RESEARCH ARTICLE

Performance and three-dimensional kinematics of bipedal lizards during obstacle negotiation

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SUMMARY

Bipedal running is common among lizard species, but although the kinematics and performance of this gait have been well characterized, the advantages in biologically relevant situations are still unclear. Obstacle negotiation is a task that is ecologically relevant to many animals while moving at high speeds, such as during bipedal running, yet little is known about how obstacles impact locomotion and performance. We examined the effects of obstacle negotiation on the kinematics and performance of lizards during bipedal locomotion. We quantified three-dimensional kinematics from high-speed video (500 Hz) of six-lined racerunners (*Aspidoscelis sexlineata*) running on a 3 m racetrack both with and without an obstacle spanning the width of the track. The lizards did not alter their kinematics prior to contacting the obstacle. Although contact with the obstacle caused changes to the hindlimb kinematics, mean forward speed did not differ between treatments. The deviation of the vertical position of the body center of mass did not differ between treatments, suggesting that in the absence of a cost to overall performance, lizards forgo maintaining normal kinematics while negotiating obstacles in favor of a steady body center of mass height to avoid destabilizing locomotion.

Key words: locomotion, running, *Aspidoscelis sexlineata*.

INTRODUCTION

An animal's ability to successfully move through its habitat during predator escape, prey capture and territory defense can have strong impacts on its fitness (Garland and Losos, 1994). Animals live in complex habitats where they face many different challenges to locomotion, resulting in strong selection for the ability to deal with these challenges (Garland and Losos, 1994). Although locomotor performance is commonly viewed as an interaction of individual traits on which selection may act (Arnold, 1983), it is often quantified under laboratory conditions that are not entirely representative of the natural habitat where selection is active (Hertz et al., 1988; Ashley-Ross, 1994; Irschick and Jayne, 1999a). Animals may experience a number of different disruptions to steady locomotion when moving through the natural habitat, such as turns in the locomotor path (Higham et al., 2001) or uneven terrain (Daley et al., 2006). One example of a poorly examined disruption to locomotion in natural habitats is physical obstructions (obstacles) in the locomotor path (Stergiou et al., 2001; Watson et al., 2002; Kohlsdorf and Biewener, 2006). Understanding how animals deal with ecologically relevant scenarios, such as obstacle negotiation, can help us understand how selection has shaped the evolution of behavior, physiology and morphology.

Despite its relevance to many taxa, obstacle negotiation has been studied in relatively few systems, including cockroaches (Pelletier and McLeod, 1994; Watson et al., 2002), lizards (Kohlsdorf and Biewener, 2006; Kohlsdorf and Navas, 2007), cats (Drew, 1993; McFadyen et al., 1999), guinea fowl (Daley and Biewener, 2011) and humans (Chen et al., 1991; Sparrow et al., 1996; Chou et al., 1997; Begg et al., 1998; Stergiou et al., 2001; Patla and Greig, 2006).

In many of these systems, the focus has centered on the neuromuscular control of locomotion (Drew, 1993; Watson et al., 2002; Daley and Biewener, 2011) or the role of sensory systems during obstacle negotiation (Pelletier and McLeod, 1994; Patla and Greig, 2006), rather than the consequences to overall locomotor performance. Although lizards are a model system for studies of locomotor performance (Losos, 1990; Garland and Losos, 1994; Aerts et al., 2000; Higham et al., 2001; Irschick, 2003), there are few studies assessing obstacle negotiation in lizards (Kohlsdorf and Biewener, 2006; Kohlsdorf and Navas, 2007). For example, *Sceloporus malachiticus* exhibits a decrease in overall locomotor speed when negotiating obstacles (Kohlsdorf and Biewener, 2006). In addition, these lizards increasingly elevate the forelimb during obstacle negotiation to pull the center of mass (COM) up and over the obstacle (Kohlsdorf and Biewener, 2006). Although previous studies of obstacle negotiation have focused on locomotion in quadrupedally running lizards, many lizard species in several different families commonly employ bipedal gaits when running at speeds similar to those during quadrupedal running (Snyder, 1952; Snyder, 1962; Irschick and Jayne, 1999a; Clemente et al., 2008). For example, bipedal running speeds for *Dipsosaurus dorsalis*, *Uma scoparia* and *Aspidoscelis tigris* differ from average quadrupedal running speeds by only $0.1 \, \text{m s}^{-1}$ (Irschick and Jayne, 1999a).

Many groups, including cockroaches, lizards, small rodents and primates, can run using differing numbers of limb pairs, but only cockroaches and lizards must start running using all limb pairs before transitioning to a bipedal gait in the same locomotor bout (Snyder, 1952; Djawdan and Garland, 1988; Full and Tu, 1991; Thorpe et al., 2007). Despite its widespread occurrence in lizards, the

mechanisms and advantages of bipedal locomotion are poorly understood (Snyder, 1949; Snyder, 1952; Snyder, 1962; Urban, 1965; Irschick and Jayne, 1999a; Irschick and Jayne, 1999b; Aerts et al., 2003; Clemente et al., 2008; Rocha-Barbosa et al., 2008). Early studies have suggested that bipedal running in lizards is inherently faster than quadrupedal locomotion, similar to bipedal locomotion in cockroaches (Snyder, 1962; Full and Tu, 1991). However, analysis of high-speed locomotion of four different lizard species showed no significant differences in speed between bipedal and quadrupedal running (Irschick and Jayne, 1999a). Another suggested advantage of bipedality is the possible increase in environmental perception during locomotion by elevating the head and expanding the visual field, but this hypothesis has not yet been tested (Kohlsdorf and Biewener, 2006). Modeling of small lizards running at high speeds has suggested that bipedal running is the passive result of torques generated about the hips by high acceleration (Aerts et al., 2003). Australian agamid lizards achieve a bipedal gait at acceleration thresholds similar to those predicted by this model (Clemente et al., 2008), but a direct test of the model using force measurements from bipedal lizards remains to be done. A bipedal gait may be specifically advantageous during obstacle negotiation because of the increased height of the body COM, unlike quadrupedal lizards, which must elevate the body COM prior to contacting an obstacle (Kohlsdorf and Biewener, 2006). Unlike many animals that use bipedal locomotion, lizards are also capable of running quadrupedally, thus allowing for comparisons between the two gaits (Irschick and Jayne, 1999a). The ability to negotiate obstacles without suffering the same performance decrease seen in quadrupedally running lizards may be an advantage of bipedal locomotion upon which selection may act. However, these potential advantages have not been explored.

The six-lined racerunner, *Aspidoscelis sexlineata* Reeder 2002, is a small diurnal lizard that is common across the eastern United States (Fitch, 1958; Hardy, 1962). These lizards are terrestrial wide-foragers, employ a bipedal gait during high-speed running and regularly occur in habitats where vegetation and debris make obstacle negotiation an ecologically relevant task (Fitch, 1958; Hardy, 1962). Despite an early report of possible running speeds of $8\,\mathrm{m\,s^{-1}}$, few studies have examined the locomotion of this species (Hoyt, 1941; Urban, 1965; Ballinger et al., 1979). The extreme speeds attributed to this species suggest that running performance impacts its fitness and make it well suited for studies of high-speed obstacle negotiation.

By examining the three-dimensional kinematics and performance of *A. sexlineata* during unobstructed and obstructed locomotion, we address the following questions. (1) Relative to an unobstructed path, does locomotor performance during bipedal locomotion decrease with the presence of an obstacle? Mean forward speed has been commonly used as a measure of locomotor performance, but the advent of new techniques in the past decade has made it easier to measure acceleration, which may be more important in predator–prey interactions (Garland and Losos, 1994; McElroy and McBrayer, 2010). Acceleration is probably most crucial at the start of locomotion in order to initially increase the distance from a threat, but determining acceleration during a prolonged steady run may not be an accurate measure of performance. Therefore, we used mean forward speed as a measure of performance rather than mean acceleration. We expect no changes in mean forward speed in the presence of an obstacle because of the increased height of the COM during bipedal running (Kohlsdorf and Biewener, 2006). (2) Do the hindlimb kinematics of bipedal lizards change during obstacle negotiation? We expect that lizards will alter their kinematics in the presence of an obstacle in a manner similar to that of humans approaching an obstacle in the locomotor path in order to avoid disrupting locomotion (Chen et al., 1991). Specifically, elevating the hindlimb in the swing phase preceding contact with an obstacle would help to counteract the sudden increase in the surface height caused by the obstacle.

MATERIALS AND METHODS

Experiments were performed using six individuals of *A. sexlineata* [4.4–8.5 g, snout–vent length (SVL)=5.8–6.8 cm] collected in summer 2010 from Ocala National Forest, Ocala, FL, USA (US Department of Agriculture Forest Service permit SEM451 and Florida Fish and Wildlife Conservation Commission permit WX07348 to L.D.M.). Lizards were housed individually in 10 gallon aquaria, fed crickets every other day and given water *ad libitum* following arrival from the field. The lizards were encouraged to run down a 3-m-long racetrack that was either unobstructed or had a small rectangular obstacle (1.8 cm high by 5.6 cm along the length of the track) placed across its width, with a height estimated to be 50% of the hindlimb length of the lizards (Fig. 1). Because there are currently no data on the specific characteristics of obstacles encountered by these lizards in their natural habitat, we chose an obstacle height that could potentially challenge normal bipedal locomotion without requiring the use of the forelimbs (a distinct obstacle negotiation strategy) (Kohlsdorf and Biewener, 2006). The racetrack was 30 cm wide and allowed the lizards to run at an angle relative to the length of the track, but this behavior was rare. Any trials in which the lizards ran at an angle that resulted in contact with one of the walls were excluded from analysis. Two synchronized high-speed digital video cameras (1080×1080 pixels, Photron APX-RS, Photron USA Inc., San Diego, CA, USA) operating at $500\,\mathrm{frames\,s^{-1}}$ were used to obtain detailed body and limb movements. One camera was positioned perpendicular to the track to provide a lateral view, while a mirror mounted above the track at a 45 deg angle allowed us to obtain a dorsal view in the second camera. In order to calibrate the three-dimensional space that was captured by the two cameras, we placed a custom calibration object in the field of view prior to experiments; this object was composed of an aluminum sheet with a number of posts (of varying height) screwed into the sheet.
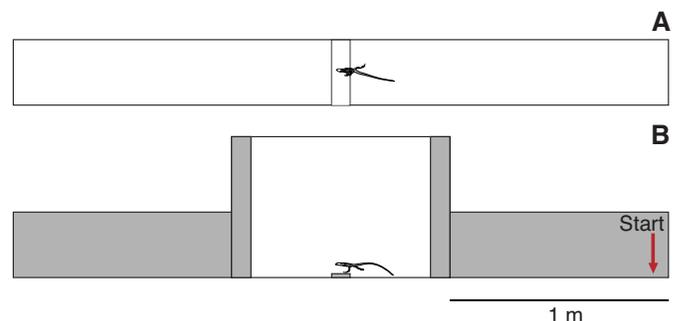

Fig. 1. (A) Dorsal and (B) lateral views of the racetrack (3 m×30 cm, length×width) with a scaled diagram of a lizard for visual reference. The obstacle consisted of a rectangular prism (1.8×5.6 cm) extending across the width of the track. All running surfaces were covered with cork to provide traction and the racetrack was enclosed with plywood on all sides except for a 76-cm-long section of track in the field of view of the camera that was enclosed with plexiglass. After being placed at one end of the track, the lizards ran towards a cardboard box located at the far end to simulate a refugium.
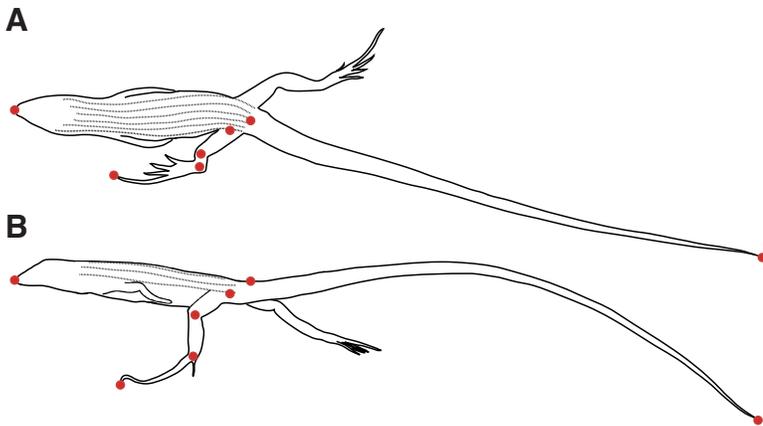
A

B

Fig. 2. Schematic diagram of *Aspidoscelis sexlineata* in (A) dorsal and (B) lateral views with red circles indicating anatomical landmarks used during digitizing. The points from left to right in the lateral view are: tip of snout, tip of fourth digit, ankle, knee, hip, base of tail and tip of tail.

We conducted trials once per day for a period of 6 weeks, collecting only three or four video sequences per day from each lizard in order to avoid any influence of fatigue. Lizards were fasted for 24 h before being randomly presented with either treatment on each day of testing. Performance between the beginning and end of the experimental period was compared to ensure no degradation in performance. Joints were marked with small circles of white paint to highlight anatomical landmarks for use during digitizing (Fig. 2). We only analyzed strides in which the lizards ran without stopping through the field of view, did not contact the wall of the racetrack and did not contact any surface with the forelimbs. Because the obstacle extended only 5.6 cm down the length of the track, the lizards were able to step over it in a single stride, resulting in a total of three treatment groups: trials without the obstacle (no obstacle), trials in which the lizards stepped on the obstacle (on obstacle) and trials in which the lizards stepped over the obstacle (over obstacle).

All housing conditions and experimental procedures were approved by the Institutional Animal Care and Use Committee at Clemson University (animal use protocol 2010-044) and Georgia Southern University (protocol I1006).

Measurements

We digitized three-dimensional coordinates of the anatomical landmarks in MATLAB (version R2009a, The MathWorks, Natick, MA, USA) using DLTdv3 (Hedrick, 2008). Coordinates were imported into Microsoft Excel 2011 (Microsoft Corporation, Redman, WA, USA) in order to calculate stride length, stride duration, trunk angle, tail elevation and hindlimb kinematics. We determined the position of the COM on the long axis of the body after Alexander (Alexander, 1983) and used a quintic spline in MATLAB to smooth the three-dimensional displacement of this point in order to calculate speed and acceleration in three dimensions (Walker, 1998). Because of the limitations in the field of view, we measured the hindlimb kinematics for a single stance phase (stance), the swing phase preceding it (swing 1) and the swing phase following it (swing 2), where the stance phase of interest was the time of limb contact on the obstacle or immediately prior to stepping over the obstacle. Either the right or left hindlimb was digitized depending on which was on the ground in the stance phase of interest, and we calculated three-dimensional: (1) ankle angle, (2) knee angle, (3) femur retraction, (4) femur depression and (5) long-axis rotation of the femur. Femur retraction was defined as the angle between the femur and a vertical plane passing through the long axis of the body, with positive values indicating greater retraction. Femur depression was defined as the angle formed between the femur and a horizontal plane passing through the hip, with positive

values indicating greater depression. Finally, long-axis femur rotation was defined as the angle formed between a plane containing the hip, knee and ankle and a vertical plane passing through the femur, with positive values indicating an anterior rotation (counter-clockwise in a left lateral view) (Irschick and Jayne, 1999a). We measured the hindlimb kinematics at lift-off, footfall, and 25, 50 and 75% of each of the phases of bipedal locomotion. We determined trunk angle as the angle between horizontal and a line drawn from the snout to the hip. Elevation of the tail was determined as the vertical distance between the tip and base of the tail and the deviation of the COM height was the total difference between the maximum and minimum vertical positions of the COM across the entire trial.

Statistical analyses

Values were averaged from all trials for a given individual in each treatment, but we were unable to obtain data for all treatments for all individuals. As a result, we analyzed five individuals from the no-obstacle group, five individuals from the on-obstacle group and three individuals from the over-obstacle group. We used JMP (version 9.0.0, SAS Institute Inc., Cary, NC, USA) to perform discriminant function analyses (DFAs) using the five hindlimb kinematic variables to assess differences between treatment groups at four time points: 75% of swing 1, footfall, lift-off and 25% of swing 2. Wilks' lambda was calculated for each DFA in order to assess overall differences between treatment groups. To determine which pairs of treatment groups differed significantly on which discriminant functions, one-way ANOVAs with Tukey–Kramer honestly significant difference (HSD) tests were performed on the standardized scoring coefficients of the first two discriminant functions, with treatment as a fixed factor.

We used Pearson's correlation coefficient to test for a relationship between mean forward acceleration across the trial and body angle, and used a one-way ANOVA with a Tukey–Kramer HSD test to determine the effects of treatment on the change in body angle across a trial. Mean sprint speed was measured rather than maximum sprint speed because the latter may only be achieved momentarily. Small changes in instantaneous speed may have some effects on energetics, but these will likely be reflected in overall performance. The effective speed with which an animal crosses a given distance is likely more relevant than momentary condition in situations where selection acts on running performance. For example, mean running speed during obstacle negotiation may determine whether a predator is able to catch up to a lizard during a predator–prey interaction. We also measured the mean forward speed from each of the three phases then performed a one-way repeated-measures ANOVA with treatment as a fixed factor to determine the effects of treatment on
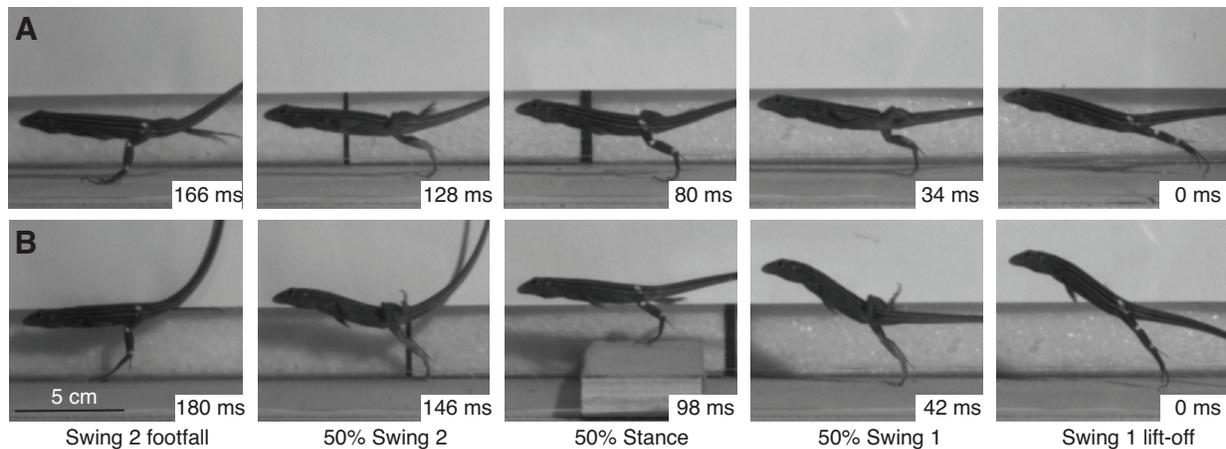
Fig. 3. Representative videos of the (A) no-obstacle and (B) on-obstacle treatments from one individual *A. sexlineata*. Time stamps indicate elapsed time from lift-off of swing 1.

the mean speed in each of the three phases. We performed one-way ANOVAs with Tukey–Kramer HSD tests on mean forward speed and the deviation of the COM height to assess differences in overall performance between treatment groups. We performed one-way ANOVAs with Tukey–Kramer HSD tests on duty factor calculated using either swing 1 or swing 2 with stance in order to assess differences in stride.

Although acceleration may not be an accurate performance measure during a prolonged steady run, it is important to differentiate between acceleration over long time scales and within-step acceleration. In steady running, every stance phase will produce a discrete acceleration event (Farley and Ko, 1997), but this does not necessarily translate into an overall acceleration of the animal over multiple steps. Measuring acceleration during the stance phase can, however, be used to estimate the force produced during that phase. The lizards in our study did not exhibit consistent patterns of acceleration during the stance phase of interest. Thus, we simply compared whether the lizards accelerated or decelerated in the stance phase of interest between treatments using a χ^2 contingency test.

RESULTS
Kinematics

Because over-obstacle and no-obstacle trials were similar for all variables at all time points, all comparisons described here are made between no-obstacle and on-obstacle trials (Fig. 3). Hindlimb kinematics were similar between treatment groups during both swing

1 and swing 2 (Fig. 4, Table 1). On-obstacle trials separated significantly along the first discriminant function 1 (DF 1) in DFAs at both footfall and lift-off, and all hindlimb kinematic variables were correlated with DF 1, with the exception of femur retraction at footfall (Fig. 5, Tables 1 and 2).

During bipedal running when unimpeded by an obstacle, lizards began the swing phase with the ankle and knee extended (133.2±6.2 and 138.9±8.4 deg, respectively) and the femur in a depressed (32.3±3.8 deg), retracted (70.5±8.6 deg) and anteriorly rotated (51.7±4.6 deg) position, placing the entire hindlimb behind and below the hip. The ankle extended initially during swing (133.2±6.2 to 154.0±7.1 deg) before flexing (154.0±7.1 to 122.9±5.6 deg) at the end of swing, whereas the knee flexed mid-way through (138.9±8.4 to 95.0±11.2 deg) before extending during the remainder of the swing phase (95.0±11.2 to 141.1±2.3 deg). The femur was protracted throughout the swing phase (70.5±8.6 to –60.4±4.2 deg) while being elevated during the first half to a position above the hip (–23.4±3.4 deg) and depressed for the second half of swing (45.1±3.1 deg). The femur remained anteriorly rotated throughout the phase (51.7±4.6 to 51.6±6.2 deg) and then rotated to a neutral position immediately preceding footfall (51.6±6.2 to –0.3±3.7 deg; Fig. 4).

At footfall, the ankle and knee were in an extended position (122.9±5.6 and 141.1±2.3 deg, respectively) and the femur was in a depressed (45.1±3.1 deg), protracted (–60.4±4.2 deg) and neutral rotational position (–0.3±3.7 deg), placing the entire hindlimb anterior to, and below, the body. The ankle reached maximal flexion

Table 1. Correlations between each hindlimb kinematic variable and the first discriminant function (DF 1) of each discriminant function analysis

Kinematic variable	75% Swing 1	Footfall	Lift-off	25% Swing 2
Variance explained by DF 1	86%	94%	91%	96%
Ankle angle	0.55	0.72*	0.84*	0.98*
Knee angle	0.80*	0.96*	0.94*	0.92*
Femur depression	0.60*	0.98*	0.96*	0.77*
Femur retraction	–0.68*	0.49	0.80*	0.54
Long-axis femur rotation	0.61*	0.78*	0.84*	–0.23

Overall differences between treatment groups are as follows: 75% Swing 1, Wilks' λ=0.16, *P*=0.2; Footfall, Wilks' λ=0.16, *P*=0.2; Lift-off, Wilks' λ=0.06, *P*=0.02; and 25% Swing 2, Wilks' λ=0.22, *P*=0.3.
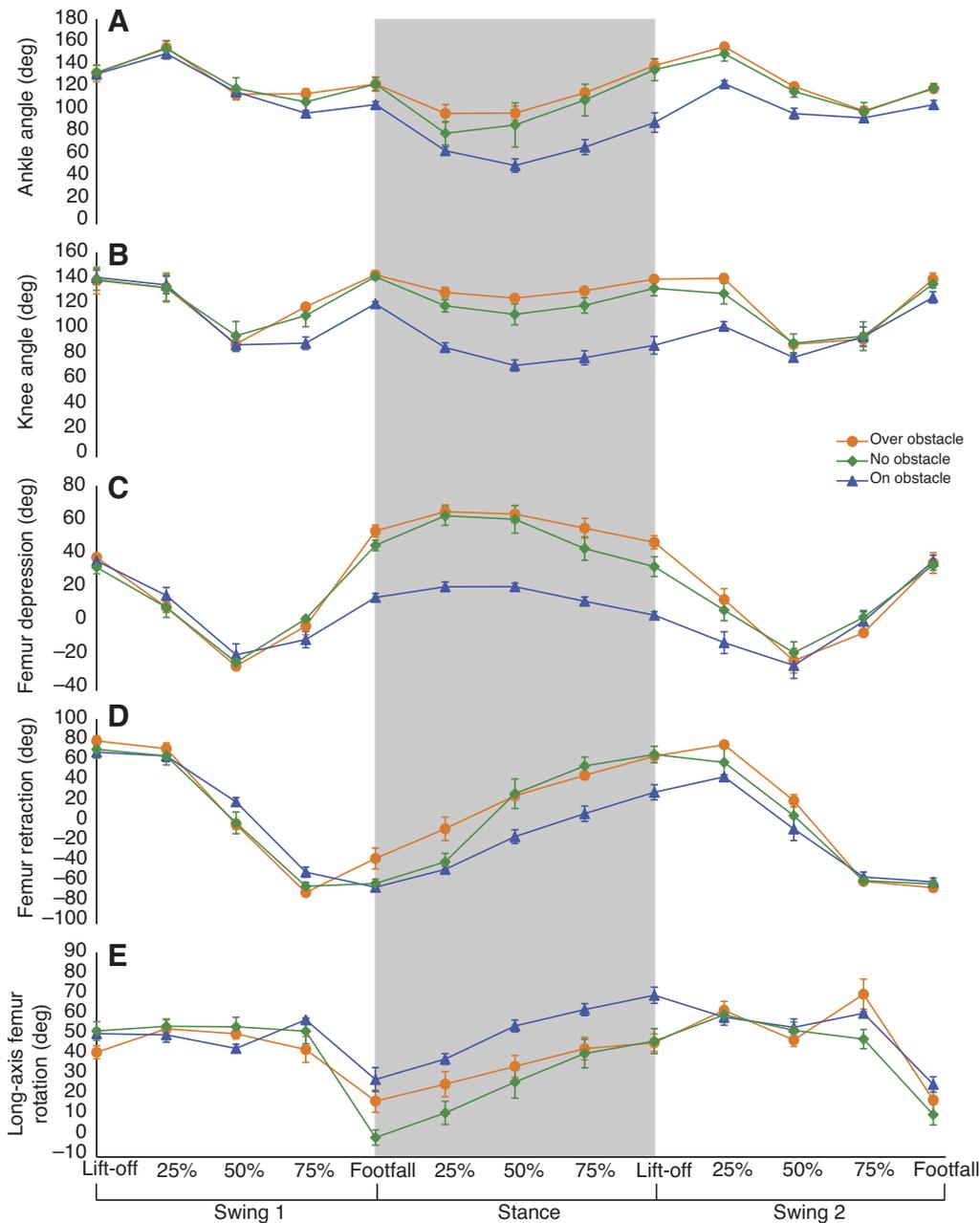*P<0.05.

Fig. 4. Hindlimb kinematics of *A. sexlineata* for each treatment group during the three analyzed phases of bipedal running. Mean values (±s.e.m.) for each treatment group are indicated for each of the following kinematic variables: (A) ankle angle, (B) knee angle, (C) femur depression, (D) femur retraction and (E) long-axis femur rotation. Each phase of the stride was scaled to 100% of that phase and values were extracted from standardized time points indicated on the *x*-axis. The grey bar indicates the time period during which the kinematics of the on-obstacle treatment group differed significantly from the no-obstacle and over-obstacle treatment groups based on discriminant function analyses. Over obstacle, *N*=3; on obstacle, *N*=5; no obstacle, *N*=5.

(79.7±10.3 deg) shortly after footfall and then extended steadily through the duration of stance (79.7±10.3 to 135.6±9.6 deg) while the knee flexed slightly during the first half of stance (111.7±8.2 deg) before extending again (132.0±5.8 deg). Depression of the femur continued from the previous swing phase, reaching a maximal value mid-way through stance (45.1±3.1 to 60.4±8.1 deg) and then elevating steadily (60.4±8.1 to 32.7±5.8 deg). The femur retracted (–60.4±4.2 to 65.5±8.0 deg) and rotated anteriorly (–0.3±3.7 to 46.7±6.1 deg) throughout stance, returning the hindlimb once again to a position behind the hip by the time of lift-off of the next swing phase (Fig. 4).

When stepping on the obstacle, the ankle and knee were less extended at footfall (104.9±2.6 and 119.9±1.7 deg, respectively) than when stepping over the obstacle or running along an unobstructed path, whereas ankle and knee flexion were greater throughout stance compared with the other two treatments (104.9±2.6 to 51.4±5.8 deg, and 119.9±1.7 to 71.7±4.4 deg, respectively). The femur was less depressed (14.6±2.3 deg) at footfall when stepping on the obstacle

compared with the other two treatments. The femur reached maximal depression (20.9±2.1 deg) midway through stance before being elevated again, but remained in a less depressed position (4.2±2.0 deg) compared with the other treatments at the beginning of swing 2. When contacting the obstacle, the stance phase began with the femur in a more anteriorly rotated position (28.0±5.8 deg) compared with the other treatments, but the femur showed an amount of rotation throughout stance similar to that of the other treatments, resulting in a correspondingly more anteriorly rotated position at lift-off (69.1±3.9 deg). The femur was in a protracted position (–64.1±1.3 deg) similar to that of the other treatments at footfall; however, retraction of the femur was reduced across the stance phase, leading to a more retracted position (28.6±7.4 deg) at the start of swing 1 compared with the other treatments. After lift-off from stance, hindlimb kinematics when lizards stepped on the obstacle returned to values similar to those of the other treatment groups and remained so for the duration of swing 2 (Fig. 4).
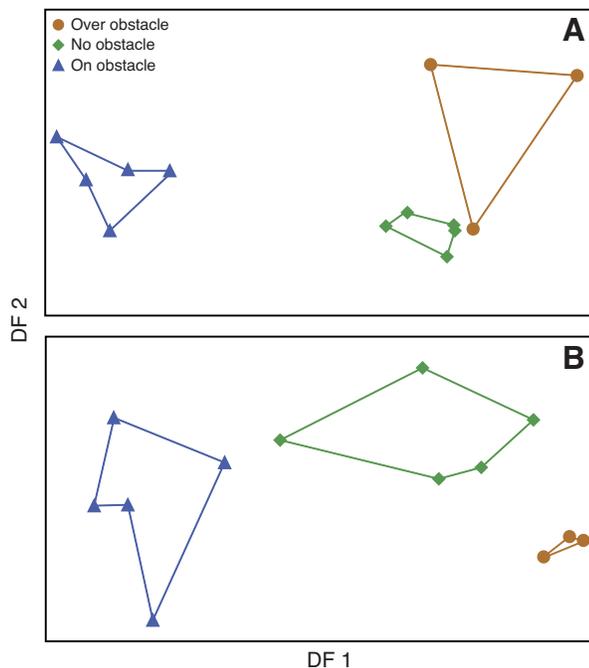
Fig. 5. Scores for the first and second discriminant functions (DF 1 and DF 2, respectively) for discriminant function analyses of hindlimb kinematics at (A) footfall and (B) lift-off showing the significant separation of treatment groups. Kinematic variables included were ankle angle, knee angle, femur retraction, femur depression and long-axis femur rotation for each individual time point. Wilks' lambda values were 0.03 (P=0.003) for footfall and 0.06 (P=0.02) for lift-off.
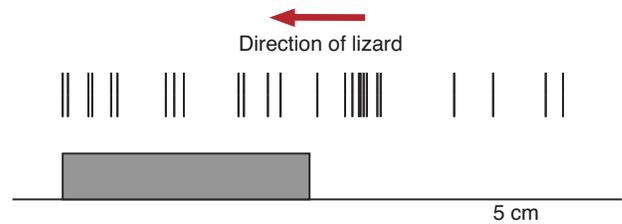

Fig. 6. The distribution of footfall location along the length of the racetrack with respect to the obstacle. Each line represents the position of the tip of the fourth digit at footfall in each of the 39 trials used in kinematic analysis. The average position for all trials was on the obstacle 0.7 cm past the front edge.

The trunk angle relative to the horizontal varied widely across trials and we found no relationship between trunk angle and mean forward acceleration during unobstructed bipedal strides (Pearson's correlation, r=–0.095, P=0.5), nor did we find any relationship between treatment and the change in body angle across a trial (Table 3). Because of the extreme length of the tail it was possible for the proximal portion of the tail to be elevated above the body COM while the distal portion dragged on the ground behind the lizard; therefore, the vertical distance between tail tip and tail base did not always completely describe the orientation of the tail and was thus excluded from analyses.

Performance

In a majority of trials (87% of 27 trials) where an obstacle was present, lizards were unable to maintain their bipedal gait after traversing the obstacle. We found no significant effects of treatment ($F_{2,10}$=1.38, P=0.3), phase ($F_{2,9}$=1.7, P=0.24) or their interaction (Wilks' λ=0.38, $F_{4,18}$=2.58, P=0.07) on mean forward speed. Changes in forward speed between swing 1 and swing 2

Table 2. *P*-values for comparisons of canonical scores for the first discriminant function (DF1) for each of the two significant discriminant function analyses, at footfall and at lift-off, based on one-way ANOVAs and Tukey–Kramer HSD tests with treatment as a fixed factor

Comparison	Footfall DF 1	Lift-off DF 1
Over obstacle *vs* on obstacle	<0.0001	<0.0001
Over obstacle *vs* no obstacle	0.2	0.05
On obstacle *vs* no obstacle	<0.0001	0.0002

were minimal (no obstacle=1.8±0.6 SVL s^{-1}; on obstacle=–0.8± 0.7 SVL s^{-1}; over obstacle=1.0±0.2 SVL s^{-1}). There was no difference in duty factor between treatments using either swing 1 or swing 2, nor did the two swing phases differ from each other overall. There were no significant differences between treatment groups in mean forward speed across whole trials or in the deviation of the height of the COM (Table 3). Lizards decelerated through stance in 25% of 12 no-obstacle trials, 21% of 14 on-obstacle trials and 38% of 13 over-obstacle trials. There were no patterns in whether a lizard accelerated or decelerated based on treatment (χ^2=1.05, d.f.=2, P=0.059) or individual (χ^2=1.98, d.f.=5, P=0.85).

DISCUSSION

We observed two distinct modes of obstacle negotiation for bipedal locomotion in *A. sexlineata*, stepping on the obstacle and stepping over the obstacle, though these modes do not appear to represent behavioral strategies. The lizards did not experience any change in performance associated with obstacle negotiation, regardless of the mode of obstacle negotiation, supporting our first hypothesis that forward speed does not change in the presence of an obstacle. However, the lizards did not modify their hindlimb kinematics in order to deal with an obstacle in the locomotor path, contrary to our second hypothesis that lizards increase the elevation of the hindlimb in preparation for obstacle contact in order to avoid disruption to locomotion.

Comparisons with other taxa

Despite the limited number of lizard species for which the kinematics of bipedal locomotion have been quantified, many interspecific differences have been reported (Irschick and Jayne, 1999a; Rocha-Barbosa et al., 2008). The bipedal gait of *A. sexlineata* does not match the described gait for any one species but it most closely resembles that observed in *Aspidoscelis tigris*, despite *A. sexlineata*'s smaller size (Urban, 1965; Irschick and Jayne, 1999a). Overall hindlimb movements were characterized by a vertical orientation of the femur and digitigrade posture throughout stance, which is consistent with previous descriptions of bipedal locomotion in *Callisaurus draconoides* (Irschick and Jayne, 1999a) and *Liolaemus lutzae* (Rocha-Barbosa et al., 2008). Trunk angle was highly variable during bipedal running, as was position of the forelimb. There was no visible lateral undulation of the body, unlike those observed during the bipedal locomotion of *Basiliscus plumifrons* (Hsieh, 2003). We did not quantify pelvic rotation because of the presence of high digitizing error in extracting these data, but visual inspection of the high-speed video reveals a vertical rotation of the pelvis accompanying femur depression, moving the hindlimb closer to midline beneath the body (Urban, 1965). Snyder suggested that

Table 3. Mean (±s.e.m.) values of locomotor variables for each of the three treatments and the *F*-ratios obtained from separate one-way ANOVAs with treatment as a fixed factor

Variable	No obstacle	Over obstacle	On obstacle	$F_{2,10}$	P
Mean forward speed (SVL s^{-1})	3.6±0.4	4.7±0.5	4.0±0.4	1.46	0.28
Deviation of COM height (SVL)	0.17±0.06	0.28±0.07	0.35±0.06	2.37	0.14
Duty factor 1	0.29±0.02	0.23±0.02	0.26±0.02	2.97	0.10
Duty factor 2	0.31±0.03	0.21±0.03	0.27±0.03	2.71	0.11
Change in body angle (deg)	5.1±2.3	1.6±3.0	4.3±2.3	0.48	0.65

COM, center of mass; SVL, snout–vent length.

habitat characteristics divide bipedal lizards into two general categories – those inhabiting open sandy areas and those in more forested areas – and reflect differences in the bipedal kinematics (Snyder, 1952). By these kinematic criteria, *A. sexlineata* could be classified in the former category. However, future studies incorporating kinematic and morphological measurements with ecological and phylogenetic data are necessary to determine whether interspecific differences in bipedal gaits are the result of multiple independent origins or divergence from the ancestral mode to meet different functional demands.

Do bipedal lizards anticipate obstacles?

We found that the hindlimb kinematics of lizards prior to contacting an obstacle did not differ from kinematics while running down an unobstructed racetrack. An alteration of stride length or frequency many strides before contact could provide an advantage by ensuring that the foot falls at a specific point on or before the obstacle, minimizing the risk of slipping due to stepping on an edge (Chen et al., 1991). However, the distribution of footfalls on and around the obstacle in the present study does not reveal any region of track where footfalls occurred more frequently (Fig. 6). Because footfall location is influenced by factors other than active aiming by the animal, we did not statistically evaluate the distribution of footfalls and looked only for any overwhelming patterns. For example, the precise location at which the animal was released could determine footfall location if stride length is held constant, which means that lizards would need to be released at the same location in order to control for this effect. Additionally, some recorded trials showed lizards stepping on either the front or back edge of the obstacle, resulting in unusual patterns of locomotion, which led us to exclude them from the present analysis. It therefore appears that whether a lizard steps on or over an obstacle is dependent on chance.

Do obstacles alter hindlimb kinematics?

The kinematics of a lizard stepping over the obstacle differed little from those of unobstructed locomotion, but the kinematics of lizards stepping on the obstacle differed significantly from those of both unobstructed running and stepping over the obstacle. The changes to the hindlimb kinematics when stepping on the obstacle seem to be a passive consequence of continuing to move the limbs as though the obstacle were not present and the surface were not elevated. However, the lizards did not adjust their kinematics throughout stance in order to deal with the disruption and, therefore, did not return the limb to the normal position (Fig. 4). Sub-optimal limb position could reduce the force generated during stance phase, possibly leading to deceleration. Although lizards sometimes produced no acceleration during the stance phase of interest, we found no patterns in whether the lizard accelerated or decelerated during stance based on treatment or individual. It may be that the momentum of the lizard from the previous step carries the animal forward and, though the hindlimb does contact the ground in the

stance phase of interest, no propulsive force is generated. Data from force plate recordings are necessary to address this.

Rather than maximizing propulsion during stance, the priority for a bipedal lizard could be maintaining a steady body COM height. The body COM of the lizards was elevated above the height of the obstacle for the duration of the trials because of the extension of the hindlimb in the parasagittal plane at stance, despite the trunk being nearly horizontal in some instances. Body COM height remained relatively steady and total deviation of the body COM height did not differ between obstructed and unobstructed trials (Table 3). Lizards may tolerate changes to hindlimb kinematics forced by contact with an obstacle in order to maintain a steady body COM height and avoid overall disruption to locomotion.

Do obstacles decrease locomotor performance?

We found no difference in the mean forward speed of bipedally running lizards between obstructed and unobstructed running and no significant effect of the obstacle on the mean forward speed of each phase. However, it is undeniable that the presence of the obstacle disrupted locomotion both in the changes to the kinematics after contact and in the fact that lizards were unable to maintain their bipedal gait after contacting the obstacle in a majority of trials. It is possible that lizards may experience a deceleration occurring after the time frame examined here that may result in a decreased mean forward speed, but more study is needed to understand how recovery after a disruption to locomotion impacts fitness. One way to assess this is by using a longer trackway and a larger camera field of view. However, this would compromise resolution when using small lizards. Additionally, quantifying escape sequences in an animal's natural habitat could reveal the relevance of any decreased performance following the interaction with an obstacle.

Is bipedality advantageous?

The lack of kinematic adjustment during obstacle negotiation along with the absence of any performance decrease suggests that bipedality provides an advantage during obstacle negotiation by allowing locomotion to continue without disruptions to the body COM. Locomotor performance is commonly quantified as mean forward speed (Garland and Losos, 1994). The ability to successfully move in order to escape predators, catch prey and defend territories is an important contributor to fitness that is likely affected by locomotor performance (Garland and Losos, 1994). In a study of obstacle negotiation during steady quadrupedal running in *S. malachiticus*, forward speed decreased in the presence of obstacles (Kohlsdorf and Biewener, 2006). This, coupled with the results of the present study, suggests that lizards may avoid performance decreases associated with obstacle negotiation by employing a bipedal gait. If performance during obstacle negotiation does indeed contribute to fitness, then selection would favor bipedal locomotion provided there are no overriding costs associated with that locomotor mode. Comparing the performance of *A. sexlineata* during

quadrupedal obstacle negotiation with that of bipedal obstacle negotiation would directly address this issue; however, *A. sexlineata* tend to exclusively employ a bipedal gait during escape situations. Kohlsdorf and Biewener noted that *S. malachiticus* sometimes used a bipedal gait when negotiating rectangular obstacles with heights equal to 31 and 65% of the length of the hindlimb (Kohlsdorf and Biewener, 2006), but the kinematics and performance of this behavior have not been analyzed. Further study of obstacle negotiation with diverse species of lizards using different gaits over a wide range of speeds is necessary to fully address the advantages of bipedal running to obstacle negotiation. In addition, presenting lizards with a variety of obstacles (size, shape and orientation) would potentially reveal slight alterations that are due to habitat structure.

Bipedality does not necessarily need to confer an increase in fitness itself. The removal of the forelimbs from the locomotor cycle could be a consequence of selection acting on some other feature of locomotion (Gould and Lewontin, 1979; Aerts et al., 2003). Models of bipedal locomotion show that the bipedal gait in lizards may be a result of the torque generated about the hips by the hindlimbs during high acceleration causing the front of the body to lift from the ground (Aerts et al., 2003). An inherent consequence of this model is that the trunk angle of a bipedally running lizard should be greater than that of a quadrupedally running lizard (i.e. elevated from horizontal) and a greater acceleration presumably produces a more elevated trunk, but we found no such relationship. Additionally, we observed some bouts of continuous bipedal running in which the lizards had a trunk angle that was nearly horizontal. Unlike models of bipedality using acceleration from a quadrupedal gait (Aerts et al., 2003), we analyzed sustained bipedal running and the mean acceleration across entire trials was only $1.5\pm0.5\,\mathrm{m\,s^{-2}}$. A bipedal gait could be maintained in the absence of high acceleration by counterbalancing the trunk COM by holding the tail horizontally to maximize the torque about the hip (Irschick and Jayne, 1999a). In general, the tail tip tended to drag on the ground during bipedal running in *A. sexlineata*, but these lizards possess extremely long tails, up to three times the SVL. Further study is needed to determine the relative position of the tail COM during bipedal running and the torque it generates about the hips.

This study provides indirect evidence that does not support a model of bipedality as a passive result of high acceleration, but data incorporating ground reaction forces with three-dimensional kinematics are necessary to directly test this model (Aerts et al., 2003). Additionally, bipedality may be a passive result of some feature of locomotion other than acceleration. For example, the height of the hip and the effective length of the hindlimb increase significantly with speed in *Dipsosaurus dorsalis* (Fieler and Jayne, 1998). Depending on the relative limbs lengths of the species in question, there could exist a speed threshold at which elevation of the hip and thus the body makes contacting the ground with the forelimb impossible. Future studies will examine the role of hip height and hindlimb extension in bipedality.

Although bipedality may have first appeared in lizards as a passive consequence of other locomotor features, lizards have evolved means to exploit the phenomenon (Snyder, 1952; Snyder, 1962; Aerts et al., 2003). Many lizard species possess adaptations to this gait that imply a selective advantage, specifically the relatively long hindlimbs and long substantial tails associated with bipedal species (Snyder, 1954; Snyder, 1962). By actively manipulating the body COM, lizards may be able to achieve a bipedal gait at lower accelerations (Clemente et al., 2008). With the tail held straight behind the body, *A. sexlineata* have a body COM located 20% of the SVL anterior of the cloaca, but this may be shifted by altering the location of the trunk COM or tail COM (Irschick and Jayne, 1999a). In addition to manipulations of the tail COM, some lizards pull the forelimbs up alongside the body during bipedal running, which may shift the COM posteriorly, suggesting that this gait is actively chosen (Clemente et al., 2008). We observed this same behavior in *A. sexlineata*, although the frequency of occurrence varied within each individual and does not appear to follow a predictable pattern. This behavior may also simply be a method to prevent the forelimbs from dragging on the ground during low-trunk-angle bipedal running (Irschick and Jayne, 1999a), though the relationship between forelimb tucking and trunk angle is not yet known.

Conclusions

Bipedally running *A. sexlineata* did not alter their hindlimb kinematics in preparation for obstacle negotiation. When stepping on the obstacle, lizards continued to move their hindlimbs as though the surface were not elevated above the trackway. Maintaining stability by preventing changes in the body COM height seemed to be more important than returning hindlimb kinematics to normal parameters. Mean speed when encountering an obstacle did not differ from that during unobstructed running. However, in the majority of trials, lizards were unable to maintain the bipedal gait after obstacle negotiation and fell into a quadrupedal gait, which warrants further investigation.

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