

RESEARCH ARTICLE

Evolution of limb bone loading and body size in varanid lizards

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SUMMARY

Geometric scaling predicts that stresses on limb bones and muscles should increase with body size. Mammals counter this size-related increase in stress partially through changes in bone geometry, but largely through changes in posture, with larger species having a more erect stance. However, the ability to counter size-related stresses in this fashion may be limited to those taxa that have a parasagittal gait (such as mammals), where legs are swung underneath the body. We examined locomotor kinematics for 11 species of varanid lizards (from 0.04 to 8 kg body mass) that have a sprawling gait, to determine how they moderate size-related stresses. Posture, as indicated by femur adduction and hip heights, did not change significantly with body size, beyond that expected from geometrical scaling. Instead, lizards mitigated size-related increases in stress by increasing duty factor and possibly reducing femur rotation. Incorporating these factors in biomechanical models predicted that both bending ($\propto M^{0.016}$, where M is mass) and torsional ($\propto M^{-0.049}$) stresses should be nearly independent of body size over the size range examined. However, increasing duty factor and reducing femur rotation probably have deleterious effects on speed, and this difference in kinematics with size may explain why speed scales lower for sprawling lizards than for parasagittal mammals ($\propto M^{0.17}$ and $\propto M^{0.24}$, respectively). Further, paralleling conclusions for the synapsid lineage, these findings suggest that evolution from sprawling to upright posture did not occur in archosaurs as a response to larger size; rather, these archosaurs likely became upright first and larger later.

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Key words: Varanidae, gait, posture, locomotion, scaling.

INTRODUCTION

The extent to which a group of animals can maintain a characteristic shape and patterning of limb movements over a range of body size, and the consequences of doing so, has been of interest for hundreds of years, at least since Galileo (Galilei, 1638). As size increases in geometrically similar animals, length (l) and diameter (d) scale to the animal's mass to the power of a third ($M^{0.33}$). Given that forces due to body mass should scale as $M^{1.0}$ and cross-sectional area should increase as $M^{0.66}$, then stress on bones and muscles is predicted to increase as $M^{0.33}$. Consequently, larger animals may have an increased risk of limb bone failure (Biewener, 1982; Biewener, 1983b). However, peak skeletal stress recorded for various terrestrial mammals, ranging in size from 0.1 to 300 kg, was fairly uniform, with a safety factor (fracture stress/peak locomotor stress) of 2 to 4 (Biewener, 1989).

Changing the biomaterial properties of bones and muscles may allow larger animals to withstand higher forces than geometrical scaling implies. However, for terrestrial mammals, the force-generating ability of striated muscle per unit fiber area (Close, 1972; Biewener et al., 1988) and the maximal strength of mammalian bone per cross-sectional area (Biewener, 1982; Currey, 2002) are fairly independent of body size. Changing the geometric shape of bones may also increase their resistance to stresses (e.g. bones might be thicker for larger animals). There is some support for this explanation: bone dimensions scale as $l \propto d^{0.89}$ (Alexander et al.,

1979; Maloij et al., 1979; Biewener, 2005), not $d^{1.0}$, for a broad size range of mammals and birds, indicating relatively greater increase in bone diameter than bone length with size. However, including this effect in bone stress models still predicts that peak bone stresses should scale with $M^{0.28}$ (Biewener, 1982).

It appears that terrestrial mammals maintain constant stresses by changing the way bones and muscles are loaded, rather than by geometric shape scaling. Larger mammals reduce stress by adopting an upright posture (Biewener, 1983a; Biewener, 1983b; Biewener, 1989; Biewener, 1990). This decreases the moment arm of the ground reaction force relative to the muscular moment arm, a ratio termed the effective mechanical advantage (EMA). This in turn reduces the muscular effort required to prevent the limbs from collapsing, and reduces the bending forces imposed on long limbs by increasing the proportion of the load borne by simple compression (Biewener, 2005). Over a size range from mice to horses, average EMA of the forelimb and hindlimb scaled as $M^{0.25}$. This scaling of EMA indicates a reduction of muscular and bone stresses that cancel the expected increase in stresses discussed above, explaining the observation that stress is independent of body size.

Most studies of biomechanical consequences of scaling have focused on terrestrial mammals, which have parasagittal kinematics where the limbs are adducted underneath the body. It is unclear whether similar patterns will apply to other taxa that use

fundamentally different forms of loading and kinematics. Lizards (Brinkman, 1981; Reilly and DeLancey, 1997) and crocodiles (Gatesy, 1991; Reilly and Elias, 1998) have a more sprawling limb posture in which the femur is highly abducted laterally and femoral motion is largely in the horizontal plane, incorporating considerable axial rotation. This difference in posture can affect the loading of appendicular bones (Blob, 2001) and presumably could alter stress loading.

Studies of *in vivo* limb bone stresses and strains indicate that these postural differences are correlated with differences in limb bone loading. In mammals that use parasagittal kinematics, limb bone torsion is minimal and bending loads predominate (Rubin and Lanyon, 1982; Biewener, 1989; Biewener, 1990). In contrast, during non-parasagittal locomotion by iguanas and alligators, limb bone torsion predominates to such an extent that safety factors in shear are approximately half those of safety factors in bending for the femur and tibia (Blob and Biewener, 1999; Blob and Biewener, 2001).

The dominance of torsion in lizard and crocodilian limb bones predicts specific differences between the scaling relationships of their limb bones compared with scaling patterns for taxa with limb bones loaded primarily in bending. As body size increases, torsional shear stress in limb bones is expected to increase more than bending stress (Blob, 2000); therefore, limb bone diameters of lizards and crocodilians might be expected to scale with relatively greater positive allometry than the limb bones of mammals. This expectation was examined by Blob (Blob, 2000), who found a significantly lower scaling for the limb bones of larger-bodied varanid lizards (femur $l \propto d^{0.84}$) compared with felids and canids (femur $l \propto d^{0.90}$). However, as for terrestrial mammals, the size-related changes in limb bone geometry do not completely compensate for size-related increases in limb bone stress. Although there is a significant difference in the scaling of lizard limb bones from geometric similarity, torsional bone stress is predicted to scale with $M^{0.166}$ for the varanid femur. Could larger lizards adjust limb posture to reduce bone stresses and thereby reduce the risk of limb bone failure, as do larger terrestrial mammals?

It is unclear whether changes for lizards in limb posture with body size will reflect those observed for terrestrial mammals, i.e. a shift to more upright posture with increases in body size. There is some evidence that larger terrestrial reptiles have a more upright limb posture, for example the 'high-walk' observed for crocodilian species (Gatesy, 1991; Reilly and Elias, 1998). However, measurements of *in vivo* strain on the limb bones of alligators (Blob and Biewener, 1999) and calculations for iguanas (Blob and Biewener, 2001) indicate that limb bone stresses and strains actually increase with the use of more upright stance, so a more sprawling posture might moderate size-related increases in limb loading for larger lizards. So if posture change is an unlikely mechanism for reptiles, what might they do instead? Or, will larger reptiles simply face a greater risk of limb bone failure?

Varanid lizards are an ideal group for such a study as they exhibit large variation in body size (by almost three orders of magnitude) within a single genus (Pianka, 1995; Thompson and Withers, 1997). In the present study, we measured three-dimensional hindlimb kinematics from 11 species of varanid to evaluate three potential mechanisms that large varanid lizards might use to mitigate size-related increases in limb bone loading: posture, duty factor and limb rotation. Body posture has been shown to reduce limb loading in mammals, duty factor may reduce peak stress based on previous studies (Alexander, 1977) and limb rotation may have significant effects on femur torsion.

MATERIALS AND METHODS

Study animals

We measured morphological and kinematic characteristics for 11 species of varanid lizard: *Varanus acanthurus* Boulenger 1885 ($N=2$, mean \pm s.d., 75.2 ± 4.6 g), *V. eremius* Lucas & Frost 1895 ($N=3$, 51.9 ± 9.8 g), *V. giganteus* (Gray 1845) ($N=3$, 2966 ± 1944 g), *V. glauerti* Mertens 1957 ($N=2$, 39.57 ± 1.60 g), *V. gouldii* (Gray 1838) ($N=7$, 381.4 ± 146.5 g), *V. mitchelli* Mertens 1958 ($N=3$, 124.8 ± 146.3 g), *V. panoptes* (Storr 1980) (adult $N=6$, 3172 ± 1771 g; juvenile $N=1$, 101.1 g), *V. rosenbergi* Mertens 1957 ($N=1$, 590 g), *V. scalaris* Mertens 1941 ($N=2$, 98.5 ± 18.4 g), *V. tristis* Schlegel 1839 ($N=1$, 154 g) and *V. varius* (White 1790) ($N=1$, 7900 g). Because of intraspecific differences in size, juvenile *V. panoptes* were included as a separate point from adult *V. panoptes* in regressions, but this did not change the results of statistical analysis.

Morphology

Length of the hind limbs and body mass were measured for each individual within 2 weeks of capture. Limb length was measured using digital calipers or measuring tape with the three segments of the limb measured separately. Total hind limb length (HLL) was the sum of these measurements. Mass was measured using either a 5 kg spring balance for large lizards ($>2000 \pm 25$ g), kitchen scales for medium-sized lizards ($1000\text{--}2000 \pm 0.5$ g) or laboratory scales for small lizards ($<1000 \pm 0.05$ g).

Kinematics

To measure the three-dimensional (3-D) kinematics of a lizard's stride, individuals were filmed while running using either the Peak Motus analysis system (Peak Performance Technologies Inc., Oxford, UK) operating at 200 Hz or the Vicon 612 motion analysis system (Vicon, Oxford, UK) operating at 250 Hz. Markers were placed on the pelvis, hip, knee and ankle joints, the metatarsals at the base of the toe, and the tip of the toe excluding the claw. The 3-D coordinates of the markers were imported into BodyBuilder software (Vicon). Further analysis of kinematic variables and joint angles were performed using MATLAB (MathWorks, Natick, MA, USA). Each stride began at footfall of the right hindlimb, and ended at its next footfall. Mean forward velocity (m s^{-1}) was the distance moved along the x -axis by the lumbar vertebrae (m) during a stride, divided by stride duration (s). The duty factor was the percentage of the stride cycle that the right hindfoot was on the ground.

Three joint angles described the movement of the hindlimb segments relative to each other. The knee joint described the posterior facing angle between the femur and tibia, such that a value of 90 deg indicated that the tibia was perpendicular to the femur, and smaller values between 0 and 180 deg indicated greater flexion of the joint. The ankle joint was between the dorsal aspect of the foot and the tibia, so that a value of 90 deg indicated that the foot was perpendicular to the tibia, and a value of 180 deg indicated that the foot was aligned with the tibia. The metatarsal joint was between a segment describing the toe (from the base of the toe to the toetip) and a segment describing the foot (from the base of the toe to the ankle) on the dorsal side of the foot, so that an angle of 180 deg indicated that the toe and foot were aligned whereas smaller values indicated greater dorsi-flexion. We were unable to measure the metatarsal angle for three species; two (*V. acanthurus* and *V. glauerti*) had feet too small to attach a marker to both the base of the toe and the toe tip, and the third (*V. varius*) had a stride that involved dragging the dorsal surface of the toe along the floor, which removed the marker from the toetip. In both cases, only the base of the toe was marked.

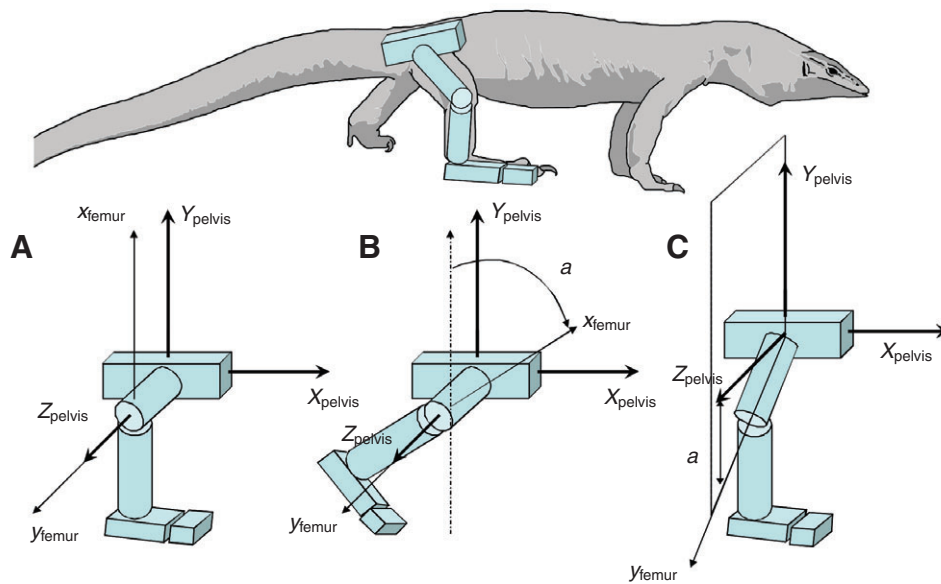


Fig. 1. Kinematic analysis of movement of the femur for the hindlimb of varanids. (A) Femur showing 0 deg of femur rotation and 0 deg of femur adduction. (B) Femur showing a deg of femur rotation and 0 deg of femur adduction. (C) Femur showing 0 deg of femur rotation and a deg of femur adduction.

Three angles described the movement of the femur relative to the hip (Fig. 1). Femur retraction was the angle between the femur and a line perpendicular to the long axis of the pelvis, passing through the hip, such that a value of 0 deg indicated that the femur was perpendicular to the long axis of the pelvis, and positive and negative values indicated greater amounts of retraction and protraction, respectively. Femur adduction described the angle between the femur and a horizontal plane passing through the hip, with positive and negative values indicating that the knee was below or above the hip respectively (Fig. 1B). Femur rotation is the angle between the plane containing the femur and the tibia (assuming dorso-flexion of the knee) with a vertical reference plane passing through the hip and knee, such that greater positive values indicate greater clockwise rotation of the knee joint (Fig. 1C).

For each kinematic variable, we used biomechanically relevant points during the stance phase for correlation with body size. Based on previous studies (Alexander, 1977; Christian, 1995; Blob and Biewener, 2001), ground reaction forces are typically largest during the midstance period, and thus this was chosen as one point of comparison for all kinematic variables. Alternatively, stresses may be maximal during excursion maxima or minima of joints. As these can often occur out of phase with midstance, we chose these maxima or minima as another point of comparison against body size.

Because of the limitations of the length of the running track during videoing, we were unable to record kinematics of all species running at maximum speed. To remove the effects of speed, we only analyzed strides that were between 30 and 45% of maximal speed based on data from Clemente et al. (Clemente et al., 2009). Within this range, there was no significant correlation between the percentage of maximal speed and body mass ($R^2=0.028$, $P=0.604$, $N=12$; supplementary material Fig. S1). To confirm that comparable patterns of variation in kinematics were present at higher speeds, we performed similar analyses on smaller data sets between 40–60% and 50–70% of maximal speed, both of which showed analogous results. These results are presented in supplementary material Fig. S2.

Statistical analyses

All data were \log_{10} transformed before analysis. Where angular values pass through 0 deg, 90 deg was added to all species estimates before log transformation. Scaling relationships for dimensions against mass

were estimated using model II reduced major axis (RMA) regression. RMA slope estimates, confidence intervals and comparison between RMA slopes (using the likelihood ratio test $-2\log\Lambda$, where Λ is the likelihood ratio) were implemented using the program SMATR (Falster et al., 2006).

Phylogenetic comparisons

We corrected our data for possible phylogenetic relatedness using independent contrasts (Felsenstein, 1985). The cladogram for

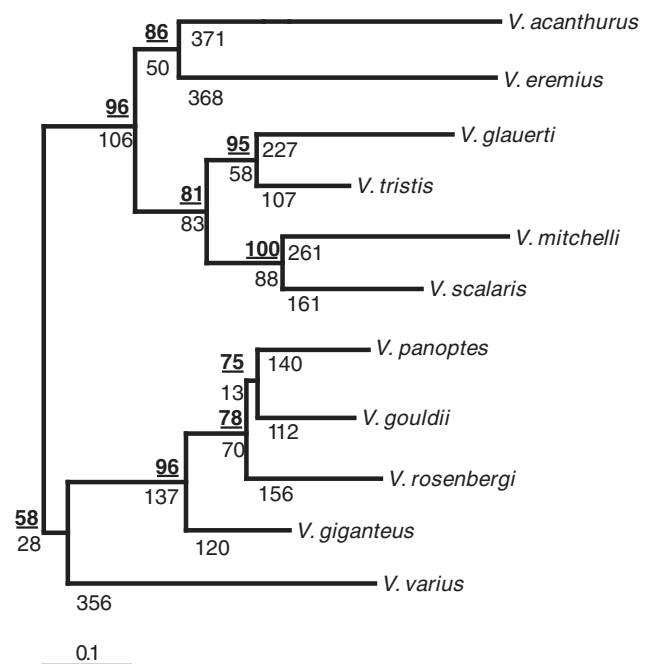


Fig. 2. Phylogenetic relationships for 11 species of *Varanus* used in this study, based on 1038 bp from the NADH-2 gene, showing the maximum likelihood hypothesis from Thompson et al. (Thompson et al., 2009). Bootstrap values >50% (percentages of 100 pseudoreplicates) are underlined and shown above branches; branch lengths are shown below the branches (substitutions per site × 1000).

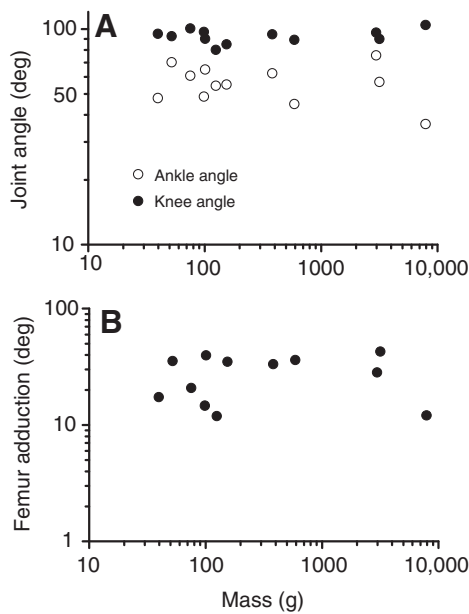


Fig. 3. Posture changes with body size for 11 species of varanid lizard. (A) Joint angles for the knee (closed circles, $N=12$) and ankle (open circles, $N=12$) versus body mass. (B) Femur adduction (deg below horizontal) versus body mass ($N=12$). Data were \log_{10} transformed.

varanids and branch lengths that we used for independent contrast analysis was based on 1038 bp of the NADH-2 gene (Thompson et al., 2009) (Fig. 2). Standardized contrasts were positivized (Garland et al., 1992) for RMA regression and forced through the origin. The index K^* was computed to quantify the strength of the phylogenetic signal, based on Blomberg et al. (Blomberg et al., 2003). Both K^* values and independent contrasts were calculated using a custom-written Visual Basic program IC-PCW (ver. 1.08; P. Withers, University of Western Australia).

RESULTS

Scaling of body posture

There were no differences between the RMA slopes for scaling of hip height at midstance and HLL with mass ($-2\log\Lambda=0.001$, $P=0.978$), indicating that the stance height of larger lizards was not relatively higher than that of smaller lizards, but instead increased in a geometric fashion with HLL. The scaling of joint angles for the ankle, knee and femur adduction was not significantly different from 0 (Fig. 3; Table 1), indicating that the limbs of larger lizards were not more or less extended than those of smaller lizards. Further, the movement of the pelvis did not change significantly with body size (Table 1). There may even be some evidence for a more sprawling posture with increased size. The minimum metatarsal angle showed positive scaling (Table 1), and when this was examined further the metatarsal angle at push off was found to be positively and significantly related to body size ($R^2=0.68$, $P=0.006$), with larger lizards tending to push off using a flatter foot, and smaller lizards showing more digitigrade (upright) foot posture toward the end of the stance phase.

Scaling of duty factor and femur rotation

Duty factor was positively related to body mass (Fig. 4A; Table 1), indicating that larger lizards had a relatively longer stance phase. Femur rotation at midstance was negatively related to mass (Fig. 4B; Table 1). As higher values indicate greater clockwise rotation of the femur, this relationship with body mass indicates that larger lizards rotated the femur less at midstance than smaller-bodied lizards.

However, to understand the functional evolution of these traits it is important to examine their variation in a phylogenetic context. The co-evolution of body size and kinematics is illustrated in Fig. 5. Both body mass and duty factor were strongly associated with phylogeny for varanids (body mass, $K^*=1.047$, $P=0.015$; duty factor, $K^*=0.661$, $P=0.004$). When both body size and duty factor values were re-analyzed in a phylogenetic context using regression of independent contrasts, the slope between duty factor and body mass increased and the relationship remained significant ($R^2=0.62$,

Table 1. Reduced major axis (RMA) regression coefficients for phylogenetically and non-phylogenetically informed data sets in varanid lizards

Variable	Mean	R^2	P	RMA slope (95% CI)	RMA slope of independent contrasts (95% CI)
Hind limb length (mm)	139.8±88.9	0.98	<0.001	0.325 (0.295–0.358)	0.311 (0.271–0.356)***
Hip height mid. (mm)	53.9±33.8	0.89	<0.001	0.324 (0.258–0.407)	0.313 (0.239–0.410)*
Hip height av. (mm)	55.8±34.3	0.89	<0.001	0.322 (0.254–0.407)	0.308 (0.238–0.399)*
Duty factor (%)	41.9±5.8	0.75	<0.001	0.079 (0.056–0.112)	0.083 (0.053–0.130)*
Metatarsal angle mid. (deg)	135.6±9.9	0.09	0.423	0.049 (0.022–0.105)	0.063 (0.026–0.150) ^{n.s.}
Metatarsal angle min. (deg)	111.5±12.6	0.47	0.041	0.075 (0.041–0.138)	0.065 (0.028–0.152) ^{n.s.}
Ankle angle mid. (deg)	56.1±11.3	0.05	0.478	–0.117 (–0.221 to –0.061)	–0.138 (–0.276 to –0.069) ^{n.s.}
Ankle angle min. (deg)	48.8±9.9	0.02	0.632	–0.119 (–0.228 to –0.062)	–0.155 (–0.311 to –0.077) ^{n.s.}
Knee angle mid. (deg)	92.4±7.7	0.07	0.395	0.048 (0.025–0.091)	0.061 (0.030–0.121) ^{n.s.}
Knee angle min. (deg)	85.0±8.0	0.02	0.696	0.055 (0.029–0.105)	–0.069 (–0.139 to –0.034) ^{n.s.}
Femur adduction mid. (deg)	27.4±11.3	<0.01	0.898	0.268 (0.139–0.516)	0.267 (0.133–0.535) ^{n.s.}
Femur adduction min. (deg)	12.2±7.6	0.01	0.741	–0.043 (–0.083 to –0.022)	–0.039 (–0.079 to –0.020) ^{n.s.}
Femur retraction mid. (deg)	0.99±7.3	0.01	0.759	0.045 (0.023–0.086)	–0.053 (–0.107 to –0.027) ^{n.s.}
Femur rotation mid. (deg)	78.6±8.9	0.34	0.046	–0.063 (–0.109 to –0.036)	–0.064 (–0.120 to –0.034) ^{n.s.}
Femur rotation max. (deg)	96.6±9.7	0.05	0.478	–0.056 (–0.107 to –0.029)	–0.053 (–0.106 to –0.027) ^{n.s.}
Pelvic yaw mid. (deg)	8.0±2.2	0.02	0.683	0.173 (0.090–0.332)	0.200 (0.101–0.398) ^{n.s.}
Pelvic yaw max. (deg)	16.4±3.8	0.09	0.339	–0.140 (–0.263 to –0.075)	–0.100 (–0.193 to –0.052) ^{n.s.}
Pelvic roll mid. (deg)	5.9±4.7	0.03	0.615	0.027 (0.014–0.053)	0.036 (0.018–0.071) ^{n.s.}
Pelvic roll max. (deg)	11.7±5.1	0.01	0.833	–0.249 (–0.480 to –0.129)	–0.347 (–0.693 to –0.173) ^{n.s.}

Means ± s.d. are shown for each kinematic variable. mid., midstance value; min., minimum value throughout stride; max., maximum value throughout stride. R^2 and P values are shown for non-phylogenetically corrected data. For phylogenetically corrected data, *** $P<0.001$, * $P<0.05$ and ^{n.s.} $P>0.05$. Bold values indicate significance.

Hip height av., hip height averaged over the entire stride; Hip height mid., hip height from a specific point during the stride.

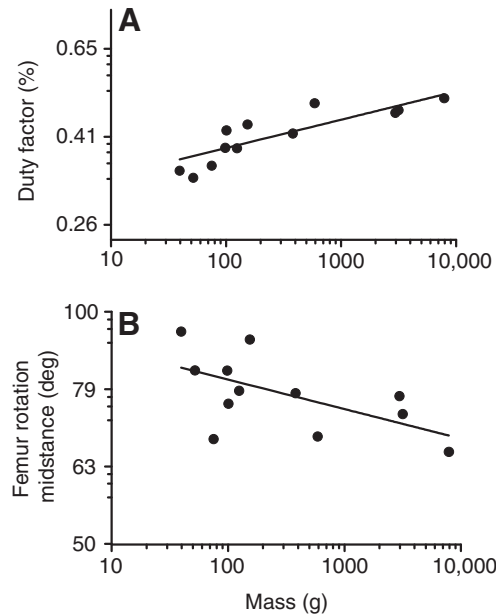


Fig. 4. Correlates of stride characteristics with body size for 11 species of varanid lizard. (A) Duty factor *versus* body mass, $N=12$. (B) Femur rotation at midstance *versus* body mass, $N=12$. Line represents least-squared regression slope. Data were \log_{10} transformed.

$P=0.031$; Table 1). This provides strong evidence that there is both an association of duty factor with body size, within the phylogenetic context, and strengthens the implication that these two traits are functionally or even causally linked. In contrast, changes in femur adduction (i.e. body posture) did not have a significant association with body mass (Table 1) or phylogeny (femur adduction, $K^*=0.643$, $P=0.103$; Fig. 5), indicating that limb posture for these varanids was independent of size or taxon.

Like body mass and duty factor, femur rotation at midstance was strongly related to phylogeny ($K^*=0.923$, $P=0.037$; Fig. 5). However, when these data were analyzed in a phylogenetic context, the relationship between body size and rotation of the femur at midstance scaled similarly, but became statistically insignificant ($R^2=0.24$, $P=0.405$). This might suggest a lack of causal relationship between the two traits (femur rotation and mass) or it could simply be due to a lack of statistical power owing to the reduction in sample size for contrasts. Only by using a wider phylogenetic range of species with a weaker phylogenetic pattern in mass will it become clear whether the femur rotation pattern is functionally related to body mass, or is just a phylogenetic correlate.

DISCUSSION

Unlike mammals (Biewener, 2005), varanid lizards do not adopt a more upright stance as they increase in size (Fig. 1). The likely explanation for the lack of upright posture in larger varanids is that upright posture in the group leads to a decrease in the EMA and, therefore, an increase in muscular stresses and bending bone stresses, as has been reported for iguanas and alligators (Blob and Biewener, 1999; Blob and Biewener, 2001; Reilly and Blob, 2003). This suggests that larger lizards may face a greater risk of limb bone failure than smaller species or that varanids use a different mechanism for coping with size-related increases in stress. By including the scaling of duty factor and femur rotation in current biomechanical models, we are able to predict that stresses on varanid limb bones may be independent of body size.

Changes in duty factor represent an increase in the proportion of the stride over which force can be distributed, lowering peak stress. Alexander (Alexander, 1977) presented a model relating the vertical and horizontal forces to stride kinematics. Forces are expected to be maximal at midstance and the peak force ($F_{\text{midstance}}$) is given by:

$$F_{\text{midstance}} = \frac{\pi m g}{4 f_d}, \quad (1)$$

where m is the body mass supported, g is the gravitational acceleration and f_d is the duty factor. As the body mass supported is $M^{1.0}$ and duty factor scales as $M^{0.083}$, then forces acting on the bones and muscles are expected to scale $\propto M^{1.0}/M^{0.083}=M^{0.917}$. This suggests that the force that must be supported is relatively lower for larger lizards. This finding is central to further considering how bone stresses should scale for animals with a sprawling posture.

Stress in limb bones is a function of both the ground reaction force (GRF) and muscular forces. If the stress induced by limb bone curvature is neglected (Biewener, 1983b), then maximum bending stress at the midshaft of the limb bone (σ_b) is given by:

$$\sigma_b = \frac{FLn}{I}, \quad (2)$$

where F is the force applied during locomotion, L is the moment arm of the force, which is proportional to the length of the femur, n is the distance from the neutral axis of bending to the cortex of

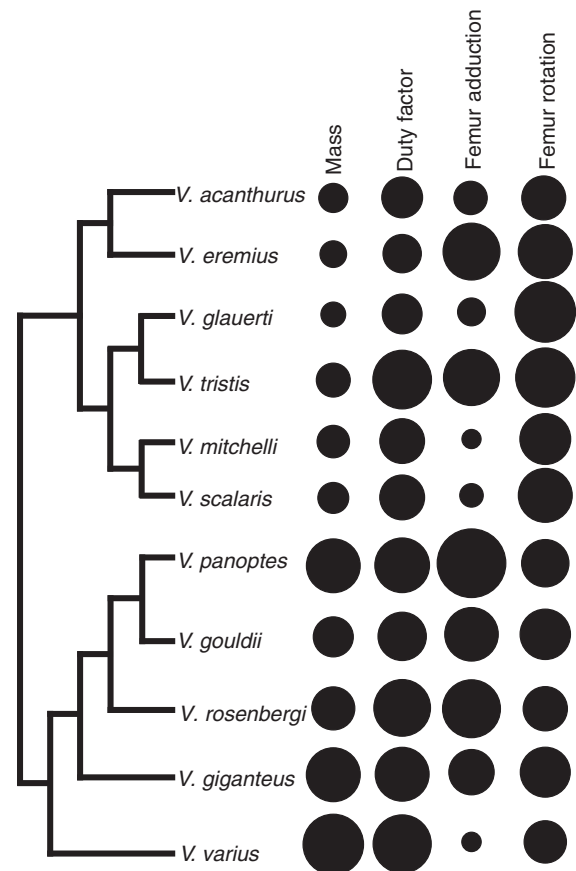


Fig. 5. The evolution of body mass, duty factor, femur adduction and femur rotation in varanid lizards. The size of the solid circle indicates the magnitude of each variable, scaled such that the largest value for each variable is the largest diameter, and other circles represent scaled fractions.

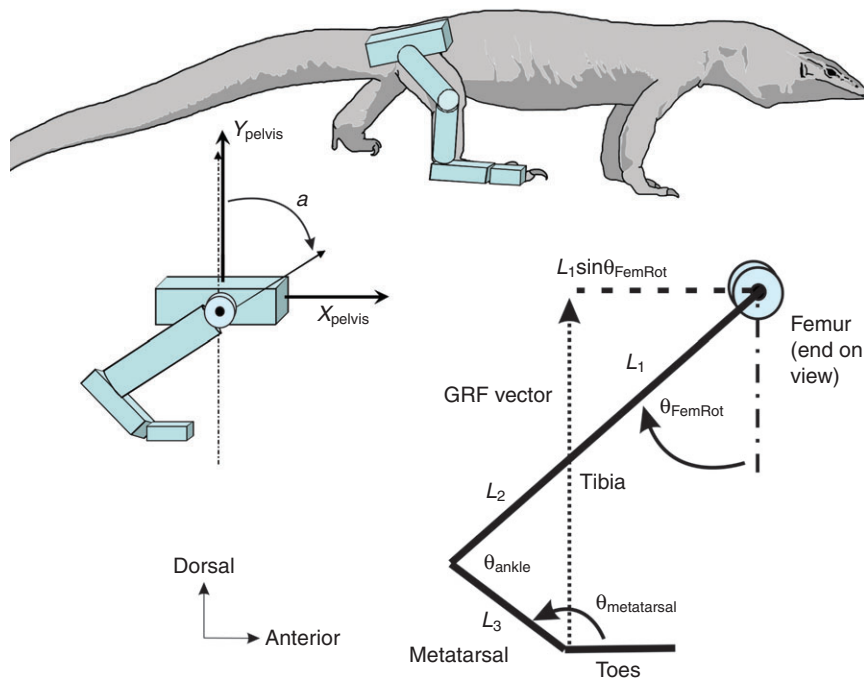


Fig. 6. Diagram describing the calculation of the torsional moment arm at midstance. The femur is shown end on, with the knee joint marked with a solid black circle. Segments of the limb are displayed as solid black lines. L_1 and L_2 are sections of the tibia that separate at the point of intersection with the vertical component of the ground reaction force (GRF). L_3 is the segment of the limb between the ankle marker and the metatarsal marker. θ_{ankle} , ankle joint angle at midstance; θ_{FemRot} , rotation of the femur at midstance; $\theta_{\text{metatarsal}}$, metatarsal joint angle at midstance.

the bone, essentially the midshaft radius of the bone, and I is the midshaft second moment of the area of the bone (Wainwright et al., 1976; Blob, 2000; Blob and Biewener, 2001). Therefore, to determine bending forces for the femur at midstance in varanids, force is expected to scale as $M^{0.917}$ (from above), L will scale with the length of the femur as $M^{0.35}$, n will scale with the diameter of the femur as $M^{0.417}$ [for consistency, both length and diameter exponents from Blob (Blob, 2000) were used here], and the second moment of area, given by $I = \pi r^4/4$ (where r is the bone radius) should scale with diameter as $(M^{0.417})^4$. This gives $\sigma_b \propto M^{0.917} M^{0.35} M^{0.417} / (M^{0.417})^4 = M^{0.016}$, suggesting that bending stress in the varanid femur scales close to zero.

Stresses in torsion may be more important than bending stresses in lizards, given that the safety factor for the former is approximately half that of the latter (Blob and Biewener, 1999; Blob, 2001). Torsional stress may result from the action of both the GRF and of muscles rotating the femur. The torsional stress (τ) acting on the femur due to GRF was estimated previously (Wainwright et al., 1976) as:

$$\tau = \frac{Tc}{J} \quad (3)$$

Here, c is the distance from the central axis of rotation to the cortex of the bone (and therefore proportional to the diameter) and J is the polar second moment of area, equal to $\pi r^4/4$; therefore, like I , J is proportional to d_{bone}^4 . T is the torsional moment applied to the bone, the scaling of which is important to the calculation of torsional stress. To simplify estimation of the torsional moment, we considered only the vertical component of the GRF, which seems justified as the vertical component is much larger than the lateral or horizontal components (Alexander, 1977; Christian, 1995; Blob and Biewener, 2001). Because neither femur retraction nor femur adduction change significantly with body size (see Table 1), we modeled the 3-D movement of the hindlimb as a 2-D model with the only degree of freedom being rotation along the femur. The lateral view of the femur is presented in Fig. 6. Considering only the vertical component of

the GRF originating from the metatarsal marker, the torsional moment (T) will be $T = L_1 F \sin \theta$. Here F is the force acting on the femur ($M^{0.917}$) and θ is the rotation of the femur at midstance, which the present study has shown to be $\propto M^{-0.064}$. Further we assumed that the length of the metatarsus, between the ankle and the metatarsal marker (L_3) scaled similarly to the length of the tibia ($L_1 + L_2$), which seems reasonable in these geometrically similar animals (Thompson and Withers, 1997; Blob, 2000). If this is the case, then L_1 is a proportion of the tibia length [i.e. $\propto M^{0.349}$ (Blob, 2000)], as neither θ_{ankle} nor $\theta_{\text{metatarsal}}$ (the ankle angle and metatarsal angle at midstance, respectively) scaled significantly different from $M^{0.00}$ at midstance (Table 1). This results in L_2 scaling similarly to L_3 and, by extension, L_1 scaling similarly to L_2 . Therefore, T is expected to scale as $M^{0.349} M^{0.917} M^{-0.064} = M^{1.202}$. Using this in Eqn 3, we get $\tau \propto M^{1.202} M^{0.417} / (M^{0.417})^4 = M^{-0.049}$, suggesting that once size-related changes in femoral rotation are accounted for, torsional stress at midstance resulting from the GRF should actually decrease as body size increases.

Torsional stress owing to the GRF may be augmented by torsional stress from muscles (Blob and Biewener, 1999). The primary femoral retractor muscle of lizards is the caudifemoralis longus (CFL) (Rewcastle, 1983; Reilly, 1995). The CFL originates from the caudal vertebrae and inserts *via* a broad tendon to both sides of the femoral trochanter, ventral to the mechanical axis of the hip joint (Rewcastle, 1983). The result of this ventral attachment is that CFL contraction during retraction of the femur can cause clockwise rotation of the femur (Snyder, 1954; Gatesy, 1997). This would suggest that rather than the torsional moment arm rotating the femur, scaling with tibia length and femur rotation as it does above, it will scale with femur radius ($\propto d_{\text{femur}} = M^{0.417}$). However, because muscle force scales much lower than body mass (approximately $M^{0.80}$) (Alexander et al., 1979; Biewener, 2005), this effect probably decreases with increasing size ($\tau_{\text{CFL}} \propto M^{-0.034}$).

Therefore, the kinematic differences we have observed with increased body size may act to reduce stresses on limb bones, suggesting alternative mechanisms for stress mitigation between

mammals and lizards, although empirical measurements of bone stresses using force plate data for large and small varanids will be necessary to confirm this. However, the different mechanisms proposed to reduce size-related stress in lizards, as compared with mammals, will likely influence scaling of performance variables such as speed. Increasing duty factor will adversely affect stride frequency, decreasing speed. Similarly, reducing the rotation of the femur at midstance probably has a detrimental effect on speed, as a previous study has shown that femur rotation may contribute positively to forward propulsion (Rewcastle, 1983). The consequences of this suggest that speed should scale with a much lower exponent for varanids and other species with a sprawling posture, compared with terrestrial mammals. This certainly seems to be the case; within varanids, speed scales as $M^{0.167}$ (Clemente et al., 2009) and scales similarly in non-varanid lizards as $M^{0.17}$ (Van Damme and Vanhooydonck, 2001), whereas speed scales at a much higher exponent for mammals and birds, $\propto M^{0.24}$ (Gatesy and Biewener, 1991). Maintaining similar stress safety factors with body size thus appears to be an important limitation to speed for taxa with a sprawling posture.

It is curious that larger lizards appear to sacrifice locomotor performance in order to maintain similar safety factors. Previous studies that have examined safety factors in lizards and alligators (Blob and Biewener, 1999; Blob and Biewener, 2001), and other animals with sprawling postures such as turtles (Butcher et al., 2008), have reported safety factors nearly twice as high as those of terrestrial birds and mammals (Alexander, 1981; Biewener, 1989; Biewener, 1993), prompting the question: why can't safety factors in large lizards simply decline? Previous studies have hypothesized that these high safety factors observed in non-avian reptilian lineages may reflect adaptations to differing demands. For example, the high safety factors in reptiles may help accommodate lower rates of bone remodeling, changes in bone structure during egg laying or higher load variability during kinematics than are found in mammals or birds (Blob and Biewener, 1999; Blob and Biewener, 2001). Higher safety factors in reptiles may also represent a retained ancestral condition from which birds and mammals independently diverged (Blob and Biewener, 1999; Blob and Biewener, 2001; Butcher et al., 2008). Alternatively, safety factors in varanids may be much lower than those previously measured in other reptilian groups. The varanids used in the present study tend to be larger (up to 7.9 kg), faster (up to 8.77 ms^{-1} for *V. giganteus*) (Clemente et al., 2009) and older than subjects of previous studies of limb bone loading in non-parasagittal lineages (Blob and Biewener, 1999; Blob and Biewener, 2001; Butcher et al., 2008). This would increase the forces acting on limb bones and muscles, and possibly lower overall safety factors (Biewener, 1982; Biewener and Taylor, 1986; Biewener, 1993). If safety factors in this group were more comparable to mammals, it may explain the requirement to maintain similar safety factors as body mass increases (Biewener, 1989). Direct measurements of bone strain in varanids are required to support this hypothesis, or may provide important evidence on the prevalence of high safety factors amongst non-avian reptilian lineages.

The implications of our findings may also have significance for studies investigating the relationship between size and performance in lineages other than varanids that have sprawling locomotion. For example, during the Triassic, 250–200 million years ago, the basal archosaurs made a transition from sprawling through semi-erect to erect posture, both in the 'crocodile' and the 'bird' lineages (Sereno, 1991; Hutchinson, 2006), and this transition is seen as a key contributor to the later success of the dinosaurs, pterosaurs and birds (Charig, 1972; Bonaparte, 1984; Parrish, 1987). Kubo and Benton,

using biomechanical modeling on the limb bones of archosaurs to estimate bone stresses and posture, predicted that femur rotation was reduced in larger-bodied archosaurs (Kubo and Benton, 2007), a prediction that is supported by the results of the present study. Further, we have shown the high stresses predicted for some species in their study (e.g. *Erythrosuchus*) could have been mediated by changes in duty factors, though probably not without a detrimental effect on speed in these species.

Finally, perhaps a key question in the evolution of posture in archosaurs is whether the erect stance evolved in response to increased body size, or whether archosaurs became erect first and larger later. The ancestral varanid was probably neither large nor small, but medium-sized, evolving into both small-bodied and large-bodied lineages (Molnar and Pianka, 2004). We have shown that this evolution of body size does not appear to be coupled with changes in posture. This refutes the hypothesis that posture evolved in response to body size for varanids, and instead suggests that the most parsimonious scenario for archosaurs, as in synapsids (Blob, 2001), is that posture became upright first, probably in small-bodied lineages. This change in posture may then have allowed the evolution of large body size characteristic of the Cretaceous period.

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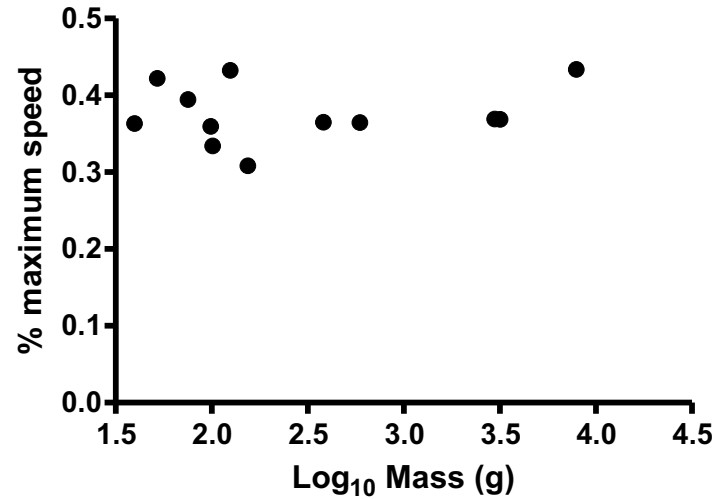
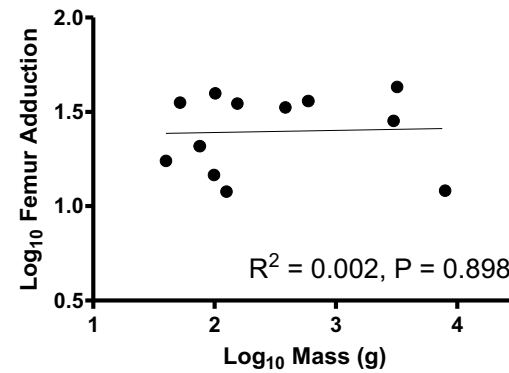
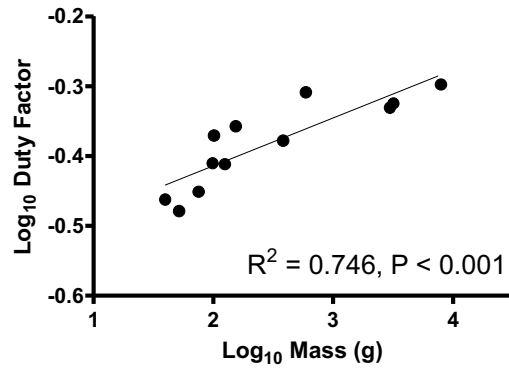
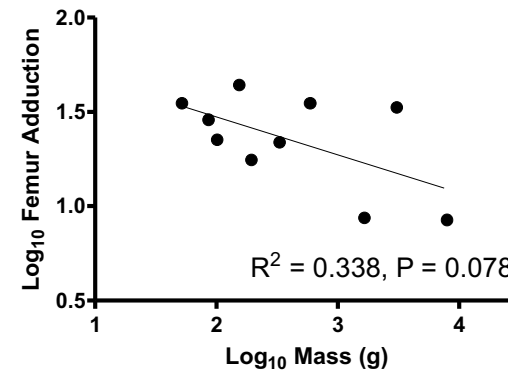
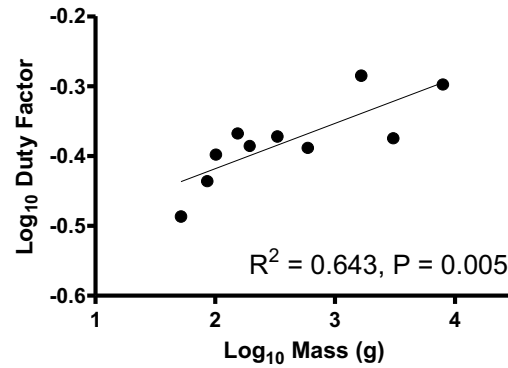


Figure S1. Plot of mass with the % of maximal speed run during kinematic trials. There was no significant relationship between the % of maximal speed and body mass ($R^2 = 0.028$, $P = 0.604$, $n = 12$).

A. 30-45% of maximal speed



B. 40-60% of maximal speed



C. 50-70% of maximal speed

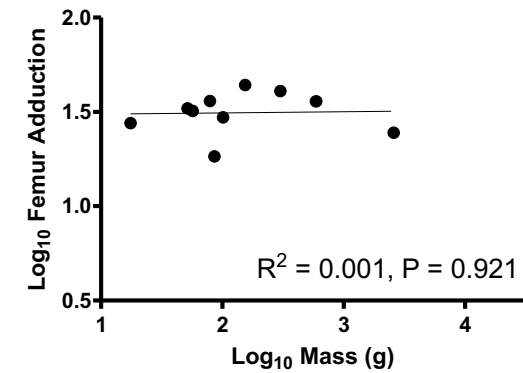
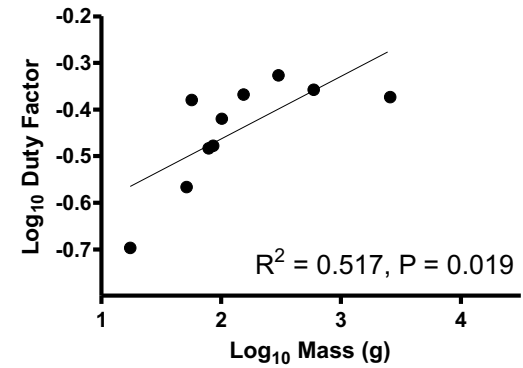


Figure S2 shows duty factor and femur adduction(depression) from three data sets, A – using strides between 30-45% of maximal speed. B – using strides 40-60% of maximal speed. C – using strides 50-70% of maximal speed. Note the largest size range (and smallest error) is for the 30-45% maximal speed which justifies its use in the study.