Biological Journal of the Linnean Society, 2009, **97**, 634–651. With 7 figures

The relationship between limb morphology, kinematics, and force during running: the evolution of locomotor dynamics in lizards

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Received 3 October 2008; accepted for publication 9 January 2009

Terrestrial locomotion occurs via the hierarchical links between morphology, kinematics, force, and center-of-mass mechanics. In a phylogenetically broad sample of seven lizard species, we show that morphological variation drives kinematic variation, which, in turn, drives force variation. Species with short limbs use a short stride–high frequency strategy when running at steady-speed and to change speeds. This link between morphology and kinematics results in relatively small vertical forces during the support phase of the stride cycle. Conversely, species with long limbs use a long stride–low frequency strategy, resulting in large vertical forces during the support phase. In view of these findings, we suggest that limb length may predict locomotor energetics in lizards because energetics are largely determined by vertical forces and stride frequency. Additionally, we propose an energetic trade-off with both long- and short-limbed species paying the most energy to move, whereas intermediate-limbed species move using less energy. Finally, when these traits are mapped onto a lizard phylogeny, we show that locomotor functional morphology exhibits both deep phylogenetic effects and contemporary patterns of evolutionary convergence. Overall, the present study provides a foundation for testing hypotheses regarding the integration and evolution of functional traits in lizards and animals in general. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **97**, 634–651.

ADDITIONAL KEYWORDS: biomechanics – force – kinematics – lizard – locomotion – morphology – running.

INTRODUCTION

When animals run, their limbs move in rhythmic cycles to propel them through the terrestrial environment. From an organismal perspective, terrestrial locomotion with limbs requires that variation of motor pattern and limb morphology will lead to a variation in stride kinematics, which, in turn, will lead to a variation in limb forces, ultimately leading to differences in center-of-mass dynamics and locomotor performance (Russell & Bels, 2001; Reilly, McElroy & Biknevicius, 2007). Thus, quantifying morphology, kinematics, and forces in an array of species provides a quantitative framework for understanding how morphology relates to locomotion, how complex functional systems evolve, and the ecological relevance of morphology (Arnold, 1983; Reilly & Wainwright, 1994). A myriad of studies have examined variation at the level of limb morphology (Alexander *et al.*, 1981; Bertram & Biewener, 1990; Miles, 1994; Miles, Losos & Irschick, 2007); limb kinematics (Sukhanov, 1968; Heglund & Taylor, 1988; Strang & Steudel, 1990; White & Anderson, 1994; Irschick & Jayne, 1999; Fischer *et al.*, 2002; Vanhooydonck, Van Damme & Aerts, 2002); and force production (Roberts *et al.*, 1998). In addition, some studies have established that morphology is related to stride kinematics (Strang & Steudel, 1990; White & Anderson, 1994; Irschick & Jayne, 1999; Vanhooydonck *et al.*, 2002) and that stride kinematics are related to forces (Heglund *et al.*, 1982; Full, 1989; Farley, Glasheen & McMahon, 1993; Farley & Ko, 1997; Chen *et al.*,

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2006; Reilly *et al.*, 2006). However, no study has simultaneously examined the inter-relationships among these three levels (morphology, kinematics, and force) across a morphologically diverse sample. The present study aimed to explain patterns of variation in the hierarchical relationship among morphology, kinematics, and force generation in a phylogenetic array of species with differing limb morphology. We note that our discussion of forces is really about the limb reaction forces that the organism produces, which must be equal and opposite to the ground reaction forces (i.e. part of the physical environment). Thus, limb reaction forces can and do evolve in response to the mechanical demands of supporting and propelling the body and are modulated by limb morphology and limb kinematics. However, to avoid confusion, and in accordance with previous studies of locomotor mechanics, we present ground reaction forces.

How must force covary with stride kinematics and limb morphology? It has been established that animals with shorter limbs have to step more often (i.e. higher stride frequency) than longer-limbed animals to attain fast speeds (Vanhooydonck *et al.*, 2002). In addition, ample studies of ground reaction forces show that, during steady speed locomotion, the sum of vertical force (i.e. vertical impulse) over a full stride divided by stride time must be equal to body weight (Biewener, 2003). However, general comparisons across speeds show that stride length, stride frequency, and float distance (kinematic speed-effects) directly influence how force is propagated during the support phase of the stride cycle (Biewener, 2003). Thus, species with short limbs should move at high stride frequency and, as a result, produce less vertical force per ground contact because they have more ground contacts (i.e. higher stride frequency) per unit time. Conversely, species with longer limbs will have fewer support phases over a given time (i.e. low stride frequency), leading to the necessity of more vertical force per support to effectively support body weight. The above arguments are based on the physical laws governing locomotion; ground reaction forces must be determined by an organism's morphology and kinematics. In view of these laws, studies of limb morphology and kinematics have made a variety of predictions regarding how morphological and kinematic variation should dictate ground reaction forces. For example, (1) species taking long strides at low frequency should generate more force than species taking shorter strides at higher frequency (Van Damme, Aerts & Vanhooydonck, 1998; Irschick & Jayne, 1999; Aerts *et al.*, 2000) and (2) the relative magnitudes of vertical and accelerative force should be good predictors of the distance travelled during the aerial phase (i.e. floating distance) of the stride cycle (Irschick & Jayne, 1999). However, empirical data testing these hypotheses, and thus clearly demonstrating that ground reaction forces are indeed determined by limb morphology and kinematics, remain scarce. In the present study, we first quantify the multivariate patterns in limb skeletal morphology, stride kinematics, and force profiles for seven species of terrestrial lizards. Forces are quantified as both impulses integrated over support duration and peak forces. Then, multivariate statistical analyses test for differences within levels and correlations among levels to suggest the morphological basis for kinematics and locomotor forces. The results obtained illustrate how the dynamics between limb length, support duration, and float distance likely influence the forces applied to the ground during the support phase of steady-state locomotion.

MATERIAL AND METHODS

STUDY SPECIES

Locomotor morphology and function were quantified in the following seven species of lizards (Fig. 1): *Laudakia stellio* Linnaeus (Agamidae), *Oplurus cuvieri* Gray (Iguanidae), *Tropidurus torquatus* Wied-neuwied (Iguanidae), *Eulamprus quoyii* Quoy and Gaimard (Scincidae), *Tracheloptychus petersi* Peters (Cordylidae), *Acanthodactylus boskianus* Daudin (Lacertidae), and *Varanus exanthematicus* Bosc (Varanidae). These species were chosen for analysis because they were all capable of high-speed running but encompassed a range of limb morphologies. All species were obtained from commercial dealers, except *Eulamprus quoyii*, which was wild

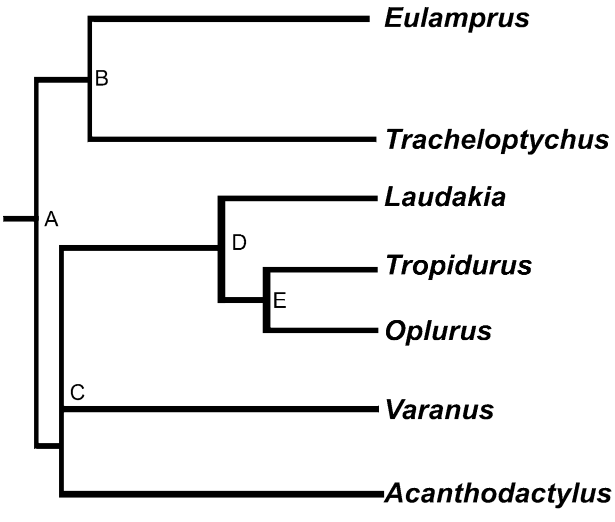

Figure 1. Phylogeny for the lizards in this study *sensu* Townsend *et al.* (2004). Branching nodes are labelled A–E for discussion of phylogenetic patterns of locomotor functional evolution.

Table 1. Species sample size for morphology (number of individuals) and locomotor function (number of trials)

	Morphology	Kinematics and force	Running speed ($m\ s^{-1}$)	Dimensionless speed
	N	*N*	Range	Range
Acanthodactylus boskianus	30	6	1.88–0.82	2.81–1.23
Eulamprus quoyii	29	10	1.92–0.71	2.87–1.06
Laudakia stellio	33	13	2.40–0.82	2.77–0.95
Oplurus cuvieri	21	17	1.69–0.59	1.80–0.63
Tracheloptychus petersi	12	6	1.41–1.01	1.97–1.42
Tropidurus torquatus	7	9	2.62–1.37	3.15–1.65
Varanus exanthematicus	13	11	2.54–0.76	2.78–0.83

caught in Brisbane, Australia. All housing and experimental procedures were conducted in accordance with approved animal use protocols.

MORPHOLOGY

To quantify locomotor morphology, we took ventral view radiographs of multiple individuals per species (samples sizes are shown in Table 1) and measured the lengths of the several morphological variables to the nearest 0.01 mm using digital calipers: snout–vent, humerus, ulna, carpal, third metacarpal, third finger, pectoral girdle width, femur, tibia, tarsal, fourth metatarsal, fourth toe, fifth metatarsal, fifth toe, pelvis length, and pelvis width (Fig. 2). These variables were used because previous studies have found that they are related to locomotor function and performance in lizards (Miles, 1994; Irschick & Jayne, 1999; Miles *et al.*, 2007). We used dissecting pins to press the lizards limbs as close to the radiograph paper as possible to minimize the effect of parallax. All specimens were obtained via museum loans (catalogue numbers are provided in the Appendix).

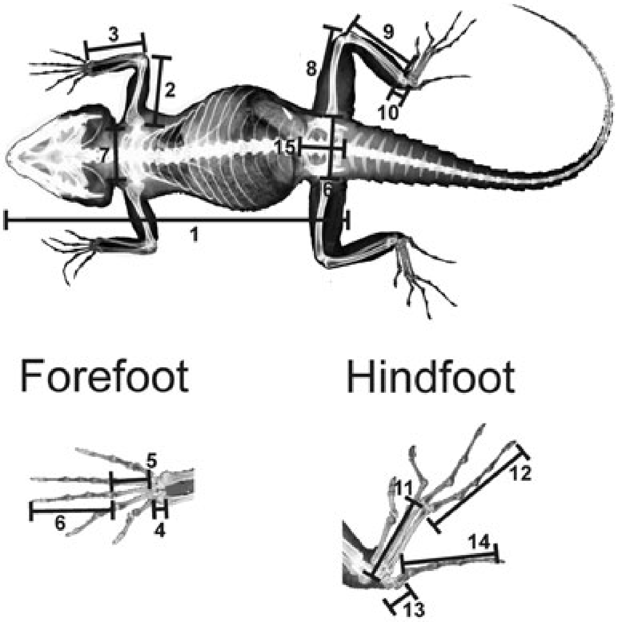

Figure 2. Ventral radiograph of *Laudakia stellio* summarizing the 16 morphological measurements. 1, snout–vent; 2, humerus; 3, ulna; 4, carpal; 5, third metacarpal; 6, third finger; 7, pectoral girdle width; 8, femur; 9, tibia; 10, tarsal; 11, fourth metatarsal; 12, fourth toe; 13, fifth metatarsal; 14, fifth toe; 15, pelvis length; 16, pelvis width. Note that when toes were bent (measurements 6, 12, and 14), we summed the measures of the length of individual phalanges.

LOCOMOTOR FUNCTION

Data collection

Stride kinematics and force were studied as lizards sprinted down a racetrack towards a dark hide box. Running was induced by gently pressing on the tail or hindlimb. Running trials were recorded over a range of running speeds, including near-maximum sprint speed for each species (Table 1). All trials consisted of lizards using running (spring-mass) mechanics and a diagonal-couplet gait. To avoid fatigue effects, each individual was run down the racetrack up to three times and then allowed to rest and recover for 24 h before subsequent trials. All species were maintained at 36–40 °C for the duration of each trial. Temperature was checked during each trial with an infra-red laser thermometer aimed along the body axis.

Stride kinematics

Lizards were filmed at 120 or 500 Hz (small, fast lizards required higher frame rates) with high-speed video cameras (JVC GRL-9800 and NAC HSV-500 C3) mounted 1 m above the surface of the force platform. Mirrors were mounted on angled walls along each side of the force platform to visualize footfalls. Kinematic analyses were conducted using APAS, version 1.0 Ariel Dynamics, INC. Speed during each trial was quantified by digitizing the tip of the snout as the lizard crossed seven evenly-spaced lines along the surface of the racetrack and calculating average speed across the entire field of view. Only trials with < 20% difference between any interval speed and the average speed were used. The timing of touch-down and lift-off for each limb was recorded in these steady speed trials. The velocity and the timing of foot touch-down and lift-off were used to calculate the kinematic variables: (1) stride length, which is the distance travelled by the center-of-mass during one entire hindlimb cycle, calculated by multiplying velocity by the amount of time between ipsilateral hindlimb touchdowns; (2) stride frequency, which comprises hindlimb strides per second, calculated as the inverse of the amount of time between ipsilateral hindlimb touchdowns (stride duration); (3) step length, which is the distance travelled by the center-of-mass when a hindlimb-forelimb couplet contacted the ground, calculated as velocity multiplied by the amount of time a couplet contacted the ground; and (4) float distance, which is the distance travelled by the center-of-mass when no limbs contact the ground, calculated as stride length minus two times step length. Support duration was defined as the time from the touchdown of the first limb of a couplet (fore or hindlimb) until the lift-off of the last of that pair of supporting limbs.

Whole body locomotor forces

Ground reaction forces were quantified and using a custom-made force platform based on a strain gauge, spring-blade design (Bertram *et al.*, 1997). Vertical (V), fore-aft (FA), and medio-lateral (ML) ground reaction forces were sampled at 500 Hz using National Instruments data acquisition hardware and LABVIEW custom designed virtual data sampling and quantification instruments. The 0.6 m (length) by 0.2 m (width) force platform surface was flush with the racetrack surface and located 3–3.6 m along its 5.2-m length. The entire surface of the racetrack and platform was covered with fine grit sandpaper to prevent foot slippage.

From the whole body ground reaction forces, we quantified 13 force variables. These variables describe the shapes and amplitudes of force profiles to be quantitatively compared across species in relation to morphology and gait (Fig. 3). Peak forces were measured as the maximum force in each direction: peak vertical, braking (negative value), accelerative, and lateral forces (absolute value for lateral to account for left versus right limb pushes). The remaining nine time-related variables were measured relative to support duration (i.e. limb morphology and kinematics affect locomotor forces applied to the substrate by the limbs). Impulses were measured as the area under the force curve (i.e. the numerical integration of the force over limb contact time) for vertical, braking, accelerative, and lateral directions. Time to peak vertical, braking, accelerative, lateral force, and time of the braking–accelerative transition point (when the fore–aft force profile switched from nega-

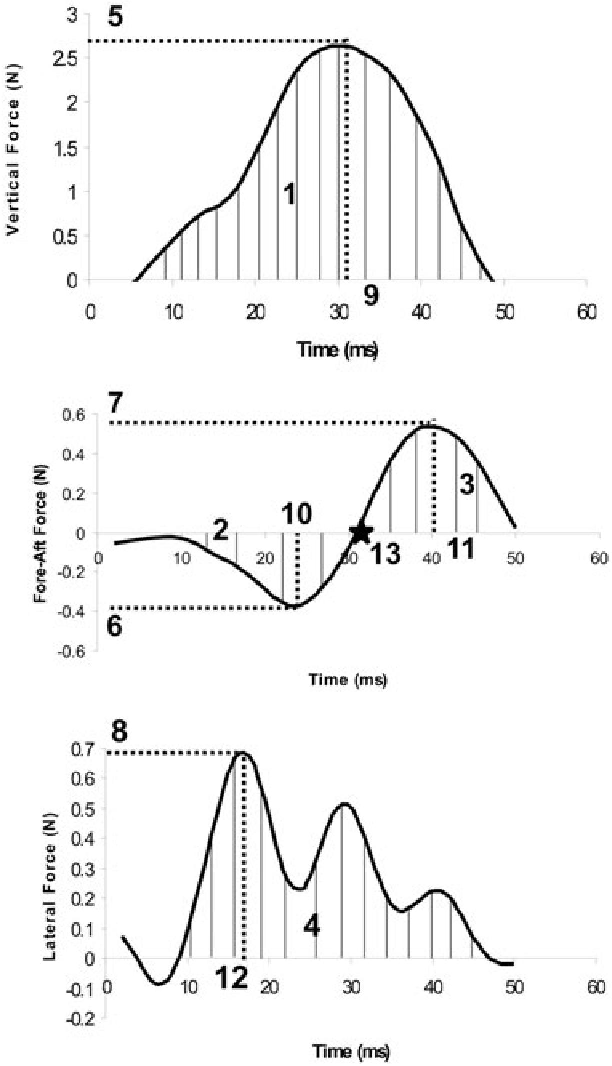

Figure 3. Representative whole body ground reaction force profiles. Impulses are represented by hatched areas; dotted lines correspond to the peak or time of peak force. 1, vertical impulse; 2, braking impulse; 3, accelerative impulse; 4, lateral impulse; 5, peak vertical force; 6, peak braking force; 7, peak accelerative force; 8, peak lateral force; 9, time to peak vertical force; 10, time to peak braking force; 11, time to peak accelerative force; 12, time to peak lateral force; 13, time of braking–accelerative transition, indicated by a star.

tive to positive values) were also measured and scaled to percentage of support duration.

STATISTICAL ANALYSIS

Prior to statistical analysis, some variables were transformed to approximate a normal distribution. All morphological variables float distance, impulses, and peak forces were $\log_{10}$ transformed. All other variables were untransformed.

Correcting for size and speed

To examine the relationship between morphology, stride kinematics, and whole body forces across species, we had to control for differences in both body size and running speeds. Morphology, stride kinematics, and forces are known to covary with body size (Mullineaux *et al.*, 2006), and they covaried with size in the species in the present study (see Supporting information, Table S1). To correct for the effect of body size, we regressed morphological variables against snout–vent length (both length measurements), kinematics against body mass, and forces against body weight (both force measurements) and used the residuals from these regression for further analysis. Preliminary analyses showed that regressing kinematics against body mass versus snout–vent length had no qualitative effect on the results.

Kinematics and force are also known to covary with running speed (Riggs *et al.*, 1993; McLaughlin *et al.*, 1996). Speed effects were evident in kinematics and force variables (see Supporting information, Table S1); thus, we corrected for speed by regressing the size corrected residuals for each variable (from the regression of body mass or weight) against speed and used these residuals for further analysis. Thus, morphological variables were corrected for body size, whereas kinematic and force variables were corrected for both body size and speed.

Finally, many studies attempt to adjust for speed and size differences across species by comparing effects at dimensionless speed (Farley *et al.*, 1993). We compared the results of using speed corrected kinematic and force variables (as described above) with a parallel analysis using dimensionless speed. Dimensionless speed was defined as $v\,g^{-1/2}\,l^{-1/2}$ (where v is speed, g is gravitation acceleration, and l is leg length) for each trial (Farley *et al.*, 1993). Leg length as opposed to hip height (Irschick & Jayne, 1999) was used because did not have detailed lateral videos from which to estimate hip height. Results from analyses based on raw speed and dimensionless speed produced the same statistical outcomes for the multivariate analyses relating morphology, kinematics, and forces. Thus, we report the results from analyses based on raw speed.

Multivariate species differences in morphology, kinematics, and force

Prior to multivariate analyses, all variables were standardized to a mean of zero and a variance of one. This placed all variables on the same scale and eliminated the problem of variables on larger scales having a larger influence on the results than variables on smaller scales (Quinn & Keough, 2002).

Each of the levels of analysis contained numerous inter-correlated variables, which can be a major problem for multivariate analyses. We addressed this issue in two ways. For, morphological and kinematic variables we conducted separate principal components analyses (PCA) to reduce the dimensionality of the data to a few PCs that describe the most of the variation in morphological or kinematic variables. By definition, the PC axes are orthogonal and uncorrelated, thus removing the effect of collinearity in the data prior to further multivariate analysis (see below). PCs were considered significant if they had eigenvalues >1 and had a percentage variation explained greater than that expected by the broken stick model (Quinn & Keough, 2002). Only significant PC axes were used in subsequent multivariate analyses.

The PCA on the force variables was not useful for addressing collinearity because it generated numerous ‘significant’ axes that explained small fractions of the variance in the force data. Therefore, we addressed collinearity in the force data by eliminating highly inter-correlated variables from multivariate analysis (Quinn & Keough, 2002). Whole body impulses were always highly correlated with whole body peak forces in each direction ($r > 0.65$) and braking impulses/forces were highly correlated with accelerative impulses/forces (because animals were moving at near-steady speed). Thus, the reduced data set included only seven variables: vertical, accelerative, and lateral impulses, and the relative time to peak vertical, braking, lateral, and braking–accelerative transition. Force variables in this reduced data set had weak correlations ($r < 0.40$), indicating that collinearity would not be problematic in subsequent multivariate analyses. Thus, the multivariate analyses of species differences used PC axes for morphology, kinematics and a reduced force data set (seven variables).

Discriminant function analysis (DFA) was used at each level (morphology, kinematics and force) to determine statistical differences among species in multivariate space. DFA extracts axes that are linear combinations of the original variables that maximize the probability of correctly assigning the data to predetermined groups (Quinn & Keough, 2002). Separate DFAs were run on morphology, kinematics, and force levels with species as the predetermined groups.

Standardized discriminant coefficients were used to determine the relationship between discriminant axes and the PC axes (morphology and kinematics) or original variables (forces). Differences among species on each level were identified by computing Mahalanobis distances (D^2) and associated F-statistics and significance tests between species centroids for DF1 and 2 (SAS Institute, 2001). Species that were not significantly different were placed in the same group; whereas species that were significantly different were placed in different groups.

Relationships between multivariate levels (morphology, kinematics, and force)

After summarizing the differences between species at each level, three approaches were used to examine relationships among species between morphological, kinematic, and force levels. First, a qualitative comparison of relationships among levels was performed visually by comparing relative positions of significantly different groups at each multivariate level (Reilly & Lauder, 1992). Second, a Mantel test assessed significant multivariate correlations between each level. The Mantel test compared matrices of Mahalanobis distances between the centroids of each species in discriminant space at one level with those of another level. The software ZT (Bonnet & Van de Peer, 2002) was used to generate 5000 randomizations to compute the correlation between: (1) morphology and kinematics, (2) kinematics and force, and (3) morphology and force. A significant Mantel tests indicates correlation between levels, but does not specify which aspects of morphology, kinematics, or force drive the correlation. Third, to identify which variables are correlated across levels, separate multiple regressions were computed between the first (and second) discriminant axes across levels. In this test, the morphology and kinematic levels were independent variables, whereas force was the dependent variable. Species values for multiple regressions were their mean scores on each discriminant axis. The combination of these three techniques allowed for the thorough examination of the relationship between species position in multivariate space at different levels of the analysis.

Finally, we employed partial least squares to test for relationships between: (1) morphology and kinematics and (2) kinematics and force. Partial least squares is ideal for this data set because (1) these data violated some of the assumptions of discriminant analysis (equal group sizes and variances) and (2) collinearity can be explicitly handled without using PCs or eliminating variables allowing for the direct estimation of the relationship of each variable between levels. Species mean values for each variable were used as input for the analysis. We used cross-validation to avoid over-fitting the data and to determine the number of factors to keep in the final analysis.

Effect of phylogeny

The evolutionary relationships among species make them non-independent data points; thus, traditional statistical analyses are clouded by phylogeny (Felsenstein, 1985). To control for the effects of phylogeny, we computed independent contrasts (Garland, Harvey & Ives, 1992) in the PDAP module of MESQUITE (Midford, Garland & Maddison, 2002; Maddison & Maddison, 2007) between species means on the first discriminant axis for each level. There was no relationship between the absolute values of standardized contrasts and their standard deviation, indicating that the contrasts were adequately standardized (Garland *et al.*, 1992). Thus, we re-ran the multiple regression with the independent contrasts of each of the first discriminant axes. The regression was forced through the origin (Garland *et al.*, 1992). A significant multiple regression would indicate that species scores on the first discriminant axis have undergone correlated evolution. The Townsend *et al.* (2004) phylogeny was use for all analyses (Fig. 3). Branch lengths were unavailable; therefore, we set all branch lengths to one, which does not substantially impact the outcome of phylogenetic comparative analyses (Diaz-Uriarte & Garland, 1998), nor the outcome of similar studies of lizard locomotor function (McElroy, Hickey & Reilly, 2008).

Finally, squared-change parsimony in MESQUITE (Maddison & Maddison, 2007) and maximum likelihood (ML) in ANCML (Schluter *et al.*, 1997) were both implemented to calculate the ancestral character states of species mean scores on DF1 and DF2 for morphology, kinematics, and force. We used both techniques because squared-changed parsimony can calculate ancestral states for phylogenies with polytomies (*sensu* the phylogeny of Townsend *et al.*, 2004; Fig. 1), whereas, ML cannot handle polytomies but can calculate standard errors and confidence intervals (CIs) around nodal estimates. For ML, we addressed polytomies by examining all possible resolutions; however, different resolutions did not materially alter the results. Squared change parsimony ancestral states were then plotted in discriminant space at each functional level. ML 95% CIs were used to infer significant evolutionary shifts (McElroy *et al.*, 2008).

RESULTS

MULTIVARIATE PATTERNS IN LOCOMOTOR LEVELS

Morphology

The PCA on size-corrected morphological variables extracted two axes that together explained 80% of

Table 2. Results of the multivariate morphological analysis

	Morphological PCA			Morphological DFA	
	PC1	PC2		DF1	DF2
Eigenvalue	10.47	1.49	Eigenvalue	9.42	1.61
% Variance	70%	10%	% Variance	79%	14%
Humerus	0.29	–0.02	PC1	9.03	0.01
Ulna	0.28	–0.11	PC2	1.97	9.50
Carpals	0.19	–0.34			
Third metacarpal	0.28	–0.20			
Third toe	0.25	–0.15			
Pectoral width	0.27	0.06			
Femur	0.30	0.06			
Tibia	0.30	–0.01			
Tarsals	0.24	–0.14			
Fourth metatarsal	0.29	0.06			
Fourth toe	0.21	0.41			
Fifth metatarsal	0.27	–0.13			
Fifth toe	0.28	0.15			
Pelvic width	0.27	0.21			
Pelvic length	0.04	0.73			

Loadings are calculated between each principal component and each kinematic variable. Standardized coefficients are calculated between each discriminant axis and each principal component.
PCA, principle componemt analysis; DFA, discriminant function analysis.

the morphological variance (Table 2). PC1 explained 70% of the variance in morphology and had uniform moderate positive loadings for all morphological variables (except pelvic length), indicating that all limb segmental lengths and pelvic width increased in relative size along PC1. Thus, species with positive values on PC1 had relatively longer fore and hindlimbs, whereas species that had negative values had relatively shorter fore and hind limbs relative to their size. PC2 explained 10% of the morphological variance and had a strong positive loading for pelvis length. Thus, species with positive values on PC2 had relatively long pelves, whereas species with negative values had relatively short pelves.

The DFA on these two morphological principal components extracted two axes (Fig. 4) explaining 93% of the variation in morphology (Table 2) and indicated that there are statistically significant differences between species (Wilk's $\lambda = 0.020$, $F_{18,388} = 65.1$, $P < 0.0001$). DF1 and 2 closely approximated principal components 1 and 2, respectively, because each PC axis variable scored large and positively on only one discriminant axis (Table 2). Thus, PC loadings for morphological variables could be interpreted along the DF axes with DF1 being related to relative limb length (relatively longer limbs had large positive values) and DF2 being related to relative pelvic length (relatively longer pelves had large positive values). Mahalanobis distances between species centroids revealed that all of the species were significantly different in morphospace (all $P < 0.0001$). Species lined up on DF1 from the relatively shortest limbs of the skink (*Eulamprus*) through the relatively average limbs in the Varanid (*Varanus*), Lacertid (*Acanthodactylus*), and the Gerrhosaurid (*Tracheloptychus*), to the relatively longest limbs in the Iguanians (*Tropidurus*, *Oplurus*, and *Laudakia*). *Tracheloptychus* (a Gerrhosaurid) had a relative limb length similar to *Acanthodactylus* and *Varanus* but was significantly different on DF2, revealing its novel extremely long pelvis.

Kinematics

The first two PCs of the size- and speed-corrected kinematic variables together explained 96% of the variance in kinematics (Table 3) and were clearly correlated with different variables. PC1 explained 58% of the kinematic variance and had a large positive loading for stride length and a large negative loading for stride frequency. Thus, species with positive values on PC1 took relatively long strides at low stride frequencies, whereas species with negative values took relatively short strides at high stride frequencies. PC2 explained 38% of the kinematic variance and had a strong positive loading for float dis-

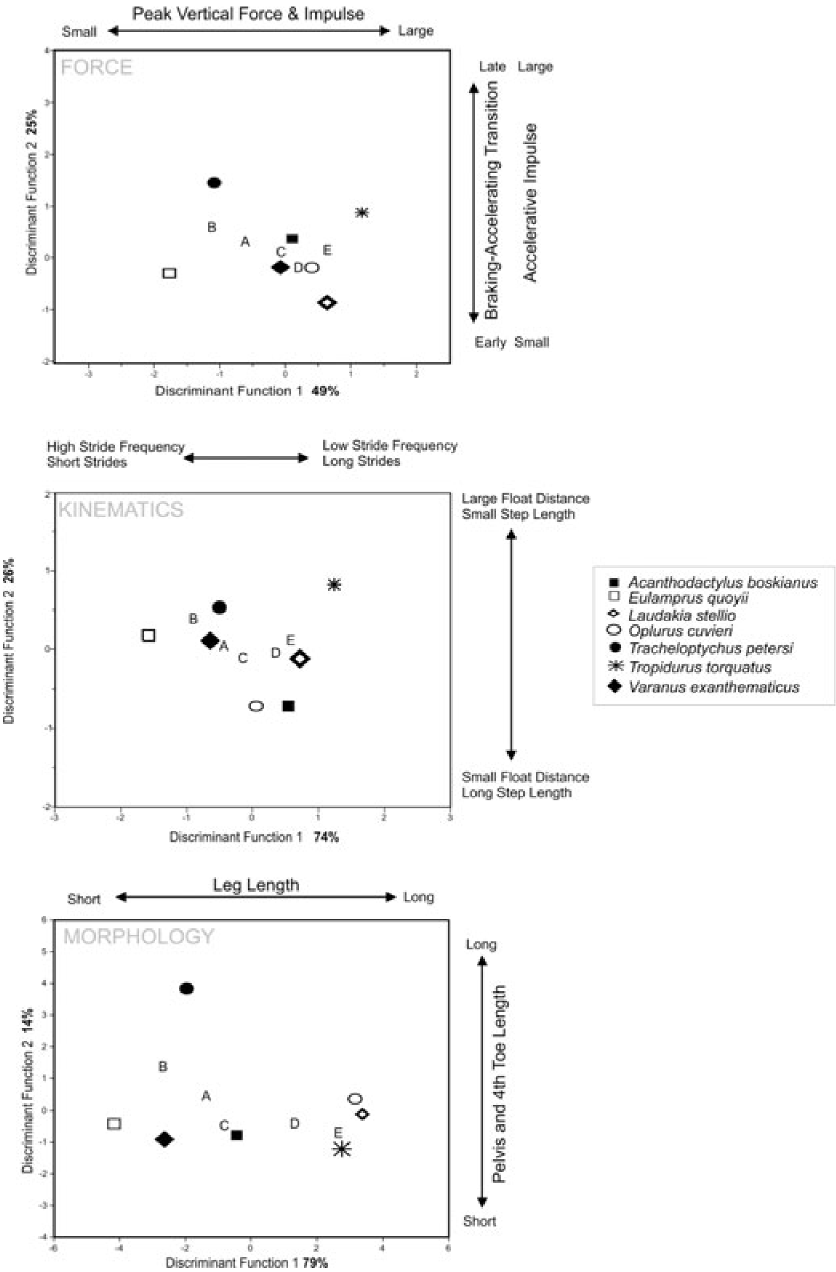

Figure 4. Bivariate plot of the first two discriminant axes for morphology, kinematics, and forces. The percentage variation explained is labelled with each axis. The variables with the largest coefficients on each axis (from Tables 3, 4, 5) are labelled opposite to that axis. Values for squared change parsimony ancestral reconstructions for nodes (A–E from Fig. 1) are plotted on each level.

tance and a strong negative loading for step length. Thus, species with positive values on PC2 had strides characterized by relatively short steps and long float distances, whereas species with negative values had strides characterized by relatively long steps and short float distances.

The DFA on these two kinematic principal component axes extracted two axes that explained 99% of the kinematic variation (Table 3). DF1 and 2 were highly correlated with PC1 and 2, respectively, based on large and positive loadings (Table 3). Therefore, the DF axes could be related to the original kinematic variables: large scores on DF1 depict species with relatively long stride length and low stride frequency, whereas small scores represent relatively short strides at high frequency. Large scores on DF2

Table 3. Results of the multivariate kinematic analysis

	Kinematics PCA			Kinematics DFA	
	PC1	PC2		DF1	DF2
Eigenvalue	2.32	1.53	Eigenvalue	0.83	0.29
% Variance	58%	38%	% Variance	74%	26%
Stride length	0.64	0.03	PC1	0.85	0.06
Stride frequency	–0.64	–0.04	PC2	–0.04	0.88
Step length	0.26	–0.73			
Float distance	0.34	0.68			

Loadings are calculated between each principal component and each kinematic variable. Standardized coefficients are calculated between each discriminant function axis and each principal component axis.
PCA, principle componemt analysis; DFA, discriminant function analysis.

describe species taking relatively short steps with long floats, whereas small scores depict species taking relatively long steps with short floats. Species were significantly different in DF (Wilks' $\lambda = 0.423$, $F_{12,124} = 5.54$, $P < 0.0001$) with Mahalanobis distances showing that the species clustered into two different groups [P within groups, range 0.071–0.725 (except *Tropidurus*, see below), P between groups, range 0.001–0.047] in kinematic space (Fig. 4). *Eulamprus*, *Varanus*, and *Tracheloptychus* clustered together and were characterized by relatively short strides at high frequency and intermediate step length and float distance. *Oplurus*, *Acanthodactylus*, *Laudakia*, and *Tropidurus* clustered in the other group with relatively long strides at lower frequency with longer steps and shorter float distances, except *Tropidurus* that exhibited relatively short steps with large float distances (Mahalanobis significance, *Tropidurus* to *Laudakia*, $P = 0.071$; to *Oplurus*, $P < 0.001$; to *Acanthodactylus*, $P = 0.0120$).

Forces

The DFA on the reduced set of size- and speed-corrected whole body force variables extracted two axes that explained 74% of the force variance (Table 4). DF1 accounted for 49% of the force variance and had a large positive score for relative vertical impulse and relatively weak scores for all other variables (Table 4). Because of the significant correlation between relative vertical impulse and relative peak vertical force ($r = 0.709$, $P < 0.00001$), we interpreted this axis as representative of both of these vertical force variables. Time to peak vertical force was not correlated with vertical impulse ($r = -0.182$, $P = 0.132$) or peak vertical force ($r = -0.054$, $P = 0.658$). DF2 accounted for 25% of the force variance and had a large positive score for the relative timing of the braking–accelerative transition and accelerative impulse (Table 4). Species were significantly different (Wilk's $\lambda = 0.246$, $F_{42,271} = 2.25$, $P < 0.0001$) with Mahalanobis distances demonstrating that species clustered into two different groups in force space (Fig. 5). *Eulamprus* with the smallest relative vertical forces and impulses was significantly different from the rest of the species (Mahalanobis' significance P range = 0.001–0.038), which exhibited relatively larger vertical forces and impulses. The extremes of this group on DF1 (*Tropidurus* and *Tracheloptychus*) also had large scores on DF2. These two species positions approached significance (Mahalanobis' significance: *Tropidurus* to *Acanthodactylus* $P = 0.133$, to other species $P < 0.050$; *Tracheloptychus* to *Acanthodactylus* $P = 0.095$, to other species $P < 0.050$), indicating larger times to the braking–acceleration transition and larger accelerative impulse compared to the rest of the group.

Table 4. Results of the discriminant function analysis of whole body force data

	DF1	DF2
Eigenvalue	0.85	0.45
% Variance	49%	25%
Vertical impulse	1.29	0.05
Accelerative impulse	0.05	0.57
Lateral impulse	0.07	0.04
Time peak vertical force	0.22	–0.01
Time braking–accelerative transition	–0.45	1.12
Time peak braking force	–0.01	0.02
Time peak lateral force	–0.31	–0.05

Standardized coefficients are calculated between each discriminant function (DF) axis and each whole body force variable.

RELATIONSHIPS BETWEEN MULTIVARIATE LEVELS

The a priori model of the causal links for terrestrial locomotion predicted that morphological variation

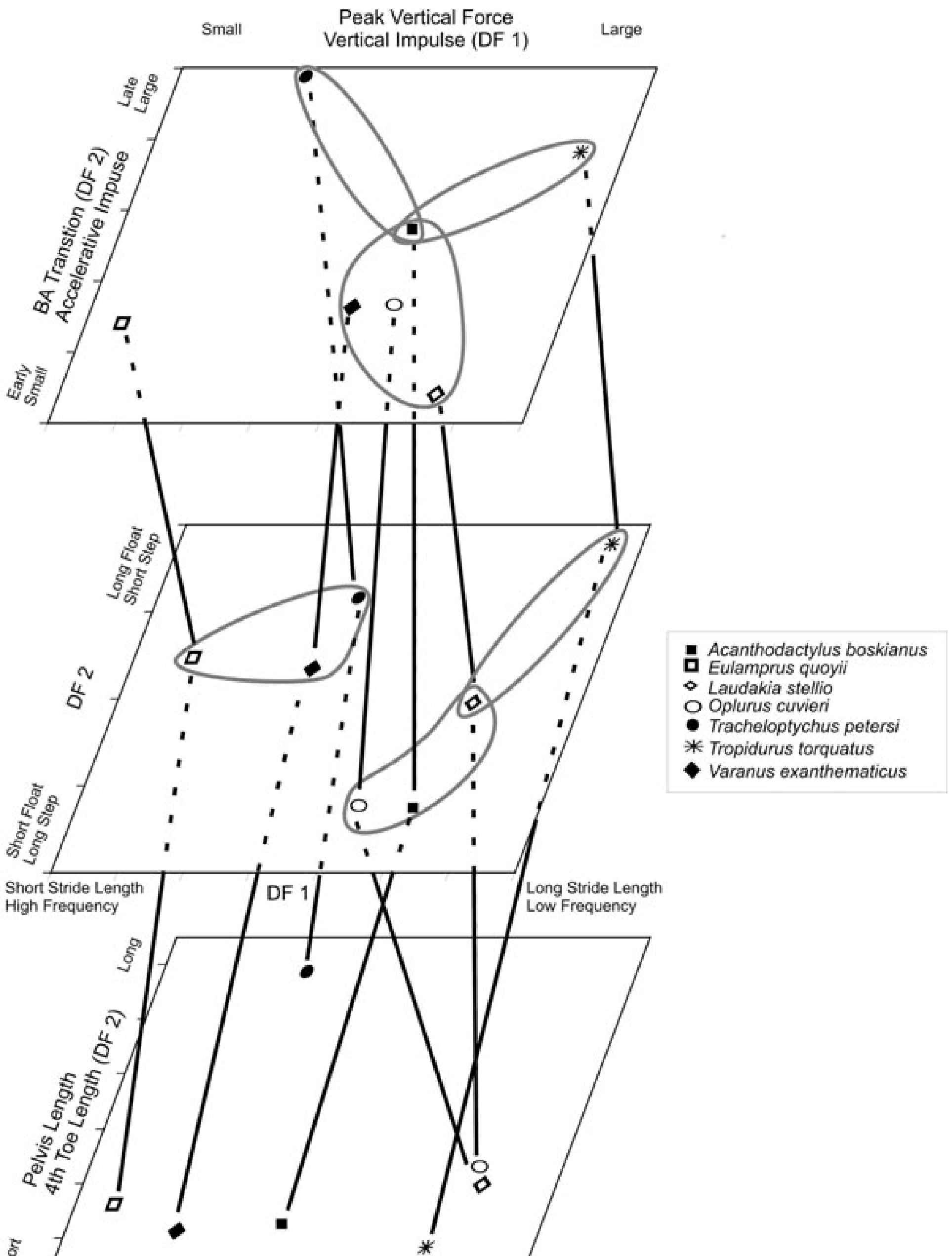

Figure 5. Visualization of the multivariate relationship between morphology, kinematics, and whole body force in the seven lizard species from the present study plotted *sensu* Reilly & Lauder (1992). Each plane represents the discriminant space for each level of analysis (from Figs 4, 5, 6) with ellipses surrounding groups at each level determined by Mahalanobis distances and associated *F*-tests. Lines connect species' centroids between levels. Note that the lines are almost vertical, indicating correspondence in species position on the first discriminant axis on each level. DF, discriminant function.

should predict kinematic variation, which, in turn, should predict force variation (Reilly *et al.*, 2007). To examine the relationships between levels, we created a three-dimensional plot that stacked each of the multivariate levels (Reilly & Lauder, 1992) aligned along the major axes of variation (DF1 and 2). Then, we examined relative positions of species between morphological and kinematic levels and then between

Table 5. Results of Mantel tests for the multivariate correlation between species position in discriminant function space at each level of analysis

	Kinematics (velocity)	Kinematics (dimensionless)	Force (velocity)	Force (dimensionless)
Morphology	0.66**	0.66**	0.84***	0.60**
Force (velocity)	0.79***	–	–	–
Force (dimensionless)	–	0.59**	–	–

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Statistical significance was determined by a randomization procedure. Correlations were calculated for velocity corrected and dimensionless speed corrected data (indicated in parentheses).

kinematic and force levels (Fig. 5). Specifically, species were related between levels:

1. *Eulamprus* was positioned on the left at each level and was significantly different from all other species on the morphology and force, but not kinematics levels. It was characterized by relatively short limbs, short stride length, high stride frequency, and small vertical impulse and peak force.
2. *Tropidurus* was positioned on the right at each level and approached statistically significant differences from other species at the kinematic and force levels. It had relatively long limbs, long stride length, low stride frequency, and large vertical impulse and peak force. *Tropidurus* also had that longest float distance and shortest step length of any species in the present study.
3. *Varanus* and *Tracheloptychus* were positioned centrally on all three levels, with both having legs of moderate length and intermediate stride length and frequency. However, *Varanus* had a short pelvis and fourth toe whereas *Tracheloptychus* had a long pelvis and fourth toe and this difference seemed to manifest itself in *Varanus* having relatively smaller accelerative impulse and an earlier braking–accelerative transition than *Tracheloptychus*.
4. *Oplurus* and *Laudakia* were positioned on the right in morphological space but centrally in kinematic and force spaces. These species had relatively long legs, long stride length and low stride frequency, and moderate-to-large vertical impulse and peak force. Although it had relatively shorter limbs, *Acanthodactylus* clustered together with these species at both the kinematic and force levels.

More generally, comparisons across levels suggest a linear spread corresponding to the first discriminant axis on each level. Species position on DF1 at the morphological level generally corresponds to their position on DF1 at the kinematic level and at the force level. Thus, increasing relative limb length corresponds to increasing relative stride lengths and lower stride frequencies. Similarly, increasing relative stride lengths and lower stride frequencies are related to increasing relative vertical forces and impulses. Three statistical tests of these relationships confirm these integrative patterns.

First, the Mantel tests indicated a significant positive correlation between the morphological and the kinematic levels ($R = 0.78$, $P = 0.0120$; Table 5) and between the kinematic and force levels ($R = 0.88$, $P = 0.0004$, Table 5). Together, these results show that there is a significant multivariate correlation between species position in morphological, kinematic, and force discriminant spaces.

Second, to uncover which variables were driving the multivariate correlations between levels, we ran a multiple regression testing for the effects of morphology and kinematics as a predictor of force. The multiple regression revealed that the morphological and kinematic levels combined to become an excellent predictor of species' centroid position on the force level for DF1 ($R^2 = 0.87$, $F_{2,4} = 13.26$, $P = 0.017$) but not on the DF2 ($R^2 = 0.44$, $F_{2,4} = 1.58$, $P = 0.38$). Thus, there is a statistically significant relationship between limb length, stride length and frequency, and vertical forces and impulses (Fig. 6).

The results of the partial least squares analysis were similar to the results of the multiple regressions based on DF axes. There was one factor between morphology and kinematics. This factor accounted for 81% of the morphological variation and explained 14% of the kinematic variation. The model coefficients indicated a positive relationship between limb skeletal element lengths (except pelvis length) and stride length but a negative relationship with stride frequency. Thus, species with longer limbs ran with longer strides at low frequency, whereas species with shorter limbs ran with shorter strides at high frequency. There was one factor between kinematics and force. This factor accounted for 49% of the kinematic

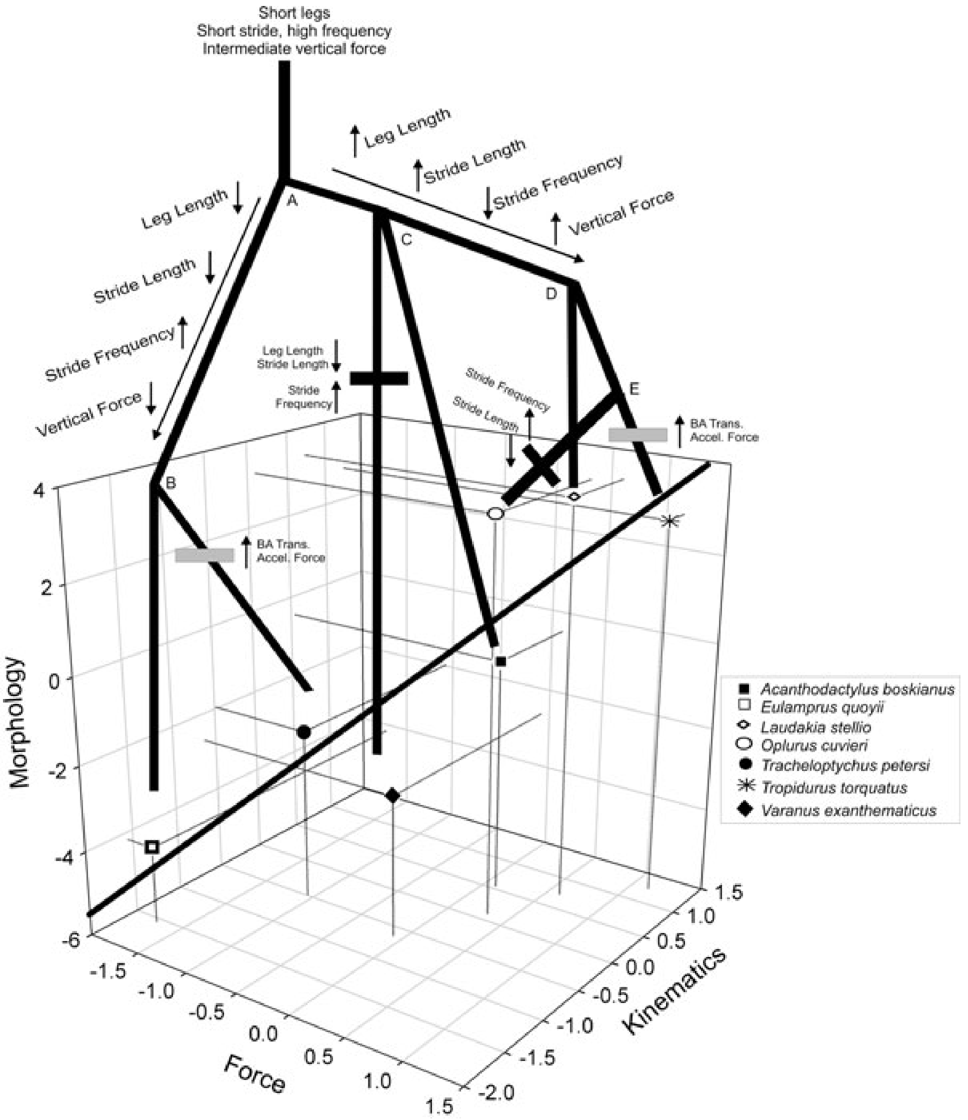

Figure 6. Three-dimensional plot of the first discriminant axis at each level of analysis. Data points are species' means on the first discriminant axis for each level. The bold line (best fit line from multiple regression) highlights the tight relationship between species position along on the first discriminant axis. Long-limbed species take longer strides at lower frequency, resulting in large vertical whole body forces and impulses. We plotted an inverted version of the phylogeny of Townsend *et al.* (2004), in which tips corresponded to species position in multivariate space, and then mapped multivariate patterns of character evolution onto the phylogeny; for further details, see Discussion. Black and grey bars are evolutionary trends on DF1 and 2, respectively, which have resulted in convergence. *Statistically significant evolutionary transition.

variation and explained 32% of the force variation. The model coefficients described a positive relationship between stride length and float distance and all impulses and peak forces, and a negative relationship between stride frequency and all impulses and peak forces. The signs of the coefficients between force variables and stride length were equal in magnitude and opposite in sign to those for stride frequency. Thus, species taking longer strides with longer float distances at low stride frequency exhibited greater magnitude ground reaction forces, whereas species with shorter strides and float distances at higher stride frequency exhibiter smaller ground reaction force.

Finally, to account for the effect of phylogeny on the relationship between multivariate levels, we re-ran the multiple regression on the independent contrasts for species centroids position on DF1. The indepen-

Table 6. Regression parameters showing the relationship between raw stride kinematics and speeds for the seven lizard species

	Stride length		Stride frequency	
Species	Slope	Intercept	Slope	Intercept
Acanthodactylus	0.65*	–0.60	0.89*	0.66
Eulamprus	0.02	–0.82	1.99*	1.10
Laudakia	0.80*	0.37	0.52*	–0.51
Oplurus	0.40*	0.24	0.86*	–0.32
Tracheloptychus	1.38*	–0.94	–0.17	1.13
Tropidurus	1.27*	–0.27	0.23	–0.01
Varanus	0.37*	1.02	0.89*	–1.02

*Statistical significance ($P < 0.05$) of regression slopes was determined by *t*-tests.

dent contrasts multiple regression revealed that morphology, kinematics, and force have undergone correlated evolution ($R^2 = 0.86$, $F_{2,3} = 9.35$, $P = 0.050$). This indicates that species evolving relatively longer limbs also evolve relatively longer strides at lower frequency and relatively large vertical forces and impulses. This relationship is plotted in Figure 6 with the phylogeny of the study species superimposed on the three-dimensional relationships of morphology, kinematics and force on DF1. Squared change parsimony ancestral node reconstructions are plotted in multivariate space in Figure 4. CIs from ML ancestral character estimates indicated that only the transition to a longer pelvis and fourth toe in *Tracheloptychus* from its ancestor was statistically significant. This significant transition and other nonsignificant trends suggested by ancestor character reconstruction are plotted in Figure 6.

DISCUSSION

The present study aimed to examine the multivariate relationships between morphology, kinematics, and force variation during running in lizards. These analyses show that these three levels of locomotor function have tight functional and evolutionary relationships (Figs 4, 5, 6, Table 6). In lizards, morphological variation predicts kinematic variation, which, in turn, predicts force variation.

LIMB MORPHOLOGY AND STRIDE KINEMATICS

A key issue in animal locomotion is how limb length effects stride kinematics after accounting for differences in size and speed (Strang & Steudel, 1990). One of our principal findings was that lizard limb length has a significant linear relationship with stride kinematics: as limb length increases, stride length increases and stride frequency decreases (Figs 5, 6). This finding was not surprising given that it is generally known across a range of animals (Strang & Steudel, 1990) and has been shown within several lizard families (Teiidae: White & Anderson, 1994; Phrynostomatidae and a single teiid: Irschick & Jayne, 1999; Lacertidae: Vanhooydonck *et al.*, 2002). However, the present study shows that a strong relationship between limb morphology and stride parameters holds across a greater portion of the morphological diversity of lizards even after controlling for phylogeny.

These data also allowed us to examine potential trade-offs between how differing morphologies modulate stride length and stride frequency to move quickly. To examine potential trade-offs in the relationship between limb length and speed modulation, we calculated the slopes from least-squares linear regressions for the relationships between running speed and raw stride length or stride frequency (Table 6). This analysis shows that the relatively shortest-limbed species (*Eulamprus*) modulates only stride frequency, whereas the one of the longest-limbed species (*Tropidurus*) modulates only stride length to increase speed, and the intermediate limbed species (except *Tracheloptychus*) modulate both. *Tracheloptychus* has average size limbs but relies on a stride length only modulation strategy. This may be because *Tracheloptychus* has an inordinately longer pelvis and fourth toe length (Fig. 4) and the fourth toe has been shown to be an important correlate of stride length (Irschick & Jayne, 1999). *Oplurus* has relatively long limbs but modulates both stride frequency and stride length. Although it is unclear why this species does not use primarily stride length modulation, it is clear that, for its size, *Oplurus* exhibits relatively slow maximum sprint speed and the shortest float distance, and this may be related to its deviation from the proposed pattern in lizards. Overall, these data support previous studies indicating the tight correlation between limb length and stride length/frequency modulation in lizards (White & Anderson, 1994; Vanhooydonck *et al.*, 2002). In addition, our data support the hypothesis that animals modulate stride frequency, stride length, or both, to increase speed (Biewener, 2003). However, this is the first time that the extremes in limb length are found to be related to the reliance on modulation of only one parameter to increase speed. Our data do not address exactly how limb kinematics (e.g. limb protraction and retraction) are related to species differences in speed modulation strategy; future studies should address this issue.

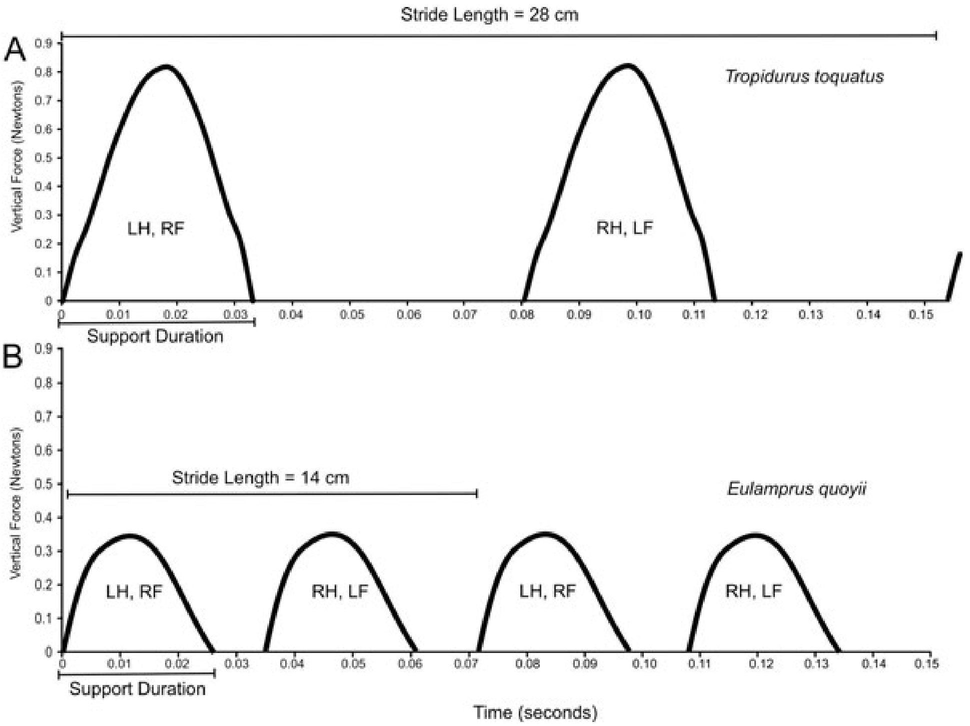

Figure 7. Sample vertical forces that illustrate how the longest and shortest-limbed lizards in the present study varied in kinematics and whole body forces. These two trials were from individuals of similar mass and speed; thus, the raw data are presented. L and R, left or right leg supports, respectively. A, *Tropidurus torquatus*: speed = 1.99 m s^{-1}; mass, 27 g; snout–vent length = 91 cm; hindlimb length, 70 cm; forelimb length = 49 cm; stride frequency = 6.2 s^{-1}; stride length = 28 cm; peak vertical force = 0.82 N; vertical impulse = 0.0163 Ns. (B) *Eulamprus quoyii*: speed = 1.92 m s^{-1}; mass = 21 g; snout–vent length = 98 cm; hindlimb length = 46 cm; forelimb length = 31 cm; stride frequency = 14 s^{-1}; stride length = 14 cm; peak vertical force = 0.36 N; vertical impulse = 0.0061 Ns. The approximate doubling of stride frequency in the short-limbed *Eulamprus* results in an approximate halving of the peak vertical force and vertical impulse compared to the long-limbed *Tropidurus*. LH, RF: left hindlimb – right forelimb couplet step; RH, LF: right hindlimb – forelimb couplet step.

STRIDE KINEMATICS AND WHOLE BODY VERTICAL FORCE

It is important to emphasize that we are discussing vertical impulses integrated over the support phase for a limb pair, as opposed to over the entire stride (Fig. 3). Although it is well known that vertical forces summed (vertical impulse) over the entire stride divided by stride time must equal body weight (Biewener, 2003) and they do over a stride in all species in this study (Fig. 7), this was not a focus of the present study. Rather, we were interested in how species modulate forces over the support phase of diagonal couplets during running to effectively support and propel the body in a sample of morphologically and kinematically diverse lizard species.

The multivariate analyses of locomotor function revealed a significant correspondence between limb length, stride kinematics, and whole body forces (Figs 5, 6; Table 5). Species with longer limbs took relatively longer stride lengths, shorter stride durations, and applied relatively larger peak whole body vertical forces and vertical impulses to the ground. For example, among lizards moving at speeds across their locomotor scope, the long-limbed *Tropidurus* had stride frequencies that were approximately one-half less, and vertical impulses (summed over support duration) that were approximately two-fold greater, than those of short-limbed but equivalently-sized *Eulamprus*. Figure 7 presents the representative vertical forces from these two species that clearly illustrate how morphology, kinematics, and force are related. The other species of lizards fell between these two extremes. As expected, the relationship between stride kinematics and vertical forces exhibited by these lizards is in accordance with well-known biomechanical principles (Biewener, 2003). However, these data on running lizards clearly demonstrate that the relationship between kinematics and vertical force is driven by the underlying limb morphological variation and that lizards with shorter limbs must use kinematic compensation to effectively support body weight. Overall, short-limbed species cycle their short legs more often with less force per support whereas long-limbed species cycle their long legs less often with more force per support all so that each species have their forces sum to body weight over their entire strides (Fig. 7). This finding supports the prediction of previous studies based on kinematics (Van Damme *et al.*, 1998; Aerts *et al.*, 2000).

Energetic implications

These findings may have important implications for the energetics of lizard locomotion. Van Damme *et al.* (1998) suggested that short stride –high frequency species should expend a large amount of metabolic energy because they must perform a lot of internal mechanical work to cycle the limbs. However, Van Damme *et al.* (1998) also point out that long stride–low frequency species will produce a large amount of force, which will also increase metabolic expenditure. Although we did not directly measure metabolic expenditure, our species appear to fall along a continuum, with the Iguanians (*Tropidurus*, *Oplurus*, and *Laudakia*) producing very large forces but moving at very low stride frequency, *Acanthodactylus*, *Varanus*, and *Tracheloptychus* producing intermediate amounts of force and stride kinematics, and the skink (*Eulamprus*) moving at very high stride frequency but with small forces (Figs 5, 7). This continuum suggests that species with very long (Iguanians) or very short (skinks) limbs may engage in the most costly locomotion; whereas species with intermediate-sized limbs (*Acanthodactylus*, *Varanus*, and *Tracheloptychus*) may engage in less costly locomotion. The cost of locomotion for running in erect birds and mammals is largely determined by these three levels of locomotor function (Roberts *et al.*, 1998; Pontzer, 2005, 2007). Examination of how the cost of locomotion is related to these levels of locomotor function in sprawling animals (i.e. turtle, lizards, alligators, and salamanders) would be a useful.

It is intriguing that, given the above argument, the high-cost species are sit-and-wait foragers (*Tropidurus*, *Oplurus*, *Laudakia*, and *Eulamprus*) whereas the low-cost species are wide foragers (*Acanthodactylus*, *Varanus*, and *Tracheloptychus*). This suggests that, similar to so many other phenotypic traits (Reilly *et al.*, 2007), foraging behaviour may be driving locomotor functional morphology and energetics in lizards (see also, Clemente, Withers & Thompson, 2009).

Propulsive forces

Interestingly, *Tracheloptychus* and *Tropidurus* had the largest values on both kinematic and force DF2, indicating that they had the largest relative float distances, the latest braking–acceleration transition times, and the largest accelerative impulses. Thus, these two species are delivering larger, more focused accelerative forces late during the support phase in order to generate longer float distances. Morphologically, this may be related to the novel longer pelvis in *Tracheloptychus* but there is no clear morphological explanation for the same pattern in *Tropidurus*. In addition, the ability to deliver more focused accelerative forces to produce longer float distances appears to be a correlate of a stride length speed modulation strategy because *Tropidurus* and *Tracheloptychus* were the only two species to rely solely on stride length to increase speed. Aside from these multivariate patterns, longer float distances were correlated with both larger vertical whole body force (Pearson product-moment correlations data pooled across all species: float distance – vertical impulse: $r = 0.38$, $P = 0.001$; maximum vertical whole body force: $r = 0.23$, $P = 0.05$) and larger accelerative whole body forces (accelerative impulse: $r = 0.33$, $P = 0.006$; maximum accelerative whole body force: $r = 0.34$, $P = 0.004$). Species that incorporate float phases are essentially producing alternating single-leg jumps in which the centre of mass (COM) is propelled ballistically with each step. This suggests that species that float over greater distances produce more vertical and accelerative forces to effectively propel the COM up and forward during this 'jump'.

EVOLUTIONARY TRENDS IN LIZARD LOCOMOTOR FUNCTIONAL MORPHOLOGY

The mapped characters on the inverted phylogeny in Figure 6 summarize the evolutionary trends in limb length, stride kinematics, and ground reaction forces in lizards. The putative ancestor (node A) had relatively short limbs, short high-frequency strides, and intermediate vertical forces (Figs 4, 6). From these ancestral traits, lizards diverged along two separate evolutionary trajectories, principally based on divergence along DF1. *Tracheloptychus* and *Eulamprus* exhibit an evolutionary trend towards even shorter limbs, shorter strides at higher frequency, and lower vertical forces (Node B and tip values); whereas the nodes (C, D, E) leading to *Varanus*, *Acanthodactylus*, and the Iguanians showed an evolutionary trend towards longer limbs, longer low-frequency strides, and larger vertical force. From its ancestor (node D), the Iguania evolved to the functional extreme with the longest limbs, longest strides at the lowest frequency, and largest vertical forces, whereas *Eulamprus* has evolved to the extreme in the opposite direction. Taken together, these data show that deep phylogenetic effects (locomotor functional split at node A) are imprinted upon locomotor functional evolution, which is a finding in agreement with evolutionary transitions in many other lizard traits (e.g. head morphology: McBrayer & Corbin, 2007; diet: Vitt & Pianka, 2007; feeding kinematics: Reilly & McBrayer, 2007; chemosensory systems: Cooper, 2004).

Several cases of evolutionary convergence are evident within the two overarching trends in lizard locomotor evolution. *Varanus* has converged with *Tracheloptychus* and *Eulamprus* with its short limbs and short high-frequency strides. However, *Varanus* has not converged in vertical force dynamics, which may be related to morphological changes that enhance bipedal posturing in this genus (Schuett, Reiserer & Earley, 2009). *Oplurus* has reverted to having somewhat shorter, faster strides and this may be related to this species being relatively slow for its size. Finally, *Tracheloptychus* and *Tropidurus* exhibit convergence along DF2, evolving longer float distances, shorter steps, later braking–accelerative transition times, and larger accelerative impulses than their ancestors and, although this may be related to longer pelvic and fourth toe morphology in *Tracheloptychus*, the reason for this in *Tropidurus* remains unclear. Thus, locomotor functional evolution clearly exhibits some flexibility. Future studies should explore how evolutionary flexibility in finer details of locomotor function is related to specific aspects of ecology, habitat use, and behaviour.

ACKNOWLEDGEMENTS

We thank Emily Bevis, Caityln Cato, Alex Fotis, Bailey Miles, Andy Parchman, and Kristen Stover for their assistance with data collection and analysis. John Bertram, David Lee, Audrone Biknevicius, Andrew Lammers, and Kay Earls provided advice and encouragement regarding the function of our force platforms. Robbie Wilson kindly helped us acquire permits from the Queensland government of Australia to work on *Eulamprus*. Jens Vindum and Bob Drewes (California Academy of Sciences), Robert Espinosa (California State University at Northridge), Steve Rodgers (Carnegie Museum), and Jim McGuire (University of California Museum of Vertebrate Zoology) kindly loaned lizard specimens for morphological study. Joe Eastman graciously allowed us to use his X-ray machine and developing equipment. Audrone Biknevicius, Angela Horner, Mike Jorgenson, Don Miles, and Gar Rothwell provided comments that greatly improved the quality of the manuscript. This research was funded by NSF grants (IBN 9727212; IBN 0080158; IOB 0520100) and Ohio University Research Challenge grant to S.M.R., and an Ohio Center for Ecology and Evolutionary Biology Fellowship to E.J.M.

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APPENDIX

MUSEUM CODES

Museum abbreviations: CAS, California Academy of Sciences; CSUN, California State University at Northridge; CM, Carnegie Museum; UCMVZ, University of California Museum of Vertebrate Zoology.

Acanthodactylus boskianus: CAS (9723, 9709, 9710, 9708, 138659, 138657, 138658, 138655, 138120, 138724, 138722, 138723, 138721, 13871, 138718), CM (56708, 56648, 56569, 56662, 56756, 56597, 56566, 56643, 56761, 56760, 56612, 56649, 56567, 56661, 56568).

Eulamprus quoyii: CAS (76873, 76879, 76830, 76822, 76850, 76868, 76827, 76853, 76845, 76826, 76860, 76880, 76843, 76824, 76868, 76867, 76847, 76877, 76825, 76872, 76858, 76862, 76842, 76852, 76835, 76851, 76848, 76818, 76854).

Laudakia stellio: CAS (217951, 217952, 217874, 217875, 217683, 217876, 217685, 217684, 218089, 218090, 217985, 218091, 5007, 2992, 217987, 217984, 217711, 217709, 217807, 217808, 217809, 217710, 217712, 217803, 217802, 217804, 218005, 218092, 217680, 217980); three uncataloged individuals from Ohio University.

Oplurus cuvieri: CAS (12777, 126358, 135152, 13951, 13957, 13953, 13950); UCMVZ (238792, 128904, 238791, 238793, 117597, 21117, 238794, 247486, 238795, 238790, 238796); three uncataloged individuals from Ohio University.

Tracheloptychus petersi: CSUN (1213); UCMVZ (238766, 238764, 238765, 238768, 238767); six uncataloged individuals from Ohio University.

Tropidurus torquatus: CM (136154, 136117, 64888, 943, 4594, 7411); one uncataloged individual from Ohio University.

Varanus exanthematicus: CM (24705, 24700, 15163); CSUN (1417, 2903); UCMVZ (75661); CAS (169935, 139504, 103109, 130091); three uncataloged individuals from Ohio University.

SUPPORTING INFORMATION

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

Table S1. Pearson product-moment correlation coefficients between morphological, kinematic, and whole body force variables and body size (snout–vent length or mass) and speed.