

Integration and dissociation of limb elements in flying vertebrates: a comparison of pterosaurs, birds and bats

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Abstract

Flapping flight has evolved independently in three vertebrate clades: pterosaurs, birds and bats. Each clade has a unique flight mechanism involving different elements of the forelimb. Here, patterns of limb integration are examined using partial correlation analysis within species and matrix correlation analysis across species to test whether the evolution of flapping flight has involved developmental dissociation of the serial homologues in the fore- and hind limb in each clade. Our sample included seven species of birds, six species of bats, and three species of pterosaurs for which sufficient sample sizes were available. Our results showed that, in contrast to results previously reported for quadrupedal mammals, none of the three clades demonstrated significant integration between serial homologues in the fore- and hind limb. Unexpectedly, there were few consistent patterns of within-forelimb correlations across each clade, suggesting that wing integration is not strongly constrained by functional relationships. However, there was significant integration within the hind limbs of pterosaurs and birds, but not bats, possibly reflecting the differing functions of hind limbs (e.g. upright support vs. suspension) in these clades.

Introduction

The evolution of flapping flight is regarded as a key innovation that has led to the outstanding success and longevity of several vertebrate groups, allowing them to explore and exploit new ecological niches. This ability has evolved independently in three vertebrate clades in the last 200 million years. These clades are the bats, birds and extinct pterosaurs, which died out during the Cretaceous – Paleogene boundary extinction event approximately 65 million years ago (Padian, 1985; McGowan & Dyke, 2007). Each of these three clades has a distinct approach to flapping flight using different skeletal elements of the forelimb (Fig. 1).

Pterosaurs date from the Late Triassic and were the first true flying vertebrates (Padian, 1985; Wellnhofer, 1991;

Unwin, 2006). They have been historically divided into two groups: the paraphyletic long-tailed ‘Rhamphorhynchoidea’ (now termed nonpterodactyloid pterosaurs) and the monophyletic short-tailed Pterodactyloidea (Howse, 1986). The pterosaur wing was membranous and supported by an elongated fourth digit or wing finger (Cuvier, 1801; Marsh, 1882; von Zittel, 1882). They are presumed to have lost their fifth digit, and their first three digits were used as grasping claws (Fig. 1a) (Padian, 1984). Pterosaurs are usually portrayed as quadrupedal animals with relatively weak hind limbs and varying amounts of terrestrial or arboreal capability (Unwin, 2006; Witton & Habib, 2010). However, the condition of the hind limbs is a topic of controversy, with some authors suggesting that pterosaurs were capable of upright bipedality (Padian, 1983a), while others contend that terrestrial locomotion was only possible through quadrupedality incorporating the forelimbs, as is supported by most trackway evidence (Wilkinson, 2008).

In contrast to the condition in pterosaurs, the flight surface of the modern bird (Neoornithes) wing is formed

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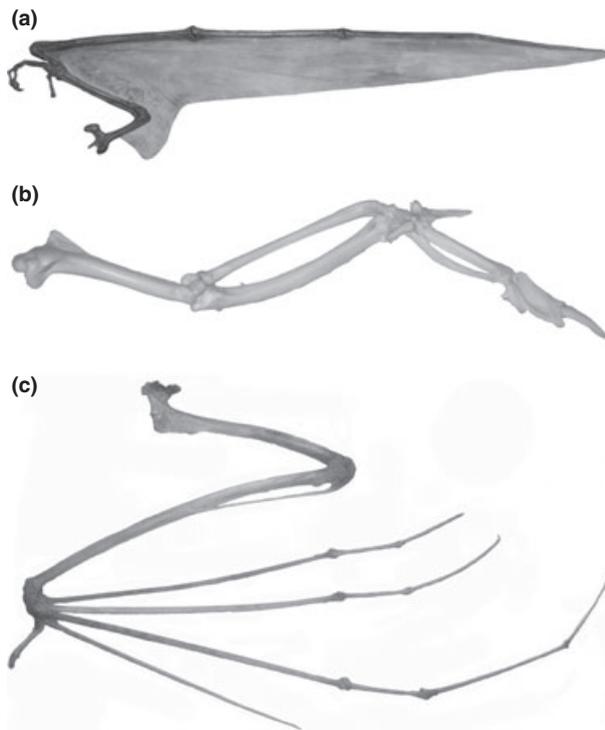


Fig. 1 A diagrammatic comparison of the forelimbs of the three clades of flying vertebrates: (a) pterosaurs, (b) birds and (c) bats shown in a ventral view. Not to scale.

from long feathers that are anchored to the skin along the posterior border of the humerus, ulna and hand (Liem *et al.*, 2001; Pough *et al.*, 2009). The forelimb is highly modified with many bones in bird wings fused, and only the first, second and third digits remaining (Fig. 1b) (Liem *et al.*, 2001). The fourth and fifth digits fused to form the long carpometacarpus bone (Liem *et al.*, 2001). The first digit, the alula, has a small tuft of feathers and can move independently to vary lift during different stages of flapping flight (Liem *et al.*, 2001).

The hind limbs of birds have also undergone significant modifications. Most modern birds have anisodactyl feet, the plesiomorphic condition for modern birds in which three toes point forwards and one behind (Lockley *et al.*, 2007). The metatarsals are fused with the distal tarsal forming a structure called the tarsometatarsus, and the proximal tarsal bones and tibia have fused to form the tibiotarsus (Pough *et al.*, 2009). This form has been modified in many lineages; some lineages have evolved zygodactyly or heterodactyly, in which two toes are forward facing and two are backwards, while others have all four toes facing forward, called pamprodactyly (Lockley *et al.*, 2007). Still others have webbed feet, whether partially webbing the front three digits (semipalmate), fully webbing the front three digits (palmate), or with four fully webbed forward facing digits (totipalmate) (Van Tuinen *et al.*, 2001).

Bats are the only mammals to have developed true flapping flight and have done so through a number of morphological specializations in the forelimb, including elongation of skeletal elements of the wing as well as the reduction of the ulna, which helps in reducing the overall weight of the animal (Sears, 2007). Bat wings are membranous but are supported by the four elongated digits unlike the single wing digit of pterosaurs (Altringham, 1996; Liem *et al.*, 2001). The first digit is free from the membrane to function as a grasping claw (Fig. 1c) used for climbing and grooming (Altringham, 1996; Liem *et al.*, 2001). Elongation of bones is most profound in the third, fourth and fifth digits (Fig. 1c) which are the primary supporting elements (Sears *et al.*, 2006; Sears, 2007). The wing membrane is flexible (Sears, 2007) and stretches over the digits attaching to the side of the body and hind limb (Liem *et al.*, 2001). The membrane acts as an aerofoil for flapping flight, but limits terrestrial locomotion because it incorporates the hind limb (Sears, 2007).

Limbs as serially homologous structures and their development

Serially homologous structures have a fundamental similarity in different organisms as a result of common developmental history (Young & Hallgrímsson, 2005; Schmidt & Fischer, 2009; Young *et al.*, 2010). They occur when a developmental program for a structure is duplicated and expressed in multiple locations, as has happened in the evolution of tetrapod limbs. The presence of four limbs at defined points along the body is a general feature of the tetrapod body plan, although some burrowing and swimming tetrapods have reduced or lost some or all of their limbs (Tickle, 2002). Tetrapod limbs have the same basic plan, being divided into three segments in the fore- and hind limbs (Table 1), with the corresponding segments in each limb representing serial homologues (Tickle, 2002): the proximal stylopod (humerus, femur), middle zeugopod (radius/ulna, tibia/fibula) and the distal autopod (manus, pes) (fig. 1b in Young & Hallgrímsson, 2005). As serial homologues, fore- and hind limbs share the many of the developmental pathways that regulate growth and positioning of limbs along the body (Young & Hallgrímsson, 2005; Gilbert, 2006).

Morphological integration

Morphological integration refers to the relationships among morphological elements that results from the interaction of biological processes, such as development or function (Marroig & Cheverud, 2001; Klingenberg, 2008) and can be identified by quantifying patterns of covariation or correlation among multiple traits (Goswami & Polly, 2010b). Serial homologues may display integration that reflects their shared developmental

Table 1 Skeletal elements measured from the stylopod, zeugopod and autopod of pterosaur, birds and bats species.

	Stylopod	Zeugopod	Autopod
Pterosaurs			
Forelimb	Humerus	Radius, ulna	Metacarpal IV, phalanges 1–4 of digit IV
Hind limb	Femur	Tibia (partial autopod)	Metatarsal III
Birds			
Forelimb	Humerus	Radius, ulna	Ulnare, radiale, first digit, carpometacarpus, 2nd digit phalanx 1 and 2nd and 3rd digit phalanx 1
Hind limb	Femur	Tibiotarsus (partial autopod)	Tibiotarsus (partial zeugopod), tarsometatarsus
Bats			
Forelimb	Humerus	Radius, ulna (if not fused)	Metacarpals and phalanges of digits 1–5
Hind limb	Femur	Tibia, fibula	Metatarsals digits 1–5

history. Such relationships can be conserved over the course of evolution, as has been demonstrated in the limbs of quadrupedal mammals (Young & Hallgrímsson, 2005; Schmidt & Fischer, 2009; Bennett & Goswami, 2011; Kelly & Sears, 2011).

In the first comparative study of limb covariation, Young & Hallgrímsson (2005) conducted a morphometric study to investigate serial homology and patterns of limb covariation structure in six species of placental mammals including rodent, primate, bat and carnivoran species. Their results demonstrated a common pattern of limb covariation across taxa but reduced covariation between the limbs of nonquadrupedal species, most noticeably in the bat, likely reflecting the divergent functions of the fore- and hind limbs. This result supported the limb integration model suggested by Hallgrímsson *et al.* (2002) that predicted specialization of limbs would favour the reduction of covariation between limbs (Young & Hallgrímsson, 2005). A reduction in covariation could allow selection to act separately on fore- and hind limb morphology and thus promote the evolution of tetrapod limb disparity (Hallgrímsson *et al.* (2002)).

Bennett & Goswami (2011) followed up on Young & Hallgrímsson's 2005 study by investigating whether differences in developmental strategies are reflected in limb integration in marsupials and monotremes. Their results indicated that covariations were high only among forelimb elements within diprotodontid marsupials, whereas monotremes only showed significant covariation between serially homologous structures, i.e. between the limbs, rather than within limbs. The high covariation within forelimb elements and lack of significant covariation between fore- and hind limb elements of diprotodontid marsupials might relate to their early mechanical requirements to crawl into the pouch (Bennett & Goswami, 2011). Their results also showed

that monotremes are notably different from other mammals, likely also reflecting unusual heterochronic shifts in skeletal ossification (Weisbecker, 2011). A similar study focusing on limb integration in New World marsupials found concordant results, with marsupials showing greater within limb integration and less integration between limbs than observed in placentals or monotremes (Kelly & Sears, 2011).

In another study, Schmidt & Fischer (2009) carried out correlation analyses on the limbs of 189 mammalian species. They suggested that higher covariation observed among elements of the hind limb was due to the common demand of propulsion, while the dissociation between limbs and the lower covariation within forelimbs was ascribed to functional divergence of forelimb roles among the different taxa in their sample (Schmidt & Fischer, 2009). This observed dissociation between limbs and the lower forelimb integration relative to the hind limb is of interest because the hypothesized primitive tetrapod condition is strong integration between serially homologous developmental modules, which could constrain limb variation (Wagner & Altenberg, 1996; Goswami & Polly, 2010a; Young *et al.*, 2010). Therefore it has been hypothesized that during the evolutionary history of organisms with functionally divergent limbs the integration between fore- and hind limb serial homologues was reduced, allowing independent selection on the limbs and an observable shift in the patterns of element covariation (Young *et al.*, 2010).

Here, we test whether the evolution of flapping flight, which requires extreme functional divergence of the fore- and hind limbs, has resulted in the dissociation of the limbs in pterosaurs, birds and bats, as has been previously hypothesized for birds (Gatesy & Dial, 1996). As each of these clades has independently evolved flapping flight, we have three separate tests of whether functional divergence of the limbs necessitates a breakdown in limb integration, specifically between serially homologous structures (Table 1). Furthermore, we can assess whether the changes in the forelimb skeleton within each clade, described above, have affected their respective patterns of forelimb integration. Specifically, we can identify whether elements with an important functional role in the support of the wing show stronger integration with each other than with elements with a reduced functional role in the wing. To quantify patterns of integration within and between the fore- and hind limbs, we conduct partial correlation analyses, separately for each species. We then assess whether patterns of limb integration are conserved within each clade by using matrix correlation analysis to compare the similarity of limb element correlations across all of the sampled species in each clade.

Between limbs

Pterosaurs, birds and bats have morphologically and functionally divergent forelimbs. Therefore, in contrast to results for quadrupedal mammals, it is hypothesized that

there will not be significant correlation between serial homologues (Table 1) in each limb in any of these clades, consistent with the previous analysis of bats (Young & Hallgrímsson, 2005) and previous hypotheses for birds (Gatesy & Dial, 1996).

Within limbs

While we hypothesize low between-limb correlations, we expect that each of these three clades will show strong within limb correlations, which we will assess with partial correlation analyses. Because species within each of these three clades share a unique wing morphology and structure, we further expect that species within each clade will show a common pattern of forelimb integration, which we test with matrix correlation analysis. As hind limbs are highly integrated in most mammals (Schmidt & Fischer, 2009), we hypothesize that they will be similarly significantly integrated in the taxa studied here.

Methods and materials

Specimens

Species with significant samples were chosen from each of the three volant clades to sample the maximum possible diversity of each clade. Data was collected from specimens housed at the Natural History Museum in London, Natural History Museum at Tring, Grant Museum of Zoology, University College London and Oxford University Museum of Natural History. Data for pterosaurs was obtained from an existing source of length measurements (Andres, 2010).

Pterosaurs

Species were selected based on the numbers of specimens and representation of skeletal elements from an existing database of morphometric data. Pterodactyloid and non-pterodactyloid pterosaur species were included to provide a broad sample of pterosaur phylogeny. Two nonpterodactyloid species, *Rhamphorhynchus muensteri* ($n = 14$), *Dorygnathus banthensis* ($n = 10$), and one pterodactyloid species, *Pterodactylus kochi* ($n = 9$), were used.

Birds

A recent robust molecular phylogeny for 169 species of birds (Hackett *et al.*, 2008) was used to select a representative sample of avian diversity. Data was collected for seven bird species from the following avian orders: Passeriformes, *Emberiza citrinella* (Yellowhammer; $n = 30$); Falconiformes, *Accipter nisus* (Eurasian Sparrowhawk; $n = 31$); Charadriiformes, *Vanellus vanellus* (Northern Lapwing; $n = 19$); Pelecaniformes, *Phalacrocorax aristotelis* (European Shag; $n = 21$); Apodiformes, *Apus apus* (Common Swift; $n = 27$); Columbiformes, *Columba palumbus* (Common Woodpigeon; $n = 30$); and Anseriformes, *Somateria molissima* (Common Eider; $n = 16$).

As noted above, birds vary in their hind limb morphology, with most having the plesiomorphic anisodactyl foot. Our dataset includes taxa with that most common morphology (*E. citrinella* and *C. palumbus*), as well as pamprodactyl (*A. apus*), semipalmate (*V. vanellus*), palmate (*S. molissima*), and totipalmate (*P. aristotelis*) forms.

Bats

Teeling *et al.* (2005) generated a highly resolved molecular phylogeny for the traditional extant bat families, resulting in two large clades (Yinpterochiroptera and Yangochiroptera) and four smaller clades of traditional families (Rhinolophoidea, Emballonuroidea, Noctilionoidea and Vespertilionoidea). We collected data for five bat species and attempted to represent each of the four smaller clades. However, a bat species from within the Emballonuroidea could not be included in this study due to limited specimens. The species included were as follows: Rhinolophoidea; *Rhinolophus ferrumequinum* (Greater Horseshoe Bat; $n = 14$); two species from within Noctilionoidea: Phyllostomidae; *Anoura caudifera* (Tailed Tailless Bat; $n = 9$), and Mormoopidae; *Pteronotus quadridens* (Sooty Moustached Bat; $n = 7$); one species from within the Vespertilionoidea; *Nyctalus noctula* (Common Noctule; $n = 7$); and one from Pteropodidae, *Pteropus giganteus* (Indian Flying Fox; $n = 8$).

Data collection

Data from the stylopod, zeugopod and autopod was obtained from the forelimbs of all specimens from the three clades. Full hind limb skeletal elements were not available for some species due to incomplete specimens. Thus bird specimens contained hind limb data of only the stylopod and zeugopod, whereas bats and pterosaurs had data from stylopod, zeugopod and autopod (Table 1). The landmarks for pterosaur specimen measurements in Andres (2010) were taken from Bennett (2001) and obtained using Mitutoyo 200 mm callipers. Length measurements of forelimb and hind limb skeletal elements of the birds and bats were obtained using LiMiT[®] 150 mm callipers. Landmarks for birds and bats were first selected for each bone in the limbs to measure the interlandmark distance. Positions were kept as comparable as possible across all species. The greatest length of each bone was measured. Each skeletal element was measured three times and averaged using JMP statistical software (version 5.0.1a, SAS Institute Inc., Cary, NC, USA).

Proximal forelimb and hind limb landmarks in all bird species were taken according to Von Den Driesch's guide to the measurement of animal bones from archaeological sites (humerus: fig. 54; radius: fig. 55; ulna: fig. 56c; carpometacarpus: fig. 57b; digit II: fig. 58; femur: fig. 60a; tibiotarsus: fig. 61a; tarsometacarpus: fig. 62b. Von Den Driesch, 1976). Landmarks for measuring distal forelimb

elements (radiale, ulnare, first digit phalange 1, second digit phalanx 2 and third digit phalanx 1) were not included in Von Den Driesch (1976) and are as shown in Figure S1. Landmarks for bat forelimb and hind limb skeletal elements are shown in Figure S2. Landmarks for pterosaur forelimb and hind limb elements are shown in Figure S3.

Statistical analysis

Covariance matrix repeatability

Because limb integration reflects genetic and developmental relationships among elements, it is important to identify patterns of integration independently for a single species, if not for a single population, and thus sample sizes become a key limiting factor for analyses. Prior to analysis, we assessed the strength of our datasets using covariance matrix repeatability. For the forelimb, hind limb and between forelimbs and hind limbs in each species, the covariance matrix repeatability was assessed for both raw and log-transformed data using a Monte Carlo simulation with 10 000 replicates in Poptools (version 2.7.5) (Hood, 2006). Covariance matrix repeatability measures the likelihood that the same covariance matrix would be identified from a different sample of the same population, by repeatedly resampling the original dataset and generating a mean vector correlation between the original and resampled datasets (Goswami & Polly, 2010b).

Partial correlation analysis

Patterns of limb correlation were examined separately in each species using partial correlation analysis carried out using multivariate methods in JMP (version 5.0.1a, SAS Institute Inc.). Partial correlation analysis measures the strength of association between two variables whilst controlling for the effects of the other variables within the data matrix making it well suited for complex systems such as the appendicular skeleton (Marroig & Cheverud, 2001; Young & Hallgrímsson, 2005). For simplicity, results reported below are from partial correlation analyses of untransformed data only, but results of analyses using log-transformed data are consistent and do not change the interpretation.

To test the significance of partial correlation results, a theoretical measure known as the edge exclusion deviance (EED) was calculated (Magwene, 2001; Young & Hallgrímsson, 2005):

$$\text{EED} = N \ln \left(1 - \rho_{ij\{K\}}^2 \right),$$

where N is the number of specimens and $\rho_{ij\{K\}}$ is the partial correlation of the variables i and j with the other variables in the data set held constant $\{K\}$ (Magwene, 2001). The value of each partial correlation between two variables is tested against the χ^2 -distribution with 1 degree of freedom (Magwene, 2001). Any EED value less

than 3.84 (corresponding to $P = 0.05$, d.f. = 1, from the χ^2 -distribution) is considered to be nonsignificant, meaning that the two variables in question are statistically independent from one another (Magwene, 2001).

Partial correlation analysis was conducted within the forelimb, within the hind limb, and between the forelimbs and hind limbs of each species. A number of specimens were excluded prior to data analysis because key skeletal elements were missing. However, because partial correlation analysis will not work with singular matrices, it is imperative that the number of specimens exceeds the number of variables being analysed (in this case, skeletal elements). To maximize the number of specimens that could be used, skeletal elements were also removed if they were unavailable for the majority of specimens within each species. This was the case among a number of bird species, as such analyses were conducted to include either all available elements or to maximize specimen or element number. In each case details of the number of specimens and elements used in any analysis is detailed in results and in all supplementary tables and results.

Due to limited availability of bat specimens, partial correlation analyses were conducted in two halves, firstly, among proximal skeletal elements of the stylopod and zeugopod and secondly among distal elements of the autopod. The phalanges of all bat species digits could not be included within any analyses.

Paucity of bat specimens is primarily due to preferred preparation methods; most museum specimens preserving wings with the membrane intact, making direct measurement of bony elements problematic and thus limiting specimen availability for this project.

Matrix correlation analysis

Species-specific correlation matrices incorporating all available elements were generated, using Pearson's product moment correlation coefficient and Fisher's z -transformation to correct for sample size effects in PALaeontological STATistics (PAST) (version 1.99) (Hammer *et al.*, 2001). These species-specific correlation matrices were then subjected to matrix correlation analysis and Mantel's test to identify significant similarity in limb integration among species. First, fore- and hind limb skeletal elements were analysed and second, between elements of both fore- and hind limb were analysed together. Significance of the matrix correlation above the 95% confidence interval was computed using Mantel's test with 10 000 replicates in Mathematica 7.0 (Wolfram Research Inc., Champaign, IL, USA). Mantel's test is a permutation test that assesses the similarity between two matrices against random expectation by holding one matrix constant, while randomly permuting the second matrix to generate a null distribution of matrix correlations against which to compare the original matrix correlation (Goswami & Polly, 2010b). It is important to note that, because Mantel's test uses only the distribution

of data in the matrices being compared, a lower correlation may have stronger significance in one analysis than a higher correlation does in another. All pterosaur and bird species were included in both analyses. Due to limited available hind limb data *P. quadridens* was excluded from the fore- and hind limb analysis of bat species.

Results

Covariance matrix comparisons

Covariance matrix repeatability was carried out using both raw and logged data for each species separately. Overall, repeatabilities were high, indicating a robust data set for most species. Covariance matrix repeatabilities for raw and logged data were high in all pterosaur species, ranging from 0.962 in *R. muensteri* to 0.999 in *D. banthensis* (Table S1). Matrix repeatability was generally higher for raw data than logged data in all species.

In all bird species, the covariance matrix repeatabilities were high, ranging from 0.882 in *A. apus* to 0.998 in *A. nisus* (Table S1). Again, raw data matrix repeatability was higher than log data matrix repeatability in all species both within and between limbs of all species with the exception of between hind limb skeletal elements of *A. apus* ($n = 21$).

Matrix repeatabilities were comparably lower within bat species than in pterosaur and bird species, ranging from 0.612 in *R. ferrumequinum* to 0.977 in *P. giganteus* (Table S1). This may have been due to smaller specimen numbers in bats compared to birds and pterosaurs.

Partial correlation analysis

Between limbs (Fig. 2, Table 2)

Pterosaurs. There were few significant partial correlations between the fore- and hind limb elements observed in both *R. muensteri* and *D. banthensis* (Fig. 2a), with the exception of the tibia and wing phalanx 3, which was positively significant in both (Table S2). However, seven out of the 12 between limb element comparisons in *P. kochi* showed a significant partial correlation, many of them positive relationships (Table S2). In terms of serially homologous elements, *P. kochi* and *R. muensteri* showed positive significant correlations between the humerus and femur, but no other significant correlations were observed between serial homologues.

Birds. Most bird species showed relatively weak relationships between skeletal elements of the fore- and hind limb (Table S3). *A. apus* ($n = 15$) had the highest number of significant positive partial correlations, with 9 out of the 24 partial correlation values showing a significant positive relationship. In contrast after maximizing the number of specimens of *A. apus* ($n = 21$), there were only two significant positive results (Table S3). *E. citrinella* ($n = 30$) showed no significant partial correlations between the skeletal elements of fore- and hind limbs.

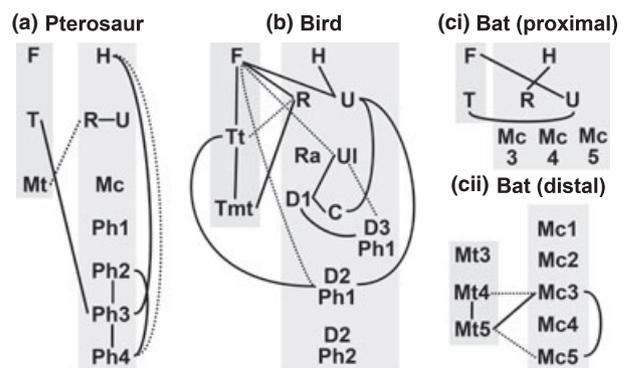


Fig. 2 Schematic diagram of one pterosaur, bird and bat species showing the significant edges ($P < 0.05$) of between fore- and hind limbs. Solid lines indicate significant positive partial correlations and dashed lines indicate significant negative results. (a) pterosaur, *D. banthensis*; (b) bird, *C. palumbus*; (c) bat, *R. ferrumequinum*, (i) $n = 11$, (ii) $n = 9$. Abbreviations: H, humerus; R, radius; U, ulna; Mc, digit 4 metacarpal; Ph1, digit 4 phalanx 1; Ph2, digit 4 phalanx 2; Ph3, digit 4 phalanx 3; Ph4, digit 4 phalanx 4; UL, ulnare; Ra, radiale; C, carpometacarpus; D1, digit 1; D2Ph1, digit 2 phalanx 1; D2Ph2, digit 2 phalanx 2; D3Ph1, digit 3 phalanx 1; Mc1, metacarpal digit 1; Mc2, metacarpal digit 2; Mc3, metacarpal digit 3; Mc4, metacarpal digit 4; Mc5, metacarpal digit 5; F, femur; T, tibia; Tt, tibiotarsus; Tmt, tarsometatarsus; Mt3, metatarsal digit 3; Mt4, metatarsal digit 4; Mt5, metatarsal digit 5.

Focusing on partial correlations among serial homologues, the humerus and femur showed significant correlations in *A. apus* and *A. nisus*. The radius and tibiotarsus showed a significant correlation in *A. apus* and *C. palumbus*, while the carpometacarpus and the tarsometatarsus are significantly correlated in *A. apus*. It is worth noting that the significant correlations between serial homologues found in *A. apus* fall below the significance threshold when specimens, rather than variables, are maximized (Table S3).

Bats. Bat species partial correlations between limbs were carried out in two halves due to small specimen numbers. First, analyses were conducted with the most proximal elements of the stylopod and zeugopod and second with autopod elements only. The vast majority of between limb partial correlation values had either a nonsignificant or significantly negative result (Table S4) indicating little integration between fore- and hind limb skeletal elements in bat species. Among serial homologues, the humerus and femur were significantly correlated in *N. noctula* and the radius and tibia were significantly correlated in *P. quadridens*. In *P. giganteus*, the humerus, radius, and ulna were all significantly correlated with their serial homologues in the hind limb.

Within forelimbs (Fig. 3, Table 3)

Pterosaurs. There were few consistent patterns of partial correlations within the forelimb of the pterosaurs. There was a significant relationship between the metacarpal IV and wing phalanx 2 and 3 of both *P. kochi* and

Table 2 Results of partial correlation analysis between limbs for one pterosaur, one bird and one bat species (same as those shown in Fig. 2).

<i>D. banthensis</i> (n = 20)	H	U	R	Mc4	Ph1	Ph2	Ph3	Ph4	F	T	Mt3
H	.										
U	-0.098	.									
R	0.436	0.819	.								
Mc4	-0.31	0.126	0.209	.							
Ph1	0.016	-0.115	0.027	0.341	.						
Ph2	0.057	-0.19	0.295	-0.148	0.696	.					
Ph3	0.514	-0.111	-0.113	0.37	0.119	-0.202	.				
Ph4	-0.499	0.119	0.222	-0.357	-0.304	0.474	0.802	.			
F	-0.048	-0.076	0.2	0.326	-0.354	0.203	0.162	-0.353	.		
T	-0.149	0.109	0.076	-0.339	-0.088	0.297	0.613	-0.719	0.112	.	
Mt3	0.099	0.455	-0.613	0.367	-0.059	0.299	-0.069	0.153	0.057	0.338	.

<i>C. palumbus</i> (n = 24)	H	R	U	Ra	Ul	C	D1	D2Ph1	D2Ph2	D3Ph1	F	Tt	Tmt
H	.												
R	-0.103	.											
U	0.476	0.15	.										
Ra	0.03	0.306	-0.297	.									
Ul	0.14	0.126	0.314	0.041	.								
C	-0.213	0.018	0.601	-0.043	-0.234	.							
D1	0.175	-0.13	-0.525	0.09	0.41	0.461	.						
D2Ph1	0.002	0.156	0.51	0.337	-0.03	0.027	0.348	.					
D2Ph2	-0.251	0.103	0.284	0.118	-0.058	-0.071	0.243	-0.131	.				
D3Ph1	0.007	0.174	0.314	0.067	-0.399	-0.171	0.431	-0.195	-0.029	.			
F	0.169	0.353	0.498	0.268	-0.361	-0.237	0.299	-0.533	-0.091	-0.479	.		
Tt	-0.147	-0.668	-0.062	0.105	0.189	-0.019	-0.262	0.378	0.023	0.321	0.598	.	
Tmt	0.346	0.476	-0.567	-0.23	-0.005	0.291	-0.287	0.301	0.216	0.261	0.205	0.381	.

<i>R. ferrumequinum</i> (n = 9)	H	R	U	F	T
Proximal elements	H	.			
	R	0.563			
	U	0.13	-0.382		
	F	0.144	0.103	0.814	
	T	0.138	0.502	0.524	-0.377

	Mc3	Mc4	Mc5	Mt3	Mt4	Mt5
Digits only	Mc3	.				
	Mc4	0.16	.			
	Mc5	0.745	-0.216	.		
	Mt3	-0.409	-0.338	0.259	.	
	Mt4	-0.62	-0.06	0.477	-0.259	.
	Mt5	0.687	-0.169	-0.745	0.141	0.826

Bold indicates significance ($P < 0.05$) as calculated from the edge exclusion deviance (EED). (A) Pterosaur, *D. banthensis*; (B) bird, *C. palumbus*; (C) bat, *R. ferrumequinum*. Abbreviations: *H*, humerus; *R*, radius; *U*, ulna; *D4Mc*, digit 4 metacarpal; *Ph1*, digit 4 phalanx 1; *Ph2*, digit 4 phalanx 2; *Ph3*, digit 4 phalanx 3; *Ph4*, digit 4 phalanx 4; *Ul*, ulnare; *Ra*, radiale; *C*, carpometacarpus; *D1*, first digit phalanx 1; *D2Ph1*, digit 2 phalanx 1; *D2Ph2*, digit 2 phalanx 2; *D3Ph1*, digit 3 phalanx 1; *Mc1*, metacarpal digit 1; *Mc2*, metacarpal digit 2; *Mc3*, metacarpal digit 3; *Mc4*, metacarpal digit 4; *Mc5*, metacarpal digit 5; *Mt 3–5*, metatarsals digits 3 to 5; *Tt*, tibiotarsus; *Tmt*, tarsometatarsus.

R. muensteri (Table S5). Other significant partial correlations were seen between the humerus and wing phalanx 3 of *P. kochi* and *D. banthensis* and between phalanx 2 and phalanx 3 of *P. kochi* and *R. muensteri*.

Birds. Some consistent patterns across birds were found the within-forelimb partial correlations. The humerus and radius had significant positive partial correlations in *A. nisus*, *E. citrinella* (after maximizing measurements $n = 22$)

and *P. aristotelis* (Table S6). There were significant positive partial correlations between the radius and ulna of *A. nisus*, *C. palumbus*, *E. citrinella* (after maximizing specimens $n = 30$), *P. aristotelis* and *V. vanellus*. The carpometacarpus and first digit had a significant positive partial correlation in *A. apus* (after maximizing measurements $n = 15$) and *C. palumbus*. Second digit phalanx 1 and second digit phalanx 2 had significant positive partial

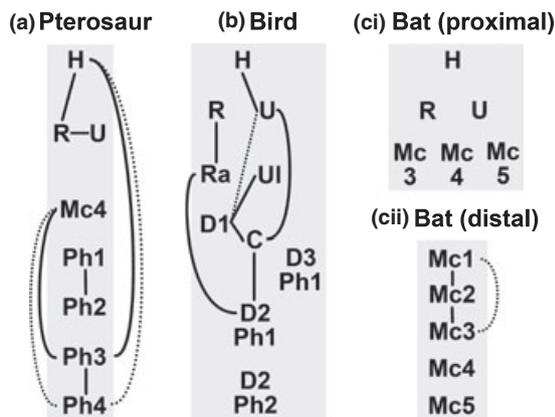


Fig. 3 Schematic diagram of one pterosaur, bird and bat species forelimb showing the significant edges ($P < 0.05$). Solid lines indicate significant positive partial correlations and dashed lines indicate significant negative results. (a) Pterosaur, *D. banthensis*; (b) bird, *C. palumbus*; (c) bat, *R. ferrumequinum*. Abbreviations: *H*, humerus; *R*, radius; *U*, ulna; *Mc*, digit IV metacarpal; *Ph1*, digit IV phalanx 1; *Ph2*, digit IV phalanx 2; *Ph3*, digit IV phalanx 3; *Ph4*, digit IV phalanx 4; *UI*, ulnare; *Ra*, radiale; *C*, carpometacarpus; *D1*, digit 1; *D2Ph1*, digit 2 phalanx 1; *D2Ph2*, digit 2 phalanx 2; *D3Ph1*, digit 3 phalanx 1; *Mc1*, metacarpal digit 1; *Mc2*, metacarpal digit 2; *Mc3*, metacarpal digit 3; *Mc4*, metacarpal digit 4; *Mc5*, metacarpal digit 5.

correlation in *A. apus* (after maximizing measurements $n = 15$), *A. nisus*, *S. molissima*. The radiale and ulnare had few comparable significant partial correlations between species. No single pattern was common across all bird species.

Bats. In three of the five bat species (*N. noctula*, *P. giganteus* and *P. quadridens*) there were a relatively large number of significant partial correlations, both positive and negative, between digits only and digits and more proximal elements (Table S7). This was particularly evident in *P. giganteus*, which had a strong positive partial correlation values between the metacarpal of digit V, and metacarpals of digits III and IV. A significant positive relationship was also found between the metacarpal of digit III and metacarpals of digits IV and V in *N. noctula*.

Overall, as with the birds, there were few consistent patterns of significant partial correlations across all bat species. The only similar patterns appear within digit only comparisons of *N. noctula*, *P. giganteus* and *R. ferrumequinum* between metacarpal of digit I and metacarpals of digits II and III, and the metacarpal of digit II and metacarpal of digit III.

Within hind limbs

Pterosaurs. There was a significant relationship between the femur and tibia in all three species of pterosaurs, as well as between the tibia and metatarsal III in *D. banthensis*, although this element was not available for comparison with the other two pterosaur species due to a limited number of measurements (Table S8).

Birds. All bird species had predominantly positive partial correlations among hind limb elements. Significant positive relationships between the femur and tibiotarsus were evident in all species (Table S9). Five out of the seven bird species also showed a significant positive partial correlation between the tibiotarsus and tarsometatarsus. The other two species (*A. nisus* and *A. apus*) had a weak negative relationship between these skeletal elements (Table S9). Four bird species also showed a negative relationship between the femur and tarsometatarsus (Table S9).

Bats. Within the hind limbs of bats, the femur and tibia had a significant positive partial correlation in *P. giganteus* and *N. noctula* (Table S10). In all other species, the partial correlation values were close to 0 suggesting little integration among these elements. The femur and metatarsal digit III had a significant positive partial correlation in *N. noctula* and *P. quadridens* only (Table S10).

Matrix correlation analysis

In general, fewer significant matrix correlations were observed when forelimb skeletal elements were analysed alone than in analyses incorporating both fore- and hind limb correlations. There were no significant matrix correlations between pterosaur species when only forelimb skeletal elements were analysed (Table 4), but there were significant matrix correlations among all pterosaur species when fore- and hind limb correlations were analysed together.

Among bird species there were only six significant correlations across species in forelimb-only analyses, compared to eight significant correlations in all-element analyses (Table 5). Significant matrix correlations between species of both forelimb only and between fore- and hind limb elements were concentrated in comparisons involving *S. molissima* and three other species (*A. nisus*, *C. palumbus* and *E. citrinella*).

Four different matrix correlation analyses were conducted for bat species: (i) humerus, radius, and three metacarpals of the forelimb (Table 6, upper right triangle); (ii) all five metacarpals of forelimb, excluding *P. quadridens* (Table 6, lower left triangle); (iii) distal hind and forelimb elements (Table 7, upper right triangle); and (iv) proximal hind and forelimb elements (Table 7, lower left triangle). The only significant matrix correlations among bat species were between *N. noctula* and *P. quadridens*, and *P. giganteus* and *R. ferrumequinum* (Table 7, upper right triangle), when fore- and hind limb distal elements were analysed.

Discussion

The results of the analyses conducted here support the hypothesis that functional divergence of the limbs during the independent evolution of flapping flight in pterosaurs, birds and bats is reflected in the reduction of the

Table 3 Results of partial correlation analysis of forelimbs for one pterosaur, one bird and one bat species (same as those shown in Fig. 3).

<i>D. banthensis</i> (n = 20)	H	U	R	Mc4	Ph1	Ph2	Ph3	Ph4
H	.							
U	-0.104	.						
R	0.509	0.774	.					
Mc4	-0.319	0.316	0.097	.				
Ph1	0.052	-0.219	0.033	0.291	.			
Ph2	0.022	0.089	0.153	-0.067	0.707	.		
Ph3	0.573	0.047	-0.216	0.452	-0.063	0.188	.	
Ph4	-0.719	-0.008	0.404	-0.639	-0.131	0.222	0.614	.

<i>C. palumbus</i> (n = 24)	H	R	U	Ra	Ul	C	D1	D2Ph1	D2Ph2	D3Ph1
H	.									
R	0.188	.								
U	0.630	0.168	.							
Ra	0.095	0.354	-0.139	.						
Ul	0.007	-0.058	0.217	-0.082	.					
C	-0.228	0.111	0.517	-0.194	-0.153	.				
D1	0.104	-0.064	-0.367	0.149	0.408	0.399	.			
D2Ph1	0.053	-0.043	0.188	0.367	0.268	0.355	-0.033	.		
D2Ph2	-0.195	0.217	0.189	0.106	-0.047	0.007	0.14	0.012	.	
D3Ph1	0.043	0.057	-0.046	-0.043	-0.318	-0.009	0.225	0.292	0.099	.

<i>R. ferrumequinum</i> (n = 9)	H	R	U	Mc3	Mc4	Mc5
Proximal elements and metacarpals 3–5	H	.				
	R	0.403	.			
	U	0.541	0.327	.		
	Mc3	0.161	-0.161	-0.222	.	
	Mc4	-0.45	0.213	0.343	0.372	.
	Mc5	0.189	0.451	-0.379	0.429	-0.049

	Mc1	Mc2	Mc3	Mc4	Mc5
Digits only	Mc1	.			
	Mc2	0.688	.		
	Mc3	-0.678	0.926	.	
	Mc4	0.106	0.171	-0.041	.
	Mc5	-0.202	0.047	0.124	-0.058

Bold indicates significance ($P < 0.05$) as calculated from the edge exclusion deviance (EED). (A) Pterosaur, *D. banthensis*; (B) bird, *C. palumbus*; (C) bat, *R. ferrumequinum*. Abbreviations: *H*, humerus; *R*, radius; *U*, ulna; *Mc4*, digit 4 metacarpal; *Ph1*, digit 4 phalanx 1; *Ph2*, digit 4 phalanx 2; *Ph3*, digit 4 phalanx 3; *Ph4*, digit 4 phalanx 4; *Ul*, ulnare; *Ra*, radiale; *C*, carpometacarpus; *D1*, first digit phalanx 1; *D2Ph1*, digit 2 phalanx 1; *D2Ph2*, digit 2 phalanx 2; *D3Ph1*, digit 3 phalanx 1; *Mc1*, metacarpal digit 1; *Mc2*, metacarpal digit 2; *Mc3*, metacarpal digit 3; *Mc4*, metacarpal digit 4; *Mc5*, metacarpal digit 5.

integration between skeletal elements of the fore- and hind limbs, as previously hypothesized in the evolution of birds (Gatesy & Dial, 1996). In particular, the strong correlations between serially homologous structures found in quadrupedal mammals were observed in only a few taxa in this study. Among the pairs of serial homologues included here, the humerus and femur displayed significant correlations in the most species (two pterosaurs, two birds, and two bats), although this number still represents a minority of the 15 species sampled. That the most proximal elements show the strongest correlations possibly reflects a proximal-distal pattern in the strength of integration, which may further

relate to developmental timing, as proximal limb elements generally ossify before proximal ones (Weisbecker *et al.*, 2008; Weisbecker, 2011).

Unexpectedly, there were few consistent patterns in forelimb element integration in any of these clades, suggesting that developmental integration of the wing elements is not requisite for their functional coordination. In general, bird species showed higher and more significant partial correlations among forelimb elements and more significant matrix correlations among species than observed in pterosaurs or bats. For example, more patterns of forelimb integration were shared found across bird species, such as between the radius and ulna of

C. palumbus, *A. nisus*, *V. vanellus*, *P. aristotelis*, and *E. citrinella*, than the other two clades. However, even within birds, there was little consistency in forelimb partial correlations across different species, and thus little evidence that skeletal elements of the wing are subject to significant integration due to their shared function.

Results for pterosaurs and bats, both of which suffered from lower sample sizes than were available for most bird species, were largely similar to those for birds, with large variation in forelimb integration among taxa. Acquiring

larger sample sizes of relatively complete specimens for pterosaur species, which are limited by preservation, discovery, and preparation, will likely remain a confounding factor in examining their morphological integration. Various partitions of the dataset were used for bats to circumvent the low sample sizes and maintain a reasonable specimen to measurement ratio. Robust covariance matrix repeatabilities indicate that results for most species were not greatly affected by small sample sizes, but it is likely that the higher significances observed for analyses of bird species are at least partially due to better sampling.

In contrast to the results for forelimb integration, the relatively consistent patterns of partial correlations within the hind limb of the pterosaur and bird species may suggest that an ancestral developmental integration pattern is maintained in this structure, or that the hind limb elements are under similar functional constraints across these taxa. There was conserved hind limb integration across all bird and pterosaur taxa, with significant partial correlations between the femur and tibia of all pterosaur species (Table S8) and between the

Table 4 Results of matrix correlation analysis and Mantel's test for pterosaurs, conducted among only forelimb elements (lower triangle) and between fore- and hind limbs (upper triangle).

	<i>D. banthensis</i>	<i>P. kochi</i>	<i>R. muensteri</i>
<i>D. banthensis</i>	1	0.833**	0.723**
<i>P. kochi</i>	0.913	1	0.739*
<i>R. muensteri</i>	0.719	0.744	1

* $P < 0.05$. ** $P < 0.01$.

Table 5 Results of matrix correlation analysis and Mantel's test for birds conducted among only forelimb elements (lower triangle) and between fore- and hind limbs (upper triangle).

	<i>A. apus</i>	<i>A. nisus</i>	<i>C. palumbus</i>	<i>E. citrinella</i>	<i>P. aristotelis</i>	<i>S. molissima</i>	<i>V. vanellus</i>
<i>A. apus</i>	1	0.379	0.453	0.560*	0.375	0.520	0.357
<i>A. nisus</i>	0.555	1	0.412	0.389	0.042	0.720**	0.219
<i>C. palumbus</i>	0.463	0.752**	1	0.803	0.776**	0.694**	0.284
<i>E. citrinella</i>	0.516	0.773*	0.706	1	0.634**	0.651**	0.103
<i>P. aristotelis</i>	0.532	0.407	0.559	0.179	1	0.303	0.450*
<i>S. molissima</i>	0.495	0.857**	0.901**	0.755*	0.481	1	0.168
<i>V. vanellus</i>	0.477	0.268	0.453	0.079	0.943**	0.363	1

* $P < 0.05$. ** $P < 0.01$.

	<i>A. caudifera</i>	<i>N. noctula</i>	<i>P. giganteus</i>	<i>P. quadridens</i>	<i>R. ferrumequinum</i>
<i>A. caudifera</i>	1	0.243	0.182	–	0.588
<i>N. noctula</i>	0.499	1	0.931	–	0.673
<i>P. giganteus</i>	0.025	0.157	1	–	0.638
<i>P. quadridens</i>	0.391	0.664	0.41	1	–
<i>R. ferrumequinum</i>	0.492	0.765	0.347	0.567	1

Table 6 Results of matrix correlation analysis and Mantel's test for bat forelimbs, conducted among humerus, radius and metacarpals 3–5 (lower triangle), and only metacarpals 1–5 (upper triangle). *P. quadridens* was removed from the sample due to insufficient complete specimens.

	<i>A. caudifera</i>	<i>N. noctula</i>	<i>P. giganteus</i>	<i>P. quadridens</i>	<i>R. ferrumequinum</i>
<i>A. caudifera</i>	1	0.636	0.646	0.384	0.721
<i>N. noctula</i>	0.372	1	0.737	0.897**	0.876
<i>P. giganteus</i>	0.232	0.236	1	0.731	0.868**
<i>P. quadridens</i>	0.216	0.641	0.291	1	0.805
<i>R. ferrumequinum</i>	0.536	0.762	0.191	0.904	1

** $P < 0.01$.

Table 7 Results of matrix correlation analysis and Mantel's test for bat limbs, conducted among proximal elements, humerus, radius, femur and tibia (lower triangle), and distal elements 3rd and 4th metacarpals and 3rd and 4th metatarsals (upper triangle).

femur and tibiotarsus of all bird species (Table S9). Although the sample of birds included aquatic, terrestrial, and arboreal species, bearing four different hind foot morphologies, most species showed similar partial correlations among hind limb elements. Differences in hind limb partial correlations among bird species did not correspond to differences in hind limb morphology or habitat. Bats showed greater variation in hind limb integration across species than observed in birds and pterosaurs. No significant correlations among hind limb elements were consistently found across bat species.

Previous work in different mammalian groups has suggested that conservation of limb proportions is largely driven by similar biomechanical requirements for propulsion (Schmidt & Fischer, 2009). Differences in within limb integration may thus reflect differences in clade ecology, behaviour or different biomechanical requirements. For example, high within hind limb integration could be due to similar requirements that birds and pterosaurs have for upright support, or at least that each clade was subject to consistent, if different, constraints on hind limb integration. The lack of such integration in bats may reflect morphological changes associated with their alternative use of their hind limbs in hanging and climbing rather than in upright support.

Our knowledge of the locomotory abilities of extinct animals is limited and often relies on analogies to similar extant organisms (Padian, 1983a). Pterosaurs have been reconstructed as either bipedal (Padian, 1983a,b; Bennett, 1990, 2001) or quadrupedal animals with sprawling (Bramwell & Whitfield, 1974), semi-erect (Wellnhofer, 1988; Wilkinson, 2008), or erect posture (Henderson & Unwin, 1989; Chatterjee & Templin, 2004). The evidence of a number of trace fossils seems to support quadrupedal reconstructions (Unwin, 1999), although this may not be true of all pterosaur species (Padian, 1983) and pterosaurs have also been suggested as having varying posture over phylogeny (Unwin, 2006; Padian, 2008; Witton & Habib, 2010). The similar patterns in hind limb integration within the skeletal elements of pterosaurs and birds shown here suggest some similarity in the construction of their limbs. Whether this is due to common ancestry or bipedality is unknown; however, these results suggest that, pterosaur hind limbs functioned more like the hind limbs of birds than bats, and thus were likely used in upright support. It is likely that pterosaurs had to maintain some bipedality during takeoff and landing while wings were extended (Wilkinson, 2008), and so this may have imposed functional constraints on hind limb integration, but see Habib (2008) for an alternative takeoff method. Whatever the posture of pterosaurs, the similar integration within the hind limbs of the pterosaur species analysed further suggest that these limbs had a similar function over phylogeny.

Results of the matrix correlation analyses among species within each clade were consistent with the partial

correlation analyses. There were few significant correlations between species when only forelimb correlations were considered, reflecting the lack of consistent patterns of forelimb integration. In contrast, both pterosaurs and birds, as well as some bats, showed significant matrix correlations when both fore- and hind limb correlations were analysed. This result corresponds well with the consistent lack of between limb integration in all three clades found in the partial correlation analysis. Despite differences in within-forelimb partial correlations, species in each clade showed a consistent dissociation of the forelimb and the hind limb, resulting in the observed significant matrix correlations among species. That more significant results of the matrix correlation analyses were observed in birds and pterosaurs than in bats is likely due to stronger and more conserved hind limb integration in those clades, in contrast to the greater variation in hind limb integration found in bats.

Our results for between limb integration were generally consistent with the findings of Young & Hallgrímsson (2005), Bennett & Goswami (2011), and Kelly & Sears (2011). These studies found that increased functional divergence between the forelimbs and the hind limbs resulted in reduced integration between limbs compared to the more highly integrated limbs of quadrupedal mammals. We similarly found dissociation and reduced integration between the limbs of pterosaurs, birds and bats. These results may correspond with reduced, or at least not reinforced, developmental constraints of serially homologous structures, allowing increased morphological variation between limbs (Wagner & Altenberg, 1996; Goswami & Polly, 2010a). Selection acting upon the elements of the forelimb for their role in flapping flight may have necessitated a fragmentation of the ancestral limb integration structure (Young & Hallgrímsson, 2005). Quadrupeds use their limbs in a similar and coordinated fashion, and consequently their development is highly constrained, reflected in the strong between-limb integration observed in quadrupedal mammals. The results presented here suggest that, at some point during the evolution of the pterosaur, bird and bat lineages, the integration between homologous elements of the fore- and hind limb was reduced, allowing the fore- and hind limbs to vary and evolve relatively independently of each other. It is possible that disassociation occurred in an ancestor of birds and pterosaurs, in which case the dissociation of the fore- and hind limb may reflect common ancestry rather than convergence. In this regard, it will be important for future studies to assess limb integration in nonmammalian quadrupeds, as no data outside of mammals, with the exception of this study, is currently available.

Many of our initial hypotheses in this study were based on the results for a single bat species in Young & Hallgrímsson (2005). In their study, the bat showed higher within forelimb covariation when compared to the hind limb and consequently we expected that a similar pattern would be observed across other flying

vertebrates. However, this was not the case. Young & Hallgrímsson's (2005) study used only one bat species (*C. brevicauda*), and our results for each of the three clades examined here demonstrate that results for a single species cannot be necessarily generalized to higher taxonomic levels, as has been noted in other comparative studies of morphological integration (Goswami, 2006, 2007; Wilson & Sánchez-Villagra, 2009).

One possibility for the lack of conserved patterns of integration in the forelimb is that the increased functional importance of the nonskeletal elements of the forelimb in flapping flight reduces the integration, or at least the necessity for integration, of the skeletal elements. For example, the primary feathers of birds form a significant part of the aerofoil surface, and so assessing the integration of shape between feathers and skeletal elements may be more informative than looking at skeletal elements in isolation. Studies of integration across tissue types are uncommon, and this may be one promising avenue of research to better understand the evolutionary significance of functional and developmental integration. As our bird sample consisted entirely of members of Neornithes, it would also be interesting to incorporate early fossil birds. Some key structures in the modern bird wing, such as a fully fused carpometacarpus and an alula, are not present in *Archaeopteryx* (Padian, 1998) and quantifying patterns of integration in transitional forms may provide unique information on the fragmentation of forelimb integration in birds. Unfortunately, early fossil birds are rare, and sufficient sample sizes for measuring integration are not currently available.

As mentioned above, the greatest consistency of our results with previous studies (Magwene, 2001; Young & Hallgrímsson, 2005; Bennett & Goswami, 2011; Kelly & Sears, 2011) is in the observed dissociation of functionally divergent serial homologues. There are a number of areas in which this investigation could be developed to further study the effect of function dissociation on integration and variation. This study documents changes in limb integration associated with one type of major transition in vertebrate evolution, the origin of flapping flight. Other ecological transitions, such as the fish to tetrapod transition, aerial to terrestrial transition of flightless birds, or the terrestrial-aquatic transition of aquatic mammals or reptiles would be equally interesting to examine for changes in limb integration.

Ontogenetic changes in limb function present another promising area of research. The few studies of ontogenetic integration in mammalian crania (Zelditch & Carmichael, 1989; Goswami & Polly, 2010b) have demonstrated that patterns of integration change substantially during growth. Many young birds and bats are not capable of immediate flapping flight after hatching or birth (Heers *et al.*, 2011); thus one may hypothesize that flight elements show increased integration during development for these altricial species. Wing-

assisted inclined running is used in some species after hatching before young are capable of flapping flight (Dial, 2003; Heers *et al.*, 2011), and it would also be interesting to test if age-related transitions between locomotory styles is related to ontogenetic shifts in between or within limb integration.

In conclusion, we have shown that coincident with the evolution of vertebrate flapping flight, the functional divergence of fore- and hind limbs in pterosaurs, birds and bats has resulted in reduced covariation between limbs, allowing increased independent variation in serially homologous structures. Our results demonstrate that the development of hind limbs is relatively conserved in pterosaur and bird clades and may reflect their utility in upright support. The most surprising result was the lack of significant forelimb integration in most of the species studied here. This breakdown of within limb integration during the extensive modification of the forelimb in these three clades suggests interestingly that flapping flight elements need not be highly integrated to be functional in flapping flight. The interplay between developmental and functional influences on element variation and covariation has been a recurrent theme in studies of morphological integration. These results suggest that function and development can be, but not are necessarily, coordinated, with implications for our understanding of developmental constraints and their importance for morphological evolution.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Location of landmarks of distal skeletal elements of the bird forelimb, shown on *Columba palumbus*.

Figure S2 Location of landmarks in the fore- and hind limbs of bats, shown on *Pteropus giganteus*.

Figure S3 Location of landmarks in the fore- and hind limbs of pterosaurs, shown on *Pterodactylus kochi*.

Table S1 Covariance matrix repeatabilities from Monte Carlo simulations for all sampled species.

Table S2 Results of partial correlation analyses between limbs for pterosaurs, using raw length data.

Table S3 Results of partial correlation analyses between limbs for birds, using raw length data.

Table S4 Results of partial correlation analyses between limbs for bats, using raw length data.

Table S5 Results of partial correlation analyses of forelimbs for pterosaurs, using raw length data.

Table S6 Results of partial correlation analyses of forelimbs for birds, using raw length data.

Table S7 Results of partial correlation analyses of forelimbs for bats, using raw length data.

Table S8 Results of partial correlation analyses of hind limbs for pterosaurs, using raw length data.

Table S9 Results of partial correlation analyses of hind limbs for birds, using raw length data.

Table S10 Results of partial correlation analyses of hind limbs for bats, using raw length data.

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