doi:10.1111/j.1558-5646.2009.00935.x

ALTERNATE PATHWAYS OF BODY SHAPE EVOLUTION TRANSLATE INTO COMMON PATTERNS OF LOCOMOTOR EVOLUTION IN TWO CLADES OF LIZARDS

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Received May 6, 2009
Accepted December 10, 2009

Body shape has a fundamental impact on organismal function, but it is unknown how functional morphology and locomotor performance and kinematics relate across a diverse array of body shapes. We showed that although patterns of body shape evolution differed considerably between lizards of the Phrynosomatinae and *Lerista*, patterns of locomotor evolution coincided between clades. Specifically, we found that the phrynosomatines evolved a stocky phenotype through body widening and limb shortening, whereas *Lerista* evolved elongation through body lengthening and limb shortening. In both clades, relative limb length played a key role in locomotor evolution and kinematic strategies, with long-limbed species moving faster and taking longer strides. In *Lerista*, the body axis also influenced locomotor evolution. Similar patterns of locomotor evolution were likely due to constraints on how the body can move. However, these common patterns of locomotor evolution between the two clades resulted in different kinematic strategies and levels of performance among species because of their morphological differences. Furthermore, we found no evidence that distinct body shapes are adaptations to different substrates, as locomotor kinematics did not change on loose or solid substrates. Our findings illustrate the importance of studying kinematics to understand the mechanisms of locomotor evolution and phenotype-function relationships.

KEY WORDS: **Body shape, kinematics, locomotion, performance, substrate.**

A major component of animal diversity is variation in body shape (Carroll 1997)—the relative dimensions of the body, head, tail, and limbs. How this variation translates into functional diversity is a fundamental question in evolutionary biology (Losos 1990b; Melville and Swain 2000; Young et al. 2007). A related, and equally fascinating question, is whether this variation is adaptive for certain ecological situations. For example, it is well documented that *Anolis* lizard species with different relative hind limb lengths are able to run faster on perches with certain characteristics, and they exploit those optimal habitats in nature (Losos et al. 1989; Losos 1990b; Irschick and Losos 1999). Toward these ends, there have been many detailed studies of the evolution of morphological diversity within certain clades (Schmitz et al. 2005; Stayton 2005; Wiens et al. 2006; Ward and Brainerd 2007; Brandley et al. 2008), and considerable attention has been devoted to understanding how variation in morphology translates into variation in organismal performance (Losos 1990a; Macrini and Irschick 1998; Bonine and Garland 1999; Vanhooydonck and Van Damme 1999; Melville and Swain 2000; Braña 2003; Wainwright 2007). However, we still lack a complete understanding of the functional and ecological ramifications of variation in body shape because most of these studies focus on taxa with a narrow range of body shapes. This potential limitation may hamper the ability to effectively link patterns of body shape evolution with patterns of functional evolution and understand how different habitat characteristics interact with morphology to influence function.

Arnold's (1983) classic paradigm relating morphology to performance to fitness has been a valuable framework for studying the implications of variation in body shape on locomotion intraspecifically because selection primarily acts on whole-organism performance traits such as sprint speed, and secondarily on morphological traits (Arnold 1983; Garland and Carter 1994; Bonine and Garland 1999; Calsbeek 2008; Irschick et al. 2008). This paradigm has been expanded to the interspecific level to test for adaptation of morphological and performance traits to different habitats (Emerson and Arnold 1989; Garland and Losos 1994; Fieler and Jayne 1998; Aerts et al. 2000; Melville and Swain 2000). Such an approach can be bolstered by understanding how different aspects of body shape (e.g., trunk vs. limbs) are used during a functional task, such as locomotion. The inclusion of kinematic variables into any interspecific functional performance analysis is crucial because animals can exhibit different kinematic strategies that can alter morphology-performance relationships (Lauder and Reilly 1996; Irschick and Jayne 1998), akin to many-to-one mapping (Wainwright et al. 2005). Kinematic variation is, therefore, a behavioral mechanism through which either morphologically similar species can achieve dissimilar levels of performance or morphologically distinct species can achieve similar levels of performance (Wainwright et al. 2005; Kohlsdorf and Navas 2007).

Lizards and snakes (Squamata) exhibit a remarkable diversity of body shapes, and thus are an ideal model system for investigating the implications of these body shapes for locomotion. A snake-like, or elongate and limb-reduced body shape, has evolved in all major vertebrate clades (Lande 1978; Jockusch 1997; Bejder and Hall 2002; Caldwell 2003; Ward and Brainerd 2007), and squamates have evolved this body shape at least 26 times independently (Greer 1991; Wiens et al. 2006; Brandley et al. 2008). Body shape transitions have been studied extensively in snake-like squamates (Stokely 1947; Presch 1975; Lande 1978; Greer 1987, 1990; Caputo et al. 1995; Greer et al. 1998), and more recently, in a phylogenetic context (Pellegrino et al. 2001; Wiens and Singluff 2001; Whiting et al. 2003; Schmitz et al. 2005; Kohlsdorf and Wagner 2006; Wiens et al. 2006; Ward and Brainerd 2007; Brandley et al. 2008). In contrast, a broad and short, or stocky, body shape has also evolved several times in vertebrates (Brainerd and Patek 1998; Sherbrooke 2003; Bergmann et al. 2009), but is little studied. Of particular interest to understanding the evolution of these two extreme morphologies concerns which body parts have evolved to give these different morphologies, and whether the ancestral body shape for each clade is similar. The direction and magnitude of evolutionary change of different body parts can only be fully assessed with an understanding of their starting points. We address these questions first, which is necessary for determining how patterns of body shape evolution translate into patterns of locomotor evolution and, ultimately, different locomotor strategies.

Although many comparative studies have related locomotor performance to morphology among species, these have not included species with a wide range of body shapes from stocky to snake-like (e.g., Losos 1990b; Bonine and Garland 1999; Irschick and Jayne 1999; Melville and Swain 2000; Vanhooydonck et al. 2002). Studies of locomotion in snake-like vertebrates are also limited in scope because they are not comparative or lack taxa with intermediate body shapes (Gans 1962, 1973, 1986; Walton et al. 1990; Gans and Fusari 1994; Renous et al. 1995; Renous et al. 1998). Here, we consider a broad range of species, representing both extreme body shapes and many intermediates, in a phylogenetic context. With this approach, we are able to gain an evolutionary perspective of how variation in body shape translates into variation in locomotion. More specifically, we are interested in whether evolution of different body shapes is related to locomotor evolution, which can be defined as the direction of change in locomotor variables that include both performance and kinematic measures. We differentiate locomotor evolution from kinematic strategy, which consists of the actual values that a given species has for the locomotor variables studied.

Directly related to relationships between body shape and locomotor evolution is the issue of whether interspecific differences in such traits represent adaptations to different habitats. An elongate, limb-reduced body shape has been variously hypothesized to be an adaptation to (1) a fossorial habitat (Gans 1973, 1975; Shine 1986; Bejder and Hall 2002), (2) a structurally complex habitat (Melville and Swain 2000; Van Damme and Vanhooydonck 2002), (3) a loose sand substrate (Gans 1986; Greer 1990), and (4) an aquatic habitat (Caldwell 1999). However, only the second hypothesis has been tested functionally (references above), but not over a wide range of body shapes. The third hypothesis is predicated on the argument that an elongate body allows for a greater surface area to push on the loose substrate, minimizing substrate displacement (Gans 1986; Greer 1990; Mueth et al. 1998), and is tested here. There is some evidence that locomotor kinematics and performance are influenced by solid versus loose substrates in both limbed and limbless squamates (Carothers 1986; Gans 1986; Jayne 1986; Kelley et al. 1997). In contrast, few such hypotheses have been made for the evolution of a stocky body shape. A few studies have suggested that a stocky body shape may promote the evolution of mechanical defenses, such as spines, and documented

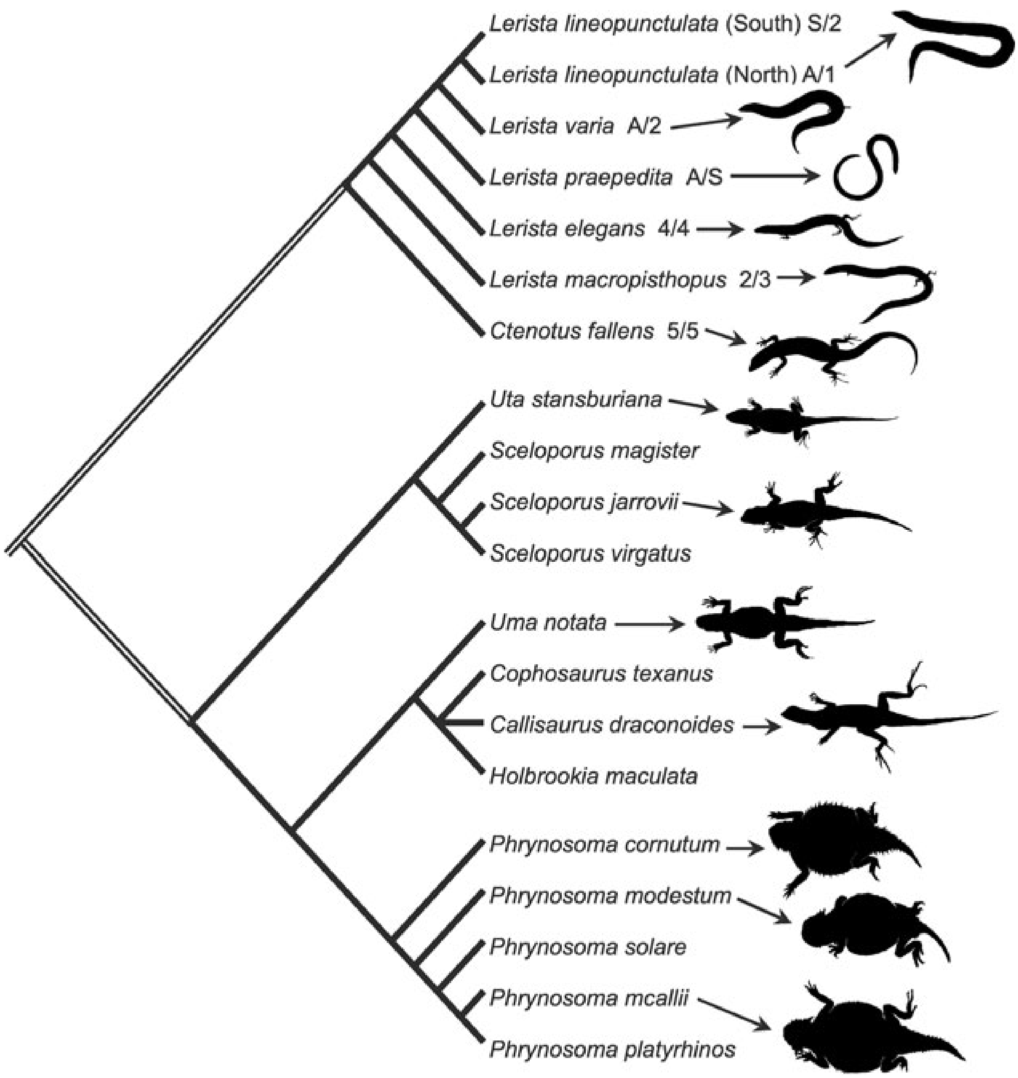

Figure 1. **Phylogeny of the species studied based on published topologies (see text). Letters and numbers after species names of skinks indicate the development of the limbs (front/hind), where numbers indicate number of digits on each limb, "S" indicates a reduced, digitless stump, and "A" indicates absence. All phrynosomatines have two pairs of fully formed limbs with five digits on each (5/5). Open phylogenetic branches signify that the two clades are not closely related.**

that stocky animals often exhibit slow locomotion (Brainerd and Patek 1998; Bergmann et al. 2009).

We examine how locomotor kinematics and performance are related to body shape on two different substrate types (solid and loose sand) using 20 lizard species from two clades (Fig. 1). One clade is the scincid genus *Lerista*, which has repeatedly evolved body elongation and limb reduction (Greer 1987, 1990; Kendrick 1991; Reeder 2003; Wilson and Swan 2005). The other clade is the Phrynosomatinae, which includes the stocky horned lizards, the gracile sand lizards, and the intermediate fence lizards (Reeder and Wiens 1996; Bonine and Garland 1999; Losos and Miles 2002; Harmon et al. 2003; Sherbrooke 2003). Our goal is to relate the evolution of locomotor kinematics and performance to body shape evolution, examining how differently shaped species modulate locomotor performance through changes in kinematics. Specifically, we are interested in: (1) which body parts have been involved in the evolution of stocky and snake-like body shapes in these clades; (2) whether patterns of body shape evolution coincide with patterns of locomotor evolution; and (3) whether differently proportioned animals run differently on loose versus solid substrates.

Materials and Methods

FIELD SPECIMEN AND DATA COLLECTION

We collected live specimens of 13 species of the Phrynosomatinae in southern Arizona during May and June 2006, and six species of *Lerista* and *Ctenotus fallens* (Scincidae) in coastal Western Australia during September and October 2006. Both areas exhibit peak diversity of these lizards (Greer 1987; Kendrick 1991;

Gans and Fusari 1994; Sherbrooke 2003; Stebbins 2003). Species names appear in Figure 1, sample sizes and locality information are in Appendix A1. Phrynosomatine species and *Ctenotus* were collected with nylon noose or by hand. *Lerista* were collected by raking leaf litter of *Acacia ramulosa* under which they live and catching them by hand once uncovered (Pough et al. 1997).

Specimens were processed and released unharmed at site of collection within two days of capture. We recorded sites of capture using a Garmin VistaC GPS unit (Olathe, KS) and marked the sites with flagging tape before transporting the lizards back to camp. We collected, euthanized, preserved, and deposited 12 individuals of *Lerista* in the Western Australian Museum to serve as voucher specimens (listed in Supporting information).

We measured head length (HL), height (HH), and width (HW); body height (BH) and width (BW); tail length (TL); front (FLL) and hind limb (HLL) lengths; and snout-vent length (SVL) for each specimen using a Mitutoyo digital caliper (Kanagawa, Japan) to 0.01 mm. We also marked the occiput, pectoral girdle, pelvis, mid-back, level of the cloaca, elbow and knee on each lizard using nontoxic white paint (Vanhooydonck et al. 2002) (see Supporting information for descriptions of these measurements and points).

We ran lizards on a 15 cm wide, 1-m-long racetrack while video recording them from dorsal view at 250 Hz using a Fastec Troubleshooter 250 camera (San Diego, CA). The length of the racetrack allowed us to record locomotor performance and kinematics in an equivalent manner for lizards that run long distances as well as those that move intermittently. Although this did not allow some species (i.e., the sand lizards) to attain maximum velocities, this was a secondary concern because we were interested primarily in how body shape relates to kinematics to mediate performance, and not on how fast each species can move. By statistically accounting for differential velocity among species when appropriate (see below), we were able address how body shape influences kinematics. We conducted trials at ambient temperatures on warm days, with the lizards' body temperatures averaging 30°C in Arizona, and 26°C in Australia. Although these body temperatures are somewhat cooler than optimal body temperatures for both clades (Pough et al. 1997; Bonine and Garland 1999), lizards run at a high percentage of maximal capacity over a broad range of temperatures (Huey and Bennett 1987; Bergmann and Irschick 2006). We believe that the advantages of conducting performance trials on freshly captured specimens outweigh the drawbacks of having weaker temperature controls. This is particularly the case when the study is not focused on the maximal sprinting performance of the subjects, as is the case here.

We ran each lizard twice each on substrates of 120-grit sandpaper and 1-cm-deep play sand with a mean particle diameter of 882 μm in Arizona and 824 μm in Australia. The layer of sand used to run the skink species was ∼0.5 cm deep to prevent burrowing. We randomized the order in which substrate was presented and rated the trials as excellent, good, fair, or poor, based on motivation of lizards, straightness of path, and whether they came in contact with the walls of the racetrack. No trials rated as poor were analyzed. We also excluded any trials in which lizards either ran bipedally (occurred in 8/290 trials) or when the majority of the tail was missing (18/290 trials) because both factors influence kinematics (Gans and Fusari 1994; Irschick and Jayne 1999).

VIDEO DIGITIZATION AND DATA CALCULATION

In the laboratory, we imported all videos into Motus Peak Performance software (2000; Peak Performance Technologies, Englewood, NJ) and digitized all seven points painted on the lizards frame-by-frame. We smoothed the resulting two-dimensional (xy) raw coordinates using a quintic spline and a generalized cross-validatory algorithm, which was then differentiated to obtain instantaneous velocity data (Walker 1998). We calculated axial and hind limb angles as measures of axial bending and limb protraction and retraction. The axial angle was formed by the pectoral girdle–mid-back–pelvic points, and the hind limb angle was formed by the cloacal–pelvic–knee points.

Using the highest-rated video for each substrate and individual, we calculated maximal recorded instantaneous velocity, average recorded velocity, maximal axial angle, the angles of the hind limb at maximal protraction and retraction, stride and step durations and lengths, and duty factor. We calculated stride and step lengths as the displacement of the occiput point during a stride or a step, and duty factor as the proportion of time of a stride occupied by the step (Bergmann and Irschick 2006). Upon compilation of the data, we removed outliers, defined as two standard deviations from the mean, and calculated species means for all variables (presented in the Supporting information). None of the kinematic variables were significantly related to SVL, when phylogeny was taken into account with phylogenetically independent contrasts (PICs), so the raw species mean values were used in subsequent analyses.

MORPHOMETRIC ANALYSIS

Because species were the units of comparison, we accounted for phylogeny in our analyses. For the phrynosomatines, we used the combined data phylogeny of Reeder and Wiens (1996), consistent with the mtDNA phylogeny of Leaché and McGuire (2006). For *Lerista,* we used the allozyme phylogeny of Kendrick (1991), consistent with the DNA phylogeny of Skinner et al. (2008), with *Ctenotus* the sister group to *Lerista* (Reeder 2003). We set all branch lengths to one and then ultrametricized the tree because these branch lengths fit the assumptions of PICs better than empirically determined branch lengths, as evaluated using standard diagnostic plots of the absolute value of the contrasts against their standard deviation (Garland et al. 1992). Unless otherwise stated, analyses for the two clades were conducted separately because they diverged ~170 million years ago (Wiens et al. 2006).

We conducted most statistical analyses with SYSTAT version 10.2 (Wilkinson 2001). To address whether body shape evolution in both the phrynosomatines and *Lerista* involved similar relationships among body parts, we ran an evolutionary principal component analysis (ePCA) on each clade separately, using log-transformed species mean data. Evolutionary PCAs were done using a correlation matrix calculated from regressions of PICs forced through the origin (Wiens and Singluff 2001) and without subsequent rotation of axes. The correlation matrices were calculated using the program IDC (available from L.J. Revell at http://anolis.oeb.harvard.edu/~liam/programs/), which allows for their more simple calculation than using PDAP, where all pairwise relationships among variables would have to be calculated and their R-values compiled. The resulting correlation matrices were used as input for a standard PCA. We added 1 to *Lerista* front limb data prior to transformation to avoid zero values (Hill and Gauch 1980). For PCAs, we interpreted components only if they were not spherical with subsequent PCs, as evaluated using Bartlett's test for sphericity (see Jackson 1993a for discussion). To determine which PCA eigenvector elements (loadings) to interpret, we calculated 95% confidence intervals for each using asymptotic standard errors calculated by SYSTAT following Jackson (1993b) and interpreted only those loadings whose confidence intervals did not overlap zero.

We calculated two ratios for use as indices of body shape for subsequent analyses. The relative degree of elongation was represented with the ratio BW/SVL, and the relative length of the hind limb was represented with HLL/SVL. Given that the two clades under study are different morphologically and have evolved along different trajectories (see Results), we deemed the use of regression residuals or PC factor scores more difficult to interpret from a functional standpoint than these ratios (for full justification see Supporting information). To address whether the ancestors of each clade had a similar body shape, we calculated PICs for these ratios and their ancestral reconstructions for the root of each clade using the PDAP module for Mesquite version 2.5 (Garland et al. 1992; Maddison and Maddison 2006). Ancestral reconstructions calculated in this way are equivalent to maximum likelihood and squared-change parsimony reconstructions (Schluter et al. 1997; Garland et al. 1999; Losos 1999). No additional outgroups were used in this analysis so that reconstructions of ancestral morphology were not biased by outgroup selection.

SUBSTRATE EFFECTS

To investigate the effects of substrate (sandpaper vs. sand) on locomotion, we calculated standardized PICs for each locomotor variable from trials on each substrate using IDC. We then analyzed the contrasts for each variable using paired-sample *t*-tests. We used an alpha of 0.05 and sequential Bonferroni corrections to account for multiple comparisons (Rice 1989).

RELATIONSHIPS BETWEEN MORPHOLOGY AND LOCOMOTION

We used both multivariate and bivariate approaches to investigate relationships between morphometric and locomotor variables. For the multivariate approach, we reduced the dataset to include only individuals for which there were data for all kinematic and performance variables, combining data from both substrates because of a lack of substrate effects, and including both clades. Branch lengths connecting the two clades were scaled relative to our within-clade branch lengths following the phylogeny of Wiens et al. (2006). As both a northern and a southern morph of *L. lineopunctulata,* and *L. praepedita* had hind limbs that were too short to digitize, we excluded them from this analysis. Morphometric data were analyzed using PCA. A second PCA included all of the kinematic and performance variables. These two PCAs did not take phylogeny into account, as this was done during the subsequent partial Mantel test (see below). For both PCAs, we calculated species means for standardized factor scores for the first four PCs and used these to calculate pairwise Euclidean distances among species for each PCA using the BioDist version 1.8.0 Package for R (R Core Development Team 2008). We represented the phylogeny as pairwise distances by summing all branch lengths connecting each pair of species. We used partial Mantel tests (Anderson and Legendre 1999) to test for correspondence between morphospace and locomotor space while accounting for phylogeny (similar to Irschick and Jayne 1999; Glor et al. 2003; Young et al. 2007) using zt software (Bonnett and Van de Peer 2002). We ran Mantel tests for 1×10^6 iterations.

For the bivariate approach, we used PICs to examine relationships between morphological indices and each locomotor variable using the PDAP module in Mesquite (Felsenstein 1985; Garland et al. 1992; Maddison and Maddison 2006). We regressed standardized contrasts, positivized appropriately, for pairs of variables through the origin (Garland et al. 1992). We used ordinary least squares regression because variation in morphometric indices was far less than that present in the locomotor variables (McArdle 1988). When relationships between the two clades coincided, a single regression was done for both, but without including the root contrast. When relationships were in opposite directions and one or both were significant, separate regressions were done for each clade. Finally, to determine how kinematic variables influenced performance, we ran PIC regressions for each kinematic variable against maximal recorded velocity. Some kinematic variables (see Results) were significantly related to maximal recorded velocity, and to evaluate the relationships of these aspects of kinematics to our body shape indices while accounting for velocity, we did multiple regressions of PICs through the origin, using maximal

Table 1. **Standardized loadings for PC-1 and PC-2 from separate ePCAs including phrynosomatines or skinks.**

Variable	Phrynosomatines		Skinks	
	PC1	PC2	PC1	PC2
LSVL	**0.3658**	**0.2270**	**0.2870**	**0.5867**
LBW	**0.3401**	**−0.5283**	**0.3889**	0.0763
LBH	**0.3545**	−0.1986	**0.3906**	0.0469
LHL	**0.3524**	0.0536	**0.3806**	0.0976
LHH	**0.3572**	−0.2129	**0.3855**	0.0471
LHW	**0.3605**	**−0.2988**	**0.3884**	0.1024
LFLL	**0.3597**	**0.3550**	**0.2522**	**−0.6457**
LHLL	**0.3373**	**0.6054**	**0.3253**	**−0.4568**
Eigenvalue	7.1362	0.4764	6.5341	0.9898
Variance	89%	6%	82%	12%

Note: Loadings whose 95% confidence interval excludes zero are in bold. *n*=13 species for phrynosomatines, seven species for skinks. All variables were log-transformed prior to analysis.

recorded velocity as a covariate. By conducting these multiple regression analyses as well as ones that did not account for velocity, we were able to determine which kinematic variables were (1) unrelated to body shape, (2) related to body shape but dependent on velocity, or (3) related to body shape irrespective of velocity.

Results

CHARACTERIZING BODY SHAPE EVOLUTION IN *LERISTA* AND THE PHRYNOSOMATINAE

The ePCA of morphometrics indicated that body shape has evolved through changes in different body parts in the two clades. For both clades, PC-1 represented a size component, having all high, positive loadings; it explained 89% and 82% of total variance for the phrynosomatines and skinks, respectively. PC-2 represented a shape axis, explaining 6% and 12% of variance, respectively, but standardized loadings between the two clades differed among variables (Table 1). For the phrynosomatines, PC-2 represented a trade-off between relative (size-corrected) length of front and hind limbs on the one hand, and relative body width on the other. In contrast, for the skinks, PC-2 represented a trade-off between relative front and hind limb length on the one hand, and relative SVL on the other (Table 1).

Kolmogorov–Smirnov tests indicated that PICs (excluding the root; $n = 18$) of log(BW/SVL) were normally distributed (MaxDiff $= 0.139$, $P = 0.485$) whereas those of the untransformed ratio were not (MaxDiff $= 0.206$, $P = 0.042$), and PICs of HLL/SVL were normally distributed (MaxDiff $= 0.116$, $P = 0.845$) whereas those for the transformed ratio were not (MaxDiff $= 0.240$, $P = 0.007$). Hence, we used log(BW/SVL) and HLL/SVL for all analyses relating morphology to locomotor performance or kinematics.

Regressions of PICs for these ratios (Fig. 2A) supported ePCA results, with the phrynosomatines tending to have relatively shorter limbs as their bodies became broader ($n = 12$, $r^2 = 0.452$, $t = -3.01$, $P = 0.012$), and the skinks tending to have relatively longer limbs as their bodies got broader ($n = 6$, $r^2 = 0.823$, $t = 4.82$, $P = 0.0054$). Ancestral reconstructions for the root nodes for the phrynosomatine and skink clades for these ratios clearly show that the two clades had morphologically distinct ancestors (Fig. 2B).

THE EFFECTS OF SUBSTRATE ON LOCOMOTION

Locomotor kinematic and performance variables were not significantly affected when lizards were run on sandpaper or loose sand

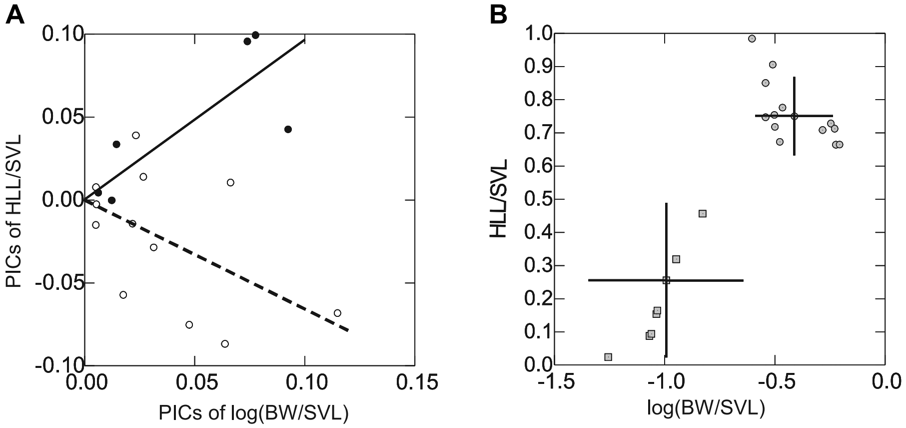

Figure 2. **The relationship between the log (BW/SVL), an index of body stockiness/elongation, and HLL/SVL, an index of relative hind limb length. (A) Plot of phylogenetically independent contrasts for all phrynosomatine and skink species included in this study. Closed circles—skink contrasts, open circles—phrynosomatine contrasts. Solid line is a regression through the origin for the skinks, dashed line is a similar regression for the phrynosomatines. (B) Raw species means for indices plotted with the basal ancestral reconstruction for each clade and associated 95% confidence intervals. Squares—skinks, circles—phyrnosomatines, shaded for better visibility of overlapping symbols.**

Table 2. Results from paired-sample *t*-tests of phylogenetically independent contrasts comparing locomotor variables from lizards running on sandpaper and sand.

Variable	*n*	M_{diff}	*t*	*P*
Maximum velocity	19	0.082	0.60	0.556
Average velocity	19	−0.008	−0.05	0.957
Axial angle	19	−0.059	−0.83	0.418
HLL protraction angle	17	−0.446	−1.60	0.130
HLL retraction angle	17	−0.098	−0.81	0.431
Stride duration	13	0.518	1.99	0.070
Step duration	13	0.445	1.92	0.079
Stride length	13	−0.028	−0.19	0.852
Step length	13	0.471	1.29	0.220
Duty factor	13	0.528	2.00	0.069

Note: *n* is number of species, M_{diff} is the mean difference.

substrates (Table 2). Therefore, all bivariate PIC analyses used only trials run on sandpaper, the substrate that provides for easier comparison to previous studies of lizard locomotion (e.g., Bonine and Garland 1999; Irschick and Jayne 1999).

THE EFFECTS OF BODY SHAPE EVOLUTION ON LOCOMOTOR EVOLUTION

The PCA of morphometric variables for the reduced dataset (including the 16 species with limbs large enough to be digitized) defined a single morphospace for phrynosomatines and skinks. PC-1 was a size component whereas PC-2 represented a trade-off between SVL and hind limb length, PC-3 explained a positive association between SVL and hind limb length, and PC-4 explained only 3% of total variation (Table 3). In the PCA of locomotor variables, PC-1 explained 48% of variation, which was associated with a trade-off between velocity and stride length on the one hand, and axial bending and stride duration on the other. PC-2 explained variation associated with stride length, and stride and step duration, PC-3 characterized a trade-off between hind limb protraction and retraction angles, and PC-4 characterized a trade-off between hind limb protraction angle on the one hand, and axial bending and velocity on the other (Table 4). The first two PCs most clearly separated *Lerista* from all other lizards. Mantel tests that accounted for phylogenetic relationships indicated a significant correspondence between morphology and kinematics for all species ($r^2 = 0.667$, $P < 0.001$; Fig. 3A,B).

Table 3. Morphometric PCA results for the reduced dataset. Standardized loadings, eigenvalues, and percent explained variance for each PC are presented.

Variable	PC1	PC2	PC3	PC4
Snout-vent length	**0.187**	**−0.721**	**−0.606**	−0.083
Body width	**0.394**	0.008	**0.166**	**0.460**
Body height	**0.399**	−0.101	0.010	**0.138**
Head length	**0.395**	−0.059	0.003	**−0.458**
Head height	**0.403**	0.031	**0.146**	**0.218**
Head width	**0.405**	−0.005	**0.088**	**0.260**
Hind limb length	**0.174**	**0.655**	**−0.732**	0.067
Front limb length	**0.372**	0.191	**0.202**	**−0.658**
Eigenvalue	5.866	1.000	0.733	0.237
Variance explained	73%	13%	9%	3%

Note: Loadings whose 95% confidence interval excludes zero are in bold. *n*=149, 16 species. All original variables were log-transformed. Front limb length is a presence/absence character, HLL is hind limb length.

Table 4. Locomotor PCA results for the reduced dataset. Standardized loadings, eigenvalues, and percent explained variance for each PC are presented.

Variable	PC1	PC2	PC3	PC4
Maximal velocity	**0.429**	−0.071	**0.176**	**0.368**
Average velocity	**0.441**	−0.054	**0.143**	**0.316**
Axial angle	**−0.359**	−0.154	0.052	**0.665**
HLL protraction angle	−0.023	−0.143	**0.801**	**−0.419**
HLL retraction angle	0.312	**0.297**	**−0.437**	**−0.243**
Stride duration	**−0.358**	**0.372**	0.133	0.191
Step duration	**−0.281**	**0.579**	0.133	0.046
Stride length	**0.374**	**0.187**	**0.219**	0.219
Step length	**0.218**	**0.592**	0.177	0.032
Eigenvalue	4.359	1.712	1.250	0.704
Variance explained	48%	19%	14%	8%

Note: Loadings whose 95% confidence interval excludes zero are in bold. *n*=149, 16 species.

Analysis of PICs of kinematic variables on maximal recorded velocity showed that stride duration and stride length were significantly related to maximal velocity, but angle of axial bending and duty factor were not (Table 5). Hence, maximal velocity among species was modulated by both increases in stride length and decreases in stride duration. Regression analyses of PICs of locomotor variables on log(BW/SVL) and HLL/SVL showed different patterns between the two functional morphological indices (Fig. 4; see Supporting information for all analyses). Both clades exhibited coincident patterns of evolution between morphological indices and all locomotor variables except maximal axial bending. The stockiness index was negatively related to maximal axial bending only in skinks (Fig. 4A), with more elongate species bending their body axis more. In phrynosomatines, there was very little variation in axial angle and no relationship with body shape (Fig. 4A). This index was also positively related to stride length in all species, with broader species taking longer strides (Fig. 4B). However, this relationship became nonsignificant when maximal recorded velocity was included as a covariate ($R^2 = 0.5448$; $n = 12$; $t = 1.655$; $P = 0.129$). In both clades, species with relatively longer hind limbs achieved higher maximal recorded velocity

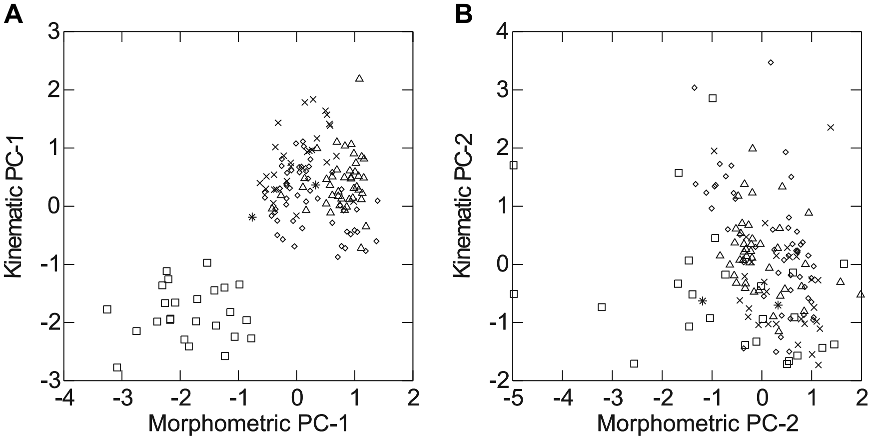

Figure 3. Plots of factor scores for PC-1 (A) and PC-2 (B) from PCAs of morphometric and kinematic variables for the reduced dataset (see text). Squares—*Lerista* spp., asterisks—*C. fallens*, triangles—*Phrynosoma* spp., "x"—sand lizards, diamonds—*Sceloporus* spp. and *Uta stansburiana* (see Fig. 1 for complete species list).

(Fig. 4C). Skinks but not phrynosomatines with longer limbs bent their body axes less (Fig. 4D). In general, species with relatively longer hind limbs also took quicker (Fig. 4E) and longer strides (Fig. 4F). However, when the effects of velocity were accounted for, the relationship with stride duration became nonsignificant ($R^2 = 0.5665$; $n = 12$; $t = -0.570$; $P = 0.581$), but the relationship with stride length remained significant ($R^2 = 0.7587$; $n = 12$; $t = 3.746$; $P = 0.004$).

Discussion

HOW HAVE STOCKY AND SNAKE-LIKE BODY SHAPES EVOLVED?

How morphology evolves is of fundamental importance to understanding how function evolves (Carroll et al. 2005; Wainwright 2007). Our results indicate that stocky and snake-like body shapes have evolved from different starting points and in divergent, but not opposite directions in phrynosomatine and *Lerista* lizards (Fig. 2). These alternative trajectories of body shape evolution, nevertheless, have resulted in similar patterns of locomotor evolution between the two clades (Fig. 4), ultimately translating into different kinematic strategies being adopted by differently shaped species (Fig. 3). These findings speak directly to the question of whether the repeated evolution (Stayton 2006) of similar body shapes arises through correlated changes in the same set of characters every time (Greer 1991), or through different yet predictable pathways that involve somewhat different body parts (Wiens et al. 2006). Both possibilities suggest adaptation to similar selective pressures (e.g., Stayton 2006), and functional information is important for understanding the implications of these morphological changes.

Table 5. Regressions through the origin of phylogenetically independent contrasts for kinematic variables on maximal velocity.

Variable	n	R^2	t	P
Axial Angle	18	0.086	−1.27	0.222
HLL protraction	16	0.003	0.23	0.823
HLL retraction	16	0.002	0.16	0.874
Stride length	12	0.420	2.82	**0.017**
Step length	12	0.264	1.99	0.073
Stride duration	12	0.552	−3.68	**0.004**
Step duration	12	0.011	0.35	0.733
Duty factor	12	<0.001	0.01	0.996

Note: Skinks and phrynosomatines are combined in each analysis, but the root contrast is excluded because of the distant divergence time of the two clades, and only data collected from a sandpaper substrate are included. Significant P-values are in bold. The direction of each relationship is shown by the sign of the test statistic, t.

Body elongation and limb reduction have traditionally been viewed as evolving repeatedly following the same patterns in many clades (Lande 1978; Gans 1986; Caputo et al. 1995; Greer et al. 1998). For example, some authors have surmised that the elongation of the squamate body involved the addition of presacral vertebrae (Greer et al. 1998; Polly et al. 2001; Schmitz et al. 2005), and the loss of digits occurred only when a certain minimum threshold of digit size was reached (Lande 1978; Alberch and Gale 1985). Recent analyses of body shape evolution in vertebrates indicate that the evolution of a snake-like body shape can occur via multiple pathways. The evolution of a snake-like shape in squamates has occurred in at least two major ways, leading to either short-tailed species that tend to burrow (including *Lerista* skinks) or long-tailed species that tend to be surface-dwellers (Wiens et al. 2006). Although these two ecomorphs have evolved in similar ways across squamates, the burrowing morph typically arises through trunk elongation whereas the surface-dwelling morph arises through tail elongation (Brandley et al.

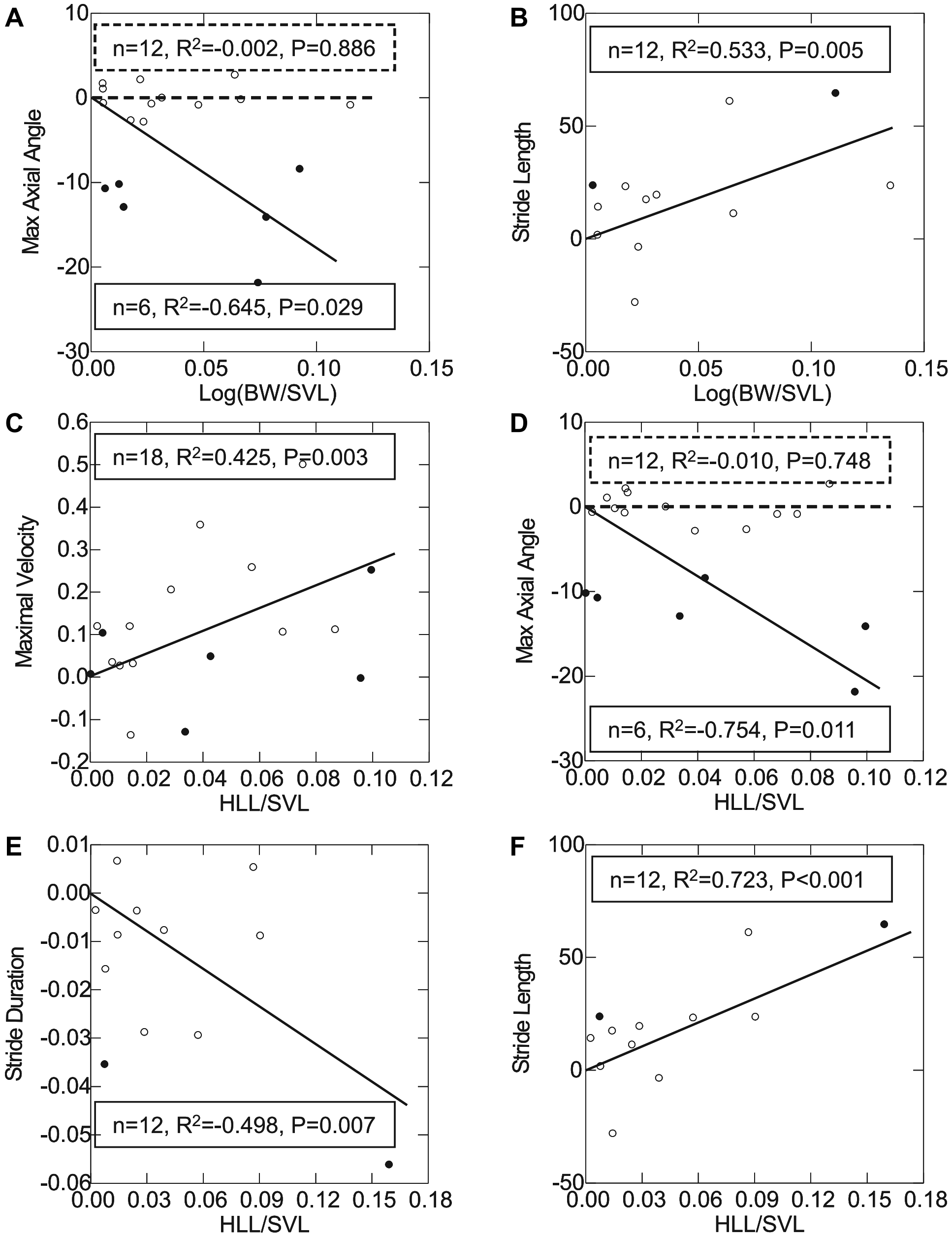

Figure 4. Plots of phylogenetically independent contrasts for maximal axial angle (A) and stride length (B) with log(BW/SVL), and maximal velocity (C), maximal axial angle (D), stride duration (E), and stride length (F) with HLL/SVL. In all cases: closed circles—skink contrasts, open circles—phrynosomatine contrasts. When two lines appear, the solid line represents a regression through the origin for contrasts for the skinks, a dashed line is a similar regression for the phrynosomatines. When only one line appears, it represents a regression for all contrasts. Maximal axial angle is in degrees, velocities are in meters per second, stride duration is in seconds, and stride length in millimeters. For each analysis, number of species (*n*), R^2, and *P* values are presented.

2008). In the spectacled lizards (Gymnophthalmidae), some lineages have front limbs that are more reduced than hind limbs whereas others exhibit the opposite trend, and these two morphs also differ in habitat use (Pellegrino et al. 2001). In various clades of fish, elongation occurs through the addition of vertebrae, the elongation of vertebrae, or both (Ward and Brainerd 2007).

Our results show how two seemingly opposite body shapes have evolved from different starting points, through changes in different sets of body parts following quite different trajectories. One might expect that stockiness arises through the broadening and shortening of the body (e.g., Brainerd and Patek 1998) whereas a snake-like body shape arises through the narrowing and lengthening of the body (e.g., Caputo et al. 1995) (with no predictions about the limbs made here). However, our findings show that the evolution of stockiness in the phrynosomatines involves the widening of the body and head, but not the shortening of the body whereas the evolution of elongation in *Lerista* involves the lengthening but not the narrowing of the body (Table 1). Limb lengths also have become shorter both as the phrynosomatines become stockier and as *Lerista* species become more elongate. Therefore, our data clearly indicate that the two clades are evolving morphologically in very different ways. This may be partially explained by the fact that the ancestral body shape of the skinks that we studied had a significantly longer, narrower body and shorter hind limbs than the ancestral phrynosomatine (Fig. 2B). Such different starting morphologies may predispose the two clades to evolve their body shapes along different trajectories.

HOW DO FUNCTIONAL ASPECTS OF BODY SHAPE INFLUENCE LOCOMOTION?

From our findings on the morphological evolution of stocky and snake-like body shapes, one might expect very different pathways of locomotor evolution for these two clades, leading to different kinematic strategies being adopted by the different species studied. The difference between locomotor evolution and kinematic strategy is subtle but important. The former refers to the changes in locomotor performance and kinematics that have occurred as body shape has evolved. For example, as body elongation has evolved in *Lerista*, axial bending has also evolved, such that more elongate species bend their bodies more during locomotion. This has not been the case in the Phrynosomatinae. Kinematic strategy refers to the actual values of the locomotor variables exhibited by the different species. For example, *Callisaurus draconoides* takes longer strides than *Sceloporus jarrovii* on account of its relatively longer hind limbs. We showed that despite having very different body shapes and patterns of morphological evolution, the two focal clades of lizards had similar patterns of locomotor evolution (Fig. 4), which ultimately resulted in different kinematic strategies (Fig. 3).

Evolutionary trends in locomotor variables were largely coincident between the two clades. For example, as relative hind limb length increased, sprinting velocity and stride length and frequency also increased (Fig. 4C,E,F). These patterns of locomotor evolution resulted in differently shaped species using different kinematic strategies: the species studied moved with different velocities, stride lengths and frequencies, a finding consistent with previous work on a variety of vertebrates (Irschick and Jayne 1998; Bonine and Garland 1999; Hoyt et al. 2000; Biewener 2003; Braña 2003). However, some, but not all of these patterns were directly dependent on differences in velocity among species. Species with relatively longer limbs moved faster by taking longer and quicker strides, but the relationship between stride duration and limb length was a function of velocity whereas the relationship between stride length and relative hind limb length was not dependent on velocity. Hence, when differential velocity among species is accounted for, species with relatively longer hind limbs take longer, but not quicker, strides. This finding concurs with the reasoning that one would expect longer strides to be taken by animals with relatively longer limbs.

We also found a positive evolutionary trend between relative body width and stride length (Fig. 4B), which likely occurs because species with broader bodies had more robust limbs (Fig. 2B). However, this relationship disappeared when the differential velocity among species is accounted for. Nevertheless, more elongate species taking shorter strides is consistent with expectations of decreased reliance on limbs in the transition from limbed to limbless locomotion (Gans 1975; Shine 1986; Greer 1987; Renous et al. 1998). Furthermore, one might expect that species in the midst of this locomotor transition might move more slowly than those that are either well-limbed or completely limbless. In addition to having similar patterns of evolution to their limb kinematics as the phrynosomatines, *Lerista* modified their axial kinematics, with species with shorter limbs and narrower bodies bending their body axes more (Fig. 4A,D; Gans and Fusari 1994). The positive relationship between the degree of elongation and axial bending in the elongate skinks, but not phrynosomatines, occurs because the degree of bending is proportional to the number of vertebrae (Brainerd and Patek 1998; Moon 1999; Van Damme and Vanhooydonck 2002), and skinks vary markedly in presacral vertebral number (Greer 1987, 1990), whereas the phrynosomatines do not (P. J. Bergmann, unpubl. data).

The distinct kinematic strategies exhibited by different species are best identified by our multivariate analyses. Both morphologically and kinematically, *Lerista* skinks cluster well apart from the other species (Fig. 3A). This is partially due to the differential role of the body axis between the two clades, and partially because the short-limbed species of *Lerista* move relatively slowly, taking short and slow strides whereas long limbed species, such as *C. draconoides*, move very quickly, taking long and quick strides. However, although more elongate *Lerista* bend their body axes more, this does not translate into faster speeds compared to less-elongate species. We also note that *C. fallens*, the skink outgroup of *Lerista*, clustered with the phrynosomatines in both the morphometric and locomotor PCAs, as opposed to with its phylogenetic relatives (Fig. 3A). This suggests that there exists a link between body shape and locomotion in lizards that overrides phylogenetic effects. Taken together, these results show how focusing on morphology alone does not reveal the mechanisms by which different levels of performance are achieved, and that inclusion of kinematics is important for understanding phenotype-function relationships when examined at a broad macroevolutionary level.

EVOLUTION OF FORM AND FUNCTION: ADAPTATION TO DIFFERENT SUBSTRATES?

A general finding in studies of locomotion of terrestrial animals is that differences in body shape and morphology often represent adaptations to different habitats and that substrate characteristics often profoundly affect locomotion (Carothers 1986; Jayne 1986; Kelley et al. 1997; Macrini and Irschick 1998; Melville and Swain 2000; Zani 2000; Claussen et al. 2002; Vanhooydonck et al. 2002). For example, in elongate squamates, lateral undulation on sand is intermediate between lateral undulation underneath the surface and on a firm substrate (Gans 1986). Substrate effects are also seen in eels (*Anguilla rostrata*), which undulate using more of their bodies when moving on land versus through the water (Gillis 1998). Lizards with shorter bodies and longer limbs tend to inhabit more open habitats, whereas elongate species inhabit more cluttered habitats that would interfere with freedom of movement of long limbs (Melville and Swain 2000; Kohlsdorf et al. 2001; Herrel et al. 2002; Van Damme and Vanhooydonck 2002; Newbold 2005).

Despite these a priori expectations, we did not find any significant differences among species in locomotor parameters on sand versus sandpaper substrates for the phrynosomatines or *Lerista*. In the only other study of locomotion in *Lerista*, which included only three species, lizards moved more slowly on a smooth Plexiglas substrate than on a sandpaper substrate (Gans and Fusari 1994). Our findings show that differently shaped species can move effectively on different substrates, and so may actually be substrate generalists. Our manipulation of substrate shows little evidence of specialization, and substrate effects may be less of a factor for small animals than large ones. This line of research should be expanded in the future to include habitat use in the field, comparing between used and available substrates to test the degree to which differently shaped species are substrate specialists or generalists (e.g., Vanhooydonck et al. 2005).

A NEW VIEW OF THE FUNCTIONAL IMPLICATIONS OF THE EVOLUTION OF BODY SHAPE

Although it is recognized that any comprehensive theory of morphological evolution must integrate genetic, developmental, functional, and evolutionary approaches (Alberch and Gale 1985), many of these components have been studied in isolation in explaining body shape evolution. A phylogenetic approach has been notably absent in many otherwise strong studies of body shape development (reviewed by Carroll et al. 2005; also see Raynaud 1990; Cohn and Tickle 1999; Shapiro 2002; Shapiro et al. 2003; Pilbeam 2004) and function (e.g., Gans 1975; Gans and Fusari 1994; but see Melville and Swain 2000). Through our integrative approach, we have shown that morphological evolution, locomotor evolution, and locomotor kinematics and performance do not coincide in an intuitive way, demonstrating that all must be considered to understand their interrelationships. Given that the phrynosomatines and *Lerista* exhibit different patterns of body shape evolution, we expected that they might also have different patterns of locomotor evolution, which would then lead to considerable variation in kinematic strategies. However, what we found was that the two clades had very similar patterns of locomotor evolution—stride characteristics, and especially stride length, were the principle factors involved in locomotor evolution with axial characteristics playing a secondary role. This is suggestive of many-to-one mapping that is seen in fish feeding (Wainwright et al. 2005; Westneat et al. 2005). Ultimately, similar patterns of locomotor evolution occurring in clades with different body shapes and patterns of morphological evolution lead to distinct kinematic strategies. Although body shape can evolve along different pathways in different taxa, the evolution of locomotion may be more conserved because there are a limited number of ways of changing how the body moves.

ACKNOWLEDGMENTS

We are grateful to K.P. Bergmann, U. Kawai, and J.J. Meyers for indispensable help in the field. We thank A. Trombecky, H. Li, and H.-J. Kim for help with digitizing videos, and to C.R. Buckley, J. Henningsen, D.M. McMillan, and S.E. Vincent for helpful discussions. We thank R.A. Duckworth, J.J. Wiens, and R.R. Weaver and two reviewers for thoughtful comments on earlier drafts of the manuscript. C.J. Bell (University of Texas Austin), C. Clemente (University of Western Australia), P. Doughty (Western Australian Museum – WAM), P. Kendrick (Conservation and Land Management – CALM), B. Maryan (WAM), and M. True (CALM) gave valuable information for work in Australia. D. Stefoni (CALM) issued permits CE001255 and SF005318 for Western Australia. T.R. Jones and M. Swain issued permit SP720856 from Arizona Fish and Game. B.L. Morrill and R.L. Pearce facilitated work on the US Marine Corps Barry Goldwater Range, Arizona. M. Hutchinson and A. Skinner (Southern Australian Museum) provided prepublication phylogenies of *Lerista* to help with analysis. This research was conducted under IACUC approval 0189-2-16-0301 (Tulane University). It was supported by funding from a Natural Sciences and Engineering Research Council of Canada PGS-D scholarship, a Gaige Award from the American Society of Ichthyologists and Herpetologists, a Grant-in-Aid of Research from Sigma Xi, and a Grant-in-Aid of Research from the Society for Integrative and Comparative Biology to PJB, and an NSF grant to Duncan J. Irschick (IOB 0421917).

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Associate Editor: F. Galis

Appendix A1

LOCALITY INFORMATION FOR SPECIMENS COLLECTED

No specimen numbers are given because most specimens were released at site of capture. Numbers include voucher specimens that appear in Appendix S1. Some species were collected at multiple localities. Sample sizes are in parentheses.

Arizona, U.S.A.: Barry Goldwater Range, Yuma Co.: *Callisaurus draconoides* (1), *Phrynosoma mcallii* (20), *P. platyrhinos* (3), *Uma notata* (2); Agua Caliente Road, Maricopa Co.: *C. draconoides* (20), *P. platyrhinos* (22), *Sceloporus magister* (1), *Uta stansburiana* (20); Chiricahua Mountains, Cochise Co.: *P. cornutum* (3), *P. modestum* (11), *S. jarrovii* (21), *S. virgatus* (22); Santa Rita Mountains, Pima Co.: *Holbrookia maculate* (22), *P. solare* (3); Tucson, Pima Co.: *S. magister* (14); Wet Beaver Creek, Yavapai Co.: *Cophosaurus texanus* (10).

Western Australia: Hamelin Station, Shark Bay: *Lerista macropisthopus* (3); Peron Station, Shark Bay: *L. macropisthopus* (21), *L. varia* (15); Nanga Station, Shark Bay: *L. lineopunctulata* (northern form) (8), *L. macropisthopus* (1), *L. varia* (1); Perth area: *C. fallens* (2), *L. elegans* (4), *L. praepedita* (2); Tamala Station, Shark Bay: *L. elegans* (2), *L. lineopunctulata* (northern form) (21), *L. praepedita* (8); Yanchep area: *L. lineopunctulata* (southern form) (6).

Supporting Information

The following supporting information is available for this article:

Appendix S1. Alternate pathways of body shape evolution translate into common patterns of locomotor evolution in two clades of lizards.

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