously maintaining flexibility during flying (Papadimitriou et al. 1996).

In gliders, we found evidence that forelimb bone length optima are largely intermediate between ground-dwellers and bats. Gliders tend to exhibit relatively greater length optima in most forelimb bones compared to ground-dwelling and arboreal mammals (Thorington and Heaney 1981; Grossnickle et al. 2020; Linden et al. 2023) but do not reach the lengths of bat forelimb bones. Nevertheless, like bats, the longer bones expand the surface area of the patagium (Thorington and Heaney 1981) and enable further adjustment of their patagium during flight, leading to more efficient aerodynamics, increased agility, and maneuverability (Thorington et al. 1998; Zhao et al. 2019). Forelimb bone widths in gliders, while clearly intermediate between those of ground-dwellers and bats for some traits (Fig. 3h, i) share an optimum with arborealists (shown by the OUM_{loc3b} support for these traits; Fig. 3g-j) and overlap with ground-dwelling optima mammals for other traits (Fig. 3g, j). Together, these results suggest gliders may maintain relative widths while continuing to elongate forelimb bones, supporting previous findings that the evolutionary elongation of glider forelimbs is not also accompanied by slimming of the bones (Grossnickle et al. 2020).

Hind limb trait evolution

We found mixed evidence that mammal glider hind limb morphologies are intermediate to those of arborealists and bats. Using our multivariate modeling approach, we found distinct optima for ground-dwellers, arborealists, gliders, and bats that best fit the first three PCs of our hind limb data (Table 1b). The trait loadings indicate that the autopodial bones dictate the distribution of PC scores in PCs 1 and 2 (Table S1b). However, there is much overlap among the optima of ground-dwellers, arborealists, and gliders in morphospace (Fig. 2d).

The univariate models uncovered further patterns: 1) width optima of hind limb bones tend to be narrower in bats and gliders compared to arboreal mammals (Fig. 4f-j), and 2) there was no pattern from arboreal to glider to bat in the hind limb length optima. Univariate modeling revealed that the widths of hind limb bones tend not to differ between bats and gliders (Fig. 4f-j) because the widths of hind limb bones in bats and gliders exhibited either a single optimum (i.e., OUM_{loc3a}) separate from ground-dwelling and arboreal mammals (Fig. 4 f, i, j) or an overlap in confidence intervals of optima between bats and gliders (Fig. 4 g, h). This shared bat-glider adaptive zone suggests that bats and gliders exhibit narrower hind limb optima, especially in the autopod region (Fig. 4f-j). In contrast, univariate modeling revealed distinct optima in long bone lengths of the hindlimb among the four locomotor regimes, in which gliders exhibited relatively longer femoral and tibial optimal lengths compared to bats, arborealists, and ground-dwellers (Fig. 4b-c). Unlike bat forelimbs, bat long bones in the hindlimb (i.e., the femur and tibia) are not the longest compared to the other locomotor regimes; in fact, the confidence intervals of optimal lengths largely overlap between bats and arborealists (Table S3b). Furthermore, bats exhibit relatively shorter metatarsals but relatively longer pedal intermediate phalanges compared to all other locomotor regimes, whereas these autopodial lengths do not differ between gliders and

arborealists (Fig. 4d-e). The different evolutionary patterns of hindlimb bones demonstrate the unique locomotor diversity among the four regimes. Although gliders and bats are both aerial, these behaviors require different functional morphologies. In gliders, relatively longer hindlimbs are needed to increase the surface area of the patagium in tandem with relatively longer forelimbs to generate lift during gliding (Thorington and Heaney 1981). In contrast, in bats, the relative lengthening of the forelimb supports the majority of the wing membranes whereas the hindlimb, specifically the ankle, serves as an anchor to the wing membrane. Bat hindlimbs are still used for flight by helping modulate lift, drag, or pitch (Cheney et al. 2014).

Overall, gliders appear to be evolving relatively lengthened hindlimb bones while bats are evolving more gracile hindlimb bones. This finding, while in opposition to our predictions, may point to the fact that modern gliders are completely distinct evolutionarily and potentially even morphologically from the gliding ancestors of bats. These results may highlight the pitfalls of modeling modern gliders as proxies for ancestral ones instead of invalidating our hypothesis that bats evolved from ancestral gliders.

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. 2a). 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 overlap clearly with the morphospace of non-bats, a higher proportion compared to that of extant 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, *Onychonycteris 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 placement of these species in morphospace leaves many possibilities open for ancestral locomotor behaviors.

Including fossil bats in our model-fitting analyses as the closest morphologies we have to bat-glider intermediaries did not significantly alter estimations of the adaptive zone between bats and gliders; the inclusion of fossils appeared to make the bat-glider trait optima marginally more similar for the forelimbs and had no change for the hind limbs (Table S7). Fossil addition to the multivariate model-fitting analysis shifted support from $mvOUM_{loc3b}$ to $mvOUM_{loc4}$, suggesting that the glider optimum is potentially distancing itself from arborealists' and towards bats' (Table S6a). Unfortunately, we can only speculate about this result because the optima resist comparison to extant-only $mvOUM_{loc3b}$ optima due to their extremely negative values, which represent unrealistic morphologies (Table S6b). Fossil addition to univariate forelimb model-

fitting analyses yielded overall slightly more support for OUM_{loc3a} models compared to extant-only modeling (Table S4a). Fossil addition to hind limb model-fitting analyses yielded overall no change in support for the $mvOUM_{loc4}$ model or OUM_{loc3a} models compared to extant-only modeling (Table S6, S4b). Regardless, 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 S5).

Limb trait optima and the adaptive landscape

Our results provide insight on the potential adaptive landscape connecting arboreal, gliding, and flying locomotion, which is represented in Figure 5 with the z-axis describing the qualitative summation of optima from the univariate model results. Overall, we found that glider forelimb optima fall intermediate between those of arborealists and bats in their lengths but only partially in their widths. Except for long bone lengths, glider hind limb optima are intermediate between ground-dweller/arborealist and bat optima or even overlap with bat optima in their lengths and widths. Together, these results emphasize that selection on key length/width traits may be “pulling” glider traits towards a flying adaptive zone yet that certain glider traits (e.g., femur and tibia lengths) are derived specifically for gliding morphology. 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 (species retain unique morphologies even while evolving some similarities; Leal et al. 2002; Stayton 2006). For gliders, there is 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. 2a) may represent a narrow

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 extant glider lineages, dermopterans (colugos) and anomalurids (scaly-tailed squirrels), have evolved farthest from ancestral arborealists (Grossnickle et al. 2020; although some extinct gliders may have experienced relatively greater change from ancestral arborealists, e.g., Meng et al. 2017; Luo et al. 2017). Here, the dermopteran species *Cynocephalus volans* and *Galeopteras variegatus* are the gliders closest to the bat forelimb morphospace (Fig. 3a; Fig. S1a), 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), reflects hypotheses that interdigital webbing was key to evolving powered flight (Anderson and Ruxton 2020 but see Gardner and Dececchi 2022 for an alternate view).

Our results also suggest that both bats and gliders exhibit broad adaptive optima in the hind limb due to gliders sharing an optimum or overlapping optima with bats, or being intermediate between non-bats and bats for many traits (Fig. 2d, Fig. 4). These broad adaptive optima could represent overlapping peaks for gliders and bats separated only by a shallow fitness valley driven by the gliders' elongated femur and tibia lengths (Fig. 5b). The hypothetical adaptive landscape is therefore valuable because the transition from quadrupedalism to hind limb suspension has been hypothesized to enable forelimbs to evolve more freely since they would no longer be weight-bearing structures (Bishop 2008). Indeed, 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). In the PC1 and PC2 hind limb morphospace, *G. variegatus* is the only glider that overlaps with the bat morphologies (Fig. 2b; 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, to complete the tentative adaptive landscape presented here (Fig. 5), these results could be strengthened by more comprehensive coverage of the lengths/widths across more mammal species and biomechanical studies tying these traits to specific functions and selective pressures.

Conclusions

Our findings complement earlier work investigating the evolution of bat powered flight by testing the selective regimes acting on bone dimensions across the mammalian forelimb and hind limb. Our results reveal that the relatively lengthened forelimbs in gliders are intermediate to those of bats and arborealists, whereas bats and gliders exhibit similarly relatively narrower hind limbs compared to arborealists. 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.

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.

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.

Anthwal, N., D. J. Urban, A. Sadier, R. Takenaka, S. Spiro, N. Simmons, R. R. Behringer, C. J. Cretekos, J. J. Rasweiler, and K. E. Sears. 2023. Insights into the formation and diversification of a novel chiropteran wing membrane from embryonic development.

BMC Biology 21:101.

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.

Boettiger, C., G. Coop, and P. Ralph. 2012. Is your phylogeny informative? Measuring the power of comparative methods. Evolution 66:2240–2251.

Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. Am. Nat. 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.

Cheney, J. A., N. Konow, K. M. Middleton, K. S. Breuer, T. J. Roberts, E. L. Giblin, and S. M. Swartz. 2014. Membrane muscle function in the compliant wings of bats. Bioinspir. Biomim. 9:025007. IOP Publishing.

Clavel, J., G. Escarguel, and G. Merceron. 2015. mvMORPH:anR package for fitting multivariate evolutionary models to morphometric data. Methods Ecol Evol 6:1311–1319.

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.

Feigin, C. Y., J. A. Moreno, R. Ramos, S. A. Mereby, A. Alivisatos, W. Wang, R. van Amerongen, J. Camacho, J. J. Rasweiler, R. R. Behringer, B. Ostrow, M. V. Plikus, and R. Mallarino. 2023. Convergent deployment of ancestral functions during the evolution of mammalian flight membranes. Science Advances 9:eade7511. American Association for the Advancement of Science.

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.

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.

Leal, M., A. K. Knox, and J. B. Losos. 2002. Lack of Convergence in Aquatic Anolis Lizards. Evolution 56:785–791.

Linden, T. J., A. E. Burtner, J. Rickman, A. McFeely, S. E. Santana, and C. J. Law. 2023. Scaling patterns of body plans differ among squirrel ecotypes. PeerJ 11:e14800. PeerJ Inc.

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.

Luo, Z.-X., Meng, Q.-J., Grossnickle, D. M., Liu, D., Zhang, Y.-G., Neander, A. I., and Q, Ji. 2017. New evidence for mammaliaform ear evolution and feeding adaptation in a Jurassic ecosystem. Nature. 548: 326-329 (doi:10.1038/nature23483).

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.

Meng, Q.-J., Grossnickle, D. M., Liu, D., Zhang, Y.-G., Neander, A. I., Ji, Q., and Z.-X. Luo. 2017. New gliding mammaliaforms from the Jurassic. Nature. 548: 291-296. (doi:10.1038/nature23476).

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. Chapter 8. Flying, Gliding, and Soaring. Pp. 148–152 *in* M. Hildebrand, D. M. Bramble, K. F. Liem, and D. B. Wake, eds. Functional Vertebrate Morphology. Harvard University Press.

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.

Papadimitriou, H. M., S. M. Swartz, and T. H. Kunz. 1996. Ontogenetic and anatomic variation in mineralization of the wing skeleton of the Mexican free-tailed bat, Tadarida brasiliensis. Journal of Zoology 240:411–426.

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.

Rickman, J., A. E. Burtner, T. J. Linden, S. E. Santana, and C. J. Law. 2023. Size And Locomotor Ecology Have Differing Effects on the External and Internal Morphologies of

Squirrel (Rodentia: Sciuridae) Limb Bones. Integrative Organismal Biology 5:obad017.

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.

Stayton, C. T. 2006. Testing hypotheses of convergence with multivariate data: morphological

and functional convergence among herbivorous lizards. Evolution 60:824–841.

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.

Swartz, S. M. 1997. Allometric patterning in the limb skeleton of bats: Implications for the mechanics and energetics of powered flight. J Morphol 234:277–294.

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., K. Darrow, and C. G. Anderson. 1998. Wing Tip Anatomy and Aerodynamics in Flying Squirrels. Journal of Mammalogy 79:245–250. [American Society of Mammalogists, Oxford University Press].

.

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.

Zhao, F., W. Wang, J. Zhang, J. Wyrwa, and F. Sun. 2019. Aerodynamic Characteristics and Pitching Adjusting Mechanism of the Flying Squirrel With Deployed Patagium. IEEE Access 7:185554–185564.

Table 1: AICcW scores from multivariate model-fitting analyses (via mvMORPH). Comparisons of the fits of multivariate models to the first three principal components of the a) forelimb dataset and b) hind limb dataset.

a) forelimb	AIC	ΔAICc	mAICcW	**b) hind limb**	AIC	ΔAICc	mAICcW
mvBM1	-94.42	225.50	0.00	mvBM1	-134.64	230.05	0.00
mvOU1	-248.73	71.20	0.00	mvOU1	-330.72	33.97	0.00
$mvOUM_{loc3a}$	-306.82	13.10	0.00	$mvOUM_{loc3a}$	-358.41	6.28	0.04
$mvOUM_{loc3b}$	**-319.93**	**0.00**	**0.99**	$mvOUM_{loc3b}$	-353.51	11.17	0.00
$mvOUM_{loc3c}$	-308.77	11.16	0.00	$mvOUM_{loc3c}$	-358.10	6.59	0.03
$mvOUM_{loc4}$	-302.35	17.57	0.00	**$mvOUM_{loc4}$**	**-364.69**	**0.00**	**0.92**

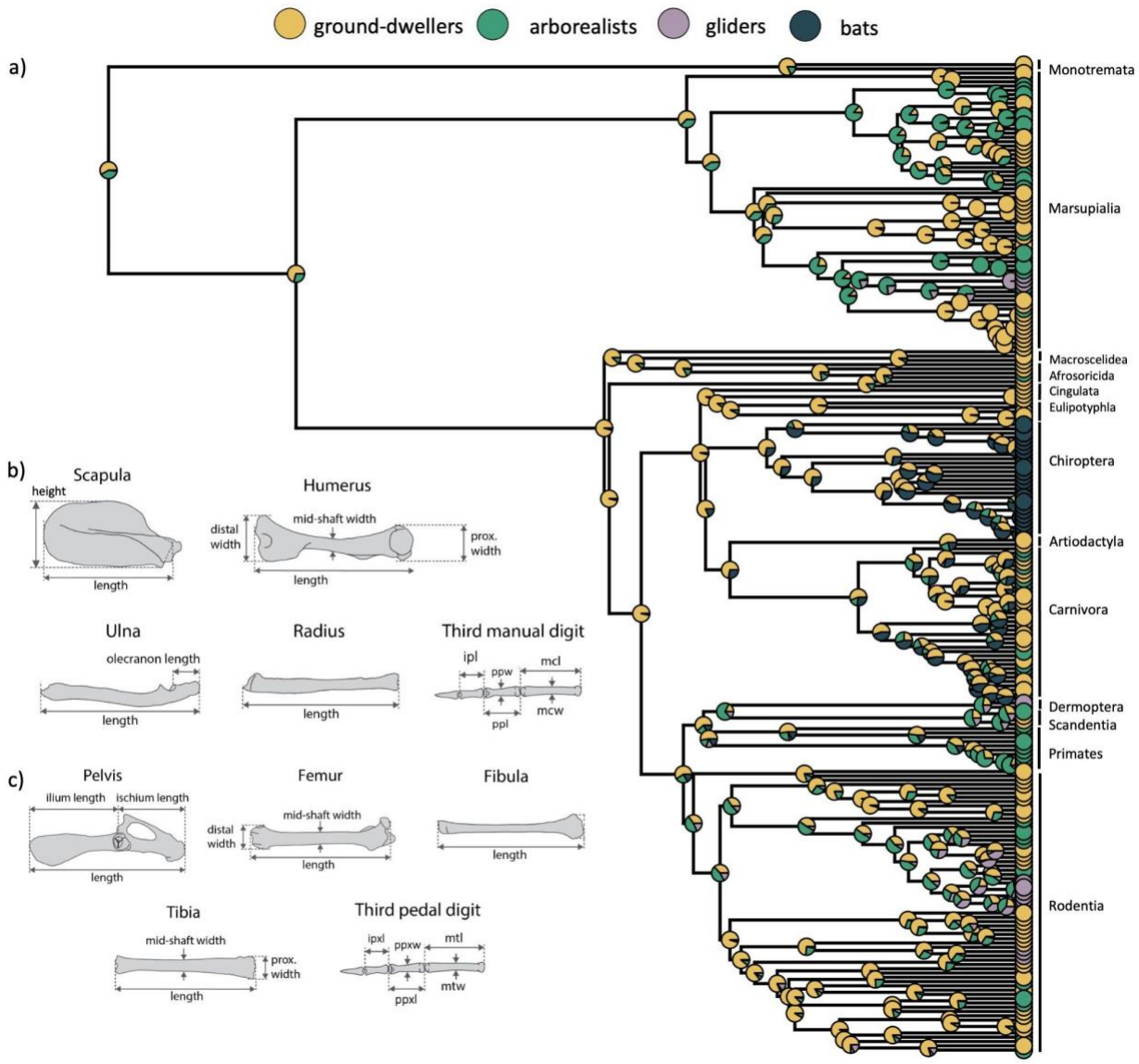

Figure 1. Morphological measurements and phylogeny. We conducted analyses based on this a) phylogeny (Upham et al. 2019) across b) 14 forelimb linear measurements for each specimen of scapula height and length (sh, sl), humerus length (hl), distal width (hdw), proximal width (hpw), and mid-shaft width (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.

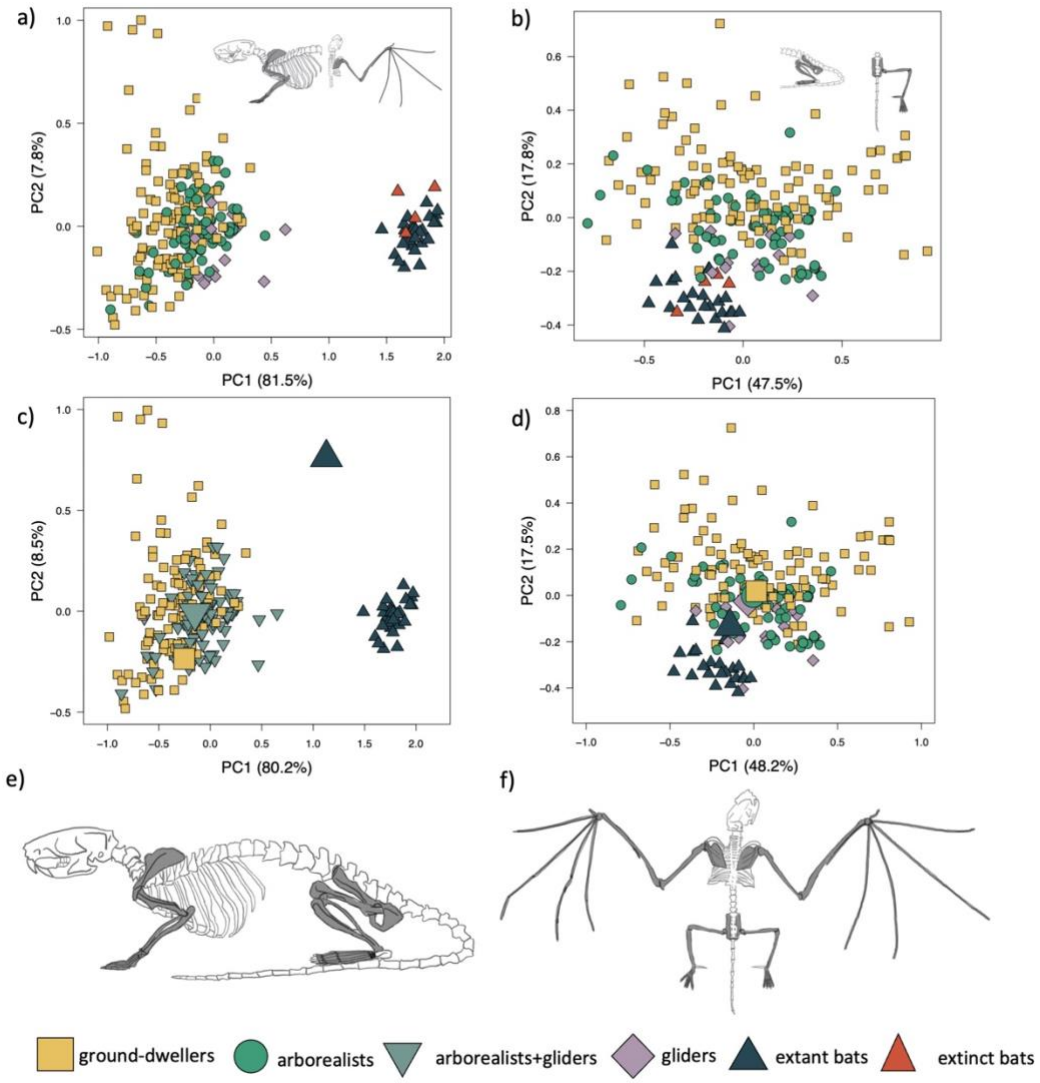

Figure 2. Forelimb and hind limb PCAs. Morphospaces consist of PC1 and PC2 of 14 forelimb traits (left panels) and 15 hind limb traits (right panels). The extant+extinct morphospace is shown for the a) forelimbs and b) hind limbs. The extant-only morphospace of c) the $mvOUM_{loc3b}$ optima (large icons) overlaid on the forelimb morphospace and the d) the $mvOUM_{loc4}$ optima (large icons) overlaid on the hind limb morphospace. Cartoons of a e) quadrupedal mammal skeleton (*Rattus norvegicus*) versus a f) bat skeleton (*Pipistrellus abramus* are shown where dark gray shaded bones on the cartoon skeletons correspond to those measured to generate the morphospace.

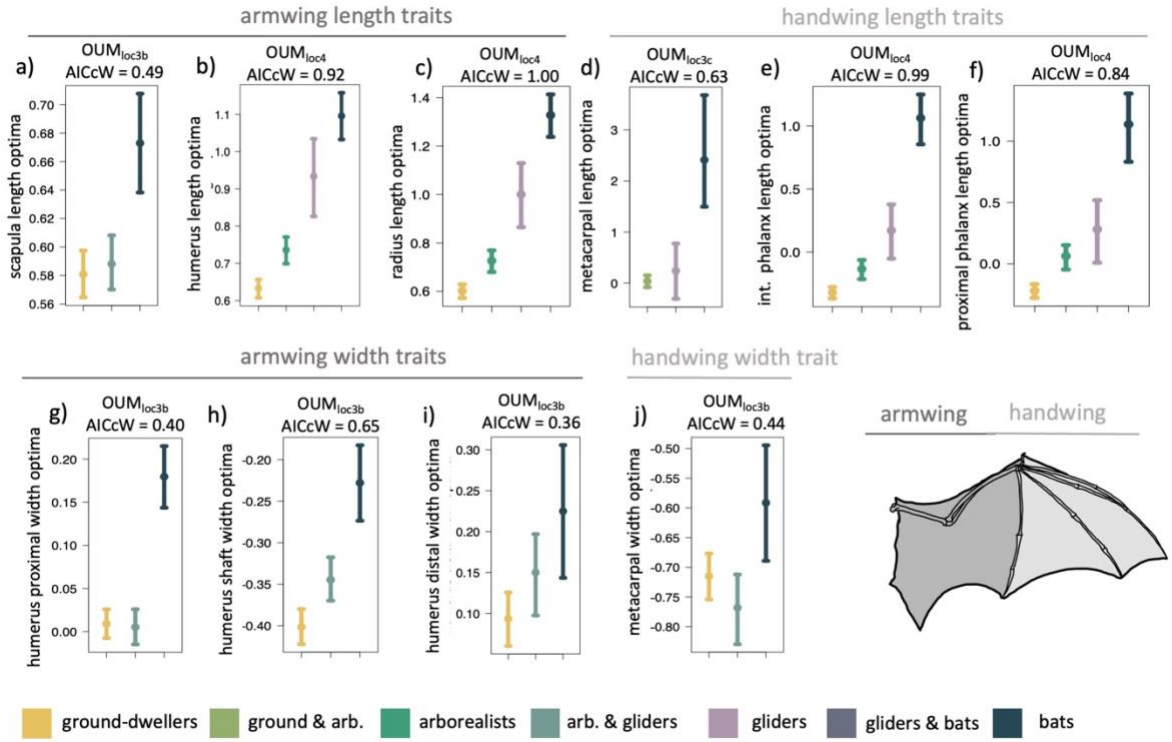

Figure 3. Forelimb trait optima from model-fitting of univariate traits. Forelimb OUM optima (θ) as a size-corrected trait value (log-shape ratio) with 95% confidence intervals (Tables S3a) for the best-fitting models (ΔAIC = 0) for 10 traits. The AICcW of the model is on top of each panel (Table S2a).

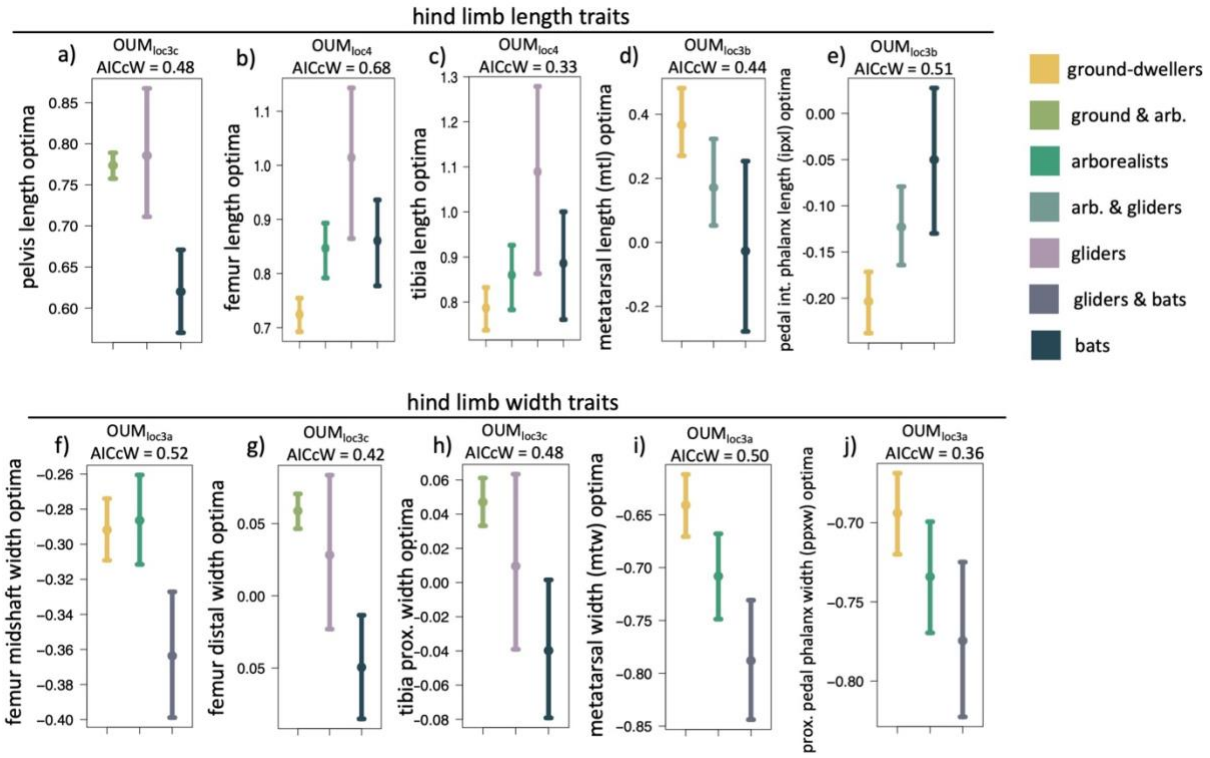

Figure 4. Hind limb trait optima from model-fitting of univariate traits. Hind limb OUM optima (θ) as a size-corrected trait value (log-shape ratio) with 95% confidence intervals (Tables S3b) for the best-fitting models (ΔAIC = 0) for 10 traits. The AICcW of the model is on top of each panel (Table S2b).

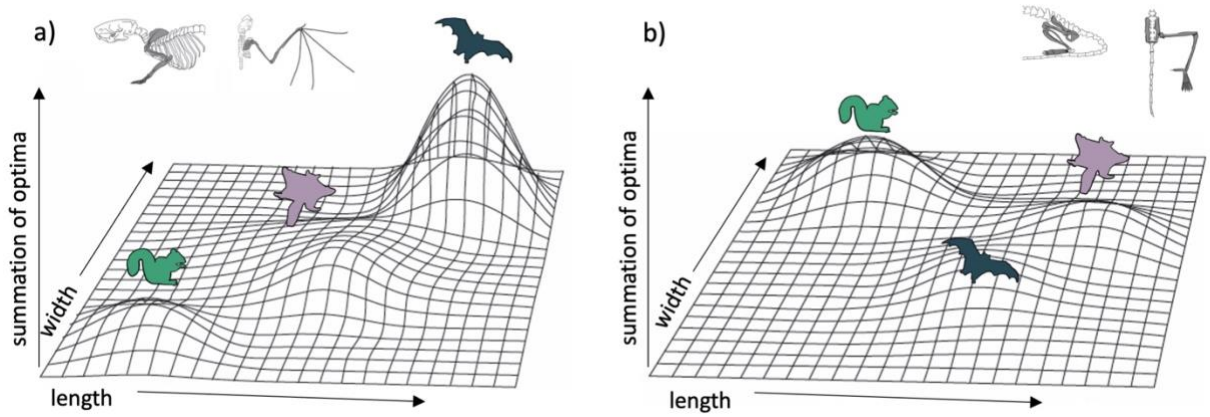

Figure 5. Hypothetical adaptive landscapes of forelimb and hind limb skeletal relative length and width evolution. Hypothetical fitness peaks are shown for arborealists, gliders, and bats for a broad summary of our conclusions from the skeletal trait optima results discussed. The "length" and "width"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