A COMPARATIVE STUDY OF SINGLE LEG GROUND REACTION FORCES IN RUNNING LIZARDS

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The role of different limbs in supporting and propelling the body has been studied in many species with animals appearing to have either similarity in limb function or differential limb function. Differential hind vs. fore limb function has been proposed as a general feature of running with a sprawling posture and as benefiting sprawled postured animals by enhancing maneuvering and minimizing joint moments. Yet only a few species have been studied and thus the generality of differential limb function in running animals with sprawled postures is unknown. We measured the limb lengths of seven species of lizard and their single limb three-dimensional ground reaction forces during high speed running. We found that all species relied on the hindlimb for producing accelerative forces. Braking forces were forelimb dominated in four species and equally distributed between limbs in the other three. Vertical forces were dominated by the hindlimb in three species and equally distributed between the fore- and hindlimb in the other four. Medial forces were dominated by the hindlimb in four species and equally distributed in the other three, with all Iguanians exhibiting hindlimb biased medial forces. Relative hind- to forelimb length of each species was related to variation in hind- vs. fore limb medial forces; species with relatively longer hindlimbs compared to forelimbs exhibited medial forces that were more biased towards the hindlimbs. These results suggest that the function of individual limbs in lizards varies across species with only a single general pattern (hindlimb dominated accelerative force) being present.
INTRODUCTION

Different limbs of multiple-legged animals (e.g. forelimb vs. hindlimb in a quadruped) appear to exhibit disparity in morphology and function. For example, many species can be roughly divided into those with hindlimb vs. forelimb dominated body support (i.e. vertical ground reaction force and impulse) (Rollinson and Martin 1981; Demes et al. 1994). Additionally, some species seem to rely on hindlimb ‘drive’ wherein the hindlimb produces the majority of the accelerative ground reaction force while the forelimb produces the majority of the braking ground reaction force. However, in other species the forelimb and hindlimb share a more equal role for accelerative-braking ground reaction force (Full et al. 1991; Demes et al. 1994; Witte et al. 2002; Lee et al. 2004; Willey et al. 2004; Autumn et al. 2006). Finally, medio-lateral ground reaction forces are not similar across species or legs. Large erect mammals, primates, and small crouched eutherians produce small medio-lateral ground reaction forces during steady speed running locomotion (Biewener 1990); however, semi-erect mammals, alligators, lizards, and cockroaches produce greater medio-lateral ground reaction forces, with these forces typically being greater in the hindlimbs (Full et al. 1991; Farley and Ko 1997; Willey et al. 2004; Lammers and Biknevicius 2004; McElroy and Reilly 2009).

Chen et al. (2006) developed a hypothetical framework of how different limbs could function during steady speed locomotion. This framework posits that animals broadly fall into one of two limb functional categories 1) animals whose forelimbs and hindlimbs both contribute equally to moving the center of mass over the support phase exhibit “similarity” in limb function (see also, Alexander and Goldspink 1977); and 2) animals whose forelimbs and hindlimbs have different roles in moving the center of mass exhibit “differential” limb function (Chen et al. 2006; Deban et al. 2012). Based on data from the house gecko (Hemidactylus garnotii, Chen et al., 2006) and previous data from a cockroach (Blaberus discoidalis, Jindrich and Full, 1999), Chen et al. (2006) argue that there are both maneuvering and mechanical benefits for coupling differential leg function with a sprawling limb posture. Additionally, bipedal running in lizards, which is an extreme form of differential limb function, has been hypothesized to confer enhanced maneuvering and obstacle negotiation (Aerts et al. 2003; Olberding and Higham 2012). However, differential limb function is not universal among sprawling animals; single limb ground reaction forces in iguanas (Iguana iguana) suggests similarity of leg function in this
species with a sprawling limb posture (Blob and Biewener, 2001). Additionally, American alligators (Alligator mississippiensis) use differential limb function but are not maneuverable or efficient; they walk slowly with a semi-erect posture and a massive, dragging tail (Willey et al. 2004). Taken together, these data suggest a general pattern of limb function for all or most sprawling quadrupedal trotting animals may not exist. To allow any generalities in limb function and its functional benefits in sprawling animals - or even lizards, it would be useful to examine a comparative sample of additional species.

Herein, we present data on seven fast-running lizards that represent a diversity of body forms, limb morphologies, and phylogenetic positions among limbed squamates. Using these data we address the two questions: i) is differential limb function a general characteristic of steady speed running in lizards? We expected differences in limb function across species given the diversity of species in our sample and ii) does limb function reflect limb morphology? We expected species with more similarly sized forelimbs vs. hindlimbs to exhibit greater similarity in limb function and conversely we expected species with hindlimbs that were relatively long compared to the forelimbs to display a greater disparity in the function between the hindlimbs and forelimbs.

MATERIALS AND METHODS

Study species

The following lizard species were studied: Laudakia stellio Linnaeus 1758, Leiocephalus schreibersi Gravenhorst 1837, Oplurus cuvieri Gray 1831, Tropidurus torquatus Wied-Neuwied 1820, Eulamprus quoyii Quoy & Gaimard 1824, Cordylus warreni Boulenger 1908 and Varanus exanthematicus Bosc 1792. All species were obtained from commercial suppliers except E. quoyii. E. quoyii was wild caught in Brisbane, Australia and released at the point of capture within 36 hours. Sample sizes are in Table 1. All housing and experimental procedures followed approved animal use protocols (IACUC U-99-03).

Data collection

Prior to each trial we measured body mass to the nearest 0.1 g for each individual. Single limb locomotor forces were quantified when lizards travelled down a racetrack towards a dark hide box. We focused on high-speed sprinting locomotion, with lizards induced to sprint down the racetrack by gently pressing on the tail or hindlimb. Each individual was induced to move
down the racetrack 2-3 times in rapid succession. Trials in which signs of fatigue or poor effort were noted (uncoordinated limb movements, dragging belly, or refusal to move after 3 tail pinches) were immediately discarded. Individuals were allowed to rest and recover for 24 hours before subsequent trials. Each individual of each species was chased down the racetrack numerous times to capture a range of the fastest speeds for each species. All species were maintained between 36-40 °C for the duration of each trail. To achieve these temperatures, lizards were warmed under heat lamps and temperature was checked via an infra-red thermal laser directed on the abdomen.

Ground reaction forces were quantified using a custom-made force platform based on a strain gauge, spring-blade design described in Bertram et al. (1997). Vertical, fore-aft, and medio-lateral ground reaction forces were sampled at 500 Hz using National Instruments data acquisition hardware and a LABVIEW custom designed virtual data sampling instrument following Parchman et al. (2003). The platform was calibrated such that it produced a linear response over the measurement range and had a minimum resolution of 15 mN in all three directions. The 0.6 m long by 0.2 m wide force platform surface was flush with the racetrack surface and located 3–3.6 meters along its 5.2-meter length. The entire surface of the racetrack and platform was covered with fine grit sandpaper to prevent foot slippage.

Analysis of gait parameters and ground reaction forces

Kinematic analyses were conducted using APAS (version 1.0). First, we determined speed for each trial by digitizing the tip of the snout as the lizard crossed seven evenly spaced (10 cm apart) lines along the surface of the racetrack. Next, we recorded the timing of touch-down and lift-off for the focal limb and computed support duration as time of lift-off minus time of touch-down and stride time as the time of touch-down until the time of the next touch-down of the same limb. Duty factor was defined as support duration divided by stride time.

To record individual limb forces we analyzed only the first limb on (forelimb), and the last limb off (hindlimb), the racetrack (Figure 1). For each trial, we reviewed high-speed video recordings of sprint sequences (120 fps or 500 fps, depending on species) to determine if the isolated forelimb or hindlimb footfalls were “clean”. A “clean” footfall was defined as when: i) the entire foot is in contact with the substrate on the surface of force platform (trials in which the foot landed on the gap between the racetrack and force platform were discarded), and ii) no other
limbs were in contact with the force platform during the entire contact phase (touch-down to lift-off) of the focal limb. These two criteria resulted in isolated individual limb contacts and ground reaction forces for both the forelimbs and hindlimbs for several trials per species (Table 1). It should be noted that forelimb and hindlimb contacts did not always come from the same run. There were no statistical differences in the distributions of speeds or masses in forelimb vs. hindlimb data within each species; which shows that our method of choosing contacts from different runs did not bias the analyses. We also note that the mixed model used to analyze the data accounted for the effect of speed and mass on ground reaction forces and impulses (see below).

Individual limb ground reaction forces were numerically integrated over limb contact time to calculate impulse (i.e. the area under the force vs. time curve). Impulses were calculated separately for vertical, braking (negative fore-aft), accelerative (positive fore-aft), and medial and lateral directions. Medial and lateral impulses were summed and the resultant impulse was always directed medially. Therefore, we refer to this summed impulse as medial impulse for here onward. In addition, peak forces were measured as the maximum force in each direction: peak vertical, braking, accelerative, and medial forces.

We only analyzed steady speed trials which were defined according to the following criteria. First, we ensured that speed over any 10 cm interval as measured by digitizing the lizards snout was < 20% different than the average speed down the racetrack; by this definition the lizard moved with only small speed fluctuations down the entire surface of the force platform (see also, McElroy et al. 2008; McElroy and Reilly 2009). To further ensure that trials were steady speed down the length of the force platform, we compared the magnitude of the braking vs. accelerative impulse within each trial for the portion of the trial that included whole body forces (Fig.1). We discarded trials that exhibited greater than 20% difference in digitized speed or impulses. The value of 20% was chosen based on previous studies of lizard locomotion (Farley and Ko 1997; McElroy and Reilly 2009; McElroy et al. 2008; Chen et al 2006). Thus, subsequent analyses only included trials that were relatively steady-speed as judged by both kinematic and kinetic recordings. We also computed the braking-propulsive bias for the forelimb and hindlimb. For the forelimb we computed this as the ratio of absolute value of braking (numerator) to accelerative (denominator) impulse. For the hindlimb we computed the braking-propulsive bias as the ratio of accelerative (numerator) to the absolute value of braking
We computed the ratio differently for forelimb vs. hindlimb so that larger values indicated a greater bias towards that limbs predominant type of impulse (forelimb:braking, hindlimb:accelerative).

Finally, we computed the timing of peak vertical ground reaction force as a percentage of support duration.

**Morphology**

To quantify morphology we took dorsoventral whole-animal radiographs of multiple individuals per species (Table 1) and measured the total length of the forelimb and the hindlimb by summing the length of their constituent elements: forelimb (humerus, ulna, carpal, 3\textsuperscript{rd} metacarpal, 3\textsuperscript{rd} finger) and hindlimb (femur, tibia, tarsal, 4\textsuperscript{th} metatarsal, 4\textsuperscript{th} toe). Snout-vent length (SVL) was also measured from each radiograph. All specimens were obtained via museum loans.

**Data Analysis**

Prior to analyses all data were log transformed. We constructed 10 separate general linear mixed models with component impulses (vertical, braking, accelerative, medial) or maximum ground reaction forces (vertical, braking, accelerative, medial) or duty factor or the braking-propulsive impulse bias as the response variable in each respective model. For each model, species and limb (hindlimb, forelimb) were entered as main effects, speed and mass were entered as covariates (speed and mass are correlated with ground reactions forces in all species, see McElroy and Reilly 2009), species*limb was entered as an interaction term and individual was entered as a random effect. Within each species*limb interaction term, we constructed planned linear contrasts to test the hypotheses that forelimb and hindlimb forces were different within each species (e.g. forelimb vs. hindlimb vertical force for *Eulamprus quoyii*). All tests were on means adjusted for the covariates in the model (speed and mass); this effectively removed the effect of these confounding variables. The planned linear contrasts were tested using F-tests with a significance level of $p < 0.05$. We did not control for multiple testing as the linear contrasts were planned prior to analysis (Quinn and Keough 2003). We note that phylogeny was not taken into account for this analysis because the test of each contrast was within species not between.
To explore differences in forelimb vs. hindlimb morphology we regressed hindlimb length on forelimb lengths. Residuals from this analysis were plotted to compare species differences in forelimb vs. hindlimb lengths and were used in the next analysis (Figure 2).

To examine the relationship between limb morphology and ground reaction forces we computed mean ground reaction forces and impulses for forelimbs and hindlimbs for each species. Then ordinary least squares (OLS) regressions of hindlimb \(y\) on forelimb \(x\) forces and impulses (vertical, accelerative, braking, and medial, separately) were generated. Residuals from each regression were saved for further analyses as a measurement of the relative role of forelimbs vs. hindlimbs in contributing to ground reaction forces and impulses. Regression and Pearson product moment correlations were then computed between the residual ground reaction forces/impulses and residual limb morphology.

Species are not independent data points due to their phylogenetic relationships, which violates a fundamental assumption of independence assumed by traditional statistical analyses (Felsenstein, 1985). To account for this non-independence we calculated phylogenetically independent contrasts for residual limb morphology and residual ground reaction force and impulses using the PDAP module in Mesquite (Midford et al., 2002; Maddison and Maddison, 2007). The phylogeny (Figure 3) was based on Townsend et al. 2004 and Bergmann and Irschick (2012); branch lengths were set to 1 and then the tree was made ultrametric. Appropriate standardization was checked by plotting the absolute value of each set of contrasts vs. their standard deviations; in all cases no relationship was detected indicating adequate standardization of the contrasts (Garland et al. 1992). We then recomputed the regression (force through the origin) and Pearson product-moment correlations between the independent contrasts for residual limb morphology and the independent contrasts for residual ground reaction forces/impulses.

**RESULTS**

**Vertical Forces and Impulses**

The maximum vertical force was significantly larger in the hindlimb when compared to the forelimb for *Eulamprus quoyii* (\(F_{1,12.3} = 9.5, p = 0.009\)), *Oplurus cuvieri* (\(F_{1,12.2} = 5.1, p = 0.043\)), and *Tropidurus torquatus* (\(F_{1,14.4} = 10.8, p = 0.005\)) whereas the other four species had similar forelimb vs. hindlimb maximum vertical forces (\(p > 0.10\), Fig. 4A).
The vertical impulse was significantly larger in the hindlimb when compared to the forelimb for *Eulamprus quoyii* ($F_{1,13.3} = 14.6$, $p = 0.002$), *Oplurus cuvieri* ($F_{1,12.4} = 5.3$, $p = 0.039$), and *Tropidurus torquatus* ($F_{1,17.1} = 17.8$, $p < 0.001$) whereas the other four species had similar forelimb vs. hindlimb vertical impulse ($p > 0.10$, Fig. 4B).

**Accelerative Forces and Impulses**

Maximum accelerative forces were significantly greater in the hindlimbs compared to the forelimbs for *Cordylus warreni* ($F_{1,40.4} = 6.2$, $p = 0.017$), *Eulamprus quoyii* ($F_{1,9.3} = 7.5$, $p = 0.022$), *Laudakia stellio* ($F_{1,10.2} = 13.1$, $p = 0.005$), *Oplurus cuvieri* ($F_{1,8.5} = 10.6$, $p = 0.011$) and *Varanus exanthematicus* ($F_{1,13.3} = 12.9$, $p = 0.003$), marginally significantly greater in the hindlimbs for *Tropidurus torquatus* ($F_{1,12.6} = 3.9$, $p = 0.069$), and not significantly different between limbs in *Leiocephalus schreibersi* ($F_{1,8.8} = 2.3$, $p = 0.167$, Fig. 4C).

Accelerative impulses were significantly greater in the hindlimbs compared to the forelimbs for *Cordylus warreni* ($F_{1,38.1} = 5.7$, $p = 0.022$), *Eulamprus quoyii* ($F_{1,7.7} = 7.3$, $p = 0.028$), *Laudakia stellio* ($F_{1,8.7} = 24.8$, $p < 0.001$), *Leiocephalus schreibersi* ($F_{1,7.2} = 8.0$, $p = 0.025$), *Oplurus cuvieri* ($F_{1,6.8} = 19.9$, $p = 0.003$) and *Varanus exanthematicus* ($F_{1,11.8} = 44.2$, $p < 0.001$) and marginally significantly larger in the hindlimbs for *Tropidurus torquatus* ($F_{1,11} = 4.4$, $p = 0.059$, Fig. 4D).

**Braking Forces and Impulses**

Maximum braking forces were significantly larger in the forelimb when compared to the hindlimb for *Cordylus warreni* ($F_{1,60.9} = 10.6$, $p = 0.002$), *Laudakia stellio* ($F_{1,11.0} = 6.5$, $p = 0.027$) and *Varanus exanthematicus* ($F_{1,19.3} = 48.1$, $p < 0.001$), marginally significantly larger in the forelimbs for *Oplurus cuvieri* ($F_{1,5.3} = 5.4$, $p = 0.063$) and *Leiocephalus schreibersi* ($F_{1,7.6} = 3.8$, $p = 0.089$) and not different between limbs in the other two species ($p > 0.10$, Fig. 4C).

Braking impulses were significantly larger in the forelimb when compared to the hindlimb for *Cordylus warreni* ($F_{1,47.6} = 15.7$, $p < 0.001$), *Laudakia stellio* ($F_{1,15.2} = 13.3$, $p = 0.002$), *Oplurus cuvieri* ($F_{1,14.1} = 9.5$, $p = 0.008$) and *Varanus exanthematicus* ($F_{1,18.3} = 37.0$, $p < 0.001$) whereas the other three species had similar forelimb vs. hindlimb braking impulses ($p > 0.10$, Fig. 4D).
Medial Forces and Impulses

Maximum medial forces were significantly greater in the hindlimb when compared to the forelimb for *Oplurus cuvieri* (F1,14.1 = 9.5, p = 0.008) and *Tropidurus torquatus* (F1,14.1 = 9.5, p = 0.008), marginally greater in *Eulamprus quoyii* (F1,11.9 = 3.7, p = 0.079) and *Laudakia stellio* (F1,12.2 = 3.7, p = 0.076) and did not significantly differ between limbs in the other two species (p > 0.10, Fig. 4E).

Medial impulses were significantly larger in the hindlimb compared to the forelimb for *Laudakia stellio* (F1,10 = 9.4, p = 0.012), *Leiocephalus schreibersi* (F1,8.4 = 6.4, p = 0.034), *Oplurus cuvieri* (F1,7.9 = 12.1, p = 0.008) and *Tropidurus torquatus* (F1,12.6 = 6.02, p = 0.030) whereas all other species had similar medial impulses when comparing the forelimb to the hindlimb (p > 0.10, Fig. 4F).

Duty Factor, Braking-Propulsive Bias and Timing of Peak Vertical Force

Hindlimb duty factor was significantly greater than forelimb duty factor for *Cordylus warreni* (F1,50.0 = 11.5, p = 0.001), *Eulamprus quoyii* (F1,7.5 = 14.6, p = 0.006), *Laudakia stellio* (F1,9.5 = 13.7, p = 0.005), and *Leiocephalus schreibersi* (F1,7 = 16.6, p = 0.005) whereas the other species had similar hindlimb and forelimb duty factors (p > 0.10, Table 1).

All species exhibited forelimb-dominated braking and hindlimb-dominated propulsive functionality although there was wide variation (~ 2-8x bias, dependent upon species, Table 1, Fig. 4C, 4D). The forelimb was equally biased towards braking as the hindlimb was biased towards propulsion, such that the difference in forelimb vs. hindlimb braking-propulsive bias was not significantly different for any species (p > 0.40 for all linear contrasts).

Most species had peak vertical ground reaction forces near 50% of support duration; the vertical force profile was nearly symmetrical (Table 1). Only in the hindlimb of *Oplurus cuvieri* and *Leiocephalus schreibersi* was the upper 95% confidence interval less than 50%, indicating that the vertical force profile of the hindlimb of these species was consistently skewed towards the first half of support duration.

Morphology-Force Relationships

Raw residual limb morphology was significantly correlated with raw medial impulse; species with relatively long hindlimbs compared to forelimbs produced greater medial impulses.
with the hindlimbs compared to the forelimbs (Table 2, Figure 5A). The independent contrasts
for morphology and medial impulse were also significantly correlated, indicating that relatively
long hindlimbs and greater hindlimb bias of medial impulse have undergone correlated evolution
(Table 2; Figure 5B). None of the other raw impulses, maximum forces, or their independent
contrasts, was significantly correlated with raw vertical, accelerative, or braking impulses or
maximum forces (Table 2).

**DISCUSSION**

Differential limb function occurs when the forelimb and hindlimb produce different patterns of
ground reaction forces (Chen et al. 2006). Our measurements of single leg ground reaction forces
across a diverse sample of lizards indicate a diversity of ground reaction force patterns that
exhibit characteristics of both differential and similar limb function.

All species generated more accelerating force and/or impulse with the hindlimb (Fig. 4C, D). This pattern is similar to the house gecko (Chen et al. 2006; Autumn et al. 2006) and thus a
general pattern for lizards is that the hindlimb and forelimb function differentially with respect to
the production of accelerative forces during steady-speed, level running. Several previous studies
of limb kinematics (e.g. Irschick and Jayne 1999; Reilly and Delancey 1997; McElroy et al.
2012); and limb morphology (e.g. Miles et al. 2007; Herrel et al. 2008; Russell and Bauer 2008)
also suggest hindlimb is the dominant propulsor in lizards. In contrast, we found vertical,
braking, and medial forces all exhibited species-specific patterns.

Differential limb function with forelimb-dominated braking forces was evident in four
species (C. warreni, L. stellio, O. cuvieri and V. exanthematicus) for braking impulse and five
species (C. warreni, L. stellio, L. schrebersi, O. cuvieri and V. exanthematicus) for peak braking
forces (Fig. 4C, D). These species apply braking impulses and peak forces like the house gecko
(Chen et al. 2006; Autumn et al. 2006). However, two species do not show any evidence of this
pattern (E. quoyii and T. torquatus); instead their forelimb and hindlimb show similarity of
function with respect to the application of braking forces and impulses (Fig. 4C, D). One reason
for this discrepancy could be our window of a 20% difference in accelerative vs. braking impulse
for accepting `steady-speed’ trials. If one considers that all species show differential limb
function with respect to accelerative forces, then it follows that they should show the same
pattern for braking forces, because braking and accelerative impulses should be equal during
steady speed locomotion. The difference in accelerative vs. braking impulses (based on whole
body recording from the rest of the trial (Fig. 1) in these species was ~ 17%; while it was ~ 11%
in the other species. These data support the idea that for *E. quoyii* and *T. torquatus* we may have
used trials that were more biased toward ‘accelerating’ than the other species and thus would
superficially be expected to have equal forelimb and hindlimb braking forces. This issue is
always present when studying 'steady-speed' because it is quite difficult to obtain steady-speed
trials and often investigators are forced to use a broad window for accepting 'steady-speed'. This
is particularly true in species that do not routinely move at steady speed, which is true of many
small sprawling animals (Reilly et al. 2007). For example, studies of ground reaction forces in
lizards and other sprawling animals have used changes in speed anywhere from ~5% to 50% as a
criteria to define 'steady-speed' locomotion (Ahn et al. 2004; Farley and Ko 1997; Reilly et al.
2006; Full et al. 1991; McElroy and Reilly 2009; McElroy et al. 2008; Chen et al 2006; Full and
Tu 1991; Autumn et al. 2006). In fact, the study of individual limb forces in the house gecko
(Chen et al. 2006) used a speed change of < 15%, which was similar to our study. Thus, it seems
that braking forces may exhibit differential function in the forelimbs vs. hindlimbs in all lizards,
and the exception in our data may be due to a methodological artifact.

Support of the body’s weight was equally distributed between the forelimb and hindlimb
in four species we studied (*C. warreni, L. stellio, L. schrebersi*, and *V. exanthematicus*) much
like the house gecko (Chen et al. 2006). However, we found the hindlimb played a greater role in
supporting the body’s weight in *E. quoyii, O. cuvieri*, and *T. torquatus* with greater peak vertical
ground reaction force and vertical impulses in the hindlimb vs. the forelimb (Fig. 4A, B). Such
hindlimb-dominated support of the body’s weight could arise via several pathways. First, these
three species could have body dimensions that position the centre-of-mass closer to the hindlimb
than the other four species. If the centre-of-mass were closer to the hindlimb, then one would
expect the hindlimb to bear a greater proportion of body weight and thus exhibit greater ground
reaction forces (Aerts et al. 2003, Lee et al. 2004). Data on centre-of-mass position and relative
weight of different body segments would be needed to test this hypothesis, although these three
species do not seem to have body proportions that are different from the other four, in fact both
groups have a diversity of body shapes (EJM, pers. obs). Another possibility is that these species
adopt different body postures during high-speed running, such that the forelimbs have reduced
contact time with the substrate resulting in a reduced role in supporting the body (Aerts et al.
2003; Walter and Carrier 2011). This possibility seems unlikely because patterns in the other
aspects of the ground reaction force (propulsive, braking, medial) would be expected to follow
body weight support in these species (Aerts et al. 2003), but they do not. In addition, duty factors
in the forelimb are only reduced in *E. quoyii*; there other two species have statistically similar
forelimb vs. hindlimb duty factors (Table 1). A final possibility is that the forelimb maintains
contact but the hindlimb still bears more of the weight due to a simple change in body angle.
Detailed kinematic data coupled with single-limb ground reaction force over several steps would
be required to test these possibilities.

Lee et al. (2004) suggested that support of the body’s weight by the forelimb vs. hindlimb
would bias the function of the forelimb vs. hindlimb in generating accelerative-braking ground
reaction forces. Our data show that four species share an equal role of the forelimb and hindlimb
in supporting the body and thus these species would be expected to show very small difference in
the bias between limbs (i.e. the forelimbs should be as biased towards braking as the hindlimbs
are biased towards accelerative force, see Figure 7 in Lee et al. 2004). Indeed these species do
not have significantly different forelimb vs. hindlimb biases (Table 1). However, all species had
similarity in forelimb vs. hindlimb biases, even the three species with hindlimb dominated body
support (Fig. 4). Lee et al. (2004) predicted species with hindlimb dominated body support
would show 1) reduced forelimb duty factor and, as a result, 2) reduced forelimb accelerative
force (i.e. the forelimb more biased towards a purely braking role). *E. quoyii*, *O. cuvieri*, and *T.
torquatus* had hindlimb dominated body weight support (Fig. 4), but only *E. quoyii*’s duty factor
follows the prediction. The duty factor for *O. cuvieri* and *T. torquatus* and the forelimb vs.
hindlimb bias for all three species were statistically indistinguishable. If we simply examine
mean values (Table 1), ignoring the statistical tests, our data shows only partial support Lee’s
hypothesis: *T. torquatus* does have a larger mean forelimb braking bias and reduced forelimb
duty factor, but *E. quoyii* and *O. cuvieri* had the largest difference between forelimb and
hindlimb bias with a tendency towards an inflated role of the hindlimb in accelerating, and *O.
cuvieri* has essentially no difference in forelimb vs. hindlimb duty factor. Perhaps the data from
*T. torquatus* lend support to Lee et al.’s hypothesis while *E. quoyii* and *O. cuvieri* suggest a
different functional consequence of loading the hindlimb with body weight. Loading the
hindlimb with more body weight would allow the hindlimb (and its enlarged musculature and
longer bones) to play a more prominent role in generating accelerative forces (Aerts et al. 2003;
Walter and Carrier, 2011) which could be occurring in *E. quoyii* and *O. cuvieri*.

Medial ground reaction impulses were biased towards the hindlimb in four of the species
we studied (*L. stellio, L. schreibersi, O. cuvieri, and T. torquatus*). These species are all members
of the suborder Iguania, which suggests some aspect of Iguanian morphology or physiology may
elicit hindlimb-dominated medial impulses. A similar pattern is exhibited by the house gecko
(Chen et al. 2006). Three other species show an equal role of the forelimb and hindlimb in
generating medial forces/impulses (*C. warreni, E. quoyii, and V. exanthematicus*), suggesting
similarity in limb function with respect to medial forces. Two factors may help explain
differential vs. similar patterns of medial ground reaction forces. First, lizards are known to
produce both standing and traveling waveforms along the body axis during running (Ritter
1992). Ritter (1992) showed that all species shift to a traveling wave during high-speed running,
but that the details for the traveling wave were different across species. In two species with
elongate limbs (*Dipsosaurus dorsalis* and *Cnemidophorus tigris*) the traveling wave contained a
node with less lateral movement, somewhat like a standing-wave pattern (Ritter 1992). This node
was positioned just behind the pectoral girdle, suggesting that the forelimbs experience less of a
lateral excursion than do the hindlimbs. Interestingly, the four species that showed hindlimb-
biased medial impulses (Fig. 4E, F) are those with the longest limbs (McElroy and Reilly 2009).
Additionally, the relative magnitude of medial impulses is correlated with the relative hindlimb
vs. forelimb length (Table 2, Fig. 5), such that lizard species with relatively longer hindlimb
compared to forelimb produce relatively more medial impulse with their hindlimb compared to
the forelimb. This pattern coupled with data from Ritter (1992) suggest that species with
elongate limbs may use a particular type of traveling wave of lateral bending (with a pectoral
‘node’) that results in more medial effort in the hindlimb and less in the forelimb. Ritter (1992),
suggested increased medial forces due to standing waves came at a ’cost’ to accelerative force
production; however, the species in this study all produced substantial accelerative ground
reaction forces and were all equally fast. Thus, our data imply some other function for high
hindlimb medial forces coupled with low forelimb medial forces. One possibility is that this
could relate to maneuvering, as the forelimb must only produce a small change in medial forces
whereas the hindlimb must produce larger medial forces to induce a turns (Jindrich and Full
Another possibility is that the difference in medial forces does not confer a functional advantage, but is a byproduct of morphology (Gould and Lewontin 1977). In fact, the four species with hindlimb-biased medial forces have relatively large hindlimbs compared to their forelimbs (Fig. 2) and the large medially directed forces may be a by-product of the joint excursions experienced during propulsion by a long, sprawling limb (Reilly and Delancey 1997; Irschick and Jayne 1999; Russel and Bels 2001; Fuller et al. 2011; Olberding et al. 2012; McElroy et al. 2012). Studies that combine forces, kinematics and diverse ways of moving (running, turning, burst locomotion) are needed to tease the relationship between limb length and medial forces in lizards.

Steady speed bipedal running in lizards (i.e. extreme differential limb function to the hindlimbs) requires asymmetry of the vertical ground reaction force toward the first 1/2 of support duration (Van Wassenbergh and Aerts 2013). Although the species we studied were not running bipedally during this experiment, the four Iguanian species are capable of bipedal locomotion at high speeds and *Eulamprus quoyii* would often have its body pitch up during running as if it could briefly run bipedally (EJM, pers obs). Thus, one might expect these species to show asymmetry of the vertical force profile. However, we find little evidence of this, only two of the four Iguanian species (*Oplurus cuvieri* and *Leiocephalus schreibersi*) had peak vertical forces biased towards the beginning of support duration while all other species had symmetrical vertical force profiles (Table 1). The mechanism behind these differences between species is unclear, although it could be due to differences in trunk angle or body kinematics across trials, which we did not measure. Thus, while steady speed bipedal running requires vertical force asymmetry to be stable (Van Wassenbergh and Aerts 2013), steady speed quadrupedal running in lizards does not.

Do all lizards exhibit differential limb function? In short, each species exhibited differential forelimb vs. hindlimb function for at least one component of the ground reaction force (i.e. accelerative force/impulse). However, only one species (*O. cuvieri*) showed differential limb function across all four components (vertical, accelerative, braking, lateral) and most species only showed differential function for two of the four components but the exact components were species-specific (Fig. 4). Additionally, the predication that duty factor and braking-propulsive bias should conform to a lizard body plan (Lee et al. 2004) is only partially upheld by these data. This suggests that differential limb function may not be a general feature of sprawling,
quadrupedal trotters. Thus, the biomechanics of lizard locomotion seem to be better described by functional diversity that is driven by variation in morphology and physiology across species (see also, Vanhooydonck et al. 2006; McElroy and Reilly 2009). Describing and understanding this diversity is important so that we can begin to build a broader, comparative understanding of the functional morphology and biomechanics of animal locomotion.

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AUTHOR CONTRIBUTIONS
EJM, ARB, and SMR designed the study. All authors played a role in data collection and analysis. EJM wrote the paper and all authors provided input regarding the content of the manuscript and the implications of the main findings.

COMPETING INTERESTS
No competing interests

REFERENCES


Van Wassenbergh, S., and Aerts, P. (2013). In search of the pitching momentum that enables some lizard to sustain bipedal running at constant speeds. *J. R. Soc. Int.* **10**:20130241


Table 1: Sample sizes and mean (± 1 s.e.m.) body mass, limb duty factors, accelerative/braking impulses and the braking-propulsion ratio for each species. Number in parentheses after species names are (number of individuals ran, number of individuals for which morphology was measured).

<table>
<thead>
<tr>
<th>Species</th>
<th>Body Mass (g)</th>
<th>Limb</th>
<th>N</th>
<th>Duty Factor</th>
<th>Accelerative Impulse (mN s)</th>
<th>Braking Impulse (mN s)</th>
<th>B-P Ratio</th>
<th>Time to Maximum Vertical Force (% of step duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cordylus warreni (4, 11)</strong></td>
<td>41 ± 1.0</td>
<td>forelimb</td>
<td>4</td>
<td>0.39 ± 0.01</td>
<td>1.58 ± 1.01</td>
<td>-3.23 ± 0.54</td>
<td>2.04</td>
<td>51 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>10</td>
<td>0.53 ± 0.02</td>
<td>3.01 ± 0.34</td>
<td>-0.70 ± 0.22</td>
<td>4.30</td>
<td>47 ± 4.4</td>
</tr>
<tr>
<td><strong>Eulamprus quoyii (3, 29)</strong></td>
<td>29 ± 9.0</td>
<td>forelimb</td>
<td>18</td>
<td>0.39 ± 0.01</td>
<td>0.21 ± 0.04</td>
<td>-0.52 ± 0.08</td>
<td>2.48</td>
<td>44 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>10</td>
<td>0.49 ± 0.01</td>
<td>0.96 ± 0.22</td>
<td>-0.20 ± 0.05</td>
<td>4.80</td>
<td>41 ± 4.7</td>
</tr>
<tr>
<td><strong>Laudakia stellio (2, 33)</strong></td>
<td>49 ± 2.0</td>
<td>forelimb</td>
<td>7</td>
<td>0.37 ± 0.03</td>
<td>0.87 ± 0.28</td>
<td>-3.75 ± 1.21</td>
<td>4.31</td>
<td>46 ± 3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>13</td>
<td>0.48 ± 0.02</td>
<td>3.86 ± 0.34</td>
<td>-0.96 ± 0.15</td>
<td>4.02</td>
<td>54 ± 3.4</td>
</tr>
<tr>
<td><strong>Leiocephalus schreibersi (2, 19)</strong></td>
<td>35 ± 1.0</td>
<td>forelimb</td>
<td>14</td>
<td>0.41 ± 0.02</td>
<td>0.61 ± 0.14</td>
<td>-1.48 ± 0.20</td>
<td>2.43</td>
<td>43 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>7</td>
<td>0.51 ± 0.03</td>
<td>2.00 ± 0.60</td>
<td>-0.61 ± 0.16</td>
<td>3.29</td>
<td>36 ± 3.0</td>
</tr>
<tr>
<td><strong>Oplurus cuvieri (3, 21)</strong></td>
<td>49 ± 5.4</td>
<td>forelimb</td>
<td>19</td>
<td>0.43 ± 0.01</td>
<td>0.70 ± 0.17</td>
<td>-2.06 ± 0.44</td>
<td>2.94</td>
<td>49 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>10</td>
<td>0.44 ± 0.03</td>
<td>3.20 ± 0.67</td>
<td>-0.40 ± 0.10</td>
<td>8.00</td>
<td>44 ± 1.8</td>
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<tr>
<td><strong>Tropidurus torquatus (3, 7)</strong></td>
<td>26 ± 2.3</td>
<td>forelimb</td>
<td>5</td>
<td>0.29 ± 0.03</td>
<td>0.19 ± 0.06</td>
<td>-0.75 ± 0.08</td>
<td>3.95</td>
<td>52 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>5</td>
<td>0.36 ± 0.06</td>
<td>2.21 ± 0.88</td>
<td>-0.92 ± 0.31</td>
<td>2.40</td>
<td>57 ± 3.3</td>
</tr>
<tr>
<td><strong>Varanus exanthematicus (2, 13)</strong></td>
<td>94 ± 1.4</td>
<td>forelimb</td>
<td>8</td>
<td>0.43 ± 0.02</td>
<td>1.23 ± 0.39</td>
<td>-6.62 ± 1.21</td>
<td>5.38</td>
<td>46 ± 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hindlimb</td>
<td>8</td>
<td>0.41 ± 0.04</td>
<td>6.48 ± 0.69</td>
<td>-1.09 ± 0.48</td>
<td>5.94</td>
<td>53 ± 1.4</td>
</tr>
</tbody>
</table>
Table 2: Pearson product moment correlations between raw and independent contrasts for residual limb morphology (hindlimb vs. forelimb length) and ground reaction forces/impulses. P-values are based on T-tests with 6 (raw) or 5 (independent contrasts) degrees of freedom.

<table>
<thead>
<tr>
<th></th>
<th>Raw</th>
<th></th>
<th>Independent Contrasts</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Vertical Impulse</td>
<td>0.334</td>
<td>0.47</td>
<td>-0.022</td>
<td>0.96</td>
</tr>
<tr>
<td>Maximum Vertical Force</td>
<td>0.170</td>
<td>0.72</td>
<td>-0.122</td>
<td>0.80</td>
</tr>
<tr>
<td>Accelerative Impulse</td>
<td>-0.005</td>
<td>0.99</td>
<td>-0.394</td>
<td>0.38</td>
</tr>
<tr>
<td>Maximum Accelerative Force</td>
<td>0.243</td>
<td>0.60</td>
<td>-0.076</td>
<td>0.87</td>
</tr>
<tr>
<td>Braking Impulse</td>
<td>-0.141</td>
<td>0.76</td>
<td>0.302</td>
<td>0.51</td>
</tr>
<tr>
<td>Maximum Braking Force</td>
<td>-0.347</td>
<td>0.45</td>
<td>-0.020</td>
<td>0.97</td>
</tr>
<tr>
<td>Medial Impulse</td>
<td>0.850</td>
<td>0.02</td>
<td>0.746</td>
<td>0.05</td>
</tr>
<tr>
<td>Maximum Medial Force</td>
<td>0.592</td>
<td>0.16</td>
<td>0.224</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Figure 1: Example component force traces for several consecutive steps from *Leiocephalus schreibersi*. Vertical dotted lines bound isolated forelimb (forelimb) and hindlimb (hindlimb) support durations (down = foot contacting substrate, up = foot leaving substrate). Force data between these individual limb support durations represent whole body ground reaction forces.

Figure 2: Bivariate plot of hindlimb length vs. forelimb length. Solid black line is OLS regression (slope = 0.856, intercept = 27.6), vertical dotted lines represent residuals for each species. Symbols: ▲ = *Varanus exanthematicus*, ● = *Oplurus cuvieri*, ♦ = *Laudakia stellio*, x = *Eulamprus quoyii*, ■ = *Cordylus warreni*, □ = *Tropidurus torquatus*, ○ = *Leiocephalus schreibersi*.

Figure 3: Phylogeny for lizard species in this study. Nodes are labeled to correspond to other figures. Branches are not proportional to their lengths, all branch lengths were set at 1 and then the tree was made ultrametric.

Figure 4: Forces and impulses for each component of the ground reaction forces for each species. * indicates that forelimb had significantly different ground reaction force than hindlimb within a species (p < 0.05), † indicates marginal significance (0.05 < p < 0.10). Significance was assessed using the linear contrasts within the species*limb interaction term in the general linear mixed models for component impulse or ground reaction force. Values are means ± 1 s.e.m. It should be noted that significance tests are for means adjusted for the parameters in the mixed model (see methods). Panels C and D have two tests each for each species, one for accelerative force & impulse (i.e. comparing forelimb vs. hindlimb above zero) and one for braking force and impulse (i.e. comparing forelimb vs. hindlimb below zero).

Figure 5: Panel A: Residual hindlimb-forelimb length vs. residual (hindlimb vs. forelimb) medial impulse. Regression line: intercept = 4.29x10^{-9}, slope = 0.000199, r^2 = 0.722, t_6 = 3.60, p = 0.015. Symbols: ▲ = *Varanus exanthematicus*, ● = *Oplurus cuvieri*, ♦ = *Laudakia stellio*, x = *Eulamprus quoyii*, ■ = *Cordylus warreni*, □ = *Tropidurus torquatus*, ○ = *Leiocephalus schreibersi*. Panel B: Phylogenetically independent contrasts for residual hindlimb-forelimb vs. residual medial impulse. Regression is forced through the origin: slope = 0.000214, r^2 = 0.629, t_5 = 2.91, p = 0.033. Numbers correspond to node numbers from phylogeny in Figure 3.