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# Constraints on Body Mass and Ecomorphological Evolution in Birds



Top left, *Porphyrio hochstetteri* Zealandia Wildlife Sanctuary, Wellington, New Zealand; Bottom left, *Caloenas nicobarica* Taipei Zoo, Taiwan; Right, leg bones of *Mullerornis agilis* Museum Victoria, Melbourne. Photo credits: Nicholas Chan.

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## STATEMENT OF CANDIDATE

I hereby certify that the work in this thesis entitled “*Constraints in body mass and ecomorphological evolution in birds*” has not been previously submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that this thesis is an original piece of research that has been written by me. Any help or assistance that I have received in my research and in the preparation of the thesis has been appropriately acknowledged.

Finally, I certify that all information sources and literature used have been indicated within the thesis.

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## ABSTRACT

A number of constraints have been hypothesised to have affected avian evolutionary history. Testing these hypotheses is key to understanding the causal processes behind observed patterns of diversity and disparity. Four topics on this theme are examined here. The first is that egg shell strength relative to incubator body mass limits body mass. Size variation in the giant, extinct flightless bird *Genyornis newtoni* is used to determine whether sexual dimorphism may have compensated for any mismatch between mass and eggshell strength in this species. Secondly, limits to leg bone scaling are tested for using quadratic regressions of leg bone measurements from 58 species of flightless birds. Comparisons with non-avian theropods are made to see whether patterns of scaling seen in birds demonstrate limits to leg bone allometry in birds. Thirdly, the evolution of leg bone proportions in 38 species of flightless birds is explored. Factor analyses are used to quantify changes in proportions from ancestral morphologies modelled on volant relatives. Multiple regressions are used to see if these changes are related to shifts in body size, the amount of time since loss of flight, and the size of the land area upon which each lineage evolved. In Chapter 4, the hypothesis of competition between Mesozoic birds and pterosaurs during the Mesozoic is investigated using multivariate analysis of functionally analogous traits. The results of these studies provide support to hypotheses of constrained leg bone allometry, with increases in femur length relative to girth being restricted, related to posture in birds and of ecological separation rather than competition between Mesozoic birds and pterosaurs. It is also shown that the evolution of the leg bones after the loss of flight is not limited by any universal selection pressures, with changes in the proportions of these bones showing no consistent patterns through avian phylogeny. Together, these studies provide new insights into the potential constraints that have affected avian evolution for the past 160 million years.

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# CONTENTS

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<b>Statement of candidate</b>		<b>iii</b>
<b>Abstract</b>		<b>v</b>
<b>Acknowledgements</b>		<b>vii</b>

---

<b>Introduction</b>		<b>1</b>
<b>Chapter One</b>	<b>Does size variation in <i>Genyornis newtoni</i> encompass eggshell safety limits?</b>	<b>17</b>
	Abstract	18
	Introduction	19
	Methods	21
	Results	25
	Discussion	25
	Conclusion	30
<b>Chapter Two</b>	<b>Phylogenetic variation in hindlimb bone scaling of flightless theropods</b>	<b>35</b>
	Abstract	36
	Introduction	37
	Methods	40
	Results	45
	Discussion	57
	Conclusion	63
<b>Chapter Three</b>	<b>Complex evolution of leg bones in the transition to flightlessness in birds</b>	<b>73</b>
	Abstract	74
	Introduction	75

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---

	Methods	78
	Results	88
	Discussion	102
	Conclusion	107
<b>Chapter Four</b>	<b>A comparison of pterosaur and Mesozoic avian morphospaces constructed using functionally analogous traits</b>	<b>119</b>
	Abstract	120
	Introduction	121
	Methods	123
	Results	127
	Discussion	132
	Conclusion	139
<b>Synthesis</b>		<b>149</b>

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<b>Appendices</b>		<b>157</b>
	Appendix 1: Supplementary materials	157
	Appendix 2: Chan, N.R., Dyke, G.J., & Benton, M.J. 2013. Primary feather lengths may not be important for inferring the flight styles of Mesozoic birds. <i>Lethaia</i> , Vol. 46, pp. 146–152.	158

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## LIST OF FIGURES AND TABLES

### Chapter One

Figures	1. Frequency histograms of tibiotarsus circumference, tarsometatarsus circumference, tarsometatarsal length for <i>Genyornis newtoni</i> and <i>Dinornis robustus</i>	27
Tables	1. Maximum shell thickness, yield point, incubator, and average incubator mass of three ratite genera and <i>Genyornis</i> .	24
	2. Summary statistics of leg bone measurements for <i>G. newtoni</i> and <i>D. robustus</i> .	24

### Chapter Two

Figures	1. Femoral scaling of flightless birds and non-avian theropods.	51
	2. Femoral and tibiotarsal scaling of flightless bird groups.	53
	3. Femoral scaling of non-avian theropod groups.	55
Tables	1. Quadratic regressions of length versus least circumference for avian and non-avian theropod femora and tibiotarsi.	50
	2. Standard major axis regressions (SMA) of femoral dimensions for avian groupings.	50
	3. P-values of pair-wise comparisons of avian femoral SMA slopes and intercepts.	51
	4. SMA regressions of femoral dimensions for non-avian theropod groupings.	52
	5. P-values of pair-wise comparisons of non-avian theropod femoral SMA slopes and intercepts.	52
	6. SMA regressions of tibiotarsal dimensions for avian groupings.	53
	7. P-values of pair-wise comparisons of avian tibiotarsal SMA slopes and intercepts.	54
	8. SMA regressions of tibiotarsal dimensions for non-avian theropod groupings.	56
	9. P-values of pair-wise comparisons of non-avian theropod tibiotarsal	57

### Chapter Three

Figures	1. Composite phylogeny of taxa included in this study constructed using matrix representation parsimony.	91
	2. Plot of factor 1 scores against factor 2 scores for flightless and volant species.	95
	3. Alternate timing scenarios for loss of flight in moa.	95
Tables	1. Factor loadings of each variable from factor analyses of regression residuals for the total group, palaeognaths, and neognaths.	90
	2. Multiple regressions of factor 1 scores with $\Delta CF$ , land area, and estimated time since loss of flight for flightless, terrestrial birds.	92
	3. Multiple regressions of sign-less factor 1 scores with $\Delta CF$ , land area, and estimated time since loss of flight for flightless birds.	93
	4. Multiple regressions of factor 1 scores with $\Delta CF$ , land area, and estimated time since loss of flight for flightless, palaeognaths.	94
	5. Multiple regressions of sign-less factor 1 scores with $\Delta CF$ , land area, and estimated time since loss of flight for flightless, palaeognaths.	98
	6. Multiple regressions of factor 1 scores with $\Delta CF$ , land area, and estimated time since loss of flight for flightless, terrestrial neognaths.	99
	7. Multiple regressions of sign-less factor 1 scores with $\Delta CF$ , land area, and estimated time since loss of flight for flightless, terrestrial neognaths.	100

### Chapter Four

Figures	1. Diagrams of bird ( <i>Sturnus vulgaris</i> ) and pterosaur ( <i>Rhamphorhynchus muensteri</i> ) wings extended to show the method used to correct wing lengths for elbow flexion angle.	129
	2. Plot of PC1 vs PC2 for avian and pterosaur lower jaw, wing, and leg measurements.	131
	3. PC1 vs PC2 based on a PCA of wing measurements with pterosaur metacarpal IV included either in the antebrachium or the distal wing.	131
	4. Frequency histograms of wing lengths calculated by summing all	133

---

	wing elements (A) and of lengths corrected for elbow flexion angle at maximum extension.	
Tables	1. Loadings of variables on the first three principal components from PCA of lower jaw, wing, and leg measurements.	130
	2. Loadings of variables on principal components from PCA of wing element lengths only.	132

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## Introduction

Birds are the most diverse group of living tetrapods, with approximately 10,000 species found in every environment and distributed globally (Gill, 2007; Jetz *et al.*, 2012). Unsurprisingly, there has been much interest in the fossil record and evolution of this clade in the palaeontological community for over 200 years (Mlíkovský, 1996). During this time, research questions have broadened from the narrow question of bird origins (Huxley, 1868; 1870; Ostrom, 1973; 1976) into a wide array of topics including the evolution of powered flight (Ostrom, 1979; Rayner, 1988; Senter, 2006), the nature and timing of the radiation of modern birds (Cracraft, 2001; Fedducia, 2003; Lee *et al.*, 2014a), trends in body size evolution (Hone *et al.*, 2008; Butler & Goswami, 2008; Benson *et al.* 2014a; Lee *et al.*, 2014b; Puttick *et al.*, 2014), and patterns of morphological and biomechanical evolution (Jenkins, 1993; Hutchinson, 2001; Hutchinson & Allen, 2009; Benson & Choiniere, 2013; Dececchi & Larsson, 2013; Brusatte *et al.*, 2014; Puttick *et al.*, 2014).

One area of interest is the nature and effects of constraints on morphological and ecological evolution through time. Knowledge of constraints is important for understanding the causal processes behind observed patterns of taxonomic and morphological diversity (Ruta *et al.*, 2006). Intrinsic constraints may result from limitations to physiological processes (e.g. metabolic rate, thermal tolerance of proteins), developmental pathways, or the biomechanical structure of a particular body plan (Gould, 1989; Biewener, 1991; West *et al.*, 1999; Hulsey & Wainwright, 2002; Ricklefs & Wikelski, 2002; Brakefield, 2006). These mechanisms have been used to explain parallel evolution, limits to body mass, and patterns of morphospace occupation (Olson,

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1973; Alexander, 1998; Gatesy & Middleton, 1997; Middleton & Gatesy, 2000; Hulseay & Wainwright, 2002; Brakefield, 2006) Additionally, trade-offs in functional performance, with a morphological or physiological trait sometimes having opposite requirements, may restrict the degree of variation exhibited within a clade (Ricklefs & Wikelski, 2002; Wainwright, 2007). The removal or relaxation of constraints is one way in which increases in diversity and disparity can be instigated. One example is the easing of constraints on leg bone morphology with the evolution of flight in birds (Gatesy & Middleton, 1997). In the theropod ancestors of birds the hindlimbs were integral to locomotion, however, with the evolution of flight combined with changes in the mechanics of leg movement the hindlimbs of birds were freed for alternate uses such as perching, swimming, and prey capture (Gatesy, 1990; Gatesy & Middleton, 1997; Hutchinson & Gatesy, 2000; Hutchinson, 2001). This innovation led to the greater disparity of avian leg bone proportions compared to non-avian theropods (Gatesy & Middleton, 1997).

Occupation of niches by other taxa may also block a clade from diversifying morphologically and radiating into new niche space, meaning that it can act as an extrinsic constraint (Rosenzweig & McCord, 1991; Jablonski & Sepkoski, 1996). The opening of ecological niches by extinction of a clade provides opportunities for surviving groups to radiate into the vacant niche space (Benton, 1983; Rosenzweig & McCord, 1991; Alroy, 1998). As a result, tests of this hypothesis often examine changes in disparity and diversity across mass extinction events (Alroy, 1998; Brusatte *et al.*, 2008a, b; Benton *et al.*, 2014).

This thesis explores a number of topics on the theme of morphological constraints in birds. The first two chapters examine potential mechanical constraints on avian body mass and scaling of the limb bones that support the weight of the animal. Unlike previous studies, the focus



here is on flightless birds. This is for three reasons. First, the largest birds are flightless, with the heaviest estimated to have been six times heavier than the largest ever flying bird (Amadon, 1947, Vizcaíno & Fariña, 1999). Second, hypotheses of mass limitation in flightless birds have received far less attention than those put forward for flight-capable birds. This is despite the fact that the largest bipedal theropods had masses exceeding that of the largest flightless birds by over an order of magnitude (Amadon, 1947; Christiansen & Fariña, 2004; Mazzetta *et al.*, 2004). Thus, flightless birds have not approached the apparent weight limits of active bipeds. Third, terrestrial flightless birds are an excellent group in which to examine scaling relationships of the leg bones because the hindlimbs are used solely for terrestrial locomotion. As such, scaling patterns are less subject to ecological noise caused by differences in proportions to fulfil alternate uses, thereby making comparison with non-avian theropods easier (see; Zeffer *et al.*, 2003; Doube *et al.*, 2012). The second general topic is the pattern of leg bone evolution in the transition to secondary flightlessness and factors that potentially control morphological changes. Changes in hindlimb shape with loss of flight have received much less attention than shifts in the forelimbs. The third and final topic is potential competition between early birds and pterosaurs in the Mesozoic. Opinion in the recent palaeontological literature is divided as to whether competition or ecological separation occurred between these two groups (Penny & Phillips, 2004; Wang & Zhou, 2006; McGowan & Dyke, 2007; Prentice *et al.*, 2011; Benson, *et al.*, 2014b). Testing these hypotheses is key to understanding whether the presence of another group of flying vertebrates affected the early evolution of birds.

The first chapter examines the hypothesis that a widening discrepancy between eggshell strength and incubator mass with increased body mass led to the extreme reverse sexual dimorphism seen in moa, and ultimately limits avian body mass (Birchard & Deeming, 2009;

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Deeming & Birchard, 2009). Some species of moa exhibit extreme sexual dimorphism, which is reflected in high variation in leg bone size (Bunce *et al.*, 2003; Huynen *et al.*, 2003; Worthy *et al.*, 2005; Worthy & Scofield, 2012). This dimorphism has been suggested to be a response to the greater likelihood of accidental damage to eggs during contact incubation with increased size (Birchard & Deeming, 2009; Deeming & Birchard, 2009). In Chapter 1 this hypothesis is tested by examining the degree of size variation in the extinct, flightless bird *Genyornis newtoni* (Aves: Dromornithidae). Mass estimates of this species are similar to *Dinornis*, the largest genus of moa (Murray & Megirian, 1998; Murray & Vickers-Rich, 2004). As such, similarly high variance in the leg bone dimensions of adult *G. newtoni* would be a good indication of extreme dimorphism in this species as well. The results of this study show size variation in *G. newtoni* leg bones to be three times less than that seen in *Dinornis robustus*, providing little evidence for extreme dimorphism. Additionally, the discrepancy between eggshell strength and adult body mass in *G. newtoni* is far greater than in any extant, large flightless birds. Thus, the hypothesis that the relationship between eggshell strength and incubator size is a limiting factor in avian body size is not supported. The results of this chapter have been published in the Journal of Vertebrate Paleontology (Chan, 2014).

In Chapter 2, the scaling patterns of the femur and tibiotarsus in terrestrial flightless birds are compared with those of non-avian theropods. Differential scaling, whereby the bones become more robust at a higher rate in large species than in their smaller relatives, has been demonstrated in mammals and non-avian theropods (Bertram & Biewener, 1990; Christiansen, 1999a, b). Avian femora are positioned sub-horizontally during stance, thereby increasing the torsional forces applied to this bone (Gatesy & Biewener, 1991; Carrano, 1998; Carrano & Biewener, 1999). The relatively more robust femora seen in birds compared to non-avian theropods is likely

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a response to these increased torsional stresses (Gatesy, 1991; Carrano, 1998). Combined with the requirement to keep the knee under the centre of mass, this strengthens the constraints on femoral dimensions. Measurements of the femur and tibiotarsus were taken on 278 specimens representing 58 species of flightless bird. Regressions of avian femur length against circumference show differential scaling with little variation between different phylogenetic groups. This is in contrast to non-avian theropods, which show much greater phylogenetic variation. These results support the hypothesis that the femur of birds is more constrained compared to non-avian theropods due to postural differences. This manuscript is prepared for submission to *Journal of Evolutionary Biology*.

The focus shifts in Chapter 3 towards the evolution of hindlimb proportions in response to flight loss in birds. It is well documented that there was a general trend of forelimb reduction in which the bones of the hand were the first to shorten (Marples, 1930; Olson, 1973; Livezey, 1992; Gatesy & Middleton, 2000; Nudds & Davidson, 2010). However, patterns of change in the hindlimbs have received little attention. Leg bone lengths and diameters of 334 specimens representing 38 flightless species and 19 volant species were measured. Differences in scores from factor analyses of leg bone dimensions were used to quantify transitions between volant ancestral models and flightless species. These values were tested for directionality and for association with three controlling variables: changes in body size, estimated time since loss of flight, and size of land mass on which the lineage evolved. In contrast to previous studies on the wings, no evidence for directionality in leg bone evolution was found. One apparent pattern is that ratites with leg bone proportions indicative of greater cursoriality evolved on larger land masses. However, no such patterns exist in flightless neognathous birds. This manuscript has been submitted to *Journal of Anatomy*.

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Chapter 4 explores the possibility for competitive interactions between pterosaurs and Mesozoic birds. One approach to this problem is to compare morphological characters associated with particular ecological traits using multivariate analyses. Previous studies compared these groups using measurements from homologous appendicular skeletal structures as variables (Dyke *et al.*, 2006; McGowan & Dyke, 2007). However, these units are not functionally comparable and differences in these structures likely reflect separate ancestry rather than differences in ecology (Prondvai & Hone, 2008). In this study, measurements of the lower jaws, forelimbs, and hindlimbs were taken from published text figures and analysed using multivariate analyses. Unlike previous works, the wings were divided into functionally analogous units based on the descriptions of Prondvai and Hone (2008). The results indicate that complete separation between pterosaurs and Mesozoic birds does not occur on any single axis of morphospace. Instead, separation results from a combination of size and differences in relative lengths of the mandibles, brachium, and metatarsals. This conclusion differs from those of previous studies, which found separation between these groups on each axis of multivariate space to be due to differences in the relative lengths of homologous bones. These results indicate that ecological separation between Mesozoic birds and pterosaurs rather than being a result of any single factor was due to a combination of size, locomotory adaptations, and feeding strategy. Further study of feeding related traits in pterosaurs and birds and the inclusion of immature individuals of the former in future analyses is suggested as a means of testing for ecological separation between the two groups, and for expansion into niches previously occupied by pterosaurs by modern birds.

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## Estimated Contribution to Article

Thesis Data Chapters	Estimated contribution by candidate (%)			
	Concept and Design	Data collection	Analyses and interpretation of data	Writing of manuscript
<b>Chapter 1:</b> Does size variation in <i>Genyornis newtoni</i> (Aves: Dromornithidae) encompass eggshell safety limits?	80	100	90	90
<b>Chapter 2:</b> Phylogenetic variation in hindlimb bone scaling of flightless theropods.	90	50 <sup>†</sup>	90	90
<b>Chapter 3:</b> Complex evolution of leg bones in the transition to flightlessness in birds.	90	100	90	90
<b>Chapter 4:</b> A comparison of pterosaur and Mesozoic avian morphospaces constructed using functionally analogous traits.	80	100*	90	90

<sup>†</sup>Half of the non-avian theropod data was provided by Matthew Carrano, with the remainder compiled from the literature.

\* Data compiled from the published literature.

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## Chapter 1

Does Size Variation in *Genyornis newtoni* (Aves:  
Dromornithidae) Encompass Eggshell Safety Limits?

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## Abstract

The decreasing strength of eggshell relative to the mass of the incubating parent has been hypothesized to constrain large body mass in flightless birds. This in turn has been used to explain extreme sexual dimorphism in giant moas, in which males weighed a third of the mass of the females, as an adaptation to reduce the risk of egg breakage. As a result, substantial dimorphism is also predicted in similarly large taxa. This study examines intraspecific variation in the Australian giant bird, *Genyornis newtoni*, an important component of the Pleistocene megafauna in the region. Specimens from the Lake Callabonna locality, South Australia were examined and measurements were taken of hind-limb elements that correlate strongly with body mass. These were compared to measurements taken from specimens of the giant moa, *Dinornis*, from North Canterbury, New Zealand. Body mass and eggshell strength of *G. newtoni* were also estimated using published regression equations and compared to large extant birds. The results indicate that low variation in the Lake Callabonna population, particularly when compared with *Dinornis*, does not support the presence of extreme dimorphism. In addition, the difference between body mass and eggshell strength in *Genyornis* is far greater than that seen in extant large ratites. The implications for the egg strength-based hypothesis for relatively small body size in flightless birds are discussed.



SHORT COMMUNICATION

DOES SIZE VARIATION IN *GENYORNIS NEWTONI* (AVES,  
DROMORNITHIDAE) ENCOMPASS EGGSHELL SAFETY  
LIMITS?

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SUPPLEMENTAL DATA—Supplemental materials are available for this article for free at

[www.tandfonline.com/UJVP](http://www.tandfonline.com/UJVP)

Some species of moa (e.g., *Dinornis robustus*, *D. novaezealandiae*, *Pachyornis geranoides*) exhibit extreme reversed sexual dimorphism (RSD), with females measuring up to three times the weight of the males (Bunce et al., 2003; Huynen et al., 2003; Worthy et al., 2005; Worthy and Scofield, 2012). Extreme dimorphism in these species has been suggested to be a response to the likelihood of accidental damage to eggs during contact incubation with increased size (Birchard and Deeming, 2009; Deeming and Birchard, 2009; however, for an alternative explanation see Olsen and Turvey, (2013). In this scenario, the much smaller males would have incubated the egg(s) in order to decrease the risk of shell breakage (Birchard and Deeming, 2009; Deeming and Birchard, 2009). Direct evidence for male incubation was presented in a recent study that found that only male DNA was present on the outer surface of *Dinornis* eggs, suggesting that in both *Dinornis* species the males incubated the egg(s) (Huynen et al., 2010).

This explanation for extreme RSD is based largely upon a study examining the relationship of eggshell thickness and strength to body mass in birds, and the trade-off between

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the ability of the egg to protect the developing embryo and subsequent ability of the embryo to hatch out of the egg (Ar et al., 1979; Birchard and Deeming, 2009). Larger birds have eggshell strengths that are weaker relative to their body mass; thus, the safety factor (= eggshell strength/incubator body mass) of the eggs decreases with increased incubator size (Ar et al., 1979). The strength of eggshell can be estimated using the equation:

$$Y = 1718L^{2.022} \quad (1)$$

where Y is the yield point in kg, the weight applied to the egg at the point at which the resultant deformation of the shell becomes irreversible, and L is eggshell thickness in cm (Ar et al., 1979). Data from Birchard and Deeming (2009) indicate that, in large extant ratites, the incubating bird has a mass 1.9–2.7 times greater than the yield point of the eggshell (Table 1). In these species, the incubating bird sits upon large clutches, often of 10 or more eggs (del Hoyo et al., 1992), with the weight of the incubating bird spread over the nest and adjacent ground. As such, the incubator's weight is spread over an area wider than a single egg, reducing the risk of damage to the individual eggs during incubation.

The Dromornithidae are an extinct Australian lineage of large flightless Anseriformes, the largest of which were comparable in size to *Dinornis* and the elephant birds from Madagascar (Murray and Megirian, 1998; Murray and Vickers-Rich, 2004). The dromornithids are currently classified into five genera and seven species (Nguyen et al., 2010). Of these, specimens of the Pleistocene *Genyornis newtoni* are by far the most abundant (Rich, 1979; Murray and Vickers-Rich, 2004).

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Here, size variation in *G. newtoni* is evaluated in order to test for dimorphism in this species. The limb bones of adult *Dinornis* vary widely in size due to extreme dimorphism (Worthy et al., 2005). Similarly, high variation in adult *Genyornis* would indicate a high degree of dimorphism in this genus as well. For this study, variation in size is assessed using measurements taken from hind limb bones of *G. newtoni* from a single locality, Lake Callabonna, South Australia. In addition, *Genyornis* eggshell safety factor is compared with that of extant ratites.

**Institutional Abbreviations**—**AM**, Australian Museum, Sydney, New South Wales, Australia; **MNZ**, Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand; **NMV**, Museum Victoria, Melbourne, Victoria, Australia; **SAM**, South Australian Museum, Adelaide, South Australia, Australia.

## MATERIALS AND METHODS

### Size Variation

Least circumference of tibiotarsi and length and midlength circumference of tarsometatarsi were measured for specimens of *G. newtoni* from Lake Callabonna, South Australia. One specimen measured (NMV P.207016) was a cast from a tarsometatarsus found at Lake Callabonna because the original specimen (SAM P.17024) could not be located. Lengths and circumferences of the leg bones are often strongly correlated with body mass and therefore have been used to estimate the size of extinct animals (Prange et al., 1979; Anderson et al., 1985; Campbell and Marcus, 1992; Campione and Evans, 2012).

Femoral measurements are generally used for size estimation. However, few femoral specimens of *G. newtoni* are complete enough to be able to measure total length or to measure circumferences in the same place in different specimens. For this reason, distal tibiotarsal and

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tarsometatarsal measurements were used instead. Examination of complete and near-complete tibiotarsi (SAM P.18434 and P.17033) indicated that least circumference is located towards the distal end of the diaphysis, thereby allowing measurements of least circumference to be taken from those distal portions of tibiotarsi in which that section of the diaphysis was preserved. Length measurements were taken using 300 mm digital callipers; lengths longer than 300 mm were measured using a measuring tape. Circumference was measured using thin twine wrapped round the bone. The point where the two ends met was marked with an ultrafine pen, and the marked length of string was then measured using callipers. All measured leg bone specimens of *Genyornis* from Lake Callabonna are from adult individuals. Adults were identified by the complete fusion, without trace of the symphyses, of the elements that form these two compound bones.

Measurements were taken on a total of 17 distal tibiotarsi and 14 tarsometatarsi that were deemed sufficiently intact to yield accurate measurements (Supplementary Data, Tables S1 and S2). Coefficients of variation were calculated for bones from the right side (nine tibiotarsi and eight tarsometatarsi) in order to avoid counting an individual more than once. Measurements were also taken from 13 *Dinornis robustus* tibiotarsi from the Bell Hill and Pyramid Valley localities, North Canterbury, New Zealand (Supplementary Data, Table S1). From this sample the coefficient of variation was calculated for nine left tibiotarsi of *D. robustus*, again to avoid including the same individual twice. In addition, 95% bootstrap confidence intervals (CIs) were calculated for each coefficient using R 2.14.0 (R Development Core Team, 2011; see Supplementary Data). Although an equation for estimating body mass of avians from tibiotarsal least circumference is available (Campbell and Marcus, 1992), this was not used to calculate size variance. *Genyornis* was substantially larger than *Struthio camelus*, the largest extant bird

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(Murray and Vickers-Rich, 2004). As such, mass estimates of *Genyornis* using regression equations will have high error margins due to extrapolation beyond the range of the data used (Schmidt-Nielsen, 1984). This makes such estimates unsuitable for calculations of size variation in a population.

### **Eggshell Safety Factor**

Williams (1981) reported average eggshell thicknesses of 0.12 cm (N = 278) for shell attributed to *G. newtoni*. Yield point of the eggshell was calculated using Equation 1. Body masses were estimated using the following equation from Campbell and Marcus (1992):

$$\log_{10}M = 2.424\log_{10}LCT + 0.076 \quad (2)$$

where M is mass in g and LCT is least shaft circumference of the tibiotarsus in mm. A range of masses was calculated using all measured tibiotarsi from Lake Callabonna (Supplementary Data, Table S1). In addition, least shaft circumferences of five femora from Lake Callabonna (SAM P.13878, P.13864, P.17001, P.17002, and P.17004) were measured. These were also used to estimate body mass using the equation:

$$\log_{10}M = 2.411\log_{10}LCF - 0.065 \quad (3)$$

in which LCF is least shaft circumference of the femur in mm (Campbell and Marcus, 1992).

TABLE 1. Maximum shell thickness (*L*), yield point (*Y*), incubator, and average incubator mass of three ratite genera and *Genyornis*.

Genus	L (cm)	Y (kg)	Incubator	Incubator Mass (kg)
<i>Struthio</i>	0.19	59.8	S	115.0
<i>Rhea</i>	0.09	13.2	M	35.2
<i>Dromaius</i>	0.10	16.3	M	33.3
<i>Genyornis</i>	0.12	23.6	?	326.8–443.8 <sup>Fem</sup> 167.7–253.2 <sup>Tbt</sup>

*Fem* and *Tbt*, estimates based on femoral and tibiotarsal least shaft circumferences, respectively. Ratite egg shell thickness and incubator mass values taken from Birchard and Deeming (2009), *Genyornis* shell thickness taken from Williams (1981). Incubator indicates which sex typically incubates the clutch (*S*, shared; *M*, male; *?*, unknown).  $Y = 1718L^{2.022}$  (Ar et al., 1979).

TABLE 2. Summary statistics for measurements used to calculate coefficients of variance.

	N	Mean (mm)	SD	CV	95% CI CV
<i>Genyornis</i> Tbt $C_{\text{least}}$	9	144.6	7.2	5.0	2.8–6.4
<i>Genyornis</i> Tmt $L$	8	349.7	19.0	5.4	1.7–6.6
<i>Genyornis</i> Tmt $C_{\text{mid}}$	8	134.4	8.1	6.0	3.4–7.2
<i>Dinornis</i> Tbt $C_{\text{least}}$	9	128.5	21.4	16.7	6.9–19.0

**Abbreviations:** *CI*, confidence interval;  $C_{\text{least}}$ , least circumference;  $C_{\text{mid}}$ , midshaft circumference; *CV*, coefficient of variance; *L*, length; *SD*, standard deviation; *Tbt*, tibiotarsi; *Tmt*, tarsometatarsi.

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## RESULTS

### Size Variation

Measurements of tibiotarsal least circumference, tarsometatarsal length, and tarsometatarsal midshaft circumference all passed Jarque-Berra tests for normality ( $P = 0.845$ ,  $0.622$ , and  $0.691$ , respectively). *Dinornis* tibiotarsal least circumferences also passed a Jarque-Berra test ( $P = 0.545$ ). Tibiotarsal least circumference of *D. robustus* had a coefficient of variation more than three times higher than that of *Genyornis*, with no overlap of the 95% CIs (Table 2). Coefficients of variation and 95% CIs were similar for all three measurements of *Genyornis* (Table 2).

### Eggshell Safety Factor

Using the equation given by Ar et al. (1979), a thickness of 0.12 cm yields an estimated yield point of 23.6 kg for *Genyornis* eggshell (Table 1). Estimated mass of *Genyornis* using tibiotarsal least circumferences ranged from 167.7 to 253.2 kg, whereas masses estimated from femoral least circumferences ranged from 326.8 to 443.8 kg (Table 1). Mass estimates from tibiotarsal measurements give a range of 7.1–10.7 times the difference between adult mass and eggshell strength; for femoral-derived estimates, the mass range is 13.8–18.8 times eggshell strength. Measurements of two femora (SAM P.13878, P.13864) and a tibiotarsus (SAM P.18434) from a single individual produced estimates of 434.3 and 253.2 kg, respectively.

## DISCUSSION

The estimated strength of *Genyornis* eggshell is at least seven times lower than estimates of adult body mass. In contrast, eggshell strength in large extant ratites is less than three times lower than average incubator mass (Table 1). Could *Genyornis* have overcome this difference

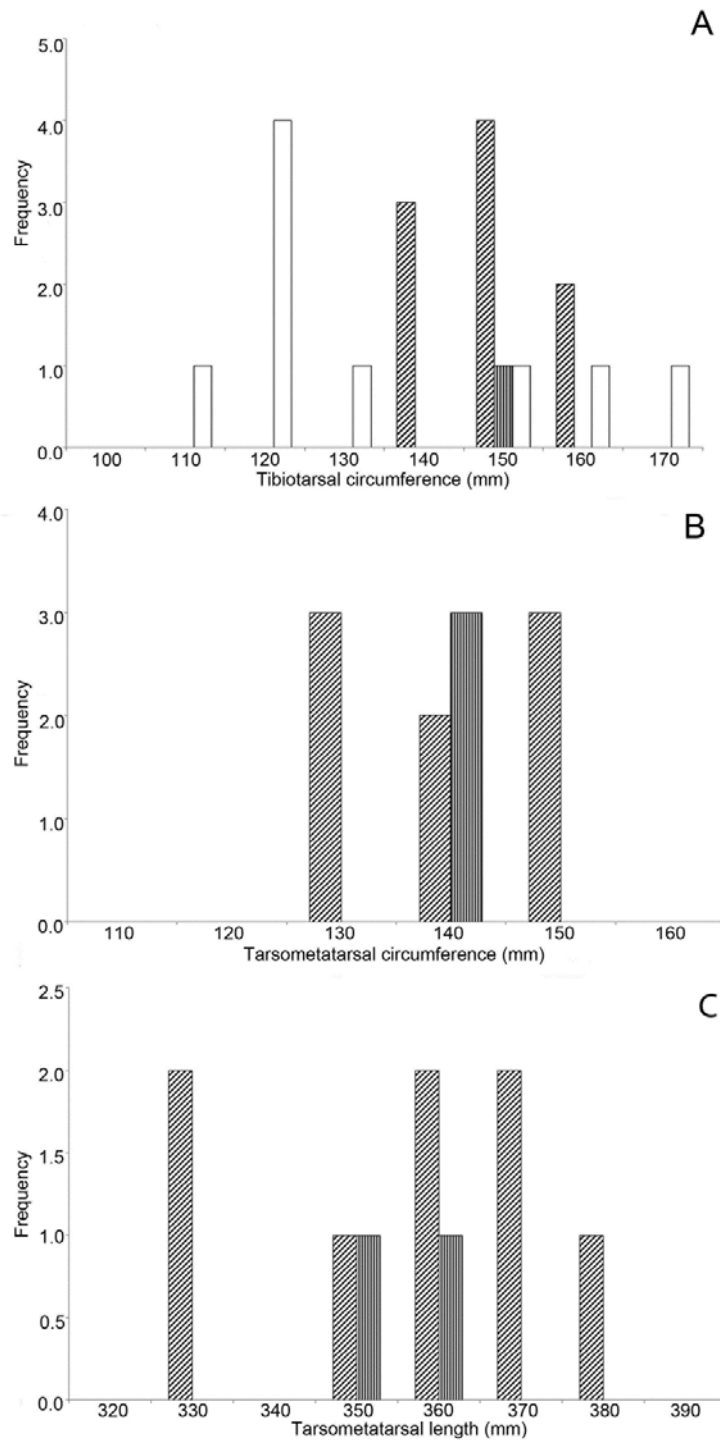
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through extreme dimorphism? Variation in the circumferences of *Genyornis newtoni* tibiotarsi is substantially lower than that of *Dinornis*, with no overlap of confidence intervals of the coefficients of variation. The amount of variation found in tibiotarsal circumference in the *D. robustus* sample is similar to that found in a larger sample of femoral lengths from Bell Hill (Turvey and Holdaway, 2005). Additionally, Worthy et al. (2005) reported similar variances for *Dinornis* femoral and tibiotarsal measurements from the Canterbury region, indicating that size variance in these bones is comparable. This suggests that despite the small sample size, the range covered is a good representation of the intraspecific variation found at this site.

The low variation in *G. newtoni* found at Lake Callabonna may either be a true biological signal or an artefact of preservational bias towards individuals of a particular size. This is plausible given that the terrestrial vertebrates found in the same deposits as *Genyornis* are all large-bodied animals that appear to have become entrapped in a clay mud (Wells and Tedford, 1995). However, the presence of *Dromaius novaehollandiae* in the deposit (Wells and Tedford, 1995) indicates the potential for the preservation of smaller individuals of *Genyornis*. Interestingly, measurements of additional specimens from Cuddie Springs, New South Wales (tibiotarsi: AM F.33402, AM E.12; tarsometatarsi: AM F.130161, AM F.112330, AM F.4481) and Lancefield, Victoria (tarsometatarsus: NMV P.41827) all fall within the range of the Lake Callabonna sample (Fig. 1; Supplementary Data, Table S1).

Alternatively, the absence of smaller individuals at Lake Callabonna may be due to a biologically biased sex ratio. Such a bias might have resulted from differential mortality, with the smaller sex experiencing higher levels of preadult mortality due to costs associated with reproduction, or due to behavioral differences. Another possibility is that the larger sex was territorial, leading to the smaller sex being excluded from the area. For example, larger female





**FIGURE 1.** Frequency histograms of **A**, tibiotarsus circumference; **B**, tarsometatarsus circumference; and **C**, tarsometatarsal length. Open bars, *Dinornis*; hatched bars (diagonal lines), *Lake Callabonna Genyornis*; hatched bars (vertical lines), *Genyornis* from Cuddie Springs and Lancefield.

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cassowaries (*Casuarius casuarius*) have been reported to scare off smaller males outside of the breeding season (Crome, 1975). Alternatively, the smaller sex may have been occupied with incubation or taking care of young, and so may have inhabited other habitats during the dry season when it is likely that most animals were entrapped at Lake Callabonna (Wells and Tedford, 1995).

A study by Allentoft et al. (2010) found that a large sample of *D. robustus* from Pyramid Valley, an area of seasonally dry forest surrounding a lake, exhibits a highly skewed adult sex ratio of 1:19 in favor of females. This provides a possible model for the Lake Callabonna *Genyornis* population. In the case of Pyramid Valley, mass-biased taphonomy can be ruled out by the presence of taxa smaller than *D. robustus* males, and a hypothesis of an uneven sex ratio in the source population can be discarded due to the much more even sex ratios at the nearby (<6 km) Bell Hill Vineyard site (Allentoft et al., 2010). The female biased sex ratio at Pyramid Valley has been suggested to have been caused by segregation of males and females (Worthy et al., 2005; Allentoft et al., 2010). This separation may have been caused by territorial exclusion from the area by dominant females or by males caring for the young in a more closed environment (Worthy et al., 2005; Allentoft et al., 2010). All of the scenarios that can be used to explain the absence of smaller individuals at Lake Callabonna are based on the following assumptions: (1) that substantially smaller adults of the opposite sex were present in the original population; (2) that all of the individuals found at Lake Callabonna are of the same sex; and (3) that the hypothesis of eggshell strength constraining body mass in flightless birds is correct. However, if it is accepted that the low size variation of *G. newtoni* is not a preservational artifact, then this would lead to the conclusion that this species was not sexually size dimorphic.

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A partial femur with the distal and proximal ends heavily eroded from Baldina Creek, SouthAustralia (SAM P.17102), was attributed to *G. newtoni* (Stirling and Zietz, 1896a, 1896b, 1900). If this specimen does belong to an adult individual of *G. newtoni*, then variation in this species would appear to be greater than that seen from specimens from Lake Callabonna, and it may represent a member of the smaller sex. The estimated body mass of this specimen based on measurement of the least circumference (185 mm) and Equation 3 is 251.9 kg. Mass estimates based on femoral circumferences are 1.8 times higher on average than estimates based on tibiotarsal circumference (Table 1), which in this case would give a mass of ~139.2 kg. This is still 5.9 times greater than the estimated yield point for *Genyornis* eggshell. It should also be noted that the Baldina Creek locality is located ~400 km from Lake Callabonna and geographic or temporal variation cannot be ruled out as potential causes for the difference in size between the specimens from these two localities.

The discrepancy between the size of *Genyornis* and the estimated yield point of *Genyornis* eggshell poses a problem for the hypothesis that the relationship between incubator mass and eggshell strength limits body mass in flightless birds. Unlike the large moas, there is currently no clear evidence for extreme sexual dimorphism that would lessen the difference between incubator mass and eggshell yield point to levels seen in extant ratites. It remains to be seen whether adult individuals of much smaller size, either of *Genyornis* or other large dromornithids, will be discovered in the future.

As the most productive locality for *G. newtoni*, Lake Callabonna provides the best opportunity to test for extreme sexual dimorphism in dromornithids and body mass constraints associated with reproductive traits in flightless birds (Rich, 1979). Further discoveries of *G.*

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newtoni at this locality are required in order to make a more comprehensive assessment of size variation in *Genyornis newtoni*.

Furthermore, it is proposed that greater investigation of the hypothesis of eggshell strength limiting size in flightless birds is required. Currently, this hypothesis does not consider whether total incubator mass is a true reflection of the pressures placed on each egg during incubation. Several factors may affect the stresses placed upon the eggshell, including the number of eggs, the brooding posture (which influences how much weight is placed upon the ground or nest as well as the eggs), and behavior, which influences the likelihood of accidental impact between neighboring eggs and/or between the eggs and the incubator. Further study to determine the actual pressures placed upon the eggs during incubation is required in order to examine whether total incubator mass is an adequate measure of the external forces experienced by the egg during incubation.

## CONCLUSION

The difference between eggshell strength and adult body mass in *Genyornis newtoni* is much higher than that of extant, large flightless birds. The low variance in *G. newtoni* leg bone measurements precludes the presence of extreme dimorphism that would overcome this. Therefore, the current evidence indicates that the discrepancy between eggshell strength and body mass was not countered by smaller individuals of one sex incubating the eggs, as has previously been suggested for the similarly sized *Dinornis*. This calls into question the hypothesis that the relationship between eggshell strength and incubator size is the limiting factor in flightless bird body size.

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## Chapter 2

# Phylogenetic variation in hindlimb bone scaling of flightless theropods

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**Abstract**

The robusticity of the weight-bearing limbs of large terrestrial animals is expected to increase at a more rapid rate than in their smaller relatives. This scaling enables large species to maintain stresses in the limb bones that are similar to those seen smaller ones. Differential scaling has previously been found in mammals and non-avian theropods but has not been demonstrated in terrestrial, flightless birds. In this study, polynomial regressions of leg bone length and circumference in flightless birds were carried out to test for a similar relationship to that seen in non-avian theropods. Flightless birds exhibit differential scaling, with the femora of large taxa becoming thicker relative to their lengths at a greater rate than in smaller taxa. Contrary to previous studies, no evidence was found for differential scaling in the leg bones of non-avian theropods. There is instead phylogenetic variation in limb bone scaling between taxonomic groups, with tyrannosaurs in particular scaling differently to other groups. Phylogenetically corrected quadratic regressions and separate analyses of taxonomic groupings found little phylogenetic variation in flightless birds. It is suggested here that the non-linear scaling seen in avian femora is due to the need to maintain the position of the knee under a more anterior centre of mass. The femur of non-avian theropods is not so constrained, so the scaling of this bone is linear with greater variability between clades. Phylogenetic variation in limb bone scaling further broadens the confidence intervals for mass-predictive scaling equations based on limb bone measurements of non-avian theropods.

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## Introduction

Aves is the most diverse tetrapod class with approximately 10,000 extant species ranging in size from the bee hummingbird (~0.002kg) to the ostrich (~110kg) (Gill, 2007; Dunning Jr., 2008; Jetz et al., 2012). There is now an abundance of evidence indicating that birds are a derived group of theropod dinosaurs (Huxley, 1868, 1870; Ostrom 1973, 1976; Padian & Chiappe, 1998). A gradual accumulation of the traits that today distinguish Aves occurred throughout the evolution of Theropoda (Padian & Chiappe, 1998; Brusatte *et al.* 2014). Much attention is paid to changes directly related to the evolution of powered flight, including shifts in the orientation of the bones of the pectoral girdle (Jenkins 1993; Senter, 2006), changes in forelimb proportions (Middleton & Gatesy, 2000; Benson & Choiniere, 2013; Dececchi & Larsson 2013), and body size reduction (Turner *et al.* 2007; Novas *et al.* 2012; Dececchi & Larsson 2013; Benson *et al.* 2014; Lee *et al.* 2014; Puttick *et al.* 2014). However, a number of key changes also occurred in the pelvic girdle and limbs (Gatesy & Middleton, 1997; Hutchinson 2001; Hutchinson & Allen, 2009). One of these is the shift in femoral orientation from a near vertical position to a sub-horizontal one, with the long axis running cranio-ventrally (Gatesy, 1990, 1991; Hutchinson & Allen, 2008).

The change in femoral orientation is associated with a more cranial centre of mass in birds compared to non-avian theropods. It is due either to the reduction in the tail and associated musculature (Gatesy, 1990), an increase in the relative size of the pectoral girdle (Allen *et al.*, 2013), or a combination of the two. The sub-horizontal orientation of the femur allows the knee to be placed under the centre of mass (Gatesy, 1990, 1991). A major implication of this construction is that the loading regime of avian femora is dominated by torsion due to the ground reaction force (GRF) crossing the long axis of the bone (Carrano, 1998; Carrano & Biewener,

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1999; Main & Biewener, 2007). Although the degree to which torsion is greater than other strains varies during the step cycle, it is only superseded by bending strain at the end of it, prior to push-off of the foot when the femur is at its most vertical (Carrano, 1998; Carrano & Biewener, 1999). As bone is less resistant to twisting than to axial compression or bending strains, avian femora are required to be more robust to torsional strain (Gatesy 1991; Biewener & Dial, 1995; De Margerie *et al.* 2005). These different factors likely impose greater constraints on femoral morphology in birds compared to non-avian theropods.

Several studies have shown that avian femora become more robust with increasing size. Specifically, diameter and the maximum second moment of area scale with positive allometry relative to femoral length (Gatesy, 1991; Carrano, 1998; Doube *et al.*, 2012). Comparisons with non-avian theropods indicate that avian femora are also relatively more robust than those of their non-avian counterparts (Gatesy, 1991; Carrano, 1998; Campione *et al.* 2014). However, the datasets used in these studies often contain large numbers of flying species (Carrano, 1998). In other cases, a separate analysis of the flightless or “ground” component of the dataset is predominantly focused on palaeognathous birds (Gatesy, 1991; Doube *et al.*, 2012). The former is an issue because flying birds often make alternative uses of the hind-limbs (e.g. prey capture, perching) that are associated with differences in femoral dimensions (Zeffer *et al.*, 2003; Doube *et al.*, 2012). These adaptations potentially make the regression slope a composite of scaling relationships from different functional groups. On the other hand, a palaeognath-dominated terrestrial data set makes it difficult to tell whether differences in scaling are due to functional or phylogenetic differences. Studies with such datasets overlook the large amount of data which could be added by including flightless Gruiformes and other avian taxa which have independently evolved a terrestrial flightless condition.

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If the function of the avian femur does limit body mass, then regressions of femoral length versus circumference should exhibit a curvilinear pattern with large taxa scaling with a lower exponent than small taxa, i.e., there should be differential scaling between large and small taxa in log-log space. A previous study found that interspecific differential scaling occurs in non-avian theropods between femoral and tibial lengths and their respective circumferences with exponents decreasing in larger theropods (>300kg) (Christiansen, 1999a). Such a scaling pattern has been interpreted as a feature which enables larger taxa to maintain similar stresses in the bones to smaller taxa despite their increased size by having relatively more robust leg bones (Bertram & Biewener, 1990; Carrano, 2001). This relationship between leg bone length and circumference has also been found in mammals (Bertram & Biewener, 1990; Christiansen, 1999b, c). Although maintaining similar stresses to smaller species in large mammals during stance can be achieved through linear allometric patterns, e.g. elastic similarity (McMahon, 1973, 1975), such a relationship alone may not be sufficient to maintain those stresses during locomotion (Alexander, 1977). However, a recent study of allometric patterns in extant quadrupeds found no difference in the scaling coefficient of body mass against femoral circumference in different size classes of mammals (Campione & Evans, 2012). Differences between size classes were found in regressions of femur circumference and length (Campione & Evans, 2012). This would suggest that the previously observed pattern of scaling between femoral circumference and length in mammals was driven by variation of the latter, rather than by changes in the relationship between circumference and body mass.

Given the greater constraints placed upon avian femora it is expected that the scaling curve of femoral circumference ( $x$ ) and length ( $y$ ) should exhibit a lower inflection point than that seen in non-avian theropods. The additional constraints on avian leg bones outlined here only

apply to the femora. As a result, curvilinear scaling of the tibiotarsus of terrestrial birds is not expected, particularly given that few avian species have attained a body mass approaching the 300 kg cut-off point between small and large theropods used by Christiansen (1999a) (Amadon, 1947; Nguyen *et al.* 2010). These predictions are tested here using a large, phylogenetically broad sample of terrestrial flightless birds fit to be compared with non-avian theropods.

## Materials and methods

Femoral length and least circumference measurements were taken for 58 species of terrestrial flightless birds. Of these, 17 species represented the Palaeognathae and 23 represented the “core-Gruiformes” (here referred to as Gruiformes). The dataset also included species of Columbiformes, Psittaciformes, Galliformes, and Anseriformes. Length was measured from the tip of the femoral trochanter to the distal-most point of the lateral condyle. Tibiotarsal length and least circumference measurements were taken from 54 species. Due to the frequency of damage to the cnemial crest in fossil and sub-fossil specimens, tibiotarsal length was measured from the interarticular area to the distal-most point of the distal condyles. Length was measured to the nearest 0.01mm using 300mm digital callipers and rounded to the nearest 0.1mm. A small number of specimens were measured using 150mm dial callipers to the nearest 0.1mm (Appendix S1). Distances over 30cm were measured to the nearest 1mm using a tape measure. Circumferences were measured with cotton twine wrapped tightly around the bone shaft and marked at the end with ultra-fine marker pen; the length of the marked section was then measured using 300mm callipers. For bones with a diameter less than 7mm, individual strands of twine were used. All measurements were repeated twice and the average was used for analysis. In

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addition, measurements of the phorusrhacid, *Psilopterus lemoinei* were provided by Federico Degrange (personal communication 2013). Tibiotarsal length for *P. lemoinei* was measured from the area interarticularis to the sulcus intercondylaris (Degrange personal communication 2013).

Only adult specimens were measured. These were identified by the following characteristics: femur exhibiting a fully formed intercondylar bridge, patella fully fused to the tibia with no trace of patellar-tibial symphysis, tibiale and fibulare fully fused to the tibia with no evidence of symphysis, and a completely formed supratendinal bridge (when present).

Non-avian theropod femoral lengths and circumferences were taken from the literature. The core of the dataset was provided by Carrano (PhD thesis 1998) and Christiansen (1999a) with additional measurements for 15 taxa added from more recent literature (Appendix S2). The former author used mid-shaft circumferences whilst the later used least circumferences. A one-way paired t-test of  $\log_{10}$  circumference measurements from specimens used in both datasets indicated that the Christiansen (1999a) measurements were significantly lower ( $t = -2.47$ ,  $DF = 21$ ,  $p = 0.011$ ) than the corresponding measurements taken by Carrano (PhD thesis 1998). Linear regression of the difference between corresponding measurements against the Carrano (PhD thesis 1998) measurements found no significant relationship between these variables (multiple  $R^2 = 0.058$ ; adjusted  $R^2 = 0.011$ ;  $p = 0.282$ ), indicating that the difference between measurements from the two sources did not increase with size of measurement. A simple transform function was therefore added to make the two data sets compatible. It involved calculating the difference between  $\log_{10}$  mid-shaft circumference and  $\log_{10}$  least circumference in specimens present in both data sets. The mean of the differences was then subtracted from mid-shaft circumferences to give an estimated least circumference. Species averages were used except when specimens were listed

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as indeterminate members of a particular genus, family, or the clades Tetanurae or Theropoda. These specimens were treated as separate data points. Taxa which have since been found to be *nomina dubia* were removed and any synonyms were merged. This protocol yielded a sample size of 76, with 54 of these being identified to species level.

Tibiotarsal length and least circumference measurements for non-avian theropods were mostly taken from Christiansen (1999a) and Carrano (PhD thesis 1998) with additional data added from Benson (2010). As with the femoral data, Christiansen (1999a) used least shaft circumferences and Carrano (PhD thesis 1998) used mid-shaft circumferences. The same procedure as outlined for femoral measurements was used to make the two data sets compatible. As with the femora, a linear regression of the differences between  $\log_{10}$  circumferences of specimens in both data sets against the equivalent Carrano (PhD thesis 1998) measurements found no significant relationship between the differences and increased circumference (multiple  $R^2 = 0.011$ ; adjusted  $R^2 = -0.065$ ;  $p = 0.706$ ). The total sample size was 51, with 42 identified to species level.

Quadratic regressions were used to test for a curvilinear relationship between femoral length and circumference and between tibiotarsal length and circumference using R.3.01.0 (R Core Development Team, 2014). All data were  $\log_{10}$  transformed and centred on the mean prior to analysis. Centring of the data was carried out in order to reduce multicollinearity between the predictor variables (Kraemer & Blasey, 2004). The second-order variable was produced by squaring the centred circumferences. Ordinary least squares was then used to regress length ( $y$ ) against circumference ( $x$ ) plus the second-order variable against length ( $y$ ), i.e., to test a quadratic model. Quadratic models were compared with linear models using Akaike's Information



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Criterion corrected for small sample size (AICc) (Akaike, 1973; Hurvich & Tsai, 1989). AICc weights were generated using the “aictab” function in the R package “AICcmodavg” (Mazerolle, 2015). Higher AICc weights indicate that a greater likelihood that the model fits the data better than other candidate models (Mazerolle, 2006). For the avian data set, species averages were used for regression, with the exception that several subspecies were treated as separate data points because they exhibited substantial differences in size. These were *Dromaius novaehollandiae ater* (Heupink *et al.*, 2011), *Dromaius novaehollandiae baudinianus* (Worthy *et al.*, 2013), *Euryapteryx curtus curtus* (Worthy & Scofield, 2012), *Euryapteryx curtus gravis* (Worthy & Scofield, 2012), *Gallirallus australis australis*, and *Gallirallus australis scotti*.

Whether relatedness between taxa affects the significance of the quadratic term was tested in non-avian theropods using phylogenetic generalized least squares (PGLS). Phylogenetic trees were constructed using matrix representation parsimony (MRP) (Baum, 1992; Ragan, 1992) in the programme PAUP 4.0 by means of a heuristic search (see Appendix S3 for source trees). All theropods in the dataset were included with the exception of those listed as Theropoda or Tetanurae indeterminate. Specimens listed as family indeterminate were coded so that they were free to be placed anywhere within the family specified. Due to the high number of permutations caused by a number of individuals being listed as family or genus indeterminate, the number of trees generated was limited to 100,000. Of these, 1000 trees were selected randomly without replacement and dated using age ranges from the Paleobiology Database. The trees were time-scaled using the “timePaleoPhy” function in the “paleotree” package in R (Bapst, 2012) with minimum branch lengths set to 1 million years. Quadratic PGLS regressions were carried out on femoral and tibiotarsal data for each tree using the default “pgls” function assuming a Brownian motion model of evolution in the “caper” package (Orme *et al.* 2013) and the *p*-values stored. To

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account for multiple comparisons, the  $p$ -values were adjusted using the Benjamini-Hochberg correction (Benjamini & Hochberg, 1995).

A modified version of this analysis was carried out using the avian dataset. Prior to tree generation, taxa of uncertain affinity and/or lacking in dating information were excluded. This protocol trimmed the dataset to 47 taxa. The dromornithids, *Genyornis newtoni* and *Dromornis stirtoni*, and *Diatryma* were coded as “?” for nodes representing the basal split of Galloanserae and the base of Anseriformes (see Murray & Vickers-Rich, 2004; Mayr, 2011). As a result, 52 trees were generated. These trees were timescaled in the same way as the non-avian theropod tree with the exception that the dates used were a combination of molecular divergence dates and stratigraphic ranges due to the former being unavailable in a number of cases. In the case of extant taxa, first appearance dates were set to the date of molecular divergence and last appearance dates to the present. To account for the fact that molecular divergence dates do not necessarily indicate the presence of the extant species but instead to the origination of the lineage leading to that species, “timePaleoPhy” was set to add terminal ranges so that tips corresponded to last appearance dates rather than first appearances.

In order to test for differences between avian phylogenetic groups separate standard major axis (SMA) regressions for the ratites ( $n= 20$ ), Dinornithiformes ( $n= 8$ ) Galloanserae ( $n= 12$ ), Gruiformes ( $n= 24$ ), and Rallidae ( $n= 22$ ) in the avian femoral dataset were carried out using the “lmodel2” package (Legendre, 2013). The 95% confidence intervals (CI) for the intercepts and slope coefficients were compared for overlap between groups. For the non-avian theropod femoral dataset SMA regressions were carried out using Allosauroidea ( $n= 9$ ), Tyrannosauroidea ( $n= 15$ ), Tyrannosauridae ( $n= 11$ ), Ornithomimidae ( $n= 9$ ) Maniraptora ( $n= 16$ ), and

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Oviraptorosauria ( $n=10$ ). For the tibial dataset SMA regressions were carried out using the Tyrannosauroidae ( $n=10$ ), Tyrannosauridae ( $n=8$ ), Ornithimimidae ( $n=8$ ), and Maniraptora ( $n=9$ ). In addition pair-wise comparisons of slope coefficients using the “slope.com” function in the “smatr” package (Warton *et al.* 2012) in R were carried out to test whether SMA regressions shared a common slope. SMA intercepts were compared using two-tailed  $t$ -tests using an R function that prevents the alteration of the true slopes that occurs in standard  $t$ -tests of intercepts (Campione & Evans, 2012). Benjamini-Hochberg corrections (Benjamini & Hochberg, 1995) were applied to  $p$ -values of pairwise tests to account for multiple comparisons.

## Results

### Flightless avian femora

Quadratic regression of femoral least circumference versus length of femur in flightless birds found a significant negative shift in the slope coefficient with the addition of the second order polynomial (Table 1, Fig. 1). AICc weights of the linear and quadratic models indicate a 100% probability that the quadratic model provides a better fit to the data (Table 1).

Prior to adjustment of  $p$ -values the quadratic term in the phylogenetic generalized least scores (PGLS) regressions indicated a negative shift in the coefficient and was always significant ( $< 0.05$ ) regardless of the tree used, with  $p$ -values varying between 0.020 and 0.045. After adjustment, all  $p$ -values were marginal (0.046). Quadratic regression of the trimmed dataset used for PGLS without phylogenetic correction also found a marginally significant, negative quadratic term ( $p=0.045$ ). These PGLS results should be interpreted with caution due to the combination of minimum and maximum dates used to timescale the tree. As a result, the different branch

lengths may not accurately reflect true divergence dates for a number of species. In addition, a sufficiently long period of evolutionary separation has occurred between certain lineages for covariance to have become minimal. For example, the closest relative of the flightless parrot, *Strigops habroptilus*, in the dataset is a cariamiform (*Psilopterus lemoinei*). These lineages have likely been separated since the Palaeocene (Mayr, 2014). Thus in these cases PGLS may have overestimated co-variation between taxa. However, in other parts of the tree lineages have been separated for relatively short periods of time. For instance, several of the flightless rallids may have diverged from their volant relatives within a few hundred thousand years (Kirchman, 2012). With these considerations in mind, the similarity between phylogenetically corrected and uncorrected regressions suggests that reduced significance of the quadratic term in the former is due to reduced sample size rather than the phylogenetic effects. This interpretation is supported by removal of Rallidae from the sample. The rails occupy the lower end of the x-axis (Fig. 2.), so to test whether differential scaling was caused by differences between the smaller rallids and other taxa uncorrected quadratic regression was repeated with this group removed. Despite removal of 22 data points the quadratic term remained significant, albeit at a weaker level (Table 1).

Confidence intervals for slope coefficients and intercepts from SMA regressions overlap for the majority of the phylogenetic groups (Table 2). The only exception was the non-overlapping 95% CIs of the slope coefficients for Rallidae and Ratitae, with Rallidae scaling with a higher slope coefficient (0.873 compared to 0.700). Pair-wise comparisons found the Rallidae coefficient to be significantly different to both the ratite and Galloanserae slopes after Benjamini-Hochberg (BH) correction of  $p$ -values (Table 3). The Gruiformes and Ratitae slopes were also found to be significantly different from each other (Table 3). These results are likely due to rails

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occupying the lower end of the x-axis (Fig. 2) and therefore scaling with a higher coefficient than the larger ratites.

The linear component and overall avian SMA regression indicates that femoral length scales with negative allometry ( $b < 1$ ) with respect to least shaft circumference.

### **Non-avian theropod femora**

Quadratic regression of femoral least circumference ( $x$ ) and length ( $y$ ) of non-avian theropods found no significant change in slope coefficient with the addition of the second order polynomial (Table 1). AICc weights indicate that the linear regression model has a greater likelihood of fitting the data better than the quadratic model (Table 1).

Prior to adjustment,  $p$ -values for slope changes in PGLS quadratic regressions were found to be significant in 775 out of 1000 cases. Thus, significance depended on the patterns of divergences in the tree. After Benjamini-Hocberg (BH) corrections of  $p$ -values, slope changes in PGLS quadratic regressions were significant ( $p < 0.05$ ) for 258 out of 1000 regressions. However, all  $p$ -values were marginally significant with none being less than 0.043.

SMA regressions show that tyrannosauroids and tyrannosaurids scale with lower slope coefficients and higher intercepts than all other theropod groupings (Fig. 3, Table 4). These two groupings exhibit similar slope and intercept values, with the tyrannosaurids exhibiting broader confidence intervals for both values, which is likely to be due to a decrease in sample size (from 16 to 11). The Ornithimimidae are the only group for which the 95% confidence intervals of the slope coefficients and intercept overlap with tyrannosauroids. However, the confidence intervals

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are extremely broad for ornithomimids (Table 4). The allosauroid regression also has broad confidence intervals for the slope. In both cases this breadth is likely to be due to small sample sizes ( $n = 9$ ). After BH corrections, pair-wise comparisons found the Maniraptoran slope to be significantly different from the tyrannosauroid and tyrannosaurid slope, and the allosauroid slope to be significantly different from the tyrannosauroid slope (Table 5).

The tyrannosauroid slope combines a substantially higher intercept and a slower increase in length with circumference compared to other non-avian theropods. In other words, smaller tyrannosauroids appear to have longer femora compared to other taxa with similar femoral circumferences, but as femoral circumference increases this situation is reversed with tyrannosauroids having relatively shorter femora compared to other taxa.

The phylogenetic position of a small number of taxa in the dataset is currently disputed. *Timimus*, which here is included as a tyrannosauroid based on the latest description by Benson *et al* (2012), has previously been assigned to Ornithomimidae (Rich & Vickers-Rich, 1994) and Maniraptora (Agnolin *et al.*, 2010). Removal of *Timimus* from the Tyrannosauroid regression produced a slope (0.699, 95% CI = 0.622–0.781) similar to that of Tyrannosauridae. There is also some doubt as to the placement of two species within the Allosauroidea femoral dataset. *Chilantaisaurus tashiukouensis* was regarded as a tetanuran of uncertain placement by Novas *et al.* (2013), whilst *Fukuiraptor kitadenensis* was found to be a tyrannosauroid in the same study (Novas *et al.*, 2013). Both of these taxa were here included in Allosauroidea based on the results of Carrano (2012), and Zanno & Makovicky (2013). Inclusion of *Fukuiraptor* within Tyrannosauroidea resulted in coefficients of 0.622 (95% CI = 0.588–0.743) and 0.727 (95% CI = 0.650–0.811) with the presence and absence of *Timimus* in the same dataset respectively. Despite

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this uncertainty in all cases the tyrannosauroid coefficient remains significantly ( $p < 0.05$ ) lower than the maniraptoran coefficient even after BH correction of  $p$ -values.

In a plot of  $\log_{10}$  least circumference ( $x$ ) versus  $\log_{10}$  length ( $y$ ) the maniraptoran data points fall at the lower end of the  $x$ -axis with the exception of *Gigantoraptor erlianensis*, which plots a considerable distance away from the next largest species, *Deinonychus antirrhopus* (Fig. 3). Given the disproportionate effect of points at the ends of the regression line and the substantial distance between *G. erlianensis* and the remaining maniraptorans, both the maniraptoran and oviraptorosaur major-axis regressions were repeated with *G. erlianensis* removed. Removal of *G. erlianensis* led to a slight reduction in slope coefficients and a broadening of confidence intervals. This led to the confidence interval of the oviraptorosaur slope coefficient overlapping with that of the tyrannosauroids. The absence of *G. erlianensis* also had little effect on the maniraptoran slope intercept (Table 4). However, the confidence interval for the oviraptorosaur intercept broadened considerably (from 0.278–0.771 to 0.022–1.026). Therefore, the gross proportions of the femur of *G. erlianensis* appear to have been attained through continuation of a linear relationship between femoral length and circumference in smaller maniraptorans.

Regression coefficients indicate that in general, the length of non-avian theropod femora scales with negative allometry with respect to femoral circumference (Table, 4). Compared to the flightless avians this relationship is closer to geometric similarity ( $b = 1$ ). Thus, femoral circumference relative to length increases more rapidly in flightless birds than in non-avian theropods.

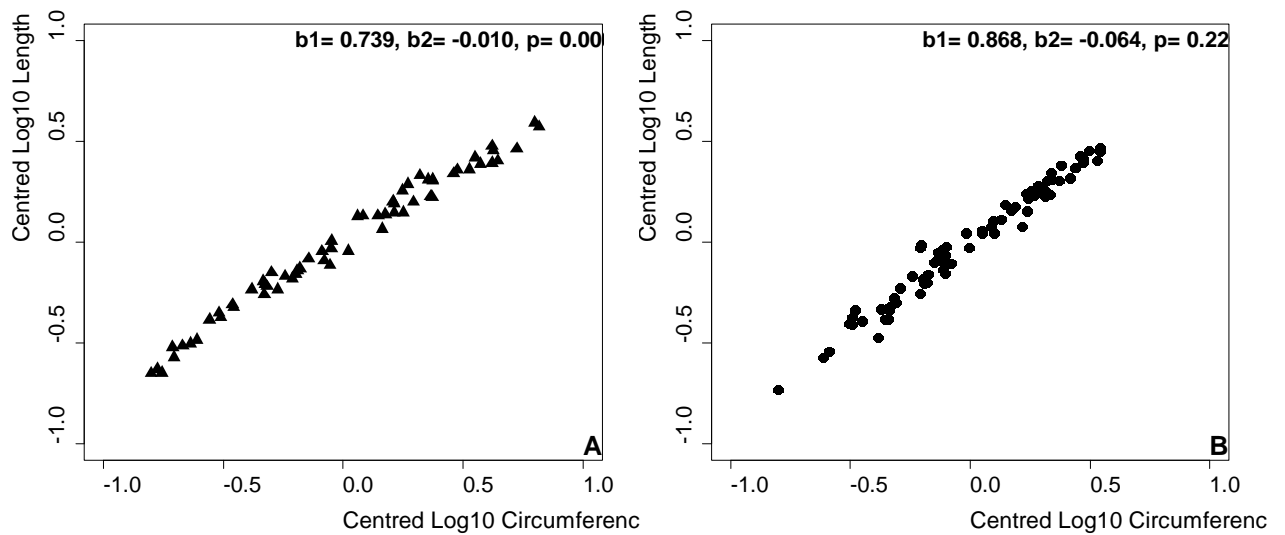
**Table 1** Quadratic regressions of length versus least circumference for avian and non-avian theropod (N-aT) femora and tibiotarsi; *b*1, linear coefficient; *b*2, coefficient for 2nd order polynomial; AICc Wt, Akaike weight. Significance level of coefficients is indicated with asterisks; \*, 0.05; \*\*, 0.01; \*\*\*, 0.001.

	N	Linear		Quadratic		
		b1	AICcWt	b1	b2	AICcWt
<b>Aves femora</b>	62	0.742***	0.00	0.739***	-0.100***	1.00
<b>Aves femora not including Rallidae</b>	40	0.723***	0.20	0.715***	-0.154*	0.80
<b>N-a Theropoda femora</b>	76	0.872***	0.59	0.868***	-0.064	0.41
<b>Aves tibiotarsi</b>	59	0.840***	0.76	0.840***	0.001	0.24
<b>N-a Theropoda tibiotarsi</b>	51	0.753***	0.15	0.721***	-0.169*	0.85

**Table 2** Standard major axis regressions of femoral dimensions for avian groupings including 95% confidence intervals for intercepts (*a*) and slopes (*b*).

	<i>a</i>	2.5%CI	97.5%CI	<i>b</i>	2.5%CI	97.5%CI
<b>Aves (n=62)</b>	0.848	0.807	0.889	0.748	0.723	0.774
<b>Ratitae (n=20)</b>	0.931	0.731	1.106	0.709	0.621	0.809
<b>Galloanserae (n=12)</b>	0.831	0.684	0.963	0.739	0.666	0.819
<b>Gruiformes (n=24)</b>	0.756	0.696	0.812	0.832	0.785	0.882
<b>Rallidae (n=22)</b>	0.711	0.641	0.780	0.874	0.817	0.936





**Fig.1** Femoral scaling of flightless birds (**A**) and non-avian theropods (**B**), “*b1*” is the linear coefficient, “*b2*” is the quadratic coefficient. The *p*-values given are for the significance of the quadratic coefficient.

**Table 3** *P*-values from pair-wise comparisons of standard major axis regression coefficients (**bold**) and intercepts (*italics*) corrected for multiple comparisons using Benjamini-Hochberg correction (Benjamini & Hochberg, 1995).

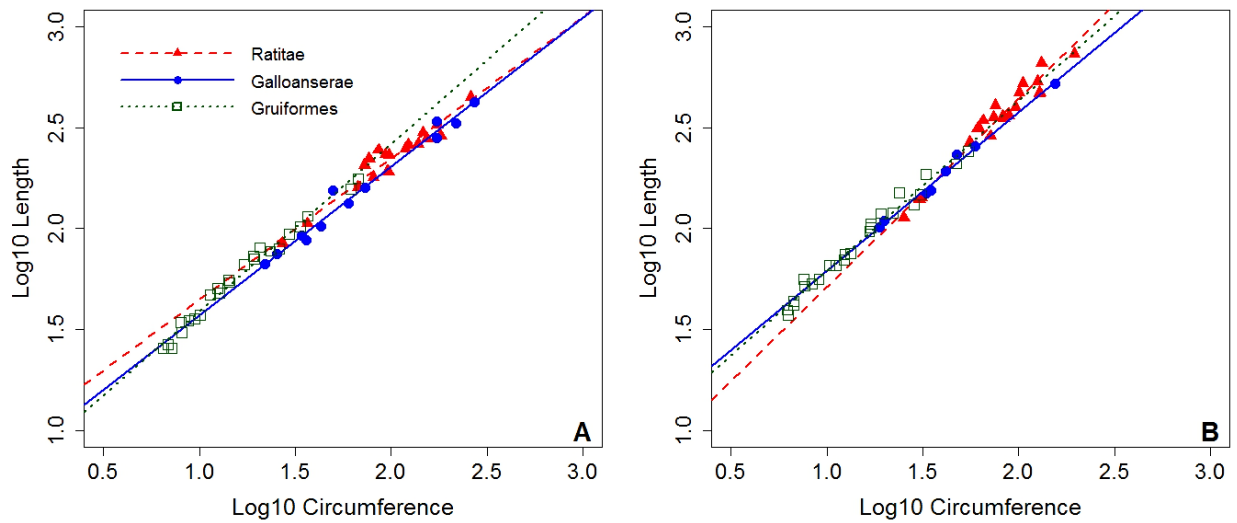
	<b>Ratitae</b>	<b>Galloanserae</b>	<b>Gruiformes</b>	<b>Rallidae</b>
<b>Ratitae</b>	-	<b>0.603</b>	<b>0.049</b>	<b>0.022</b>
<b>Galloanserae</b>	<i>0.376</i>	-	<b>0.055</b>	<b>0.022</b>
<b>Gruiformes</b>	<i>0.117</i>	<i>0.302</i>	-	-
<b>Rallidae</b>	<i>0.080</i>	<i>0.125</i>	-	-

**Table 4** Standard major axis regressions of femoral dimensions for non-avian theropod groupings including 95% confidence intervals for intercepts (*a*) and slopes (*b*).

	<i>a</i>	2.5%CI	97.5%CI	<i>b</i>	2.5%CI	97.5%CI
<b>N-a Theropoda (n=76)</b>	0.746	0.667	0.821	0.885	0.850	0.921
<b>Allosauroida (n=9)</b>	0.752	0.456	1.014	0.883	0.780	1.000
<b>Tyrannosauroida (n=15)</b>	1.336	1.161	1.493	0.645	0.580	0.717
<b>Tyrannosauridae (n=11)</b>	1.166	0.854	1.431	0.713	0.606	0.838
<b>Ornithimimidae (n=9)</b>	0.775	-0.684	1.588	0.888	0.495	1.594
<b>Maniraptora (n=16)</b>	0.623	0.478	0.758	0.941	0.869	1.019
<b>Maniraptora not inc.</b>	0.694	0.497	0.870	0.900	0.803	1.009
<b><i>Gigantoraptor</i> (n=15)</b>						
<b>Oviraptorosauria (n=10)</b>	0.540	0.282	0.767	0.981	0.863	1.115
<b>Oviraptorosauria not inc.</b>	0.587	0.059	0.994	0.956	0.719	1.240
<b><i>Gigantoraptor</i> (n=9)</b>						

**Table 5** *P*-values of pair-wise comparisons of standard major axis regression coefficients and intercepts after Benjamini-Hochberg correction for multiple comparisons (Benjamini & Hochberg, 1995).

	Allo.	Tyrannosauroid.	Tyrannosaurid.	Orni.	Mani.
<b>Allosauroida</b>	-	<b>0.004</b>	<b>0.070</b>	<b>0.983</b>	<b>0.523</b>
<b>Tyrannosauroida</b>	0.004	-	-	<b>0.467</b>	<b>&lt;0.001</b>
<b>Tyrannosauridae</b>	0.071	-	-	<b>0.565</b>	<b>0.015</b>
<b>Ornithomimidae</b>	0.962	0.259	0.598	-	<b>0.940</b>
<b>Maniraptora</b>	0.645	<b>&lt;0.001</b>	0.020	<b>0.786</b>	-



**Fig. 2** Femoral (A) and tibiotarsal (B) scaling of flightless birds from three phylogenetic groups. Fitted lines are based on MA regression results (see Table 2).

**Table 6** Standard major axis regressions of tibiotarsal dimensions for avian groupings including 95% confidence intervals for intercepts (*a*) and slopes (*b*).

	<i>a</i>	2.5%CI	97.5%CI	<i>b</i>	2.5%CI	97.5%CI
<b>Aves (n=59)</b>	0.938	0.893	0.981	0.847	0.819	0.877
<b>Ratitae (n=21)</b>	0.774	0.572	0.956	0.935	0.839	1.042
<b>Galloanserae (n=8)</b>	1.005	0.871	1.126	0.787	0.711	0.870
<b>Gruiformes (n=24)</b>	0.946	0.883	1.005	0.844	0.793	0.899
<b>Rallidae (n=22)</b>	0.912	0.838	0.985	0.878	0.816	0.946

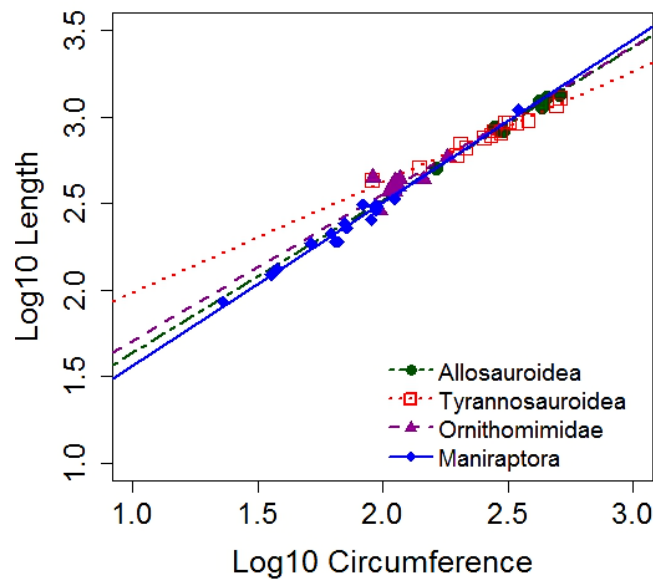
**Table 7** *P-values of pairwise comparisons of standard major axis reversion coefficients (bold) and intercepts (italics) after Benjamini-Hochberg correction for multiple comparisons (Benjamini & Hochberg, 1995).*

	<b>Ratitae</b>	<b>Galloanserae</b>	<b>Gruiformes</b>	<b>Rallidae</b>
<b>Ratitae</b>	-	<b>0.086</b>	<b>0.171</b>	<b>0.334</b>
<b>Galloanserae</b>	<i>0.201</i>	-	<b>0.241</b>	<b>0.161</b>
<b>Gruiformes</b>	<i>0.201</i>	<i>0.439</i>	-	-
<b>Rallidae</b>	<i>0.231</i>	<i>0.285</i>	-	-

### Flightless avian tibiotarsi

Quadratic regression of tibiotarsal least circumference versus length found no significant change in slope with the addition of the second order polynomial (Table 1). AICc weights indicate a 76% probability that the linear model fits the data better than the quadratic regression. Quadratic phylogenetic least squares regressions also found the quadratic term to be non-significant regardless of the tree used. The 95% CIs for slope coefficients and intercepts of SMA regressions exhibit overlap between all phylogenetic groupings with no significant differences found between slopes after BH correction of *p*-values from pair-wise comparisons (Tables 6, 7).

The scaling of tibiotarsal length against circumference is negatively allometric. However, the slope coefficient is closer to geometric similarity than the one generated for avian femora (Table 1, 2, 4).



**Fig. 3** Femoral scaling of non-avian theropod groups. Fitted lines based on results of MA regressions (see Table 3).

### Non-avian theropod tibia

Quadratic regressions of tibiotarsal least circumference versus length indicate a significant negative shift in slope coefficient with the addition of the second order polynomial (Table 1). AICc weights indicate a probability of 85% that the quadratic regression is a better model than the linear regression. However, of the 1000 PGLS regressions only 22 found the shift in slope to be significant. This count was reduced to zero after Benjamani-Hochberg correction of  $p$ -values.

Results of SMA regressions of the different phylogenetic groups suggest different scaling relationships in different taxa, with tyrannosaurids scaling with a higher intercept and lower slope coefficient than the ornithomimids and maniraptorans. However, confidence intervals for both intercepts and slope coefficients were extremely broad (Table 8). Pair-wise comparisons of SMA regression coefficients found no significant differences between slopes, even prior to BH

correction. Despite 8 out of 10 of the tyrannosauroids being also tyrannosaurids, the regression of the former indicated a lower intercept and higher slope coefficient than the latter, with some overlap of confidence intervals. This result was created by a single species, *Calamosaurus foxi*, a possible basal tyrannosauroid (Naish & Martill, 2007) which is the smallest tyrannosauroid in the dataset and falls a considerable distance away from the remaining ones. Removal of *Calamosaurus* from the tyrannosauroid dataset produces an intercept and slope coefficient similar to that of Tyrannosauridae with closely matched confidence intervals (Table 5).

Regression coefficients show that tibiotarsal length scales with negative allometry with respect to circumference in non-avian theropods (Table 1, 6).

**Table 8** Standard major axis regressions of tibiotarsal dimensions for non-avian theropod groupings including 95% confidence intervals for intercepts (*a*) and slopes (*b*).

	<i>a</i>	2.5%CI	97.5%CI	<i>b</i>	2.5%CI	97.5%CI
<b>N-a Theropoda (n=51)</b>	1.035	0.919	1.144	0.776	0.724	0.831
<b>Tyrannosauroida (n=10)</b>	0.850	0.500	1.148	0.861	0.733	1.010
<b>Tyrannosauroida not inc.</b>	1.626	1.057	2.022	0.541	0.377	0.776
<b><i>Calamosaurus</i> (n=9)</b>						
<b>Tyrannosauridae (n=8)</b>	1.643	1.023	2.062	0.535	0.362	0.791
<b>Ornithomimidae (n=8)</b>	1.288	0.410	1.823	0.681	0.416	1.116
<b>Maniraptora (n=9)</b>	1.089	0.739	1.366	0.753	0.595	0.953

**Table 9** *P-values of pairwise comparisons of standard major regression coefficients (bold) and intercepts (italics) after Benjamini-Hochberg correction for multiple comparisons (Benjamini & Hochberg, 1995)*

	<b>Tyrannosauroid.</b>	<b>Tyrannosaurid.</b>	<b>Orni.</b>	<b>Mani.</b>
<b>Tyrannosauroida</b>	-	-	0.490	0.490
<b>Tyrannosauridae</b>	-	-	0.490	0.490
<b>Ornithomimidae</b>	<i>0.441</i>	<i>0.441</i>	-	0.681
<b>Maniraptora</b>	<i>0.441</i>	<i>0.441</i>	<i>0.658</i>	-

## Discussion

With the addition of the quadratic component the already negative allometric relationship between femoral length and circumference becomes even more pronounced. This shift indicates that not only do avian femora become more robust with increased size but that length increases at a lower rate with respect to circumference in larger taxa. It is suggested here that the anterior centre of mass and the associated sub-horizontal orientation of the femur constrains the rate of femoral length increase in larger birds in order to maintain the position of the knee under the centre of mass whilst circumference continues to increase. This conclusion is supported by experimental manipulation of the centre of mass by adding artificial tails to growing chickens (Grossi *et al.*, 2014). The more posterior centre of mass caused by the addition of the artificial tail led to a more vertical orientation and a lengthening of the femur compared to non-altered individuals and to individuals which had additional weight, equivalent to the mass of the artificial tail, added near the natural centre of mass (Grossi *et al.*, 2014). The slower rate of increase in femoral length with increased size combined with variation related to leg function (Zeffer *et al.*

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2003; Doube *et al.* 2012) provides an explanation as to why femoral length performs worse than circumference as a predictor of avian body mass (Field *et al.* 2013; Campione *et al.* 2014).

Femoral length in non-avian theropods also scales with negative allometry with respect to circumference, indicating an increase in femoral robusticity with increased size. However, in contrast to flightless avians little support was found for a curvilinear relationship in femoral scaling in non-avian theropods (contra Christiansen 1999a). A quarter of the phylogenetically corrected regressions found only marginal support for differential scaling. It is possible that the finding of the previous study was caused by scaling differences between phylogenetic groups. Tyrannosaurids dominate the largest body size class of Christiansen's (1999a) data set. As shown by this study (Fig. 3; Table 5), tyrannosaurid femora increase in robusticity at a higher rate than other non-avian theropods. Thus, the prior report of curvilinear scaling of femoral proportions in non-avian theropods is likely to have been caused by the different femoral scaling in tyrannosaurs and a predominance of tyrannosauroids in the largest body class sample.

This difference between avian and non-avian theropods is probably due to the more posterior centre of mass in the latter, which would not have required the femur to re-orientate in order to reposition the knee (Gatesy, 1990, 1991). Thus, the more robust femur of large theropods was achieved simply through continuation of a linear, negatively allometric relationship between length and circumference. The restricted femoral length of birds compared to non-avian theropods means that rotation of the femur contributes much less to stride length in birds than was likely in non-avian theropods (Carrano 1998; Rubenson *et al.* 2007). In addition to lengthening and more vertical orientatation of the femur in experimental chickens, Grossi *et al.* (2014) found a concurrent increase in femoral rotation during walking. The more anterior centre



of mass, shortened femur, and low degree of femoral rotation during locomotion seen in extant birds therefore appear to be inextricably linked to each other. The differences between avian and non-avian theropods found here support the conclusion of previous studies that the scaling patterns in the former do not apply to the latter (Carrano 1998; Novas *et al.* 2014). As such the extrapolation of the scaling relationships between leg bone proportions and body mass of birds is unlikely to produce accurate estimates of body mass in non-avian theropods (Campione *et al.* 2014). Given the gradual changes in posture, morphology, and the position of the centre of mass through theropod phylogeny (Gatesy 1990, 1991; Hutchinson, 2001; Hutchinson & Allen, 2008; Allen *et al.* 2013) and the distinction between the scaling relationships between avian and non-avian members of this clade (Campione *et al.* 2014); testing whether early avians and basal neornithines fit better within the avian or non-avian scaling trends is an interesting avenue for further research.

There is no support for a curvilinear relationship between length and circumference in flightless avian tibiotarsi. This finding supports the prediction that tibiotarsal scaling is not constrained in the same way as femora. The lack of evidence for curvilinear scaling in non-avian theropods is contrary to the pattern of differential scaling between size classes found by Christiansen (1999a). Although a significant shift in slope was detected in the quadratic regression, the loss of significance after phylogenetic correction indicates this result was affected by the degree of relatedness between species.

Studies finding curvilinear scaling between limb bone length and circumference in mammals suggested that this feature allows larger species to maintain similar levels of stress on the bones as in smaller taxa (Bertram & Biewener, 1990; Christiansen, 1999b, c; Carrano 2001).

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Mammals and non-avian theropods show a straightening of limb posture with increasing body size, leading to a decrease in bending strains, an increase in the effective mechanical advantage of the extensor muscles in the legs, and a decrease in the relative muscle mass required for locomotion (Biewener, 1989; Gatesy & Biewener, 1991; Carrano, 2001; Hutchinson, 2004). These changes act in combination with a reduction in locomotor performance (i.e. reduced speed and mobility) to reduce stresses placed upon the long bones so that larger species maintain similar stresses during locomotion to those seen in smaller taxa (Biewener, 1982, 1990). Straightening of the limb bones can only occur to a certain point, after which an increase in the robustness of the limb bones through an increase in thickness or a decrease in bending moment arms through shortening of the bones is required to maintain a constant safety factor (i.e., the yield point of a structure divided by the force it encounters) (Biewener, 1990; Bertram & Biewener, 1990; Christiansen, 1999a). The lack of support for curvilinear scaling between limb bone circumference and length in non-avian theropod leg bones found here contradicts the hypothesis of size differential scaling in the case of these animals.

A previous study found increased femoral eccentricity and decreased femoral curvature in non-avian dinosaurs with increasing size (Carrano, 2001). These features in combination with a linear trend of increasing relative robusticity and decreased locomotor capability may have been sufficient to prevent increased stress on the femur during locomotion in larger theropods.

The differences between femoral scaling in tyrannosauroids and other non-avian theropods may be due to differences in the quality of sampling. Along with the oviraptorosaurs, tyrannosauroids are the most heavily sampled group in this study and this clade includes smaller basal taxa such as *Timimus* (Benson *et al.*, 2012) and *Xiongguanlong* (Li *et al.*, 2010) as well as

large, derived tyrannosaurids (Appendix S2, S3). This combination of smaller, gracile forms and gigantic, robust forms within the same clade is the probable cause of the higher intercept and lower slope coefficient in the MA regression. On the other hand, the allosauroid grouping consists of a smaller sample of mostly large forms spread across four families: Metriacanthosauridae, Allosauridae, Neovenatoridae, and Carcharodontosauridae (Carrano *et al.*, 2012). Two of the taxa in this dataset, *Chilantaisaurus* and *Fukuiraptor*, have been placed in alternative regions of the theropod tree in a different study (*contra* Carrano *et al.*) (Novas *et al.*, 2013). Maniraptora group also exhibits uneven sampling, with 10 of the 15 data points identified to genus level belonging to Oviraptorosauria (Senter, 2007; Longrich *et al.*, 2010). Four of the 15 along with an additional specimen listed as “Dromaeosauridae indet.” fall within Deinonychosauria, and one species is included from Alvarezsauridae (Senter, 2007). Additional femoral circumference data from basal tyrannosauroids, allosauroids, and more even sampling of maniraptorans would help to elucidate the patterns seen here.

Curvilinear scaling of limb bone dimensions within separate phylogenetic groups has been found in mammals (Christiansen, 1999b, c; Carrano, 2001; Campione & Evans, 2012). For example, size differential scaling in leg bone length and diameter has been identified in Carnivora and Bovidae (Bertram & Biewener, 1990). It remains possible that the different phylogenetic groupings of non-avian theropods exhibit their own size differential scaling relationships. However, greater sample sizes for each grouping are required in order to test this hypothesis. Leg bone lengths and circumferences are often used to produce body mass-predicting regression equations, which are generally applied to all non-avian theropods (Anderson *et al.*, 1985; Christiansen & Fariña, 2004; Campione *et al.* 2014). Further investigation of the changes in scaling relationships between clades and identifying these transitions within theropod phylogeny

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has the potential to improve the accuracy of body mass estimates for non-avian theropods. It would also be of interest to test whether these shifts co-occur with changes in body size through the theropod tree (Novas *et al.* 2012; Benson *et al.* 2014).

The interpretation of the results presented here partially relies on an assumption that external circumference is a reliable measure of bone robustness. A number of studies indicate that both femoral diameter and the maximum second moment of area scale with positive allometry relative to femoral length in birds (Gatesy, 1991; Carrano, 1998; Dobe *et al.*, 2012). For a given amount cross sectional area of bone, avian femora show greater second and polar moments of area (indicative of increased resistance to twisting and bending) due to the distribution of bone farther from the central axis compared to mammals (Dobe *et al.* 2012). As a result, leg bone external circumference and diameter relative both to bone length and body mass is greater (Carrano, 1998; Campione *et al.* 2014). This is an effective and efficient way to increase strength without increasing the amount of bone. In addition, femoral eccentricity increases with body size in bipedal non-avian dinosaurs, which indicates that the femora of large non-avian theropods are not as resistant to torsion as those of birds which have a circular cross section (Carrano, 2001; Margerie *et al.* 2005). However, it should be noted that variation in bone density and cross-sectional shape within both non-avian theropods and Aves may affect interpretations of robusticity based on circumference within these groups. These considerations do not detract from the observation that the lengths of avian femora are constrained by the position of the centre of mass and the resultant postural changes compared to non-avian theropods.

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## Conclusion

Curvilinear scaling of femoral length in terrestrial flightless birds leads to a more rapid relative shortening of the femur with increased size than a simple linear relationship would imply. It is suggested here that this pattern results from the femur being constrained to maintain the knee under the more anterior centre of mass. This requirement in turn restricts the length of the bone. The absence of curvilinear scaling in the tibiotarsus confirms that these restrictions only affect the proximal leg bone. On the other hand, the lack of a curvilinear relationship between femoral length and circumference in non-avian theropods indicates that the length of this bone was not so constrained in this group. Both the femur and tibia of tyrannosauroids appear to scale differently to those of other theropod taxa. However, better sampling of non-avian theropods is required to confirm these patterns.

All flightless avians are secondarily flightless, and it may be that the retention of a posture associated with the evolution of powered flight more strongly restricted mass than was the case with the ancestrally flightless non-avian theropods.

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*Psilopterus lemoinei*. The manuscript was considerably improved by comments from J. Alroy, M. Carrano, Nicolás Campione, Roger Benson, and Steven Salisbury.

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## Chapter 3

Complex evolution of leg bones in the transition to flightlessness  
in birds

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**Abstract**

Loss of flight in birds is associated with clear, patterns of change in the proportions of wing bones. However, the changes that occur in the hindlimbs have been less well studied. Measurements of the leg bones of 38 species of flightless birds and 19 of their closest volant relatives were taken. Changes in proportions from volant ancestral models to individual flightless species were quantified using differences in scores from factor analyses. These models were used instead of more conventional phylogenetic methods due to the difficulty of reconstructing traits associated with flightlessness in a small but phylogenetically broad subset of Aves. The changes in scores were tested for directionality and association with differences in body size, inhabited area of land mass, and estimated time since loss of flight using step-wise multiple regressions. No evidence was found for trends either in the directionality or degree of change in the evolution of leg bone proportions across flightless species as a whole. Splitting the dataset into palaeognaths and neognaths indicates that ratites (= flightless palaeognaths) with cursorial proportions tend to inhabit larger land masses. Contrastingly, neognaths exhibit no general patterns in leg bone evolution. The trajectories of change in leg bone proportions are far more complex than those seen in the wings, and in the case of the neognaths may reflect variation in ecology rather than any universal selection pressure.



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## Introduction

Flight has been lost multiple times in avian evolutionary history, with the majority of these losses occurring in terrestrial lineages (Roff, 1994). A variety of different hypotheses have been proposed to explain the evolution of flightlessness, including absence of predators, selection for large body size, year-round habitability of environments removing the need for migration, and adaptation of the forelimbs for uses other than flight (McCall et al. 1998). These hypotheses are not necessarily mutually exclusive, and different sets of adaptive pressures could easily apply to different flightless taxa (Nudds & Davidson, 2010).

Despite the lack of a unified theory, a number of features are common to flightless species. Both aquatic and terrestrial taxa have reduced wing lengths relative to their volant relatives (McCall et al. 1998; Livezey, 2003; Nudds & Davidson, 2010) with the latter also exhibiting reduced sternal carina, atrophied pectoral musculature (Owen, 1882; Livezey, 1992, 1993, 2003; McNab, 1994; Worthy & Olson, 2002; Maxwell & Larsson, 2007), and enlarged pelvic bones and musculature (Livezey, 1992, 1993; Worthy & Olson, 2002). However, wing-propelled swimming birds such as penguins and steamer ducks retain well-developed pectoral muscles and sternal carina (Livezey & Humphrey, 1986; McNab, 1994; Ponganis et al. 1997). Changes in feather structure or loss of flight feathers are also apparent in many flightless species (Livezey, 1989, 1992, 1993, 2003; McGowan, 1989).

Regarding the proportions of the limb bones, avian families containing flightless species tend to have shorter wings relative to body mass than families containing no flightless taxa (McCall et al. 1998; Nudds & Davidson, 2010). In flightless species the manus is the part of the wing most responsible for relative shortening (Marples, 1930; Livezey, 1992, 2003; Middleton &

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Gatesy, 2000; Nudds & Davidson, 2010). Humeral length is greater relative to the lengths of the forearm and manus compared to volant relatives (Livezey, 1992, 1993; Middleton & Gatesy, 2000). It seems, therefore, that the bones of the manus are the first to reduce during loss of flight, followed by the more proximal wing bones (Middleton & Gatesy, 2000; Nudds & Davidson, 2010).

Directionality in the evolution of the avian hind-limbs is examined in this study. Unlike the forelimbs, changes in hindlimb proportions relative to volant relatives or putative ancestors have generally been studied in individual lineages rather than throughout Aves as a whole (e.g., Livezey, 1992, 1993).

The relative proportions of the leg bones reflect habitat use (Zeffer et al. 2003) and in the case of terrestrial species have often been used to infer the degree of cursoriality (Gatesy & Middleton, 1997; Carrano, 1999). In functional terms, cursorial animals are considered to be those with specific morphological adaptations for either rapid running or traversing long distances (Gregory, 1912; Carrano, 1999). Increases in the lengths of the distal leg bones, in particular the tarsometatarsus, are associated with faster walking and running speeds, larger home ranges, and higher relative stride frequencies in terrestrial birds and mammals (Garland & Janis, 1993; Janis & Wilhelm, 1993; Bennett, 1996; Abourachid & Renous, 2000). It is important to note that cursoriality is but one end of a locomotory spectrum with graviportality, adaptations that decrease speed but potentially improve weight support, at the other end (Gregory, 1912; Carrano, 1999). Increased cursoriality with loss of flight is likely to have been advantageous on continental land masses. Specifically, the presence of mammalian predators and greater land area for dispersal are possible selection pressures that may lead to increased cursoriality in continental

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flightless birds. In addition to predation pressures, a species living on a continental land mass has a greater amount of area available for dispersal. Greater dispersal ability is potentially advantageous in reducing intraspecific competition between individuals within populations. To be able to cover larger distances, a continental flightless bird may have more cursorial proportions than a similarly sized island species. Oceanic island taxa, on the other hand, often evolve flightlessness in the total absence of mammalian predators and with land based dispersal restricted.

Differences in proportions between volant and flightless relatives may also be due to changes in body size. Previous studies indicate that femoral length increases with body mass at a lower rate than tibiotarsal and tarsometatarsal length (Gatesy, 1991; Olmos et al. 1996; Doube et al. 2012). This allometry, combined with the limited rotation in the parasagittal plane of the femur, decreases the femur's importance in increasing stride length relative to the tibiotarsi and tarsometatarsi (Carrano & Biewener, 1999; Reilly, 2000; Smith et al. 2010; Grossi et al. 2014). In addition, larger species with less crouched postures increase their speed of locomotion by increasing step frequency whereas smaller species with crouched hind-limbs do so by increasing stride length (Abourachid & Renous, 2000; Abourachid, 2001). Thus, the importance of greater distal leg bone length for achieving higher speed of locomotion appears to increase with body mass.

Alternatively, the degree of change in proportions since loss of flight may simply reflect the length of history of flightlessness. In this study 1) changes in body mass, 2) area of the land mass where flight was lost and, 3) inferred amount of time since loss of flight are used to predict the trajectories of change in leg bone proportions from volant ancestral models to flightless

descendants. Aquatic flightless species were not included because the selection pressures acting on the hind-limb proportions of these taxa are quite different and likely to combine requirements for both aquatic and terrestrial locomotion (Cubo & Casinos, 1997; Zeffer & Norberg, 2003; Zeffer et al. 2003). Given the repeated, independent losses of flight that have occurred in both the palaeognaths and rails, as well as a few other terrestrial taxa, the number of occurrences of flight loss far exceeds that seen in lineages of swimming birds (Livezey, 2003; Phillips et al. 2010; Kirchman, 2012).

## Methods

Measurements of the lengths (L), of the femur (F), tibiotarsus (Tb), and tarsometatarsus (Tm), were taken for 38 species of flightless birds along with caudo-cranial (Dcc) and mediolateral (Dml) diameters at midshaft of the tibiotarsus (Tb) and tarsometatarsus (Tm) (Supporting Information 1). These species represent a minimum of 23 independent losses of flight (Fig. 1). For tibiotarsi, the length measurements did not include the cnemial crest as it was often damaged in fossil specimens. Instead, length was measured from the interarticular area at the proximal end to the distal-most point of the condyles. For femora and tarsometatarsi, greatest lengths were measured. The point at mid-shaft was found by dividing the length of the bone by two. Measurements were taken to the nearest 0.01 mm using 300 mm digital callipers and rounded to the nearest 0.1 mm. Distances greater than 300 mm were measured to the nearest 1 mm using a tape measure. All measurements were repeated twice and the average was used for analysis. Only adult specimens were measured. These were identified by the following characters: femur

exhibiting an intercondylar bridge connecting the lateral and medial condyles, cnemial crest fully fused to the tibia with no trace of a symphysis, tibiale and fibulare fully fused to the tibia with no trace of a symphysis, and a completely formed supratendinal bridge on the tibia (when present). Femoral circumference was also measured using cotton twine wrapped tightly around the femur at mid-shaft and marked where the two ends met with ultra-fine marker pen. The length of the marked section was then measured using a 300 mm digital calliper. For bones with a diameter less than 7 mm individual strands of twine were used. Nineteen of the nearest volant relatives of the flightless species in the dataset were measured in the same way (Fig.1). These species were chosen based on published molecular phylogenies (Supporting Information 2). Morphological phylogenies were avoided because their topologies may be affected by homoplasy due to the parallel evolution of flight loss in multiple lineages (Livezey 1998, Parish 2013: p. 339). In the case of one species pair (*Chenonetta finschi*/*C.jubata*) a morphological study providing strong evidence for close relationships was used (Worthy and Olson, 2002).

To make all measurements size independent, separate regressions were carried out for each linear measurement against femoral circumference. Femoral circumference was used instead of body mass as the majority of the flightless taxa in the dataset are extinct (Supporting Information 1) and body masses were unavailable. The diameter of the glenoid facet has been shown to be a reliable predictor of body mass in volant neorthine birds (Field et al. 2013). However, this methodology cannot be applied to flightless taxa due to the relative reduction of the pectoral girdle in these species (McCall et al.1998; Livezey, 2003; Nudds & Davidson, 2010). This is especially the case for moa, in which both the humerus and its articular facet with the scapulocoracoid are absent (Owen, 1866; Worthy and Scofield, 2012). Allometric regressions containing multiple predictor variables have been used to estimate body masses in extinct avian

taxa. However, these included a number of measurements from the forelimb and so suffer the same issue as the use of glenoid diameter (Serrano et al. 2015). Femoral circumference is often used in allometric equations for estimating body mass in avians (Anderson et al. 1985; Campbell & Marcus, 1992; Field et al. 2013; Campione et al. 2014), and has low percentage prediction error (Field et al. 2013; Campione et al. 2014). As a result, it can be considered as a reliable indicator of body mass in flightless birds. Masses were not calculated for each species using allometric equations utilising femoral circumference in order to avoid extrapolation beyond the known range of the datasets used, as would be necessary for calculating the masses of the largest extinct ratites. Residuals from each regression for all species were then subjected to a factor analysis with varimax rotation using the “factanal” function in R.3.01.0 (R Core Development Team, 2014) set to produce Thompson’s scores. Chi-squared statistics showed that in all cases one factor was sufficient to fit the data ( $p < 0.05$ ). Therefore, factanal was set to extract a single factor. Like principal components analysis (PCA), factor analysis reduces the number of variables. However, unlike PCA factor analysis only computes the shared variance of the variables rather than generating a model to account for total variance (Widaman 1993). Each factor is a representation of the underlying structure of the data that accounts for the shared variance between variables. As such the amount of variance in the data explained by factor analysis will always be lower than PCA. Factor analysis was preferred to PCA as the loadings of variables on principal components may inflate the saliency of variables which would otherwise load moderately or weakly (Widaman 1993; Fabrigar et al. 1999).

In order to quantify changes in limb bone proportions between ancestral volant forms and flightless descendants the differences ( $\Delta$ ) in factor scores between each flightless species and its nearest relative were calculated (Fig. 2). In cases where multiple volant species have been

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suggested to be equally closely related to a flightless species the average factor score of the volant species was used. This rule assumes gradual evolution of the hindlimb bone proportions of the flightless species from a volant ancestor intermediate in form between the nearest flight-capable relatives. Given the links between leg bone proportions and locomotion in extant birds and mammals, non-random evolution of the leg bones of each flightless taxon was also assumed (Garland & Janis, 1993; Janis & Wilhelm, 1993; Bennett, 1996; Abourachid & Renous, 2000; Zeffer et al. 2003).

Ideally, the true ancestor of each flightless taxon would be used to quantify relative changes in morphology since loss of flight. However, putative ancestors cannot be identified for the majority of species in this analysis as the non-continuous nature of the avian fossil record precludes confident identification of ancestor-descendent relationships even when potential candidates exist (see Foote, 1996). Although methods exist for reconstruction of ancestral morphologies based on data from the tips of phylogenies, such methods face particular difficulties in mapping trait evolution in flightless birds (Phillips et al. 2010; Garcia-R et al. 2014a). In clades which contain a few volant taxa nested within an otherwise flightless group, such as the rail genus *Gallirallus*, these methods result in the last common ancestor being reconstructed as flightless with flight then being regained in some species (Slikas et al. 2002; Harshman et al. 2008; Kirchman, 2012; Garcia-R et al. 2014a). Given the absence of any direct evidence for flight being regained in any bird lineage, such a sequence of events appears improbable. This situation is exacerbated by the possible extinction of volant relatives for which there is no fossil record (Kirchman, 2012; Garcia-R et al. 2014a). The problem of spurious implied reversion to a flighted condition is not only an issue with methods based on the principal

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of parsimony because the artefact has also been seen in studies utilising maximum likelihood approaches to trait mapping on trees (Phillips et al. 2010; Garcia-R et al. 2014a).

For the ratites in the dataset, an ancestral morph was generated using the average factor score for two species of tinamou, *Crypturellus soui* and *Eudromia elegans*, which respectively represent forest and open country inhabiting forms (Bertelli & Porzecanski, 2004; Bertelli & Chiappe, 2005), and three lithornithid species, *Lithornis plebius*, *L. promiscuus*, and *Paracathartes howardae*. The Lithornithidae are an extinct group of volant palaeognaths found from the late Paleocene to the middle Eocene in North America and Europe (Houde, 1988). Combined analysis of morphological and genetic data found lithornithids and tinamous to form a monophyletic group nested within the flightless palaeognaths, thereby rendering “Ratitae” paraphyletic (Mitchell et al. 2014). The same pattern was found in a morphological study including additional cranial information (Johnston, 2011). This result indicates that flight was lost in multiple lineages within Palaeognathae (Harshman et al. 2008; Phillips et al. 2010; Haddrath & Baker, 2012; Mitchell et al. 2014). Unfortunately, there are no known fossil volant palaeognaths that fall basally to any of the ratite lineages, and so any reconstruction, regardless of methodology used, of the flighted ancestor/s of ratites relies heavily on the volant palaeognath clade nested within “Ratitae”. As there is no objective means of deciphering whether tinamous or lithornithids represent a closer fit to the ancestral forms, and given that they form a monophyletic group within “Ratitae”, an average of species from the two groups was used. The difference between this average factor score and the factor scores for the separate ratite species was calculated to give the amount of morphological change from the volant form to the flightless forms.



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Multiple regressions were used to test for relationships between changes in limb bone proportions and three potential explanatory variables:  $\Delta$  femoral circumference (as a proxy for  $\Delta$  body mass), time since loss of flight, and area of land mass on which the flightless species evolved. Models were scored using the Akaike's information criterion corrected for finite sample size (AICc, Sugiura, 1978; Hurvich & Tsai, 1989) as opposed to uncorrected AIC (Akaike, 1979) as the former is more suitable for small sample sizes (Sugiura, 1978). AICc scores and weights were generated using the "aictab" function in the R package "AICcmodavg" (Mazerolle, 2015). Regressions were listed by AICc weights, with higher weights indicating a greater likelihood that the model fits the data better than the remaining models tested. Additional multiple regressions, scored in the same way, were also carried out to test whether these explanatory variables had any relationship to the magnitude of change regardless of directionality. The amount of change was quantified by removing the sign from negative factor scores prior to step-wise regression.

Time since loss of flight was taken as the time since divergence of each flightless species from its volant relative. Admittedly, the time since divergence of two lineages does not necessarily reflect the time at which flight was lost within the lineage leading to an observed flightless species. An excellent example is of the two flightless columbiformes, i.e., the dodo (*Raphus cuculatus*) and the solitaire (*Pezophaps solitaria*) from the islands of Mauritius and Rodrigues respectively. Both species are estimated to have diverged from their closest extant volant relative (*Caloenas nicobarica*) between 17.6–35.9 Ma (Shapiro et al. 2002). However, the volcanic islands the two flightless species inhabited are considerably younger, with the emergence of Mauritius dated to 7.8 Ma and Rodrigues to 1.5 Ma (McDougal & Chamalaun, 1969). Greater relative reduction of the pectoral girdle in the dodo also suggests that it was flightless for a longer period of time than the solitaire (Livezey, 1993). Thus, it is likely that the

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ancestor of these species retained flight up until at least the formation of these two islands. As a result, the time variable for oceanic island species was informed by the time of emergence of their respective islands, either as a result of volcanic and tectonic activity or decreased sea level, as taken from published literature (Supporting Information 2). Emergence dates were preferred to molecular timings of divergence if the former post-dated the latter, as flight would likely have been retained after divergence in order to make dispersal and colonization of these islands possible.

A recent phylogenetic study by Garcia-R et al. (2014a, b) included many of the flightless rails and dated their divergence times. However, the deep external calibrations and absence of internal calibrations in this analysis may have led to overestimates of the dates of intrageneric splits (Hugall et al. 2007). As a result, a combination of previous rail phylogenies and island emergence dates was used (Supporting Information 2). Although the rail phylogenies consisted of subsets of Rallidae (e.g., Slikas et al. 2002; Kirchman, 2012), they were similar in topology to the corresponding sections of the Garcia-R et al. (2014a) phylogeny. In the case of the genus *Porphyrio*, the divergence dates from a study of the radiation of the genus (Garcia & Trewick, 2015) were used. These dates are later than those of Garcia-R et al. (2014a) and accord with previous work (Trewick 1997).

For the palaeognaths, divergence dates were taken from Mitchell et al. (2014). Current molecular phylogenies render the flightless ratites as a paraphyletic group with the volant tinamou nested within the clade, indicating that loss of flight occurred multiple times in palaeognath history (Phillips et al. 2010; Haddrath & Baker, 2012; Mitchell et al. 2014). Therefore, alternative scenarios were modelled for the timing of loss of flight in extant ratites.

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The first scenario was that all extant ratite families lost flight early in their history (i.e., soon after their divergence from the rest of the palaeognaths). The second scenario was that extant ratite families containing more than one living or recently extinct species lost flight near the point of radiation for those families. Thus, using the molecular dates of Mitchell et al. (2014) the first scenario would have the moa lineage losing flight at ~58 Ma with all species in the group listed as having been flightless since that point, whereas the second scenario would have loss of flight occurring at ~7.7 Ma, at the time when the crown group dinornithiformes appear. In the first, flight was lost early in the history of these lineages and they have been resident on their respective land masses since then (Mitchell et al. 2014). In the second, the land masses were relatively recently colonised by a volant ancestor and flight was lost subsequently near the time the crown groups radiated.

The use of these scenarios was informed by the fossil record where possible. Fossil specimens indicate that the lineage leading to extant Rheidae lost flight prior to the molecular date for divergence of the two extant species (*Rhea americana*, *Pterocnemia pennata*) given by Mitchell et al. (Tambussi, 1995). In addition, the fossil record of Casuariidae suggests that the last common ancestor of extant *Casuarius* and *Dromaius* was flightless (Worthy et al. 2014). As a result, only the first scenario was implemented for Rheidae and Casuariidae. The stem apterygid *Proapteryx micromeros* does occur prior to the estimated origin of extant kiwis. However, not enough material is available to tell whether this species was flightless or volant (Worthy et al. 2013). Remains of flightless moa have also been found which date to ~19–16 Ma (Tennyson et al. 2010). This date is much older than that given by Mitchell et al. (7.7 Ma) for the origin of crown group moa and similar to that found by a previous study (Haddrath & Baker, 2012; 19 Ma) which included *Megalapteryx didinus*, the earliest-diverging moa species (Bunce et

al. 2009). This species was not included in the molecular analysis of Mitchell et al. (2014). Therefore, a date of 19 Ma was used for the second scenario of loss of flight at the radiation of crown group moa (Fig. 3).

Based on this additional information, separate regression analyses were carried out with different scenarios for timing of loss of flight in the kiwi, moa, and elephant bird lineages. For the lineages leading to extant *Struthio*, Rheidae, and Casuariidae only scenario 1 was implemented. Ideally, the degree of morphological change measured would have been from the ancestral models to the point at which flight was lost, as indicated by the fossil record. However, the absence of fossil forms representing the transition to the flightless condition precluded such computations. Dates of divergence were not available for all species, so multiple regressions models including a time variable contained 31 species of flightless birds in total group analyses.

For the land area variable, species were separated into two groups, one consisting of those which inhabited land masses with an area less than 100,000 km<sup>2</sup>, coded with “0”, and another including species living on larger land masses, coded with “1”. This coding was used as a predictor variable. This simplification of a continuous variable was necessary due to the complex geological history of New Zealand, which has varied greatly in area since separation from Australia (Wallis & Trewick, 2009), making it difficult to assign a land area available for dispersal for New Zealand flightless birds. The cut-off point of 100,000 km<sup>2</sup> reflects the six-fold greater area of the North and South Islands of New Zealand relative to the next largest land mass in the dataset, Halmahera, in the Maluku Islands of Indonesia.

The palaeognaths in the dataset are all found on old continental land masses, and molecular phylogenies indicate divergence dates for some lineages as far back as the late

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Cretaceous or Paleocene (Phillips et al. 2010; Haddrath & Baker, 2012; Mitchell et al. 2014). On the other hand, many of the neognath species in the dataset are found on relatively recently formed oceanic islands and/or have diverged more recently from a volant relative. This is particularly the case in the many flightless species of the family Rallidae, the majority of which lost flight independently after colonisation of oceanic islands (Olson, 1973). In some cases, molecular phylogenies indicate that extant flightless species render volant species as paraphyletic (Trewick, 1997; Kirchman, 2012). Due to these differences, separate factor analyses and step-wise regressions were carried out for the total dataset, palaeognaths, and neognaths in order to see whether the relationships between variables depend on these groupings. As all palaeognaths are found on land masses greater than 100,000 km<sup>2</sup>, the palaeognath-only dataset was split into New Zealand (“0”) and non-New Zealand (“1”) species for the land area variable. New Zealand is the smallest of these land masses and contains 9 of the 15 species in the dataset.

The residuals from all regression models were tested for normality using Jarque-Berra tests (Jarque & Berra, 1980) in the R package “moments” (Komsta & Novomestky, 2015), and for equal variance using Breusch-Pagan tests (Breusch & Pagan, 1979) in the R package “lmtest” (Zeileis & Hothorn, 2002).

To test for directionality in  $\Delta$  factor scores, Wilcoxon rank-sum tests were used to show whether the medians were significantly different from zero. This was also done for  $\Delta$  femoral circumference to test for directionality in body size change from the ancestral models.

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## Results

### Total group analyses

Total variance explained by factor analysis of residuals from the complete dataset amounted to 39.1%. Tibiotarsus and tarsometatarsus length loadings were strong ( $> \pm 0.8$ ) and positive (Table 1). Femur length and tibiotarsal craniocaudal diameter had moderate, positive loadings ( $> 0.5$ ,  $\leq 0.8$ ), with all other variables loading weakly. Only tarsometatarsus mediolateral diameter had a negative loading.

Under the early loss of flight scenario for kiwi, moa, and elephant birds the best fitting model (AIC weight 0.28) included both  $\Delta$  femoral circumference and land area were retained in the regression model, with each having positive and negative relationships respectively (Table 2). Therefore, relatively increased leg bone length and mediolaterally narrower tarsometatarsi compared to ancestral forms is associated with increased size, whereas species evolving on smaller land masses tended to have the opposite relationship with shorter leg bones and broader tarsometatarsi. However, only the relationship between  $\Delta$  femoral circumference and  $\Delta$  factor scores was significant ( $p < 0.05$ ). The next best model had an AIC weight of 0.22 and included land area and time since loss of flight as variables with the relationships with  $\Delta$  factor scores being significantly negative and positive respectively (Table 2). All multiple regressions had low adjusted  $R^2$  values ( $< 0.13$ ) indicating poor model fitting to the data.

When loss of flight for kiwi, moa, and elephant birds was timed more recently, land area and estimated time since loss of flight were included in the most likely of the regression models (AIC weight 0.56). A positive relationship with estimated time since loss of flight indicates that

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species with a longer evolutionary history of flightlessness tend to have longer leg bones and a narrower tarsometatarsus relative to their volant relatives. AICc weights of the other models are substantially lower (Table 2).

The best fitting regressions using sign-less factor scores under both timing scenarios either included time since loss of flight and  $\Delta$  femoral circumference, or only  $\Delta$  femoral circumference as variables (Table 3). In all cases the relationships between independent variables and  $\Delta$  factor score were non-significant ( $p > 0.05$ ). An additional set of regressions was carried out for a larger data set including taxa with no available information for time since loss of flight. In this case, the result was similar to that under the first time scenario for the original  $\Delta$  factor scores with  $\Delta$  femoral circumference and land incorporated in the best fitting model (Table 2), whereas no significant relationships with  $\Delta$  factor score for the models using sign-less scores (Table 3). All regression residuals were normally distributed (Jarque-Berra test  $p$ -values  $> 0.05$ ) and had equal variances (Breusch-Pagan test  $p$ -values  $> 0.05$ ).

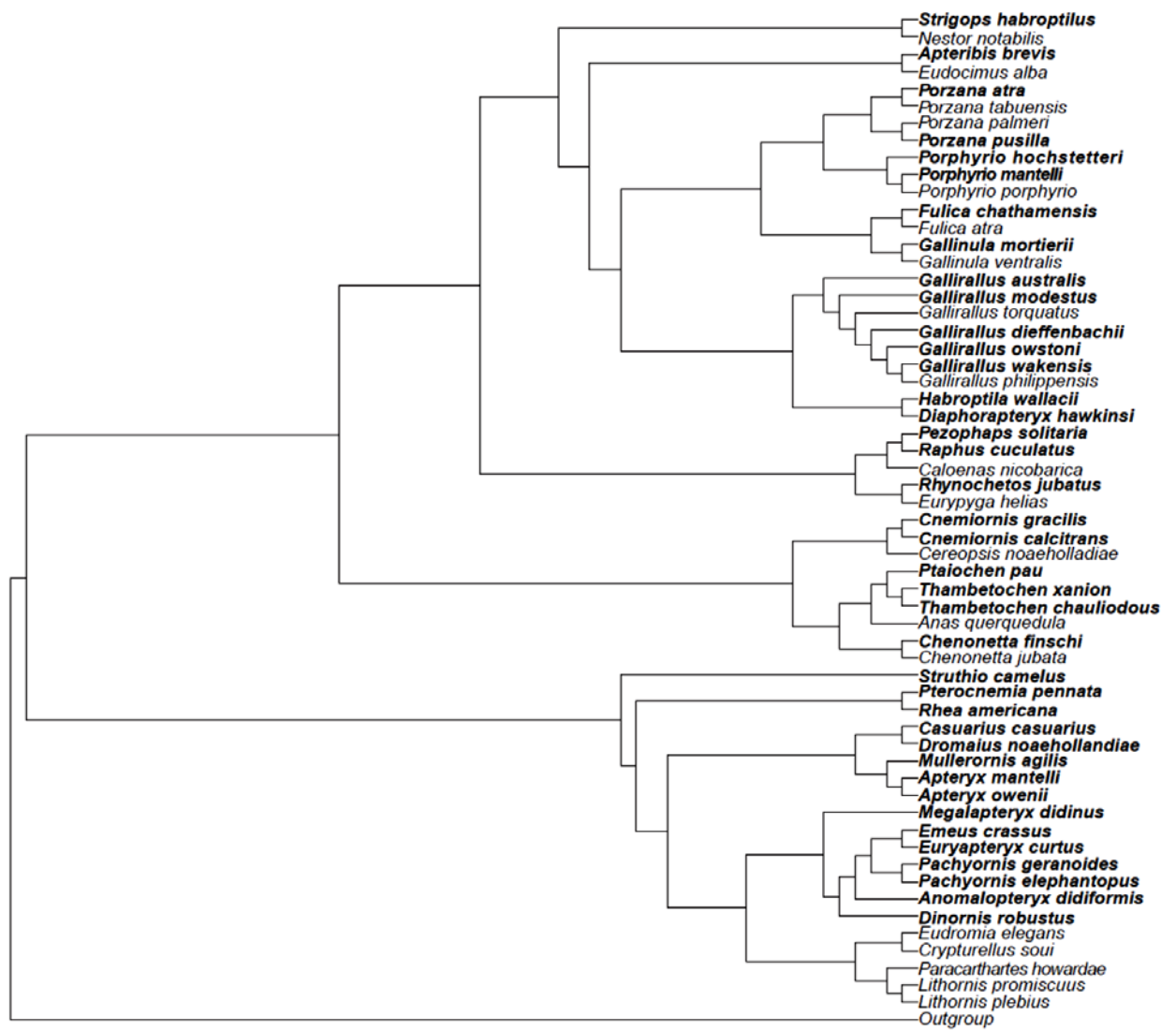
Wilcoxon signed-rank tests of  $\Delta$  factor scores and femoral circumference indicated that the median of the former was not significantly different from zero, whereas the median of the latter was significantly greater than zero. Therefore, there is no overall directionality in the changes to size-independent proportions of the leg bones from flying to flightless forms. There is a general directionality towards greater body mass, but *Gallirallus wakensis* and *G. modestus* are exceptions to this rule.

**Table 1** Factor loadings of each variable from factor analyses of regression residuals for the total group ( $n=57$ ), palaeognaths ( $n=20$ ), and neognaths ( $n=37$ ). Residuals were taken from regressions of each variable against femoral circumference.

Variable	Total group	Palaeognaths	Neognaths
Femur Length	0.642	0.841	0.621
Tbt Length	0.905	0.856	0.994
Tbt Dcc	0.725	0.913	0.636
Tbt Dml	0.168	0.679	0.616
Tmt Length	0.892	0.864	0.890
Tmt Dcc	0.281	0.462	0.188
Tmt Dml	-0.284	-0.560	0.000
<b>Proportion variance</b>	<b>0.391</b>	<b>0.576</b>	<b>0.426</b>

*Tbt*, tibiotarsus; *Dcc*, craniocaudal diameter; *Dml*, mediolateral diameter; *Tmt*, tarsometatarsus.





**Fig. 1** Composite phylogeny of taxa included in this study constructed using matrix representation parsimony (Baum 1992; Ragan, 1992). Names of flightless species are in bold italics. A hypothetical outgroup was used to root the tree. A list of source trees can be found in Supporting Information 2

**Table 2.** Multiple regressions of  $\Delta$  factor 1 ( $\Delta F1$ ) scores with  $\Delta C_f$ , land area, and estimated time since loss of flight for flightless, terrestrial birds. Models ranked by AICc weight. Time scenarios (S) are for loss of flight early in the stem of the moa, elephant bird, and kiwi lineages (S1) or for loss of flight at the point of radiation for these groups (S2). Where time is included  $n = 32$ , otherwise  $n = 38$ .  $C_f$ , femoral circumference; *Coeff.*, coefficient; *Adj R<sup>2</sup>*, adjusted  $R^2$ ; *AICcWt*, Akaike weights. Land area was coded in binary ( $<100,000\text{km}^2 = 0$ ,  $>100,000\text{km}^2 = 1$ ).

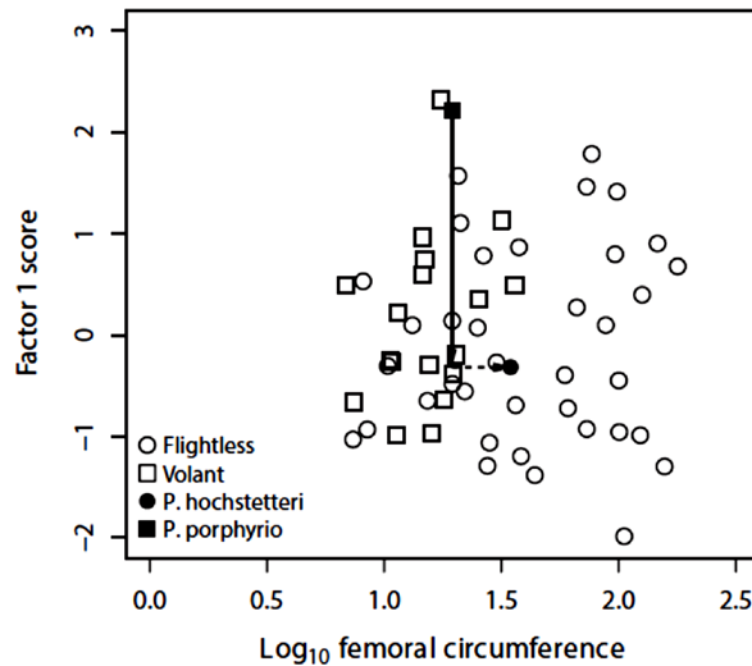
Models for $\Delta F1$	$\Delta C_f$		Land		Time S1		<i>Adj R<sup>2</sup></i>	<i>p</i>	<i>AICcWt</i>
	<i>Coeff.</i>	<i>p</i>	<i>Coeff.</i>	<i>p</i>	<i>Coeff.</i>	<i>p</i>			
$\Delta C_f$ +Land	1.861	0.022	-0.908	0.076	NA	NA	0.120	0.059	0.28
Land+Time S1	NA	NA	-1.259	0.045	0.737	0.029	0.107	0.074	0.22
$\Delta C_f$	1.096	0.114	NA	NA	NA	NA	0.050	0.114	0.18
$\Delta C_f$ +Land+Time S1	1.236	0.203	-1.284	0.040	0.438	0.271	0.128	0.078	0.14
Time S1	NA	NA	NA	NA	0.257	0.283	0.006	0.283	0.09
Land	NA	NA	-0.264	0.560	NA	NA	-0.021	0.560	0.06
$\Delta C_f$ +Time S1	1.172	0.253	NA	NA	-0.035	0.918	0.018	0.292	0.05
Land+Time S2	NA	NA	-1.341	0.014	1.008	0.003	0.224	0.009	0.56
$\Delta C_f$ +Land+Time S2	0.791	0.377	-1.409	0.011	0.816	0.040	0.218	0.019	0.21
$\Delta C_f$ +Land	1.891	0.022	-0.908	0.076	NA	NA	0.120	0.059	0.08
Time S2	NA	NA	NA	NA	0.480	0.074	0.073	0.074	0.07
$\Delta C_f$	1.096	0.114	NA	NA	NA	NA	0.050	0.114	0.05
$\Delta C_f$ +Time S2	0.442	0.649	NA	NA	0.358	0.347	0.048	0.187	0.02
Land	NA	NA	-0.264	0.560	NA	NA	-0.021	0.560	0.01
$\Delta C_f$ +Land	1.679	0.015	-0.766	0.068	NA	NA	0.123	0.038	0.59
$\Delta C_f$	1.107	0.074	NA	NA	NA	NA	0.061	0.073	0.33
Land	NA	NA	-0.284	0.468	NA	NA	-0.013	0.468	0.08

**Table 3.** Multiple regressions of sign-less  $\Delta$  factor 1 scores ( $\Delta DF1$ ) with  $\Delta CF$ , land area, and estimated time since loss of flight for flightless, terrestrial birds ranked by AICc weight. Models ranked from lowest to highest AICc weight. Time scenarios (S1 or S2) as described in Table 1. Cf, femoral circumference; Coeff, coefficient; Adj  $R^2$ , adjusted  $R^2$ . Land area was coded in binary ( $<100,000\text{km}^2 = 0$ ,  $>100,000\text{km}^2 = 1$ ).

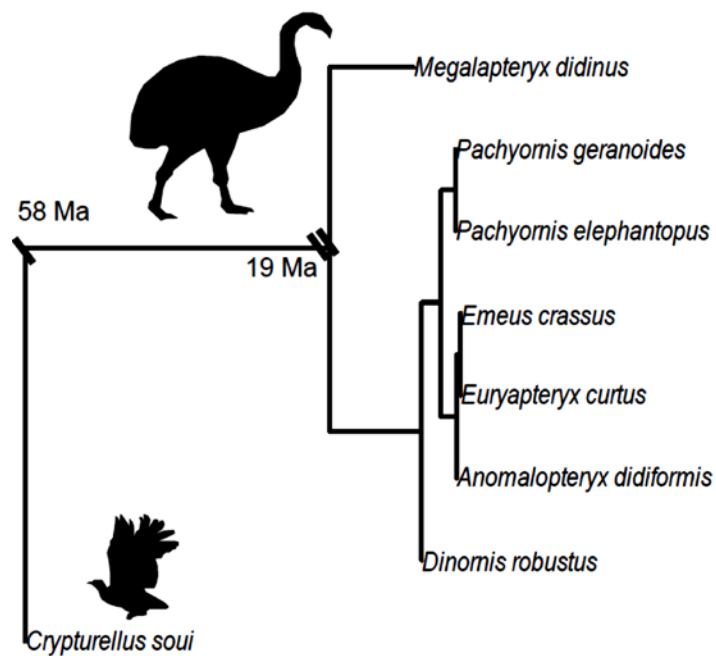
Models for $\Delta DF1$	$\Delta Cf$		Land		Time S1		Adj $R^2$	$p$	AICc Wt
	Coeff.	$p$	Coeff.	$p$	Coeff.	$p$			
$\Delta Cf + \text{Time}$	-1.028	0.064	NA	NA	0.287	0.124	0.054	0.169	0.25
$\Delta Cf$	-0.410	0.279	NA	NA	NA	NA	0.007	0.279	0.25
Land	NA	NA	0.070	0.774	NA	NA	-0.030	0.774	0.14
Time S1	NA	NA	NA	NA	0.031	0.810	-0.031	0.810	0.14
$\Delta Cf + \text{Land}$	-0.663	0.144	0.300	0.298	NA	NA	0.011	0.324	0.12
$\Delta Cf + \text{Land} + \text{Time}$	-1.032	0.067	0.078	0.820	0.259	0.257	0.022	0.315	0.06
S1									
Land + Time	NA	NA	0.058	0.871	0.009	0.961	-0.066	0.959	0.04
$\Delta Cf + \text{Time S2}$	-1.047	0.050	NA	NA	0.349	0.092	0.070	0.133	0.30
$\Delta Cf$	-0.410	0.279	NA	NA	NA	NA	0.007	0.279	0.22
Time S2	NA	NA	NA	NA	0.058	0.697	-0.028	0.697	0.13
Land	NA	NA	0.070	0.774	NA	NA	-0.030	0.774	0.12
$\Delta Cf + \text{Land}$	-0.663	0.144	0.300	0.298	NA	NA	0.011	0.324	0.11
$\Delta Cf + \text{Land} + \text{Time}$	-1.074	0.051	0.108	0.733	0.314	0.178	0.041	0.252	0.08
S2									
Land + Time S2	NA	NA	0.015	0.964	0.052	0.793	-0.063	0.927	0.03
$\Delta Cf$	-0.230	0.516	NA	NA	NA	NA	-0.016	0.516	0.44
Land	NA	NA	0.094	0.668	NA	NA	-0.022	0.668	0.38
$\Delta Cf + \text{Land}$	0.205	0.413	-0.383	0.342	NA	NA	-0.025	0.578	0.18

**Table 4.** Multiple regressions of  $\Delta$  factor 1 ( $\Delta F1$ ) scores with  $\Delta CF$ , land area, and estimated time since loss of flight for flightless palaeognaths listed in order of AICc weight. Time scenarios (S) are for loss of flight early in the stem of the moa, elephant bird, and kiwi lineages (S1) or for loss of flight at the point of radiation for these groups (S2). Land coded in binary (New Zealand = 0, >New Zealand = 1).  $N = 15$ . Cf, femoral circumference; Coeff, coefficient; Adj  $R^2$ , adjusted  $R^2$ ; AICcWt, Akaike weight.

Models for $\Delta F1$	$\Delta Cf$		Land		Time S1		Adj $R^2$	$p$	AICc
	Coeff.	$p$	Coeff.	$p$	Coeff.	$p$			
Land	NA	NA	0.904	<0.001	NA	NA	0.595	<0.001	0.75
$\Delta Cf$ +Land	0.117	0.897	0.902	<0.001	NA	NA	0.562	0.003	0.11
Land + Time S1	NA	NA	0.918	0.007	-0.412	0.942	0.561	0.003	0.11
$\Delta Cf$ +Land+Time	0.196	0.855	0.938	0.012	-1.033	0.879	0.523	0.011	0.01
S1									
Time S1	NA	NA	NA	NA	12.09	0.037	0.239	0.037	0.01
$\Delta Cf$ +Time	-0.876	0.508	NA	NA	13.63	0.036	0.206	0.099	0.00
$\Delta Cf$	0.309	0.826	NA	NA	NA	NA	-0.073	0.827	0.00
Land	NA	NA	0.904	<0.001	NA	NA	0.595	<0.001	0.42
Time S2	NA	NA	NA	NA	2.609	<0.001	0.575	<0.001	0.29
$\Delta Cf$ +Time S2	-1.185	0.211	NA	NA	2.889	<0.001	0.598	0.002	0.12
Land+Time S2	NA	NA	0.549	0.287	1.136	0.447	0.583	0.002	0.09
$\Delta Cf$ +Land	0.116	0.897	0.902	<0.001	NA	NA	0.562	0.003	0.06
$\Delta Cf$ +Land+Time	-0.938	0.501	0.184	0.805	2.337	0.326	0.564	0.007	0.01
S2									
$\Delta Cf$	0.309	0.826	NA	NA	NA	NA	-0.073	0.826	0.00



**Fig. 2** Plot of factor 1 scores against factor 2 scores. Volant and flightless species are represented by squares and circles respectively. An example of  $\Delta$  factor scores is given using the flightless *Porphyrio hochstetteri* (filled circle) and its nearest volant relative, *P. porphyrio* (filled square).  $\Delta$  Factor 1 is indicated by the solid line and  $\Delta$  femoral circumference by the dashed line.



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**Fig. 3** *Example of alternate timing scenarios using a cladogram of moa species included in this study. Cladogram is based on phylogenies of Bunce et al. (2009), Haddrath and Baker (2012), and Mitchell et al. (2014). Strikes represent alternative timings of loss of flight, with scenario 1 (single strike) being loss of flight after divergence from tinamous (represented by *Crypturellus soui*) and scenario 2 (double strike) being loss of flight at the base of the crown group. The age of the oldest remains of flightless moa was used to time the latter (Tennyson et al. 2010).*

### **Palaeognath-only analyses**

Factor analysis of the palaeognath data explained 57.6% of variance. Loadings of all leg bone lengths and tibiotarsal craniocaudal diameter were strong and positive, with other dimensions loading moderately. Tarsometatarsal mediolateral diameter was the only variable to have a negative loading (Table 1).

The best fitting regression model using earlier dates of flight loss in moa, kiwi, and elephant birds only included land area as a predictor variable (AICc weight 0.75, Table 3). The next three models all included land area as a significant predictor with the relationship between  $\Delta$  factor one scores and other variables being non-significant. Land area had a strong positive relationship with  $\Delta$  factor scores, indicating that species from land masses larger than New Zealand tend to have relatively longer leg bones, a larger craniocaudal diameter of the tibiotarsus, and a mediolaterally narrow tarsometatarsus compared to the ancestral model. Under the second time scenario land was the sole independent variable in the best fitting model (AICc weight 0.42). Time since loss of flight had a significant, positive relationship with  $\Delta$  factor scores in the next two models in order of AICc weight, thus species that have a longer history of flightlessness tend to have relatively long leg bones and mediolaterally narrow tarsometatarsi. The best models

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in both time scenarios had adjusted  $R^2$  values that were substantially higher than in the total group dataset, indicating that explanatory variables were better able to explain variance in  $\Delta$  factor scores than in the total dataset (Tables 2 and 4). No predictor variables were found to be significant in regression models using sign-less regression scores, regardless of time scenario used (Table 5).

All regression residuals were normally distributed (Jarque-Berra test  $p$ -values  $> 0.05$ ) and had equal variances (Breusch-Pagan test  $p$ -values  $> 0.05$ ).

As in the total group analysis, a Wilcoxon signed-rank test of  $\Delta$  factor scores found the median score to be not significantly different from zero.

**Table 5.** Multiple regressions of sign-less  $\Delta$  factor 1 scores ( $\Delta DF1$ ) with  $\Delta CF$ , land area, and estimated time since loss of flight for flightless palaeognaths listed by AICc weight. Time scenarios are as described in Table 4. Land coded in binary (New Zealand = 0, >New Zealand = 1).  $N = 15$ . Cf, femoral circumference; Coeff, coefficient; Adj  $R^2$ , adjusted  $R^2$ ; AICcWt, Akaike weight.

Models for $\Delta DF1$	$\Delta Cf$		Land		Time S1		Adj $R^2$	$p$	AICc Wt
	Coeff.	$p$	Coeff.	$p$	Coeff.	$p$			
$\Delta Cf$	-0.857	0.265	NA	NA	NA	NA	0.025	0.265	0.40
Time S1	NA	NA	NA	NA	1.331	0.706	-0.065	0.706	0.21
Land	NA	NA	0.025	0.890	NA	NA	-0.075	0.890	0.19
$\Delta Cf$ +Time S1	-1.148	0.178	NA	NA	3.351	0.372	0.014	0.364	0.10
$\Delta Cf$ +Land	-0.864	0.281	0.035	0.846	NA	NA	-0.053	0.540	0.06
Land+Time S1	NA	NA	-0.044	0.868	1.925	0.708	-0.151	0.921	0.02
$\Delta Cf$ +Land+Time S1	-1.353	0.149	-0.179	0.502	6.153	0.285	-0.030	0.489	0.01
$\Delta Cf$	-0.857	0.265	NA	NA	NA	NA	0.025	0.265	0.42
Land	NA	NA	0.025	0.890	NA	NA	-0.075	0.890	0.20
Time S2	NA	NA	NA	NA	0.048	0.928	-0.076	0.928	0.20
$\Delta Cf$ +Time S2	-1.004	0.238	NA	NA	0.285	0.611	-0.033	0.482	0.07
$\Delta Cf$ +Land	-0.864	0.281	0.035	0.846	NA	NA	-0.053	0.540	0.06
Land+Time S2	NA	NA	0.063	0.893	-0.122	0.930	-0.164	0.987	0.03
$\Delta Cf$ +Land+Time S2	-1.915	0.126	-0.681	0.298	2.328	0.257	-0.017	0.461	0.02



**Table 6.** Multiple regressions of factor 1 scores ( $\Delta F1$ ) with  $\Delta CF$ , land area, and estimated time since loss of flight for flightless, terrestrial neognaths listed by Akaike weight. Where time is included  $n = 17$ , otherwise  $n = 23$ . Land was coded in binary ( $<100,000\text{km}^2 = 0$ ,  $>100,000\text{km}^2 = 1$ ). Cf, femoral circumference; Coeff, coefficient; Adj  $R^2$ , adjusted  $R^2$ ; AICcWt, Akaike weight.

Models for $\Delta F1$	$\Delta Cf$		Land		Time		Adj $R^2$	$p$	AICc Wt
	Coeff.	$p$	Coeff.	$p$	Coeff.	$p$			
$\Delta Cf$	2.380	0.129	NA	NA	NA	NA	0.090	0.129	0.37
Time	NA	NA	NA	NA	0.488	0.357	-0.006	0.357	0.16
Land+Time	NA	NA	-0.538	0.106	1.227	0.078	0.112	0.171	0.14
Land	NA	NA	-0.145	0.574	NA	NA	-0.044	0.574	0.12
$\Delta Cf$ +Land	2.650	0.102	-0.225	0.365	NA	NA	0.082	0.215	0.11
$\Delta Cf$ +Time	2.142	0.228	NA	NA	0.180	0.752	0.032	0.312	0.07
$\Delta Cf$ +Land+Time	1.769	0.298	-0.488	0.142	0.905	0.222	0.123	0.207	0.04
$\Delta Cf$	2.174	0.103	NA	NA	NA	NA	0.080	0.103	0.58
$\Delta Cf$ +Land	2.353	0.082	-0.173	0.311	NA	NA	0.083	0.162	0.24
Land	NA	NA	-0.132	0.455	NA	NA	-0.020	0.455	0.18

**Table 7.** Multiple regressions of signless  $\Delta$  factor 1( $\Delta DF1$ ) scores with  $\Delta CF$ , land area, and estimated time since loss of flight for flightless, terrestrial neognaths listed by Akaike weight. Where time is included  $n = 17$ , otherwise  $n = 23$ . Land was coded in binary ( $<100,000\text{km}^2 = 0$ ,  $>100,000\text{km}^2 = 1$ ). Cf, femoral circumference; Coeff, coefficient; Adj  $R^2$ , adjusted  $R^2$ ; AICWt, Akaike weight.

Models for $\Delta DF1$	$\Delta CF$		Land		Time		Adj $R^2$	$p$	AICc Wt
	Coeff.	$p$	Coeff.	$p$	Coeff.	$p$			
$\Delta Cf$	-0.763	0.443	NA	NA	NA	NA	-0.024	0.444	0.31
Time	NA	NA	NA	NA	0.165	0.618	-0.049	0.618	0.25
Land	NA	NA	0.037	0.815	NA	NA	-0.063	0.815	0.23
$\Delta Cf + \text{Time}$	-1.211	0.282	NA	NA	0.338	0.358	-0.031	0.486	0.09
$\Delta Cf + \text{Land}$	-0.839	0.424	0.063	0.703	NA	NA	-0.086	0.699	0.06
Land+Time	NA	NA	-0.027	0.902	0.202	0.659	-0.122	0.880	0.04
$\Delta Cf + \text{Land} + \text{Time}$	-1.258	0.286	-0.062	0.778	0.431	0.394	-0.103	0.688	0.01
$\Delta Cf$	-0.562	0.530	NA	NA	NA	NA	-0.028	0.530	0.49
Land	NA	NA	-0.015	0.897	NA	NA	-0.047	0.897	0.40
$\Delta Cf + \text{Land}$	-0.556	0.548	-0.006	0.963	NA	NA	-0.079	0.824	0.11

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**Neognath-only analyses**

Factor analysis of the neognath data explained 41.6% of variance. As in the total group analysis, tibiotarsal and tarsometatarsal length both load strongly and positively. Femoral length and tibiotarsal diameters had moderate positive loadings, whilst tarsometatarsal diameters had weak or neutral loadings (Table 1).

All regression models for neognaths performed poorly with no variable having a significant relationship with  $\Delta$  factor scores and uniformly low ( $<0.13$ ) (Table 6). This was also the case in the expanded dataset including taxa without available timing information for loss of flight and when sign-less scores were used as the y-variable (Tables 6, 7).

All regression residuals were had equal variances (Breusch-Pagan test  $p$ -values  $>0.05$ ). Residuals were normally distributed (Jarque-Berra test  $p$ -values  $>0.05$ ), with the exception of the land area only model for sign-less  $\Delta$  factor scores in the expanded dataset (Jarque-Berra test  $p$ -value 0.031). For the sake of completeness this model was included in the regression table (Table 7).

Wilcoxon signed-rank tests for  $\Delta$  factor scores and femoral circumference showed only  $\Delta$  femoral circumference to be significantly different from zero.

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## Discussion

### Total Group

The two models that best fitted the data were a combination of variables including either land area and  $\Delta$  femoral circumference or land area and time since loss of flight. Both models had similar AICc weights and  $R^2$  values, thus neither model can be said to be preferred here. Land area performed poorly when used as the sole predictor with no significant relationship with  $\Delta$  factor 1 scores and this regression ranked fifth on the table based on AICc weight (Table 2). Likewise both time since loss of flight and  $\Delta$  femoral circumference had none significant relationships with  $\Delta$  factor scores when included as the only predictor. Thus, the significance of the variables in the higher AICc weight models is likely due to interactions between predictor variables. With kiwi, moa, and elephant birds estimated to have lost flight more recently, time since loss of flight and land area were significant predictors in the best fitting model. This reflects the relatively shorter, mediolaterally broad tarsometatarsi of these birds compared to other ratites, and the substantial differences in timing between the two scenarios for loss of flight in these lineages. For example, the first scenario had the kiwi lineage losing flight at the time of divergence from elephant birds at 50 Ma, whereas the second timed loss of flight at the basal split within the genus *Apteryx* at 8.6 Ma (Mitchell et al. 2014). For elephant birds, the difference in timing was 32.8 Ma (Mitchell et al. 2014), and in moa it was 39 Ma (see methods). This combination led to time being a significant predictor under the second time scenario, with taxa with relatively longer, narrower tarsometatarsi and longer tarsometatarsi estimated to have lost flight earlier. Unfortunately, the lack of unambiguous fossils of the three lineages dating between the two sets of ages means that neither of these scenarios can be ruled out at present. The

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instability of land area and  $\Delta$  femoral circumference as predictors despite neither of these variables changing between the two separate analyses indicates that interaction between variables in the multiple regressions had an effect on significance.

The absence of any significant relationship between predictor variables and the magnitudes of differences in factor scores between ancestral models and flightless taxa suggests that other factors not taken into account here may be at work. One possibility is whether there are differences in habitat preference between ancestors and descendants, for instance, whether there was shift from a closed to open habitat (e.g. forest to plains) or if the habitat remained the same. The absence of any overall directionality in  $\Delta$  factor scores also indicates that differences in selection pressures act on the leg morphology of different species. However, a trend towards increased body size was detected, with  $\Delta$  femoral circumferences being significantly greater than zero.

### **Palaeognaths**

Land area as the sole predictor was found to be the best model for explaining variation in  $\Delta$  factor scores. Among ratites there is a spectrum of leg bone proportions independent of size. Moa and kiwi tend to have short long bones and medio-laterally wide tarsometatarsi, reflected by their more negative factor scores. On the other hand the ostriches, rhea, emus, and cassowaries have relatively long leg bones, and narrow tarsometatarsi, shown by their more positive factor scores. This variation is in spite of many moa having similar femoral circumferences to ratites considered to be cursorial (Supporting Information 1). The dichotomous pattern of morphology is reflected in the distributions of the two different groups. Moa and kiwi both evolved on a smaller land mass, New Zealand, in the absence of mammalian predators, and tend to have relatively

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short and broad tarsometatarsi indicating a low degree of cursoriality (Alexander, 1983; Brassey et al. 2013). On the other hand, the other lineages (rheas, ostriches, casuariids) are present on larger land masses and have longer, narrower tarsometatarsi. This pattern is not phylogenetic as recent studies have found kiwi to be the sister group of elephant birds and more closely related to cassowaries and the emu than to moa (Phillips et al. 2010; Haddrath & Baker, 2012; Mitchell et al. 2014). The one species of elephant bird included in this study, *Mullerornis agilis*, was the only non-New Zealand ratite to have a negative factor 1 score. This variation in tarsometatarsal proportions is the likely the reason that no directionality was found in the  $\Delta$  factor scores.

### Neognaths

Multiple regressions found no significant relationship between any of the predictors and  $\Delta$  factor scores, regardless of whether or not the  $\Delta$  factor scores were sign-less. This suggests that the relationship between  $\Delta$  factor scores and  $\Delta$  femoral circumference in the total dataset was the result of differences between neognaths and palaeognaths, with the latter having relatively longer tibiotarsi and shorter femora, and not due to trends within either group. In addition, as was the case in the total dataset and the palaeognath subset, no directionality was found in  $\Delta$  factor scores. Directionality was found in body size changes with  $\Delta$  femoral circumferences significantly greater than zero. It is likely that rather than a broad overarching factor such as size driving the evolution of hindlimb morphology in flightless neognaths, the life-habits of individual species may have a greater influence. Additional studies testing for a relationship between leg morphology and ecology in flightless terrestrial neognaths within a fully phylogenetic framework would be able to confirm or deny this hypothesis. A previous study comparing the overall

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morphology of flighted and flightless rails found that the relative reduction of the forelimbs in relation to body mass was the most reliable predictor of whether a species was flightless, whereas differences in leg bone proportions tended to be more variable (Livezey, 2003). The results of the current study indicate that this variability extends to neognaths as a whole, with neither a trend in directionality nor magnitude of change in leg bone proportions being apparent. An alternative interpretation is that the evolution of leg bone proportions in flightless neognaths is random rather than the result of multiple adaptations of varying directionality in different lineages. However, given the close relationship between hindlimb morphology and locomotion and that use of the legs is the sole method of transport in terrestrial flightless birds, this interpretation is not favoured here (Garland & Janis, 1993; Janis & Wilhelm, 1993; Bennett, 1996; Abourachid & Renous, 2000; Zeffe et al. 2003).

An alternative approach to that taken here would be to reconstruct nodal values for leg bone proportions using a phylogeny including only volant birds using maximum likelihood approaches. By combining values calculated for the most recent common ancestor of the volant lineages and the flightless taxa in question with any of a number of evolutionary models (e.g. Brownian Motion, Ornstein-Uhlenbeck, Adaptive Peak) it may be possible to reconstruct nodes at the base of flightless lineages. This method would require greater sampling of volant species throughout avian phylogeny, particularly in cases where individual flightless lineages are widely separated in the tree, in order to be confident of ancestral reconstruction of volant lineages. The uncertainty of ancestral reconstructions for some lineages will potentially remain substantial for two reasons. Firstly there is evidence to indicate that the proportions of the limb bones can evolve rapidly. An extreme example is provided in a morphological study of *Chenonetta jubata* which demonstrated a 10% reduction in the length of the wing bones in the space of ~10,000 years

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(Worthy, 1988). Secondly, the gaps in the fossil record of some flightless lineages are on the order of tens of millions of years, e.g. the divergence of the *Strutio* lineage from paleognaths is estimated to have occurred 70–97 million years ago (Phillips et al. 2010; Haddrath & Baker, 2012; Mitchell et al. 2014) whilst the oldest undisputed fossil of this lineage is ~20 million years old (Mourer-Chauviré et al. 1996). This combination of high rates of evolution and long branch lengths is likely to increase the uncertainty of some ancestral reconstructions (Schulter et al. 1997). Due to the relatively small sample of volant species in the dataset this method could not be examined here and as such this remains an area for future investigation.

A number of terrestrial flightless neognaths were not included in this study simply due to the absence of well-resolved phylogenies and/or volant sister taxon candidates. These taxa range from island-inhabiting rails such as *Mundia elpenor* from Ascension Island to the large adzebills (*Aptornis*) of New Zealand and the giant dromornithids (Dromornithidae) of Australia. In many of these cases, it is likely that the volant ancestors and/or closest relatives of these species are also extinct. Further ancient DNA analyses are required to illuminate the evolutionary history of recently extinct species and new fossil discoveries are needed to elucidate the relationships of more ancient lineages. In turn, these will further improve our understanding of the changes in functional morphology of the hindlimbs with the loss of flight.



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## Conclusion

It is clear that the patterns of change in leg bone proportions in terrestrial, flightless birds are generally more complex than those exhibited in the bones of the forelimbs, with few clear trends, particularly within Neognathae. Close examination of ratite and neognath birds indicates that the patterns seen in the total group of extant Aves are merely a result of differences between the two groups.

Palaeognaths outside of New Zealand have medio-laterally narrower tarsometatarsi compared to both New Zealand species and ancestral models. This finding agrees with previous observations of leg bone morphology and cursoriality in ratites. On the other hand, flightless neognaths exhibit no obvious relationship between changes in leg bone morphology and the size of the land area inhabited. Therefore, the variation in the directionality of leg bone evolution found in both neognaths and palaeognaths most likely reflects the variability of selection pressures and differences in life habits.

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## Chapter 4

# **A comparison of pterosaur and Mesozoic avian morphospaces constructed using functionally analogous traits**

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**Abstract**

The late Mesozoic saw the origin and subsequent radiation of birds in the presence of pterosaurs. Opinion is divided as to whether these two groups competed or were ecologically separated during the Cretaceous. Comparing ecologically meaningful morphological characters using multivariate analyses is one means of testing for competitive interactions between extinct groups. Previous comparisons of Mesozoic birds and pterosaurs used measurements of homologous skeletal structures. However, these characters are not always functionally comparable and may reflect differing ancestries rather than ecologies. Here, the results from multivariate analyses of forelimb, hindlimb, and lower jaw measurements for pterosaurs and Mesozoic birds are presented. The wings were divided into three functionally analogous units; the brachium, antebrachium, and distal wing. Results of these analyses show separation of the two groups due to interaction between size and shape axes. Pterosaurs had relatively longer jaws, shorter metatarsals, and shorter brachial region compared to birds of similar size. This indicates ecological separation between the two groups by a combination of differences in size, locomotory features, and feeding adaptations. Further study of functional differences in the jaws of pterosaurs and birds is a potential means of testing for expansion by modern birds into niches previously occupied by pterosaurs.

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## Introduction

Birds appeared in the fossil record when another group of flying vertebrates, the pterosaurs, were already present. Birds and pterosaurs coexisted for at least 90 million years. Previous studies have suggested that these two groups competed for ecological space through the late Mesozoic, with birds taking over small body size niches and pterosaurs increasing in size to occupy new spaces [1,2]. The co-occurrence of birds and pterosaurs in Late Jurassic and Early Cretaceous deposits, a trend of increasing body size minima and maxima in pterosaurs from the end of the Jurassic, and similarity in diet have all been used to argue for long-term ecological competition between birds and pterosaurs [2,3].

On the other hand, studies of skeletal morphology have found the two groups to overlap little if at all [4,5]. These studies compared proportions of the skeletal elements making up the wings and legs, which may broadly reflect differences in locomotory function and habitat, and concluded that there is a lack of evidence for ecological competition between these groups due to their separation in morphospace [4,5]. Interestingly, the co-occurrence of birds and pterosaurs in Jurassic-Lower Cretaceous deposits has also been used to suggest ecological separation [6]

Several studies comparing the fundamental aerodynamic traits of wing loading (body mass/wing surface area) and aspect ratio (wing span<sup>2</sup>/wing surface area) in modern birds and pterosaurs (estimated in the case of pterosaurs) found overlap in functional morphospace [7,8]. Both wing loading and aspect ratio strongly affect flight performance. Wing loading is related to flight speed, turning radius (manoeuvrability), and turning speed (agility): high wing loading is associated with faster horizontal flight speeds and increased sinking speed, decreased manoeuvrability, and increased agility [7,9,10]. Aspect ratio affects the ability of animals to fly in

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clustered environments and manoeuvrability, as well as the amount of bending stress on the wing bones [7,9,11]. High aspect ratios (long, narrower wings) reduce manoeuvrability and increase bending stresses, whilst a longer wing restricts the ability to fly in closed spaces [9,11]. Although a number of Mesozoic avians have been found with preserved feathers (e.g. *Archaeopteryx lithographica*, *Confuciusornis sanctus*, *Hongshanornis longicresta*), many others are not so exquisitely preserved. As the wings of birds have no posterior skeletal attachments, unlike pterosaurs [12–14], wing area is difficult to estimate in specimens with only skeletal elements preserved. This fact either constrains morphospace studies to include only directly measurable features and wingspan estimates [2,4,5,15,16] or substantially reduces sample size. As such, previous studies have constructed morphospaces from measurements of measurable skeletal elements from the fore- and hindlimbs [4, 5].

This study compares the morphospaces of pterosaurs and Mesozoic birds using measurements not only from the wings but also the legs and mandible and applies multivariate analyses. A different approach is required because the wings of pterosaurs and birds are constructed in very different ways. Pterosaurs have an extremely elongated fourth manual digit, which along with the rest of the arm supports a membrane that extended down to the hindlimb [12,14]. In birds, the bones of the hand are fused to form the carpometacarpus and the flight feathers form the aerofoil surface as well as a large portion of the leading edge of the wing. Additionally, the legs are free from the wings. Previous research has used measurements from homologous skeletal structures such as the forearm (ulna/radius) and hand (metacarpals and digits) as variables in order to compare these groups [4,5]. The problem with this approach is that these units are not functionally comparable and may not accurately reflect differences in locomotion or ecology, instead being the result of differences in ancestry.



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Recent research indicates that the main distal wing joint in pterosaurs is between the fourth metacarpal and the first phalanx of the flight finger with movement at the wrist restricted [17,18]. On the hand, the distal wing joint in birds is at the wrist, meaning that all other structures distal to this point (carpometacarpus, digits, primary feathers) are functionally part of the distal wing whilst in pterosaurs this unit is made up of the phalanges of the wing finger [17]. Here I divide the wings of pterosaurs into units functionally analogous to birds, as described by Prondvai and Hone [17], and employ the measurements of these units as variables.

## Methods

For both pterosaurs and early avians, measurements of the lengths of the mandible, humerus, ulna, femur, tibiotarsus, and longest metatarsal as well as maximum mandible depth were compiled from the published literature (Supplementary Material 1). For the hand skeleton of pterosaurs, lengths of metacarpal IV and the phalanges of digit IV were taken. For the birds manus length (carpometacarpus plus digit II) was used. Measurements that were not reported in publications were taken by measuring published images.

Some pterosaur species are represented by large numbers of individuals (e.g. *Rhamphorynchus muensteri*, *Pterodactylus kochi*, *P. antiquus*). However, due to the high proportion of immature individuals in these samples [19–21] species averages were not employed. Instead, the largest complete individuals were used to represent the species. Although the largest individual in a population is not necessarily a good representative of a population as a whole, the individuals chosen were generally not the largest in the sample as completeness was

also a criterion for selection. In cases where an element from a limb was missing, the relative proportions of similarly sized individuals of the same species (if available) were used to calculate the length of the missing element (Supplementary Material 1). A number of species have been described based on individuals which were not osteologically mature according to the criteria laid out by Bennett [22], such as lack of fusion of combined bones (e.g. scapula-coracoid), grainy surface texture of long bones, and poorly ossified epiphyses. However, these criteria were used to distinguish subadults which were similar in size to osteologically mature individuals and likely filled the same niches [22]. As a result, individuals which do not exhibit full skeletal fusion were included in the dataset when no complete, fully mature individuals were available. However, those species represented only by individuals which exhibited extensive lack of skeletal fusion, poor ossification, and graining of long bones were excluded as these characters indicate the animal was still growing rapidly [22–24]. These criteria were also used for the avian dataset.

The length of the skeletal wing in birds is not representative of total wing length as the primary feathers substantially increase functional wing length by up to 100% [25]. As feathers are not preserved in all avian specimens, an equation for estimating average primary feather length was formulated using regressions of mean primary feather length against skeletal lengths in 22 Mesozoic birds. Average primary feather lengths were taken from Wang et al. [26], with some additional measurements taken from published images using the program ImageJ (Supplementary Material 2). A step-wise function in the R package “MASS” [27] based on Akaike’s information criterion (AIC) [28] was used to find the best combination of forelimb measurements for predicting primary feather length. Of the three skeletal elements (humerus, ulna, manus), manus length alone was found to be the best predictor (AIC = -94.24, Adjusted  $R^2$  = 0.743). The  $R^2$  was higher than that for regression of average primary feather length against

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total skeletal length (Adjusted  $R^2 = 0.668$ ). The equation given for estimating mean primary feather length was:

$$\log_{10}Pri_{mean} = 0.971(\log_{10}Manus\ Length) + 0.361 \quad (1)$$

Prediction error was calculated for each specimen using:

$$\frac{Predicted - Observed}{Observed} * 100 \quad (2)$$

Mean percentage error for the 23 specimens was 21.2% and varied between 1.2% (*Yixianornis grabaui* IVPP V12631) and 53.4% (*Cuspirostrisornis houi* STM A11-65). A Welch two-tailed T-test found no significant difference between predicted and observed feather lengths ( $p = 0.852$ ). As a result, equation (1) was used to estimate mean primary feather length in specimens representing 35 species of Mesozoic birds for which feather length data was unavailable.

Prior to multivariate analyses the forelimb elements were divided into three functional units: brachial, antebrachial, and distal wing. For pterosaurs, the composition of the units were brachial = humerus, antebrachial = ulna + metacarpal IV, and distal wing = digit IV. For avians, the units were brachial = humerus, antebrachial = ulna, and distal wing = carpometacarpus + digit II + mean primary feather length (Fig. 1). These units were based on the functional descriptions of Prondvai and Hone [17]. The lengths of the units and the lengths of the leg bones and mandible measurements were subjected to a principal components analysis (PCA) using the standard “princomp” function in R 3.1.0 [29]. For this analysis, 18 species of pterosaur and 22 species of early avians had sufficiently complete representatives to be included. A separate

principal components analysis was carried out using only the wing measurements. This analysis included 42 species of pterosaurs and 51 species of birds.

To examine the effect of including metacarpal IV in the pterosaur antebrachial region an additional set of analyses was carried out in which this element was included in the distal wing due to its homology to the metacarpals of birds. This approach is similar to that taken by previous studies comparing limb disparity in pterosaurs and birds in which the arm was divided into homologous units [4,5]. However, in this case primary feather length was incorporated in the distal wing unit in the avian dataset.

Whether differences in elbow angle at full wing extension affected the degree of overlap in wing lengths between pterosaurs and Mesozoic avians was also examined. The elbow angles used for both “rhamphorhynchoid” and pterodactyloid pterosaurs were 155° and 160° respectively [18,30,31]. The exception was *Pteranodon* for which the angle used was 150° [32]. To my knowledge, no studies have been carried out on the range of elbow flexion in early avians. However, maximum elbow angles have been reported for modern birds during flight with 110°-120° and 127° for the common starling (*Sturnus vulgaris*) and chukar partridge (*Alectornis chukar*) [33,34]. Therefore, the elbow angle at full wing extension for Mesozoic birds was set to 120°. Two sets of wing lengths were calculated. The first was a simple summing of the forelimb elements. The second involved estimating the true length of the wing represented from the proximal end of the humerus to the distal end of the ulna using the following trigonometric equation:

$$c^2 = a^2 + b^2 - 2ab\cos\gamma \quad (3)$$

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where  $c$  equals the distance from the proximal end of the humerus to the distal end of the ulna,  $a$  is humeral length,  $b$  is ulna length, and  $\gamma$  is equal to the angle of the elbow (Fig. 1). The corrected wing length was then calculated by summing  $c$  and the remaining wing elements (manus and feather length in birds, metacarpal and digit IV in pterosaurs). It is important to note that the angles reported *in vivo* for neornithine birds may have been further constrained by soft tissues whereas those for pterosaurs could not have been [18]. Additionally, whether the elbow angles of modern birds can be applied to early avians remains to be tested. However, the intention here is simply to examine whether differences in elbow joint angle could affect comparisons of wing length and span between pterosaurs and early avians in order to see whether there is a need for further study in this area.

## Results

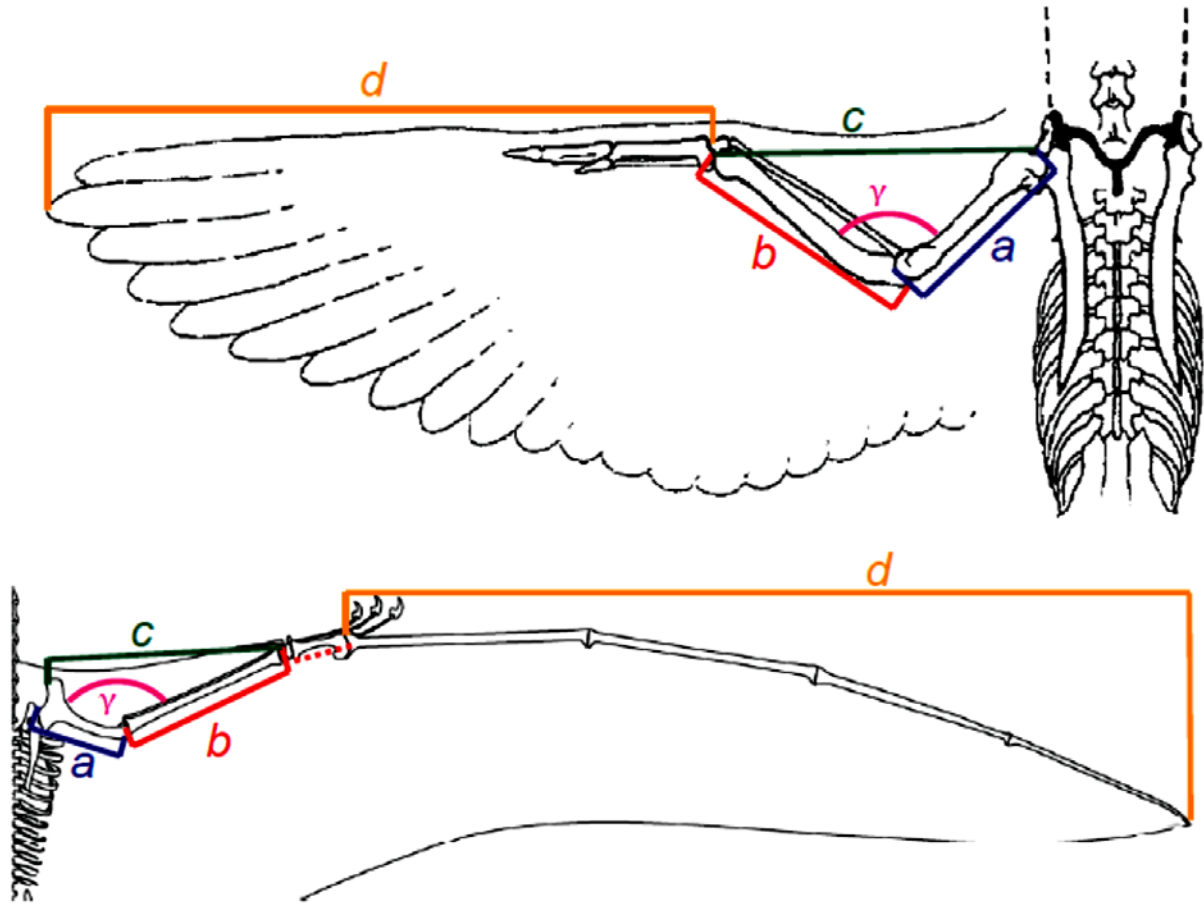
With the wing of pterosaurs divided into functional units equivalent to those of birds, the first principal component (PC) accounted for 89.5% of variance (Table 1). All variables loaded weakly ( $<0.5$ ) and negatively on this axis, indicating that first component scores primarily reflect size with larger taxa having more negative scores. However, not all loadings were equal and so some shape variation must be included in the first component. The second principal component accounted for 5.3% variance. Mandible length and metatarsal length both loaded moderately (0.5 – 0.8) with the former loading positively and the latter negatively. Brachial length also had a weak to moderate negative loading (-0.48). All other loadings were weak or neutral. Together, the first two principal components accounted for 94.7% of the variance.

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Separation between Mesozoic birds is clearly shown by plots of PC1 scores against PC2 scores, with only *Confuciusornis sanctus* falling into the 95% ellipse of pterosaurs (Fig. 2A). However, separation is not visible on either single axis as both groups have overlapping PC1 and PC2 scores. Instead, Mesozoic birds that overlap on PC1 with pterosaurs tend to have more negative PC2 scores indicating longer metatarsals, brachial region, and shorter mandibles. With the fourth metacarpal of pterosaurs included in the distal wing, the proportions of variance explained by the first three principal components were similar to those seen in the first set of PCA results (Table 1). One exception was that the antebrachial region and the distal wing had weaker and stronger loadings on PC1 respectively.

Principal components analysis of wing measurements by themselves also showed separation between Mesozoic birds and pterosaurs. When the pterosaurian metacarpal IV was included in the antebrachial region, PC1 accounted for 95.3% of variance (Table 2). All three variables loaded positively on this axis, with higher loadings for the antebrachium and distal wing than the brachial region (Table 2). The loading of the brachium was strongly positive, the antebrachium had a moderate, negative loading, and the distal wing had a neutral loading on the second principal component. This axis accounted for 3.1% of variance. Separation of Mesozoic birds and pterosaurs is apparent in a plot of PC scores with only *Yixianornis grabaui* plotting within the 95% confidence ellipse of pterosaurs (Fig. 3A). However, there is overlap between the two groups on each axis with separation due to birds with similar PC1 loadings to pterosaurs having higher PC2 scores (Fig. 3A). This indicates that compared to pterosaurs of similar size and relative distal wing length Mesozoic birds have a longer brachial region. Thus despite the low amount of variance explained by PC2, this axis is required for separation between birds and pterosaurs to become apparent.

Paired Wilcoxon signed rank tests found significant differences between the wing lengths of Mesozoic birds and pterosaurs before and after correction of wing lengths ( $p < 0.001$ ). However, correction for elbow angle had little effect on comparisons between the groups with substantial overlap between the groups both before and after correction (Fig. 4).



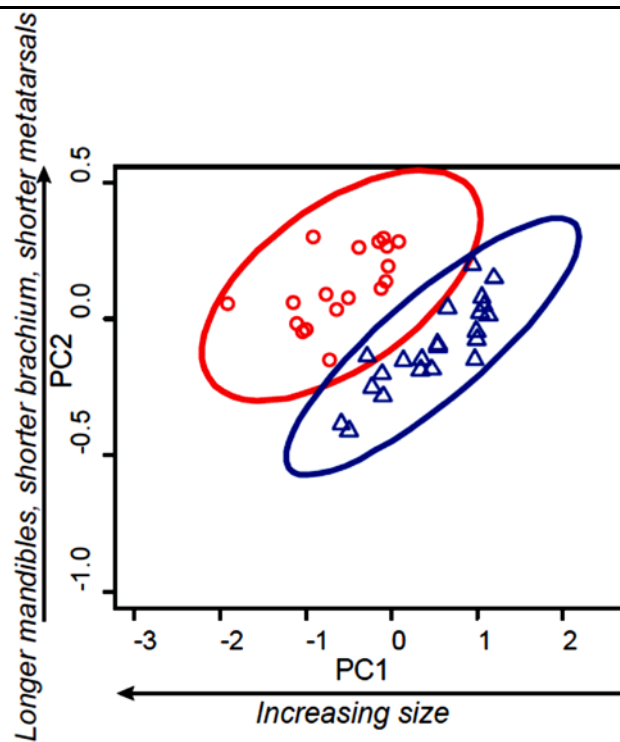
**Figure 1.** (top) Bird (*Sturnus vulgaris*) and (bottom) pterosaur (*Rhamphorhynchus muensteri*) wings extended to show the method used to correct wing lengths for elbow flexion angle. Humerus (a), ulna (b) lengths and elbow angle ( $\gamma$ ) were input into equation (3). The resultant length was summed with distal wing (d) length (manus length + average primary feather length) in the case of birds, and metacarpal IV (dotted line) plus distal wing (sum of phalanges of digit IV) in pterosaurs. Diagrams are modified from Jenkins et al. [35] and Prondvai & Hone [18].

Incorporation of the fourth metacarpal into the distal wing region in the pterosaur data changed the loadings of the three variables. All variables had negative loadings with the distal wing loaded most strongly on the first PC axis (Table 2), which accounted for 95.7% of variance. On the second PC (accounting for 3.7% of variance) brachial length loaded positively with moderate strength whereas the distal wing loaded with similar strength in the opposite direction. Antebrachial length loaded weakly on this axis. The two groups clustered more closely in a plot of PC scores compared to the analysis of functional units with four avian species (*C. sanctus*, *C. suniae*, *Y. grabaui*, and *Archaeornithura meemannae*) falling within the 95% confidence ellipse (Fig. 3B), and two pterosaurs (*Jeholopterus ninchengensis*, *Sordes pilosus*) falling in the avian ellipse. As before, birds that plotted similarly on PC1 to pterosaurs had higher PC2 scores.

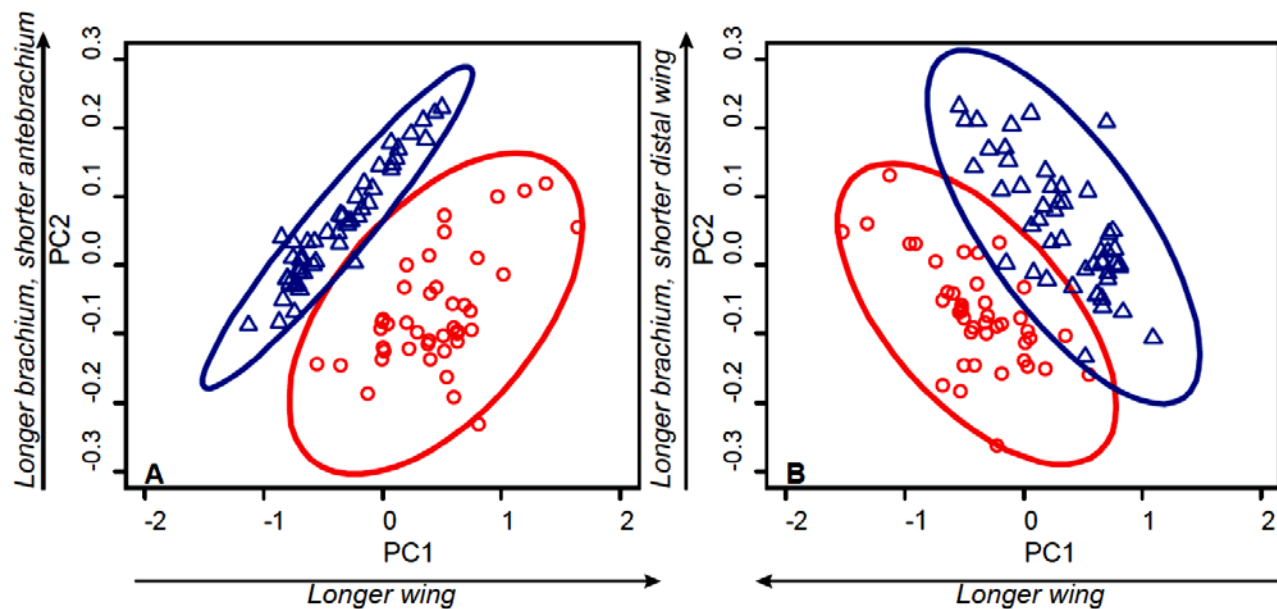
**Table 1.** Loadings of variables on the first three principal components. *Italics indicate values stemming from a PCA in which the pterosaurian metacarpal IV was included in the distal wing as opposed to the antebrachium.*

	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<i>PC1</i>	<i>PC2</i>	<i>PC3</i>
Mandible length	-0.420	0.567	-0.328	<i>-0.430</i>	<i>0.576</i>	<i>-0.375</i>
Mandible depth	-0.465	0.000	0.800	<i>-0.479</i>	<i>0.159</i>	<i>0.730</i>
Brachial length	-0.262	-0.472	-0.380	<i>-0.273</i>	<i>-0.461</i>	<i>-0.384</i>
Antebrachial length	-0.448	0.113	-0.122	<i>-0.332</i>	<i>-0.199</i>	<i>-0.335</i>
Distal wing length	-0.391	0.000	-0.281	<i>-0.451</i>	<i>0.143</i>	<i>0.000</i>
Femur Length	-0.299	-0.265	0.000	<i>-0.309</i>	<i>-0.244</i>	<i>0.000</i>
Tibia length	-0.286	-0.215	0.000	<i>-0.295</i>	<i>-0.196</i>	<i>0.141</i>
Metatarsal length	-0.115	-0.559	0.000	<i>-0.121</i>	<i>-0.522</i>	<i>0.203</i>
<b>Proportion of variance</b>	<b>89.5</b>	<b>5.3</b>	<b>1.8</b>	<b>89.1</b>	<b>5.8</b>	<b>2.0</b>





**Figure 2.** Plot of PC1 vs PC2 with the pterosaurian metacarpal IV included in the antebrachium. Mesozoic birds are represented by blue triangles and pterosaurs by red circles.



**Figure 3.** *PC1 vs PC2 based on a PCA of wing measurements with pterosaur metacarpal IV included either in the antebrachium (A) or the distal wing (B).*

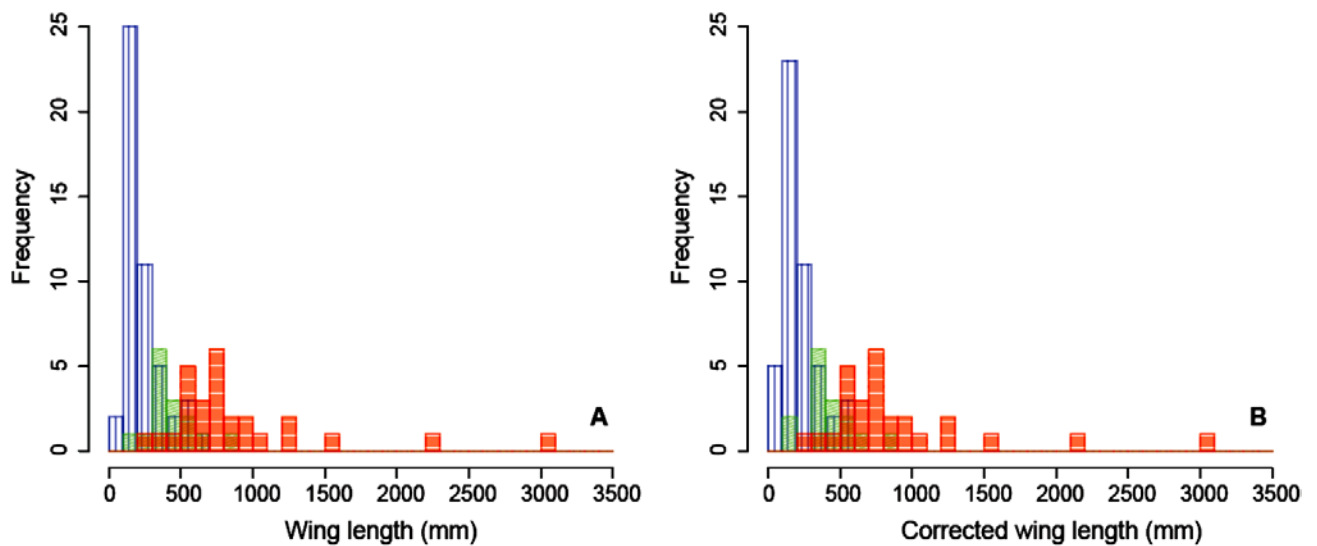
**Table 2.** *Loadings of variables on principal components from PCA of wing element lengths only. Italics indicate values stemming from a PCA in which the pterosaurian metacarpal IV was included in the distal wing, as opposed to the antebrachium.*

	<b>PC1</b>	<b>PC2</b>	<i>PC1</i>	<i>PC2</i>
Brachial	0.419	0.856	<i>-0.457</i>	<i>0.708</i>
Antebrachial	0.682	-0.517	<i>-0.540</i>	<i>0.261</i>
Distal wing	0.600	0.000	<i>-0.707</i>	<i>-0.657</i>
<b>Proportion of variance</b>	<b>95.3</b>	<b>3.1</b>	<b>95.7</b>	<b>3.7</b>

## Discussion

The results here indicate little ecomorphological overlap between pterosaurs and Mesozoic birds. Previous studies using measurements of homologous fore- and hindlimb bones found a similar pattern [4,5]. However, there are crucial differences. Firstly, there is a great deal of overlap between the groups on individual principal components axes, and so separation is instead apparent only when multiple axes are inspected. Mesozoic birds have relatively longer metatarsals, longer brachia, and shorter mandibles compared to pterosaurs of similar size. This is true regardless of whether the fourth metacarpal of the pterosaurs is included in the antebrachium (analogous position) or distal wing (homologous position). Studies comparing the lengths of homologous skeletal structures found complete separation of Mesozoic birds and pterosaurs due

to the relatively longer hands, shorter proximal forelimb bones and shorter metatarsals of pterosaurs compared to birds [4,5]. However, the distal wing had a relatively low loading on the second and third principal component axes in this study, showing that variation in this element was not a major source of separation. This is likely due to the inclusion of primary feather length in the functional distal wing unit, which reduces the difference in relative lengths of this component between birds and pterosaurs.



**Figure 4.** Frequency histograms of wing lengths calculated by summing all wing elements (A) and of lengths corrected for elbow flexion angle at maximum extension (B) for Mesozoic birds (blue, vertical hatching), “rhamphorynchoids” (green, diagonal hatching), and pterodactyls (red, horizontal hatching).

When comparing only the wing unit patterns separation between the two groups is also apparent. Mesozoic birds tend to have longer brachial regions and a shorter antebrachial region than pterosaurs of similar wing length due to the inclusion of the pterosaurian metacarpal IV in

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the functional antebrachium. With the fourth metacarpal of pterosaurs included in its homologous position within the manus overlap between the groups increases. In this case, birds tend to have relatively longer brachial lengths and shorter distal wings, which accords with previous studies dividing the wings into homologous units [4,5]. Thus, the reduced separation between Mesozoic birds and pterosaurs in the second analysis is due to the inclusion of the primary feathers in the avian distal wing.

Interpretation of the results of morphospace analyses on extinct groups should err on the side of caution. Often, the goal of comparing the morphospaces of two groups is to inform hypotheses of ecological similarity and indicate the potential for competition between taxa [36, 37]. However, a number of studies in extant animals show that there is often not a one-to-one correlation between morphology and function [36–40]. Instead, different morphologies may correspond with similar functional traits [38–39]. This problem is likely to be amplified when comparing groups with long, separate evolutionary histories, and potentially differing internal constraints on morphological change resulting in differing solutions to similar ecological pressures [2,39]. There is good reason to believe that the variables used in this study are correlated with function. As previously mentioned, the forelimb measurements are for functional units based on models of pterosaur and avian wing morphology [18]. In addition, numerous studies have found the hind-limb length and relative lengths of the leg bones to be associated with potential running ability, stride length and frequency in both bipeds and quadrupeds [41–44].

The results presented here and prior studies have indicated that the relatively longer metatarsals of birds separates them from pterosaurs in morphospace [4,5]. Birds are digitigrade

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bipeds and pterosaurs were quadrupeds with a plantigrade foot [45–47]. As a result, a lengthening of the metatarsals in birds increases stride length and the distance of the torso from the substrate (a benefit to wading birds) [41,43,48,49]. As the ventral surface of the metatarsals would have contacted the ground in pterosaurs, a lengthening of these bones would not produce the same effect.

Birds also have relatively longer brachial regions compared to pterosaurs of similar size and wing length. This is due to the extension of the antebrachial region in pterosaurs which incorporates the extended fourth metacarpal [18]. The elongate metacarpal IV of pterosaurs has been suggested to aid in a quadrupedal vaulting launch for take-off [8]. Such a mechanism for take-off is very different from the bipedal launch of birds. The differences in wing morphology between birds and pterosaurs also likely indicate differences in the kinematics of flapping flight given that differences in wing segment lengths will affect the pattern of wing folding during flapping [50].

Diet is another aspect of an animal's ecology which has yet to be quantitatively compared in birds and pterosaurs. Although stomach contents and crop remains have been identified in some pterosaurs and Mesozoic avians [51–55], such specimens remain rare. Jaw shape is a good indicator of diet in extant birds, and it seems reasonable to assume this is also the case with respect to Mesozoic avians [56–58]. As a result, jaw shape may be used not only to compare pterosaurs and early avians but also to examine whether modern birds filled in the vacant niches left after the extinction of pterosaurs and basal birds. In addition, both non-neornithine birds and pterosaurs exhibit dentulous and edentulous jaws [59–61]. Variation in tooth morphology has been used as an indicator of dietary variation within both groups, and is another source of data

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that could be used test for dietary overlap between Mesozoic avians and pterosaurs [59, 61]. It is suggested here that future studies testing for competition between these two groups should incorporate information on jaw shape and dental morphology.

The concepts of competition and ecological niches are intertwined in modern ecology [62,63]. Niches are multidimensional spaces defined by the biotic and abiotic factors required for the survival of an organism [63]. These factors may be placed in three broad categories; diet, space, and time [64]. Similarity in body size alone is unlikely to result in increased competition unless it corresponds with similarity in another aspect of ecology, e.g. diet. Like-wise, dissimilarity in body size is not a necessary prerequisite of niche separation as species of different sizes may still compete for the same resources [65]. Ecological separation may be achieved through the absence of overlap in at least one of these categories, for example species may overlap in the space inhabited and exhibit similar diets yet avoid competition by being active at different times of the day or inhabiting that space at different times of the year [64]. Due to time averaging of stratigraphic data the category of time must be broadened in scale when considering the co-occurrence of species in time to whether two taxa inhabit strata of similar geological age. Even when species show considerable overlap in niche space competition is not an inevitable conclusion [62–64]. For competition to occur at least one of the shared resources needs to be a limiting factor on population size [62,63]. As direct observation of species interactions at the population level in the fossil record is not an option, tests of niche overlap in palaeontological studies are limited to testing for the potential for competition. Such studies should focus on quantifiable species attributes that are linked to habitat use and resource utilization. This includes traits associated with feeding and substrate interaction (e.g. was the animal capable of underwater diving, wading, perching, climbing, etc.). With increasing numbers of functional traits exhibiting

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overlap and greater overlap on each dimension of hypothesized niche space, the likelihood of the taxa in question having competed over resources in the past also increases. This is also dependent on the taxa existing at the same point in the stratigraphic column (overlap in time) and in the same local beds (overlap in space). The latter comes with a note of caution that more mobile taxa, particularly those capable of flight, may traverse several different depositional environments. Fossils of birds and pterosaurs have been found to co-occur in a number of Mesozoic stratigraphic units [66, 67] and thus fulfil the criteria of spatial and temporal overlap.

Although the sample size here is relatively small, the results of multivariate analysis of the full number of measurements do suggest that pterosaurs and Mesozoic birds were ecologically separated. This separation was due to a combination of differences in body size, locomotory mode, and feeding adaptations. This conclusion comes with the caveat that the vast majority of birds used in this analysis were from Lower Cretaceous deposits due to the shortage of complete specimens from the Upper Cretaceous. Thus, the two groups may have shifted in morphospace in respect to each other in the latest Mesozoic. More specimens of Upper Cretaceous birds are required before this can be tested.

There is substantial overlap in wing lengths between birds and pterosaurs, however as previously stated similarity in size alone is not an indicator of competitive interactions between species. The largest birds in the dataset belong to the Sapeornithidae from the Jiufotang Formation of north-eastern China which exhibit wing lengths similar to contemporaneous pterosaurs such as *Sinopterus* and *Shenzhouopterus*. Sapeornithids are hypothesized to have been herbivorous due to the presence of gastroliths and the reduction of teeth, thus the relatively large body size of this family may be due to the evolution of herbivory in this clade [68,69].

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The trend of increased pterosaur body size through the Cretaceous has been suggested to be evidence of a clade wide shift in order to avoid competition with birds [2, 3]. However, the beginning of this trend in the Late Jurassic also coincides with the radiation of pterodactyloid pterosaurs [70]. The lineage leading to pterodactyloid-pterosaurs exhibits changes to the tail and limb morphology that appear to have improved the performance of terrestrial locomotion [71], take-off ability from the ground [8], and facilitated increased body size [70]. Thus, the increased average body sizes of pterosaurs in the Cretaceous may well have been due to adaptive exploration of new niches enabled by removal of intrinsic, morphological constraints to body size [70]. As such, it is plausible that the greater average size of Early Cretaceous pterosaurs compared to Jurassic species was due to an increase in the upper bounds of body masses exhibited.

However, this does not explain the absence of small sized pterosaurs for the remainder of the Cretaceous. Recent studies have demonstrated the substantial effect of Lagerstätte on estimating pterosaur diversity and disparity, with the occurrence of these deposits being an important driver of observed patterns of pterosaur diversity and disparity [70,72,73]. Konservat-Lagerstätten increase the likelihood of preservation of small bodied species from any time period in which they occur, indeed the substantial drop in avian taxonomic diversity at the beginning of the Upper Cretaceous is probably due to a reduction in the numbers of well preserved specimens due to the absence of Lagerstätte [72]. As such the absence of these deposits in the Upper Cretaceous may have at least exaggerated the observed pattern of increased minimum pterosaur body size through the Cretaceous. Of the nine exceptional pterosaur bearing deposits identified by Butler et al. [73] only one, the Niobrara Chalk, occurs within the Upper Cretaceous. The pterosaurs from this stratum are deposited approximately 200km from the nearest palaeo-



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coastline [22], as such the absence of small bodied species from these strata could well be a result of the distance away from land that these sediments were deposited. In addition, the only birds found here are the aquatic Hesperornithiformes and *Ichthyornis* [74]. Butler et al. [70,72,73] recommend caution when interpreting patterns of diversity and disparity in pterosaurs due to preservational biases caused by the heterogeneous occurrence of Lagerstätte. It is suggested here that the same caution also be applied to observed trends in body size evolution.

## Conclusion

The separation between pterosaurs and Mesozoic birds in morphospace suggests that these groups were filling different ecological niches in the Early Cretaceous. As well as differences related to mechanisms of terrestrial locomotion and flight there were also differences in feeding related traits. Separation was not merely a function of size overlap in wing lengths. Previous studies utilising similar multivariate analyses only examined appendicular traits as potential indicators of locomotory differences. However, there are many factors that contribute to the filling of an ecological niche including body size, habitat, and diet. Including measurements of the jaws adds another facet to ecomorphological comparisons of pterosaurs and birds.

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## SYNTHESIS

The evolution of diversity and disparity in birds has been greatly influenced by morphological constraints, whether imposed or relaxed. For example, the evolution of powered flight is likely to have relaxed constraints on hindlimb morphology, allowing legs to be adapted for a variety of different uses (Gatesy & Middleton, 1997; Abourachid & Höfling, 2012). In combination with the increased dispersal ability conferred by flight, this has enabled birds to colonise a wide array of environments (Abourachid & Höfling, 2012). On the other hand, the more anterior centre of mass created by a combination of tail reduction and expansion of the forelimbs and associated musculature has required the femur of birds to re-orientate in order to place the knee under the centre of mass (Gatesy, 1990; Carrano, 1998; Hutchinson, 2001; Allen *et al.*, 2013). As a result, parasagittal rotation of the femur is restricted and contributes little to stride length. Instead, rotation at the knee is the primary driver of forward motion during walking (Gatesy, 1990; Carrano, 1998; Abourachid & Renous, 2000; Rubenson *et al.*, 2007). Thus, the restrictions placed on femoral movements appear to have led to a major change in locomotor mechanics (Gatesy, 1990; Carrano, 1998; Grossi *et al.* 2014).

This thesis examined several topics on the theme of morphological constraints in birds. They included constraints on body mass, allometric scaling, and eco-morphology. The results presented provide new insights on about these themes. Firstly, the hypothesis that a widening discrepancy between eggshell strength and incubator mass with increased size limits body mass is not supported by study of one of the largest species to have existed: the difference between the mass of the smallest specimens of *Genyornis newtoni* and the mass able to be supported by the egg is far greater than reported for any large flightless bird (Chan, 2014). In addition, there is no

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evidence for *G. newtoni* exhibiting extreme reverse sexual dimorphism (RSD), a trait hypothesized to be a response to the risk of damage to the eggs during incubation in *Dinornis*, with the smaller males carrying out this task (Bunce *et al.*, 2003; Deeming & Birchard, 2009; Chan, 2014). There are two main alternative explanations for extreme RSD in *Dinornis*. Species in this genus are some of the largest within a clade which commonly exhibits RSD (Olson & Turvey, 2013). As a result extreme RSD may simply be due to allometric scaling, with the discrepancy between males and females increasing as a result of increased species size (Olson & Turvey, 2013). Another explanation is that the difference in mass between males and females represents inequality in reproductive investment and high levels of intrasexual competition between females. It has previously been hypothesised that in size-dimorphic birds the smaller sex invests more in reproduction whereas the larger competes for territories and/or mates (Olsen & Cockburn, 1993). Evidence for small clutch sizes relative to female body mass (Hartree, 1999; Werner & Greibeler, 2012), male incubation (Huynen *et al.*, 2010), and exclusion of males at certain sites (Worthy *et al.*, 2005; Allentoft *et al.*, 2010) suggest that this may have been the case in *Dinornis*. Thus, the extreme RSD seen in *Dinornis* is likely a product of these factors rather than a response to a mechanical constraint that is universal to Aves.

The second topic examined was the allometric patterns of leg bone scaling of flightless, terrestrial birds. Strong evidence was found for a curvilinear relationship between femoral length and circumference, indicating that larger species have shorter femora in relation to the girth of this bone. The pattern identified likely reflects the previously mentioned requirement to maintain the knee under the centre of mass. Contrary to previous study, no curvilinear relationship was found between femoral length and circumference in non-avian theropods (see Christiansen, 1999). This result contradicts the hypothesis that larger theropods maintained lower stresses in

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the bones by increasing their robusticity at a faster rate than smaller forms (Christiansen, 1999). Instead, it appears that may have been achieved through a linear, negatively allometric relationship between femoral length and girth, decreased locomotor performance, and an increase in resistance to mediolateral bending (Carrano, 2001).

Thirdly, the evolution of the leg bones subsequent to loss of flight was tested for directionality and correspondence with potential controlling factors. Numerous qualitative and quantitative studies have examined changes occurring in the forelimbs of birds with loss of flight (Worthy, 1998; McCall *et al.*, 1998; Livezey, 2003; Nudds & Davidson, 2010). However, changes in the leg bones have been less well studied. The results presented in the third chapter of this thesis show that there is no overall directionality to the evolution of the leg bones in flightless birds. This contrasts with the evolution of the forelimbs, which uniformly reduce in size as flight is lost (McCall *et al.*, 1998; Livezey, 2003; Nudds & Davidson, 2010). Instead, the hindlimbs of birds likely vary according to their particular environments and/or modes of life (Abourachid & Renous, 2000). This hypothesis is supported by the fact that neither the directionality nor the magnitude of change is affected by the degree of change in body size or the length of time since flight was lost. The one pattern that was found indicates that ratites living outside of New Zealand tend to have proportions indicative of greater cursoriality. This difference may be due to an increased requirement and opportunity to traverse longer distances or to increased predation pressure.

Lastly, ecomorphological comparisons between Mesozoic birds and pterosaurs were used to examine the possibility of ongoing competition between these groups. Separation between Mesozoic and pterosaurs indicates differences in locomotory and feeding adaptations. This

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finding does not support the hypothesis of competition, instead indicating ecological separation between pterosaurs and birds in the Late Jurassic and Early Cretaceous. The use of measurements of functionally analogous structures instead of homologous structures is recommended for future studies testing for the expansion of neornithines into niches opened by the extinction of pterosaurs at the Cretaceous-Palaeogene boundary. In addition, detailed examination of the morphology of the jaws and teeth of the Mesozoic groups will further clarify how niche space was divided between birds and pterosaurs during the Mesozoic.

Constraints on the morphology of birds have had a strong effect on the patterns of diversity and disparity that we see in their evolutionary history. The evolution of flight is widely seen as a removal of a constraint allowing birds to diversify into the most diverse group of tetrapods today. In particular the leg morphology of birds has been shown to be more disparate than that of non-avian theropods (Gatesy & Middleton, 1997). This release of the legs from being the primary form of locomotion has led to the adaptation of the hindlimbs for a variety of other functions (Abourachid & Höfling, 2012). However, the changes in the morphology due to the evolution of flight have also imposed new constraints on avian morphology. Not only are the kinematics of the femur restricted but the length of the bone has also become constrained in order to maintain the knee under the centre of mass (Gatesy, 1990; Carrano, 1998; Grossi *et al.* 2014). Flight also imposes limits on the maximum body mass that can be attained (Alexander, 1998). Loss of flight removes this impediment but the mark of this evolutionary history is still imprinted on flightless species as femoral length and orientation remains constrained in these species. The greater torsional stresses caused by the retention of this posture may well have limited the body mass of flightless birds preventing them from attaining the same sizes as their large non-avian

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relatives such as *Tyrannosaurus rex* (Gatesy, 1991). This provides a more plausible constraint on avian body mass than eggshell strength (Chan, 2014).

The evolution of the leg bones post-loss of flight indicates that this set of traits is quite plastic. Changes in the relative proportions of the distal limb bones do not appear to be strongly constrained to follow any particular trajectory of change due to loss of flight. This may be due to the fact that terrestrial flightless species often belong to clades in which the volant members generally spend a high proportion of their time on the ground. Limb proportions have the potential to evolve rapidly, with the distal bones often being the first to change (Worthy, 1988; Nudds & Davidson, 2010). This in turn may provide a malleable set of traits that can adapt to new selection pressures given enough time. This may be one reason why the large, flightless ratites that inhabit open spaces have evolved relatively long distal limb bones, thereby increasing their ability to cover longer distances, as this provides an evolutionary path of least resistance. It certainly requires fewer changes to morphology compared to the number of changes needed to regain flight.

By combining studies of previously neglected groups and functional characters with alternative methodological approaches this thesis provides new insights into morphological constraints on avian evolution. It is hoped that they will inform future studies of the morphological and ecological evolution of birds.

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# APPENDICES

## Appendix 1: Supplementary materials

### Chapter 1: Does size variation in *Genyornis newtoni* encompass eggshell safety limits?

Supplementary Data available free online:

<http://www.tandfonline.com/doi/suppl/10.1080/02724634.2013.826668>

All other supplementary materials are available with the digital version of this thesis stored at <http://www.researchonline.mq.edu.au/>.

### Chapter 2: Phylogenetic variation in hindlimb bone scaling of flightless theropods

**Appendix S1** Raw measurements of avian femora and tibiotarsi.

**Appendix S2** References for additional non-avian theropods.

**Appendix S3** MRP codings and phylogenetic sources.

### Chapter 3: Complex evolution of leg bones in the transition to flightlessness in birds

**Supporting Information 1** Raw measurements of flightless and volant species.

**Supporting Information 2** Phylogenetic references, land areas, and estimates of time since loss of flight.

### Chapter 4: A comparison of pterosaur and Mesozoic avian morphospaces constructed using functionally analogous traits

**Supplementary Material 1** Measurements and references for pterosaurs

**Supplementary Material 2** Measurements and references for Mesozoic birds

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**Appendix 2:** Primary feather lengths may not be important for inferring the flight styles of Mesozoic birds. Chan, N.R., Dyke, G.J., and Benton, M.J. 2013. *Lethaia*, Vol. 46, pp. 146–152.

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