



## Rock-dwelling lizards exhibit less sensitivity of sprint speed to increases in substrate rugosity

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### ABSTRACT

Effectively moving across variable substrates is important to all terrestrial animals. The effects of substrates on lizard performance have ecological ramifications including the partitioning of habitat according to sprinting ability on different surfaces. This phenomenon is known as sprint sensitivity, or the decrease in sprint speed due to change in substrate. However, sprint sensitivity has been characterized only in arboreal *Anolis* lizards. Our study measured sensitivity to substrate rugosity among six lizard species that occupy rocky, sandy, and/or arboreal habitats. Lizards that use rocky habitats are less sensitive to changes in substrate rugosity, followed by arboreal lizards, and then by lizards that use sandy habitats. We infer from comparative phylogenetic analysis that forelimb, chest, and tail dimensions are important external morphological features related to sensitivity to changes in substrate rugosity.

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### 1. Introduction

Effectively moving across variable and complex habitat structures is important to terrestrial animals for prey capture, predator evasion, and acquiring mates (Bock and Von Wahlert, 1965; Hespeneide, 1973; Arnold, 1983; Ricklefs and Miles, 1994; Calsbeek, 2008). One aspect of habitat structure, substrate, imposes varied constraints on locomotion including incline, compliance, and rugosity (the amount of unevenness). Sand, trees, and rocks are three broadly defined substrates that impose unique physical constraints on locomotion. In rocky habitats, with sparsely distributed plants, for example, distances to refuges are often great, and substrate rugosity is high (Goodman, 2007). By comparison, sand substrates are compliant, not rugose, and inclined (Jayne and Irschick, 2000). Arboreal habitats, in contrast, impose physical constraints via varying perch diameter and incline (Sinervo and Losos, 1991; Losos et al., 1993). If a central assumption in the field of functional morphology is that morphological traits are related to habitat, then measurements of tasks such as locomotion in ecologically relevant settings should yield matches between the species tested and its habitat.

Because lizards occur in many habitats and exhibit putative locomotor adaptations to habitat structure, they are a model system for tests of adaptation to substrate. Ecomorphological relationships have been examined many times in lizards that use sand, trees, and rocks; yet there exists no unambiguous support for strong ecomorphological relationships. Laterally projected, elongated toe fringes are widely believed to increase toe surface area, and thus traction, on substrates such as sand and water (Carothers, 1986; Luke, 1986). Yet no difference in sprint speed on sand was found between lizards that had toe fringes vs. those that lacked them by Korff and McHenry (2010). Therefore, differential habitat use in these two lizard species was not related to morphological differences. In the skink subfamily *Lygosominae*, the degree of rock use is correlated with increased jumping, clinging, and sprint performance (Goodman et al., 2008). Sprint performance increases may be directly related to the distance an animal has to run to escape predation, which may be comparatively greater in rocky habitats than in other terrestrial habitats (Vitt et al., 1997; Revell et al., 2007; Goodman, 2009). Furthermore, rocky habitats are challenging for animals to use because they include inclines and unsteady surfaces (Revell et al., 2007; Goodman et al., 2008). Therefore, locomotion on rocks may exert a strong selection pressure on locomotor morphology and performance (Taylor et al., 1972; Farley and Emshwiller, 1996; Goodman et al., 2008). However, Tulli et al. (2012) found no differences in sprint speeds among *Liolaemus* species that use trees, rocks, or sand. Furthermore, the same study found each ecomorph could be out-performed by other ecomorphs on its respective substrate.

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A well-supported model of habitat, morphology, and performance relationships is the radiation of *Anolis* lizards in the Caribbean. Caribbean *Anolis* lizards have been shown to partition their habitat relative to sprint sensitivity, which is the decrease in sprint speed from a perch of large diameter to a perch of smaller diameter (Losos and Sinervo, 1989; Irschick and Losos, 1999). *Anolis* species with longer legs and higher overall sprint speeds have greater sprint sensitivity (i.e., greater loss of performance on smaller diameter perches) and avoid perches where sprint performance is submaximal (i.e., smaller perches). In contrast, anoles with shorter legs, slower sprint speeds, and lower sprint sensitivity values utilize a broad range of perches in the arboreal habitats (Irschick and Losos, 1999).

Here we quantified sprint sensitivity in lizards that use trees, rocks, or sand. Rock dwelling was equated to living on highly rugose (uneven) substrates. Our analysis of sprint sensitivity was focused on an increase in substrate rugosity. Substrate rugosity was manipulated by increasing substrate particle size from sand to pebbles to cobbles. Sprint sensitivity was defined as the difference in sprint speed on a more rugose substrate (e.g., cobbles) compared to a less rugose substrate (e.g., sand). It was hypothesized that as substrate rugosity increased, sprint speed would decrease. We predicted rock-dwelling lizards would exhibit lower sprint sensitivity values than their sand-dwelling counterparts, and that tree-dwelling lizards would be intermediate along a continuum of sprint sensitivity. We predicted that arboreal lizards would have overall lower sprint speeds and that for them the variance among substrates may be low.

We also quantified variation in morphology among the study species. It was hypothesized that rock-dwelling lizards would exhibit relatively longer limb segments, as seen in *Lygosominae* skinks. This is logical because longer legs can be used to increase stride length and hip height, which would help move over highly rugose substrates (Biewener, 1990, 1991; Russell and Bels, 2001). It was also hypothesized that rock dwelling lizards would have longer tails, which aid in stability (Jusufi et al., 2008; Gillis et al., 2009; Libby et al., 2012).

Sprint sensitivity and morphology were quantified in six species of lizards: *Aspidoscelis tigris*, *Crotaphytus bicinctores*, *Gambelia wislizenii*, *Sceloporus occidentalis*, *Sceloporus undulatus*, and *Sceloporus woodi*. These species were chosen because their relative phylogenetic positions are reasonably known, the species vary in their habitat preferences (rocks, sand, trees), and they represent different body plans. *C. bicinctores* and *G. wislizenii* represent ideal study species because they are closely related (family: Crocodylidae), they are broadly sympatric, and they share a similar body plan (McGuire et al., 2007). However, *G. wislizenii* uses sandy habitats and *C. bicinctores* uses rocky habitats (Pianka, 1966, 1967). Therefore, differences in performance and morphology may represent adaptations to their habitat use. *A. tigris* is useful as an outgroup because it represents a different body plan, is distantly related to the other focal species in the study, and uses sandy substrates (Pianka, 1966, 1967; Estes et al., 1988). *S. occidentalis* lives in rocky habitats throughout much of its range and is also broadly sympatric with *C. bicinctores*. *S. woodi* is a small lizard that uses sandy substrates similar to *A. tigris* and *G. wislizenii*, although it is occasionally found near the ground on tree trunks (Branch et al., 2003). *S. undulatus* is arboreal, though it also uses rocks and other perches in portions of its range (Pounds and Jackson, 1983). The population of *S. undulatus* sampled for this study occurs in a locality in southeast Georgia where large rocks do not occur. All *S. undulatus* were collected on the boles of pine and oak trees. The three *Sceloporus* species were included because they use different habitats, are thought to share a similar body plan, and are closely related. Therefore, differences in performance among these species may also reflect adaptations to different habitats.

The present study is significant because previous research has focused on locomotor strategies while crossing obstacles (Kohlsdorf and Biewener, 2006; Olberding et al., 2012; Tucker and McBrayer, 2012) and only one other study has characterized how lizards sprint over three ecologically similar substrates (Tulli et al., 2012). We apply the concept of sprint sensitivity to lizards other than *Anolis*, and shed light on the adaptive significance of using substrates that vary in rugosity among several species.

## 2. Materials and methods

### 2.1. Study animals and capturing procedures

Performance and morphology measurements for *A. tigris*, *C. bicinctores*, *G. wislizenii*, and *S. occidentalis* were collected in the Alvord Basin and surrounding cliffs in southeastern Oregon (N 42.296097°, W 118.656414°). The Alvord Basin is characterized by sandy flats, hardpan, and dune habitats (Steffen and Anderson, 2006). *S. woodi* were collected from longleaf pine (*Pinus palustris*) forests and sand pine (*Pinus clausa*) scrub in the Ocala National Forest (N 29.257726°, W 81.778702°) and *S. undulatus* were collected from longleaf pine and American turkey oak (*Quercus laevis*) scrub in George L. Smith State Park Georgia (N 32.559930°, W 82.113771°). Lizards were found by walking haphazard transects in suitable habitats for each respective study species. Sampling occurred between 830 h and 1230 h and between 1400 h and 1800 h from June 1 to July 25, 2010 for *A. tigris*, *C. bicinctores*, and *G. wislizenii*, from June 1 to July 25, 2011 for *S. occidentalis*, and September 5 to September 20, 2011 for *S. woodi* and *S. undulatus*. Lizards were captured by noose or hand. If possible, the field active body temperature of each lizard was taken with a rapid reading cloacal thermometer immediately upon capture. This was done to ensure body temperature for each species during performance trials was similar to those experienced naturally. Only adult males were used in this study to reduce the variability in performance associated with gravidity (females) and development (subadults/juveniles). Lizards were placed in individual cloth bags and the GPS coordinates of the capture site were recorded. Lizards were released at the point of capture after all data collection had been completed. Lizards were captured and maintained according to IACUC protocol 109009.

### 2.2. Performance trials

Sprint speed in *A. tigris*, *C. bicinctores*, *G. wislizenii*, and *S. occidentalis* was measured in the field. Three straight, level runways (5 m long × 0.25 m wide × 0.4 m high) were constructed using aluminum flashing. The first runway contained coarse sand particles ranging from 0.5 to 2.0 mm diameter. The second runway contained pebbles ranging from 30.0 to 35.0 mm diameter. The third runway contained cobbles ranging from 150 to 200 mm diameter. Substrate particles were discriminated visually according to the Wentworth particle scale. Substrates were collected from nearby areas after observing the lizards moving across them. Sand was collected from sites used by *A. tigris* and *G. wislizenii*. Cobbles were collected from sites used by *C. bicinctores* and *S. occidentalis*. Pebbles were used as an intermediate substrate because they are seen in both sandy and rocky habitats. Vegetation was placed at one end of each runway to serve as a refuge that could be detected by the lizard during its escape down the runway. Each quarter meter of the runway was marked to estimate distance in video recordings. For *A. tigris*, *C. bicinctores*, *G. wislizenii*, and *S. occidentalis* sprinting ability was tested 24–48 h after capture. This time allowed

the lizards to pass any gut contents and to recover from handling stress.

*S. woodi* and *S. undulatus* were captured and returned to a laboratory setting at Georgia Southern University. *S. woodi* and *S. undulatus* were tested over a three-day period after capture. Lizards were maintained separately in glass terraria with sand substrate, a water bowl, and a hide-out. Each terrarium was heated by a UV lamp at one end to produce a thermal gradient similar to those preferred by each species (Andrews, 1998; Crowley, 1985). Terrariums were heated and lighted from 700 to 1800 h daily. Lizards were fed crickets to satiation every other day and were fasted 24 h prior to sprinting trials. Three straight, level runways (4 m long  $\times$  0.25 m wide  $\times$  0.4 m high) were constructed using the same substrates as described above. Similarly, each quarter meter was marked to calibrate distances in video recordings.

Prior to each run, lizards were placed in a thermoelectric cooler in individual cloth bags until their core body temperatures reached their field active body temperatures (35–39 °C for *A. tigris*, *C. bicinctores*, *G. wislizenii*, and *S. woodi*; 32–35 °C for *S. occidentalis* and *S. undulatus*). All performance trials were conducted during normal lizard activity hours. Substrate temperature in each runway was closely monitored so that it was the same as the temperatures the lizards experience while active in their habitat (min: 21.5 °C; max: 57.5 °C). Before the first sprint trial, non-toxic correcting fluid was painted on the occiput of each lizard to use as a tracking marker in video recordings. Lizards were placed at the zero meter mark at one end of the runway and coerced to run toward the darkened end 5 m away. Lizards were chased down the length of each runway and, if necessary, were lightly tapped on the dorsum, tail, and legs in order to encourage them to run with maximal effort. Cameras filming at 30 fps were placed above the raceway at 1 m intervals to film the lizards as they ran through the field of view. Each camera recorded a 1 m segment of the raceway. The first 3 m of each run were filmed with Sony Handycams (meters 1–2: DCR-TRV330; meter 3: DCR-SR87; Sony, Tokyo, Japan). All velocity data presented here was taken from the third meter because analysis of videos from meters 4 and 5 indicated lizards did not increase in speed after the third meter.

Three to five trials per individual were conducted in each runway. Each individual was rested for 1–3 h between trials to allow for recovery. Runs were graded on a scale of 1–5 with 1 being a “refusal to run”, 4 representing a straight, continuous quadrupedal run, and 5 representing a straight, continuous bipedal run. Runs rated 1–3 were discarded; only runs rated 4 or 5 were retained to estimate maximal velocity. Only trials from individuals with at least one good run on all substrates were retained for further analysis. The average speed of the fastest 0.25 m was used for statistical analysis. This methodology allows for comparison with other studies using photocells to estimate sprint speed, where the fastest 0.25 m increment is retained for analysis.

### 2.3. Morphology

Fourteen morphological measurements were taken on each individual following all performance trials on all substrates. The measurements were chosen because they reflect variation in body form and are relevant for locomotor performance. Measurements were taken using dial calipers (or a ruler where applicable) and included: tail length; width of the body at the chest; depth of the body at the chest; humerus length (shoulder to the elbow); radius length (elbow to wrist); manus length; length of the longest finger (IVa) measured from the manus to the tip of the claw; pelvis width; femur length (hip to knee); tibia length (knee to ankle); pes length; length of the longest toe (IVb); intergirdle length; and snout-vent length (SVL) (for raw data see the supplementary Table S1).

## 2.4. Data analysis and statistics

### 2.4.1. Sprint sensitivity

Each run rated 4 or 5 was digitized, clipped, and converted to an AVI file with Adobe Premiere software (Adobe Systems Inc., San Jose, CA, USA). Each run was subsequently viewed in DLTdv3, which was used to manually digitize the white marker on the lizards' occiput in each video frame (Hedrick, 2008). An  $x$ – $y$  coordinate from each frame was exported and saved in Microsoft Excel 2007. Velocity was estimated by measuring the linear displacement of this marker between each frame. Velocity was calculated as the first derivative of the displacement data from the digitized marker on the lizard's head. A scale bar of 25 cm was digitized in each video to calibrate the distance traversed by the lizards in each frame. To minimize the effects of manual digitization error among frames, we smoothed the data by fitting a quintic spline to the  $x$ – $y$  coordinate data using GCVSPL software (Woltring, 1986; Walker, 1998). The first derivative was calculated from the spline coefficients fitted to the  $x$ – $y$  coordinate data. We used these data as an estimate for velocity for each run.

Sprint sensitivity was calculated by subtracting the sprint speed on a more rugose substrate from the sprint speed on a less rugose substrate (e.g., sprint speed sand – sprint speed pebbles = sprint sensitivity of sand/pebbles). Small values of sprint sensitivity indicate that speed was only slightly affected by the change in substrate. Large values indicate a greater influence on sprint speed due to the change in substrate. For descriptive purposes, we used a multi-way ANOVA with Tukey–Kramer post hoc tests to test for differences in sprint sensitivity among species. Comparative analysis for these data was not needed since we were trying to detect if declines in sprint speed were significant within each species, not among species.

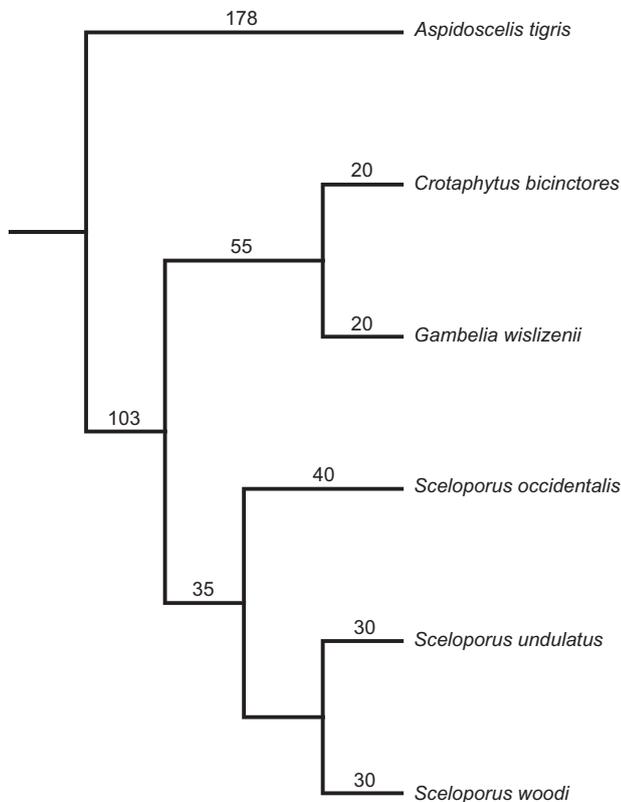
### 2.4.2. Morphology

To control for the size differences among and within species, morphological measurements were  $\log_{10}$  transformed, summed, and divided by the total number of measurements. This log-size component was then subtracted from each measurement (Mosimann and Frances, 1979). Each data point was centered by adding a value of 2 to each observation to create a new, size-adjusted measurement.

Prior to phylogenetic comparative analyses, principal components analysis (PCA) was used to ordinate the size-adjusted morphological variables into principal components. We determined the number of principal components to retain by carefully examining a scree plot and the eigenvectors of each principal component and via the broken stick rule (Jackson, 1993).

### 2.4.3. Phylogenetic analyses

We used two phylogenetic techniques to characterize the evolutionary relationship between sprint speed and morphology, as well as the differences in morphology among study species. Morphology and sprinting of *A. tigris*, *C. bicinctores*, *G. wislizenii*, *S. occidentalis*, *S. undulatus*, and *S. woodi* were examined using phylogenetic independent contrasts (PIC) (Felsenstein, 1985; Harvey and Pagel, 1991; Martins and Garland, 1991; Blomberg and Garland, 2002). Phylogenetic relationships were inferred from the available literature to generate a composite phylogenetic tree. Branch lengths (indicated in millions of years before present) were estimated based on morphological and molecular data gleaned from published data. Principal comparisons are among the two *Crotaphytidae* species and *Sceloporine* species, with *A. tigris* as an outgroup. (Estes et al., 1988; Leache and McGuire, 2006; McGuire et al., 2007; Townsend et al., 2011; Wiens et al., 2011) (Fig. 1). We used the PDAP:PDTREE module in Mesquite to calculate phylogenetic independent contrasts (PIC) on all size-adjusted morphological and sprint speed



**Fig. 1.** Phylogenetic relationships among focal species. Numbers indicate branch lengths in millions of years before present. Phylogenetic positions were derived from, and branch lengths were based on Estes et al. (1988), McGuire et al. (2007), and Wiens et al. (2011).

data (Garland et al., 1992; Midford et al., 2005; Maddison and Maddison, 2006). Absolute values of the PICs were plotted against their standard deviations to ensure the branch lengths used adequately fit the tip data. There were no significant trends ( $r^2 < 0.22$ ,  $p > 0.30$ ), so all branch lengths and tip data were retained for further analysis (Garland et al., 1992). Raw principal components PIC were then regressed with sprint sensitivity PIC (forced through to origin) to characterize evolutionary relationships between morphology and performance (Garland et al., 1999).

We used the Rhetenor package in Mesquite (Dyreson and Maddison, 2006) to ordinate morphological measurements that maximize evolutionary relationships. Rhetenor reconstructs ancestral states based on squared change parsimony and then conducts an eigenanalysis on each branch as a vector of evolutionary change. A multi-way ANOVA with Tukey–Kramer post hoc tests was applied to the evolutionary principal component (EPC) scores for each species to determine differences among species.

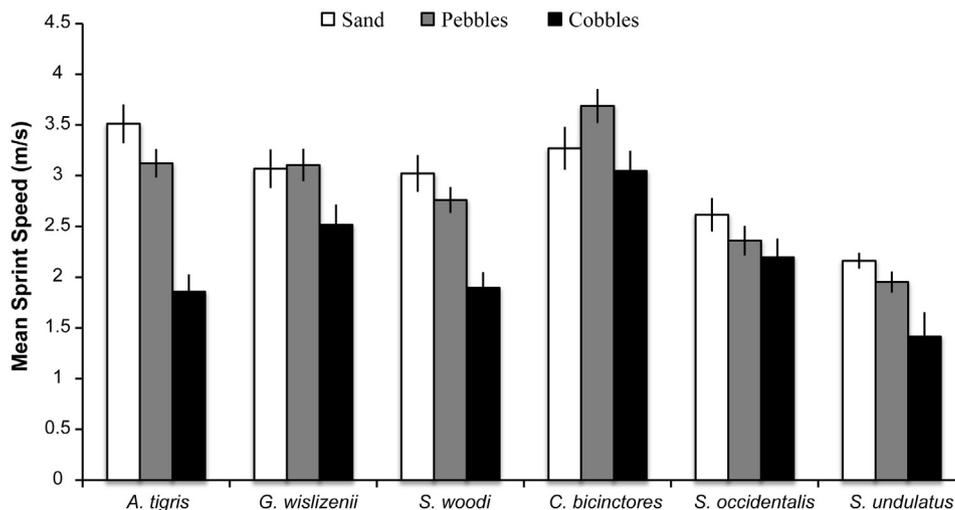
### 3. Results

#### 3.1. Sprint sensitivity

Overall, lizards in this study exhibited a decline in sprint speed as substrate rugosity increased. However, *C. bicinctores* got faster on pebbles compared to its velocity on sand. The decline in sprint speed from pebbles to cobbles in *C. bicinctores* was significant, but not between sand and cobbles (Table 1 and Fig. 2). The other rock-dwelling lizard, *S. occidentalis*, did not decrease in sprint speed between substrates (Table 1 and Fig. 2). All other lizards exhibited a significant decrease in sprint speed between sand and cobbles as well as pebbles and cobbles, but not between sand and pebbles (Table 1 and Fig. 2).

**Table 1**  
Sprint sensitivity values ( $\pm 1$  SE) for each species. Each sprint sensitivity value equals the velocity attained by each lizard on a more rugose substrate subtracted from that on a less rugose substrate.

Species (N)	<i>Aspidoscelis tigris</i> (11)	<i>Crotaphytus bicinctores</i> (14)	<i>Gambelia wislizenii</i> (12)	<i>Sceloporus occidentalis</i> (12)	<i>Sceloporus undulatus</i> (10)	<i>Sceloporus woodi</i> (9)
Sand/pebbles	0.40 $\pm$ 0.19	-0.46 $\pm$ 0.14	0.13 $\pm$ 0.19	0.35 $\pm$ 0.19	0.21 $\pm$ 0.20	0.08 $\pm$ 0.20
Sand/cobbles	1.54 $\pm$ 0.2	0.14 $\pm$ 0.14	0.76 $\pm$ 0.19	0.50 $\pm$ 0.19	0.75 $\pm$ 0.20	0.87 $\pm$ 0.20
Pebbles/cobbles	1.18 $\pm$ 0.17	0.71 $\pm$ 0.12	0.63 $\pm$ 0.16	0.15 $\pm$ 0.15	0.54 $\pm$ 0.17	0.79 $\pm$ 0.17



**Fig. 2.** Mean values ( $\pm 1$  SE) of sprint speed attained by each species on each substrate.

**Table 2**

Principal component factor loadings for the 13 size-corrected morphological traits measured on each lizard. For PC1, the eigenvalue was 4.286, with 33% of the variation in morphology explained. For PC 2, the eigenvalue was 2.99, with 23% of the variation in morphology explained. The eigenvalue for PC3 was 1.48, with 11% of the variation in morphology explained. Factor loadings >0.5 are highlighted in bold.

Morphological trait	PC1	PC2	PC3
Tail	0.42	<b>-0.81</b>	-0.23
Chest width	0.14	<b>0.75</b>	-0.25
Chest depth	<b>0.61</b>	0.47	-0.44
Humerus	-0.22	<b>0.62</b>	-0.07
Radius	0.191	<b>0.66</b>	<b>0.52</b>
Manus	0.29	0.09	-0.37
Longest finger	<b>-0.82</b>	0.25	0.23
Pelvis width	<b>-0.85</b>	0.31	0.05
Femur	<b>0.61</b>	0.32	0.38
Tibia	<b>0.63</b>	-0.18	0.43
Pes	0.81	-0.20	0.19
Longest toe	-0.48	-0.49	<b>0.50</b>
Intergirdle length	<b>-0.71</b>	-0.36	-0.29

3.2. Morphology

The first three PC axes of the PCA were most informative and explained 67% of the variation in morphology. The first PC axis described 33% of the variation. Positive loadings were associated with deep chest dimensions, long femurs, long tibias, and long pes while negative loadings were associated with long fingers, wide pelvises, and longer intergirdle dimensions (Table 2 and Fig. 3). PC 2 described 23% of the variation. Positive loadings were associated with species that have wide chests, long radii, and long humeri while negative loadings were associated with species that have long tails. PC 3 described 11% of the variation. Positive loadings were associated with species that have long radii and long toes (Table 2 and Fig. 3).

The first three EPCs were the most informative. They explained 51% of the variation in morphology after reducing the shared evolutionary history among species. The first EPC axis described 21% of the variation. Positive loadings were associated with species that have deep chest dimensions. The second EPC axis described 18% of the variation. Positive loadings were associated with species

**Table 3**

The first 3 non-phylogenetically corrected principal component factor loadings (PC1–3) averaged ( $\pm 1$  SE) for each species. These average loadings were used in the analysis of phylogenetic independent contrasts.

Species	PC1	PC2	PC3
<i>Aspidoscelis tigris</i>	0.65 $\pm$ 0.14	3.00 $\pm$ 0.14	0.61 $\pm$ 0.13
<i>Crotaphytus bicinctores</i>	-2.91 $\pm$ 0.14	0.04 $\pm$ 0.19	0.04 $\pm$ 0.34
<i>Gambelia wislizenii</i>	-2.00 $\pm$ 0.13	0.51 $\pm$ 0.20	0.08 $\pm$ 0.17
<i>Sceloporus occidentalis</i>	1.99 $\pm$ 0.11	-1.34 $\pm$ 0.18	0.18 $\pm$ 0.20
<i>Sceloporus undulatus</i>	1.81 $\pm$ 0.18	-1.17 $\pm$ 0.22	0.39 $\pm$ .024
<i>Sceloporus woodi</i>	1.24 $\pm$ 0.14	-0.07 $\pm$ 0.15	-1.71 $\pm$ 0.19

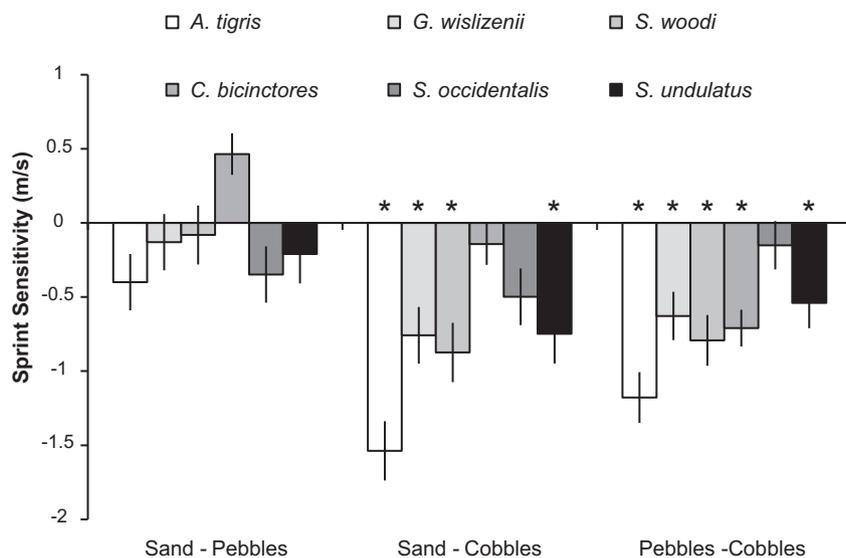
**Table 4**

Evolutionary principal component character loadings for the 13 size-corrected morphological traits measured on each lizard. EPC1 accounts for 21% of the variation in morphology, EPC2 accounts for 18% of the variation in morphology, EPC3 explains 12% of the variation in morphology. Character loadings > 0.5 are highlighted in bold.

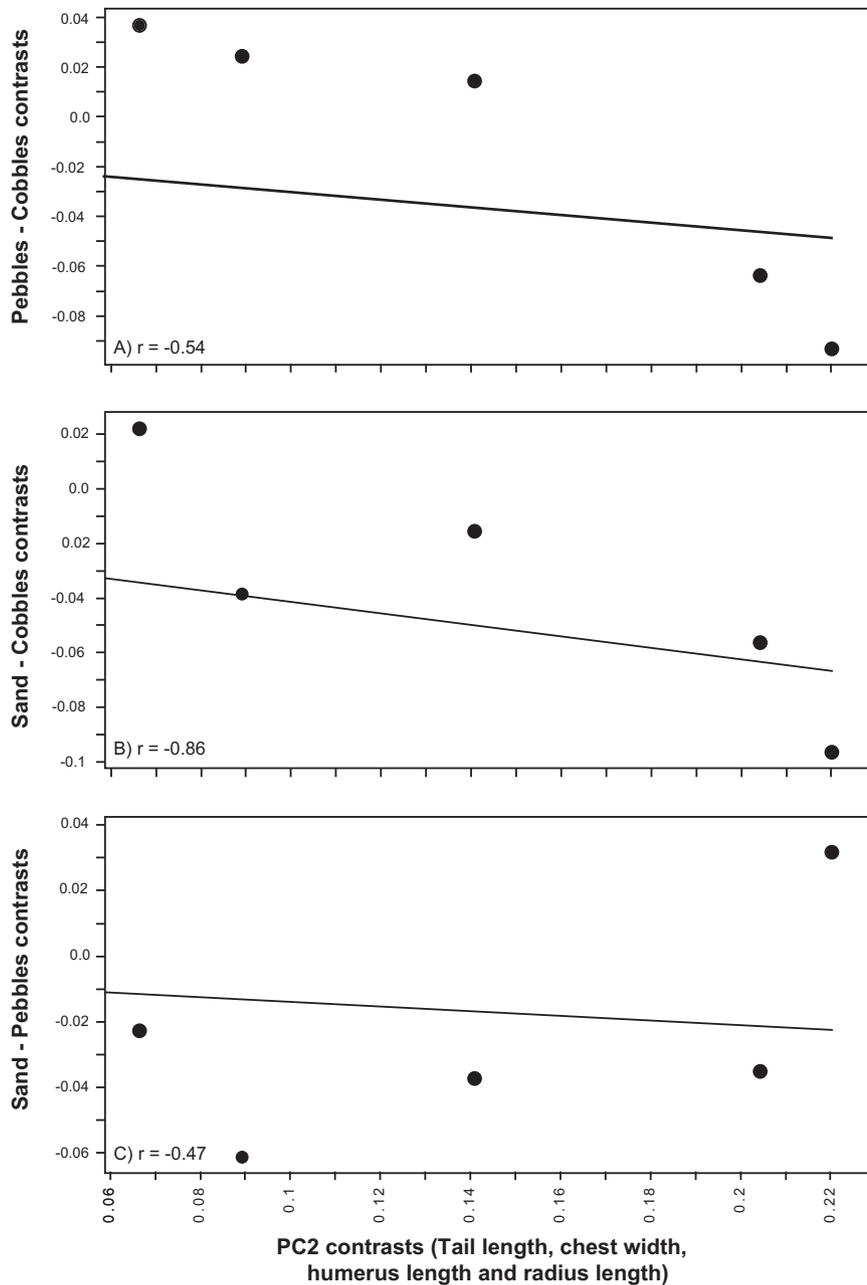
Morphological trait	EPC1	EPC2	EPC3
Chest width	-0.25	0.04	0.08
Chest depth	<b>-0.56</b>	0.05	-0.15
Humerus	0.32	-0.02	-0.08
Antebrachium	-0.01	0.11	0.17
Manus	0.23	-0.44	0.01
Longest finger	-0.21	-0.36	<b>0.56</b>
Pelvis	0.19	0.07	-0.05
Femur	-0.13	0.12	0.11
Tibia	-0.27	<b>0.69</b>	-0.14
Pes	-0.14	0.02	-0.03
Longest toe	-0.31	-0.03	0.30
Intergirdle	0.10	-0.01	0.02
Tail	-0.41	-0.41	<b>-0.69</b>

that have long tibias. EPC 3 described 12% of the variation. Positive loadings were associated with species that have longer fingers and negative loadings were associated with species with longer tails.

On EPC axis 1, *A. tigris* was significantly different from all other species. *G. wislizenii* was not significantly different from *S. woodi*, but these two species were different from all others. There were no significant differences among *C. bicinctores*, *S. undulatus*, and *S. occidentalis* (Table 3). On EPC axis 2, *A. tigris* was again significantly different from all other species. However, *S. occidentalis* was statistically different from *C. bicinctores*, but not from *S. undulatus*, *S.*



**Fig. 3.** Mean values ( $\pm 1$  SE) of sprint sensitivity for each species with respect to all substrate combinations. Bars with dots indicate species that use sand, horizontal hatching indicates rock-dwelling species, and diagonal hatching indicates arboreal species. *C. bicinctores* increased in velocity from sand to pebbles while all other lizards decreased in velocity. The two rock-dwelling species, *C. bicinctores* and *S. occidentalis*, did not exhibit a significant decline in velocity from sand to cobbles. All other species did. *S. occidentalis* did not exhibit significantly lower sprint speeds from pebbles to cobbles although all other species did. Asterisks indicate a significant decline in sprint speed for each species between the compared substrates ( $p < 0.001$ ,  $df = 5$ , two-way ANOVA with Tukey–Kramer post hoc comparisons).



**Fig. 4.** Correlation between sprint sensitivity and morphology (PC2) representing decreases in sensitivity with increased rugosity in six species of lizards: (A) pebbles/cobbles, (B) sand/cobbles, (C) sand/pebbles. Negative trends were indicated by Pearson product–moment correlations ( $r = -0.54, -0.86, \text{ and } -0.47$ ).

*woodi*, or *G. wislizenii*. There were no significant differences among *C. bicinctores*, *S. undulatus*, *S. woodi*, or *G. wislizenii* (Table 3). On EPC axis 3, there were significant differences between the *Sceloporus* species and the rest of the species (Table 3).

### 3.3. Phylogenetic relationships between morphology and performance

There was no relationship between PC1, PC2, PC3, and sprint speed on any substrate ( $p > 0.25$ ). However, the PIC analysis revealed a strong relationship between the use of rugose substrates, morphology, and sprint sensitivity. This trend was marked by contrasts on the PC2 axis, indicating tail length, wide chests, and long forelimb dimensions were important for sprinting on different substrates (Fig. 4A–C). A negative and significant trend was observed between PC2 contrasts and sprint sensitivity for sand to cobbles

( $r = -0.86, p = 0.03$ ) (Fig. 4B). Negative, but non-significant, trends were observed between the contrasts of PC2 and sprint sensitivity for pebbles to cobbles ( $r = -0.54, p = 0.16$ ) (Fig. 4A) and for sand to pebbles ( $r = -0.47, p = 0.34$ ) (Fig. 4C).

## 4. Discussion

Sprint sensitivity was valuable in diagnosing performance capabilities among species. Compared to the other species, rock-dwelling lizards exhibited reduced sensitivity to increased substrate rugosity. Evidence from the EPC analysis and sprint sensitivity analyses suggests a clear distinction between sand-dwelling and rock-dwelling animals. After accounting for phylogenetic relationships, we were able to detect morphological differences between two sets of species – *C. bicinctores*, *S. occidentalis*, and *S. undulatus*, and *G. wislizenii* and *S. woodi*. *A. tigris* was different from

**Table 5**

The first 3 evolutionary principal component factor loadings (EPC1–3) averaged ( $\pm 1$  SE) for each species. Within each PC, a shared letter indicates no significant difference between species.

Species	EPC1	Letter	EPC2	Letter	EPC3	Letter
<i>Aspidoscelis tigris</i>	1.04 $\pm$ 0.01	A	-0.21 $\pm$ 0.01	A	1.10 $\pm$ 0.01	A
<i>Sceloporus woodi</i>	0.95 $\pm$ 0.02	B	-0.30 $\pm$ 0.01	B, C	0.87 $\pm$ 0.01	B
<i>Gambelia wislizenii</i>	0.96 $\pm$ 0.01	B	-0.29 $\pm$ 0.01	B, C	1.07 $\pm$ 0.01	A
<i>Crotaphytus bicinctores</i>	0.89 $\pm$ 0.01	C	-0.32 $\pm$ 0.01	C	1.07 $\pm$ 0.01	A
<i>Sceloporus occidentalis</i>	0.85 $\pm$ 0.01	C	-0.27 $\pm$ 0.03	B	0.87 $\pm$ 0.01	B
<i>Sceloporus undulatus</i>	0.85 $\pm$ 0.01	C	-0.28 $\pm$ 0.04	B, C	0.88 $\pm$ 0.01	B

all other lizards (Table 3). Increased sensitivity to substrate rugosity was strongly correlated with longer limb dimensions and narrow bodies (Fig. 4B).

#### 4.1. Ecomorphological relationships

We found differences in morphology between those lizards that use rocks or trees, and those that use sand. However, these differences did not precisely correspond to sprint sensitivity. Whereas *C. bicinctores* and *S. occidentalis* exhibited reduced sensitivity to increased rugosity, arboreal *S. undulatus* was sensitive to the changes. Thus, the sprint sensitivity analysis was able to reveal a distinction between using rocks and trees. Hypothetically, many rock-dwelling animals use climbing to move through their environment (Vanhooydonck and Van Damme, 1999; Goodman, 2007; Goodman et al., 2008). However, the two rock dwellers in this study run over horizontal, yet rugose, surfaces. Although the number of species used in this study is relatively low, an important distinction can be made between rock and tree use in lizards. Two constraints to locomotion often associated with an arboreal lifestyle are perch diameter and climbing vertical tree trunks (Sinervo and Losos, 1991; Vanhooydonck et al., 2006). While climbing vertical surfaces seems intuitively similar to moving in rocky habitats, our observations indicate that *C. bicinctores* and *S. occidentalis* are often found on slightly inclined or horizontal, but very rugose rocky surfaces such as paleoclastic lava flows. Hence, we suggest substrate rugosity is a major constraint on locomotion in rocky habitats worthy of future research.

#### 4.2. Differences between closely related species

While *G. wislizenii* decreased in speed between sand and pebbles and pebbles and cobbles, the closely related *C. bicinctores* increased in speed between sand and pebbles, and showed no significant difference between sand and cobbles. Why would two lizards that are closely related and that share similar body plans show such dramatic differences? *C. bicinctores* is deeper bodied than *G. wislizenii* (Tables 4 and 5). Hence, it is plausible that *C. bicinctores* could slip or sink in the sand, which has higher compliance, because it is relatively heavier (Carothers, 1986; Luke, 1986; Glasheen and McMahon, 1996; Li et al., 2011). Furthermore, there is no difference in relative leg length between the two species (Tables 4 and 5). Important morphological information related to sprinting on different substrates may have been missed. For example, if *G. wislizenii* exhibits different toe morphology, the animal's mass could be distributed evenly across the sand, preventing this species from sinking (Carothers, 1986; Luke, 1986; Glasheen and McMahon, 1996). Furthermore, *C. bicinctores* almost exclusively uses bipedal running (unpublished data). Kinematic differences such as caused by bipedal running could serve two purposes for this species – raising center of mass while running, and increasing stride length. Each of these kinematic adjustments could increase stability on rugose substrates by allowing *C. bicinctores* to contact the surface without destabilizing its center of mass and by allowing the lizards to

contact the surface more efficiently (Daley et al., 2006; Kohlsdorf and Biewener, 2006; Biewener and Daley, 2007; Olberding et al., 2012).

#### 4.3. Stability in rugose habitats

In addition to sprint speed, dynamic stability is likely important in rugose habitats (Russell and Bels, 2001; Biewener and Daley, 2007; Revell et al., 2007). It is plausible that rock-dwelling animals such as *C. bicinctores* use upright body postures and/or bipedalism to increase dynamic stability over rugose surfaces of non-compliant substrates (Biewener, 2003). Clark and Higham (2011), for example, found that postural changes accounted for reduced falling over slippery surfaces in helmeted guinea fowl. A careful analysis of kinematic data could reveal why *C. bicinctores* has greater sprint speeds on rugose substrates compared to *G. wislizenii* despite their similar body plan. Furthermore, using rugose surfaces may necessitate increases in intrinsic mechanical and behavioral stability. The use of increasing muscle tension to absorb additional kinetic energy may aid in attaining high velocities (Daley et al., 2006; Biewener and Daley, 2007; Daley and Biewener, 2011). Such postural, limb angle, and mechanical adjustments have been exhibited in guinea fowl and highlight the need for such kinematic data in other terrestrial vertebrates such as lizards (Daley et al., 2006). This type of data may be especially important for closely related species that use different habitats.

#### 4.4. Conclusion

In summary, substrate rugosity plays an important, yet variable, role in terrestrial locomotion. This study revealed that there is much to be learned about how animals move over various substrates. Because animals exhibit a variety of mechanisms that serve to increase sprint speed on their respective native substrates, we suggest comparative biomechanical data should be collected to examine the evolutionary relationships between substrate, locomotion, and habitat use in terrestrial animals.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.zool.2013.01.001.

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