FORELIMB POSITION AFFECTS FACULTATIVE BIPEDAL LOCOMOTION IN LIZARDS

By

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INDEX WORDS: Sprint, Obstacle, Center of Mass, *Sceloporus*, *Aspidoscelis*

THE EFFECTS OF OBSTACLE PLACEMENT AND FORELIMB POSITION ON FACULTATIVE BIPEDAL LOCOMOTION IN LIZARDS

ABSTRACT

Recent work indicates that bipedal posture in lizards is advantageous during obstacle negotiation (Parker and McBrayer, 2016). However, little is known about how bipedalism occurs beyond a lizard's acceleratory threshold. Furthermore, no study to date has examined the effects of forelimb position on the body center of mass in the context of bipedalism. This study quantified the frequency of bipedalism when sprinting with vs. without an obstacle at 0.8 meters from initiating a sprint. Forelimb positions were quantified during bipedal running at the start of a sprint and when crossing an obstacle. Two species with contrasting body forms (and thus different body center of mass; BCoM) were studied (*Sceloporus woodi, Aspidoscelis sexlineata*) to assess potential variation due to body plan and obstacle crossing behavior. No significant difference in frequency of bipedalism was observed in *S. woodi* with or without an obstacle. However, *A. sexlineata* primarily used a bipedal posture when sprinting. Forelimb positions were variable in *S. woodi* and stereotyped in *A. sexlineata*. Caudal extension of the forelimbs helped shift the BCoM posteriorly and transition to, or maintain, a bipedal posture in *A. sexlineata,* but not *S. woodi*. The posterior shift in BCoM, aided by more caudally placed forelimbs, helps raise the trunk from the ground, regardless of obstacle presence. The body plan, specifically the length of the trunk and tail, and forelimb position work together with acceleration to shift the BCoM posteriorly to transition to a bipedal posture. Thus, species exhibit morphological and behavioral adjustments to transition to and maintain facultative bipedalism while sprinting.

INTRODUCTION

The ability to capture prey, avoid predation, and find mates is contingent on successfully navigating uneven substrates in most terrestrial environs (Vanhooydonck et al., 2007). Physical substrates such as loose rock, thick vegetation, and woody debris provide challenges to terrestrial vertebrates (Pounds, 1988). Variation in substrate characteristics directly affects locomotor performance and behavior of terrestrial vertebrates during flight from predators (Collins et al., 2003; Cooper, 1999; Losos 1990). Bipedalism – which is observed in some insects, mammals, and reptiles - is one mode of locomotion terrestrial vertebrates use to overcome obstacles (Alexander, 2004, Tucker and McBrayer, 2012, Parker and McBrayer, 2016). During predation events or social interactions, a terrestrial vertebrate's behavior, speed, and stability traversing obstacles may impinge upon their survivorship and/or fitness (Arnold, 1983; but see Garland and Losos, 1994).

Stereotyped limb movement in quadrupedal locomotion, or gait, has predictable footfalls across various speeds (Snyder, 1952; Snyder, 1954; Snyder, 1962; Irschick and Jane 1999; Farley and Christine, 1997). Some terrestrial lizards alter their gait and/or posture while sprinting (Schuett et al., 2009). Facultative bipedalism occurs in some quadrupeds when only the hind limbs contact the ground, due to a posterior shift in the body center of mass (BCoM) (Snyder, 1954). A posterior shift in BCoM during facultative bipedalism occurs in large part due to the production of high accelerative forces by the hindlimbs that would otherwise keep the forelimbs in contact with the ground (Aerts et al., 2003). Facultative bipedalism has evolved independently in numerous lizard clades as a consequence of acceleration and changes in body center of mass (BCoM) (Aerts et al., 2003; Clemente, 2014). The position of the BCoM varies depending on the length of the trunk and tail relative to the hip (Van Wassenbergh and Aerts, 2013). Lizards with an anteriorly placed BCoM are less likely to exhibit bipedalism compared to lizards with a posteriorly shift BCoM (Clemente, 2014). Thus, body shape is a key determinant in facultative bipedalism. Bipedal lizards can make small changes in the trunk and / or tail angle such that the BCoM is shifted over the hip (Van Wassenbergh and Aerts, 2013; Irschick and Jayne, 1999).

Kinematic data on the role of the hindlimb in bipedal locomotion suggest the hindlimb generates significant power, thereby effecting acceleration and maximal velocity (Wassenbergh and Aerts, 2013; Olberding et al., 2012; Snyder, 1954; Snyder 1962). Little attention has focused on the role of the forelimb during bipedal locomotion. Forelimb position may aid in obstacle navigation by shifting the BCoM posteriorly (Legrenuer et al., 2012). Snyder (1952) suggested there is no difference in limb movement between quadrupedal and bipedal locomotion. Yet, several species of lizards use various forelimb positions while moving bipedally (Irschick and Jane 1999). For example, *A. sexlineata* tends to caudally extend the limbs, despite individual variation (Olberding, et al., 2012). Varying forelimb positions may be necessary for maintaining balance, touching or pushing off an obstacle, or elevating the center of mass for obstacle clearance (Kohlsdorf and Biewener, 2006).

Certain forelimb positions during bipedal locomotion, such as caudal extension, could shift the BCoM posteriorly, while continuing to move the forelimbs in a gait cycle may aid in creation of a pitching motion via nose-up torque and high starting accelerations (Aerts et al., 2003; McElroy and McBrayer, 2010). For example, caudal extension of the forelimbs during obstacle navigation may 1) decrease contact with an obstacle by raising the distance of the limbs away from the obstacle (Self, 2012) and 2) shift the BCoM posteriorly to raise the hip height so that a lizard might clear an obstacle without losing forward speed (Olberding et al, 2012, Irschick and Jayne, 1999).

The objective of this study was to determine the role of obstacle placement and forelimb position during facultative bipedal locomotion in lizards. Two species, *Sceloporus woodi* and *Aspidoscelis sexlineata*, were selected based on their different body plans (thus BCoM), and each often exhibits bipedal locomotion. *Sceloporus woodi* run bipedally more frequently when encountering an obstacle versus without an obstacle (Parker and McBrayer, 2016). Furthermore, *Sceloporus woodi* run bipedally when an obstacle is within their acceleration threshold (0.4m), but not when multiple obstacles are present in succession (Parker and McBrayer, 2016). *Aspidoscelis sexlineata*, however, employ a bipedal posture when crossing obstacles over long distances (Olberding et al., 2012). Although many species of lizards have been documented sprinting bipedally, no published studies have examined bipedalism with an obstacle placed beyond the initial acceleration threshold, i.e. after the initial two to five steps (0.4 – 0.5 m) of locomotion (McElroy and McBrayer, 2010). Transitioning to a bipedal posture at an obstacle when already near maximal velocity suggests that bipedalism may occur as a behavioral adaptation to maintain forward speed, and is not only dependent on initial acceleration. We predict that (i) lizards use a bipedal posture more frequently with an obstacle present than without, and (ii) bipedal posture is used more at the obstacle, rather than at the start of the trial. In terms of velocity (iii), we predict that a bipedal posture will allow for maintenance of forward speed over the obstacle more than quadrupedal posture. Furthermore, we predict that (iv) caudal extension of the forelimbs shifts the BCoM posteriorly more than other forelimb positions, and (v) that forelimb positions are variable within the acceleration threshold but fixed when navigating an obstacle beyond the acceleration threshold.

<div align="center">METHODS</div>

Study Species and Field Site

This study quantifies the frequency of bipedal posture, and the position of the forelimb, when crossing obstacles during sprint locomotion. Two facultative bipedal species with differing body plans were chosen as study species: the Florida Scrub Lizard (*Sceloporus woodi*) and the Racerunner (*Aspidoscelis sexlineata*) (Fig 1A). *Sceloporus woodi* is found in open sandy habitats in peninsular Florida (Jackson, 1973). *Aspidoscelis sexlineata* has an elongated trunk and a forward BCoM compared to *S. woodi* (Clemente, 2014). *Aspidoscelis sexlineata* are found throughout the southeast

and are found in sympatry with *S. woodi* in Ocala National Forest. *Aspidoscelis sexlineata* very commonly use bipedal locomotion which is attributed in part to a posteriorly placed BCoM when sprinting bipedally (Clemente, 2014). The contrasting body plan, yet similar masses and habitat use, makes each species ideal to compare both forelimb positions during bipedal running, and when traversing obstacles outside of their acceleration threshold.

Field Collections

Field collection occurred May to August 2016 and 2017. Eighty-eight adult male *S. woodi* and 35 *A. sexlineata* were noosed using a thin filament tied in a slipknot at the end of a fishing pole. Males were retained in cloth bags and transported to the animal facility at Georgia Southern University. Each lizard was kept in a separate 10-gallon tank with sandy substrate and a hide and fasted for 24 hours to ensure digestion did not affect locomotor performance. Lizards were kept on a 12/12-hour light cycle and misted with water every morning. Crickets were fed ad libitum every three days. After sprint trails, most lizards were released at point of capture. Recaptures on subsequent trips were avoided by toe clipping and painting landmarks on each released individual. Only males greater than 42 mm SVL were used in the analyses. Females are likely to be gravid which affect locomotor performance (Iraeta et al., 2010).

Sprint trials

Landmarks were placed externally on each lizard using non-toxic paint (Appendix A) in order to visualize limb and tail position in the video. Two Mega Speed X4© high speed video cameras with RICOH lenses (50mm, F/1.4 VGA) mounted on tripods recorded sprint trials (300fps; resolution 1080 x 1024) from above along a custom-built racetrack. The racetrack substrate was lined with cork to avoid slippage. A mirror was placed at a 45-degree angle along the racetrack wall to provide dorsal and lateral views of each lizard (Appendix B). Lizards were subjected to a trial with an obstacle at 0.8 meters, and a trial without an obstacle. Trials were assigned at random to each day. Obstacles were constructed of wooden blocks which spanned the width of the track to prevent lizards from maneuvering around the obstacle. Obstacle height and width was standardized to 35% of hind limb length for each lizard (Self, 2012). Lizards with broken or regenerated tails were noted and excluded from any analysis. Lizards were warmed to field active body temperature (~36°C) in an incubator before each trial. Each lizard was held completely still at the start of the track, then released. Taps on the tail were used to coerce the individual down the racetrack to a hide. Only "successful" sprint trials were used for analysis, and each lizards had a successful trail with each trial type. A successful sprint trial was defined as avoidance of side walls, pausing, or reversing direction. Bipedal trials were defined as completion of at least one full stride without the forelimbs touching the ground. Bipedalism at the obstacle was assigned as the use of only the hind limbs for at least one full stride within four strides lengths preceding the obstacle. Bipedalism at the start of the trial was assigned as using only

the hind limbs for at least one full stride during the first four strides of a sprint. Whether a forelimb touched an obstacle when crossing was noted for each species. All video was calibrated using a 30-point calibration cube, as well as a 10-centimeter ruler painted on the race track wall (Parker and McBrayer, 2016). Videos were loaded to the computer, spliced using Microsoft Movie Maker (compressed .AVI file), and digitized in MATlab using DLTdv5 software (Hedrick, 2008). A landmark was painted at the junction of the frontal and parietal scale to calculate sprint velocity from each video. Trunk angle was quantified from video for individuals using a bipedal posture. Trunk angle was quantified from a lateral view by measuring the tangent angle of a right triangle from three points: The landmark on the hip, landmark on the scapula, and a point directly under the scapula and in line with the hip such that a right triangle was formed. Trunk angle data was examined in light of four forelimb position during bipedal runs via ANOVA. Gross limb position was easily distinguished on the video, yet image resolution prevented us from confidently collecting 3D joint kinematics with little error. Thus, three-D joint kinematics were not quantified.

Ethogram and BCoM analysis

To understand forelimb function during bipedalism, an ethogram was constructed by reviewing a subset of sprint trials of both *S. woodi* (Parker and McBrayer, 2016) and *A. sexlineata* (collected summer, 2016) (Fig 1). Images from Irschick and Jayne (1999) were also used to determine variation in forelimb positions. Forelimb positions were assigned to one of four categories: cranial extension, caudal extension, gait cycle, and cranial flexion and adduction. The frequency of each was quantified during review of high speed video for each trial. Forelimb positions were defined based on to the forelimb position relative to the trunk, with angles associated with anterior (cranial), or posterior (caudal) positions. For example, "cranial extension" was defined as having the forelimbs anteriorly abducted and flexed more than 90 degrees around the shoulder joint. "Caudal extension" was defined as forelimbs adducted and extended posteriorly at angles less than 90 degrees around the shoulder joint. Forelimbs exhibiting a "gait cycle" rotated asynchronously around the shoulder joint. Finally, limbs exhibiting "cranial flexion and adduction" were proximally adducted and flexed toward the midline. Each definition was determined from dorsal view.

After sprint trials were completed, 12 *A. sexlineata* and 20 *S. woodi* were euthanized with MS-222 to assess the change in positional BCoM due to forelimb position. Only lizards which ran bipedally in sprint trials were euthanized. The BCoM of a subset of euthanized lizards were measured using two scales (described in Clemente 2014). Two scales (0.0001g accuracy) were set parallel to each other with a wooden beam placed across each scale. The scales were tared to the mass of the beam. Each lizard was placed horizontally along the beam such that the most anterior point of the head was in line with the edge of the beam. The hindlimbs were retracted and tail extended straight for each lizard to reduce error in measuring the BCoM. The BCoM was calculated from frozen, then

slightly thawed lizards with forelimbs placed in both cranial, caudal, and alternating (gait cycle) positions to quantify the effects of the forelimb on BCoM. Cranial and caudal positions were averaged together to obtain the flexed/adducted position.

Statistical analysis

One-hundred trials of *S. woodi,* and thirty-six trials for *A. sexlineata* were retained for analysis. Statistical comparisons across species were not made due to a lack of phylogenetic inference that can be made from a two species comparison. Chi-squared tests were used to test the frequency of bipedal posture within each species in trials with or without an obstacle. Mean velocity when crossing an obstacle was calculated by averaging the velocities of the two strides before and after obstacle navigation. Sprint trials containing bipedal posture were retained to quantify the position of the forelimbs. Chi-squared tests were used to test the frequency of forelimb positions at the start of the trial with and without and obstacle, and at the obstacle. A one-way ANOVA was used to analyze variation in BCoM between caudal and cranial forelimb positions within each species. Trunk angle data was pooled from trials with or without an obstacle. A one-way ANOVA was used to test for differences in trunk angle across the four forelimb positions examine the effects of forelimb position on pitch. All analyses were conducted using JMP (v. 12.1.0 SAS institute) and figures created in Microsoft Excel (v. 16.0 Microsoft). Alpha was set to $p < 0.05$.

RESULTS

Frequency of bipedal posture with and without an obstacle

The presence or absence of an obstacle on the frequency of bipedal posture was not different in either *S. woodi* or *A. sexlineata* (Table 1; Fig 2). Whether species ran bipedally more at the start of a sprint as opposed to the obstacle was also quantified. The presence or absence of an obstacle did not affect the frequency of bipedal posture in *S. woodi* ($p = 0.64$, $\chi^2 = 0.219$, df = 1, n = 100). Also, frequency of bipedal posture was not different at the start of a trial vs. at the obstacle in *S. woodi* ($p = 0.088$, $\chi^2 = 2.905$, df = 1, n = 40). Regardless of obstacle presence, *S. woodi* primarily ran quadrupedally (Table 1; Fig 2B). The frequency of bipedal posture in *A. sexlineata* was not affected by the presence or absence of an obstacle ($p = 0.95$, $\chi^2 = 0.004$, df = 1, n = 35). Furthermore, the frequency of bipedal posture was not different at the start of a trial vs. at the obstacle for *A. sexlineata* ($p = 0.13$, $\chi^2 = 2.288$, df = 1, n = 30). *Aspidoscelis sexlineata* primarily used a bipedal posture regardless of obstacle presence (Table 1; Fig 2A).

Sprint velocity as lizards crossed an obstacle with a bipedal or quadrupedal posture was quantified on a subset of lizards (Table 2). The mean velocity of *S. woodi* was significantly faster when crossing an obstacle with a bipedal posture (2.57 m/sec $\pm$ 0.12) compared to a quadrupedal posture (2.01 m/sec $\pm$ 0.09) ($p = 0.0003$, df = 1, n = 22). Although bipedal posture was used in 89% of trials, the posture used by *A. sexlineata* did not affect sprint velocity during obstacle navigation ($p = $

0.12, df = 1, n = 7). A trend was observed towards faster mean velocities using a bipedal posture (3.03 m/sec ± 0.24), but only two of seven trials contained lizards using quadrupedal posture (1.75 m/sec ± 0.45) thus this conclusion is speculative.

Effects of Forelimb Position on BCoM

Four forelimb positions were common during bipedal locomotion: limbs adducted and extended posteriorly (caudal extension), limbs abducted and extended anteriorly (cranial extension), limbs adducted and flexed proximally (cranial flexion and adduction), and a gait cycle where limbs rotate around the shoulder axis (Fig 1). In *S. woodi,* cranial extension shifted the BCoM 13.51 (±0.56) mm anterior of the hip while caudal extension moved the BCoM 12.25 (± 0.56) mm posterior of the hip (Fig 4) (p = 0.04, t = 2.02, n = 46). While using a bipedal posture, *S. woodi* with limbs flexed and adducted has significantly higher trunk angles, and thus pitch, than *S. woodi* using a gait cycle, but not than *S. woodi* with limbs cranially extended (p = 0.0003, F = 12.76, n = 20). In *A. sexlineata*, cranial extension moved the BCoM 9.80 (± 2.25) mm anterior of the hip while caudal extension moved the BCoM 8.47 (± 2.50) mm posterior of the hip (Fig 4) (p = 0.01, t = 2.03, n = 36). Trunk angle was significantly higher when limbs were caudally extended as opposed to a gait cycle in *A. sexlineata* during a bipedal run, leading to greater pitch (p = 0.0013, F = 19.686, n = 12).

Forelimb position in S. woodi

In trials without an obstacle present, there was no difference between the use of the four categories of forelimb position either at the start, or at 0.8 meters (p = 0.4513, χ^2 = 1.591, df = 1, n = 23) (Figs 3A, 3B). When running bipedally at the start of a sprint trial, *S. woodi* used a gait cycle motion of the forelimbs in 47.1% of the trials and limbs were flexed and adducted in 41.2% of trials. Limbs were cranially extended 11.7% of trials, while caudal extension was never observed. Thus, limbs were in a gait cycle or flexed and extended more often than they were cranially or caudally extended (p = 0.0028, df = 3, n = 17). During bipedal locomotion 0.8 meters from the start (yet without an obstacle present), lizards used a gait cycle motion in 66.7% of trials, while limbs were flexed and adducted in 33.3% of trials. Cranially and caudally extended limbs were not observed, thus gait cycle and flexion adduction were used significantly more cranial or caudal extention (p = 0.03, df = 3, n = 6).

With an obstacle present, there was a significant difference between the use of the four forelimb positions at the start of a trial and at 0.8 meters (p = 0.0074, χ^2 = 9.811, df = 1, n = 28). When using a bipedal posture at the start of the trial, *S. woodi* used a gait cycle motion in 56.2% of trials and limbs were flexed and adducted in 43.8% of the trials. Caudally and cranially extended limb positions were not observed. Thus gait cycle and flexion adduction were used significantly more than cadual or

cranial extension ($p < 0.0001$, df = 3, n = 16). When running bipedally at 0.8 meters over the obstacle, limbs were flexed and adducted in 75% of the trials, cranially extended in 16.7% of the trials, and a gait cycle motion was used in 8.3% of the trials. Caudally extended limbs were not observed, therefore, limbs were flexed and adducted, or cranially extended more frequently than a gait cycle or caudal extension ($p = 0.001$, df = 3, n = 12). *Sceloporus woodi* touched the obstacle with their forelimbs 19 out of 51 trials, and all instances were with a quadrupedal posture (Table 1; Fig 5) ($p = 0.07$, $\chi^2 = 3.35$, df = 1, n = 51).

Forelimb position in A. sexlineata

The frequency of forelimb position during bipedal locomotion at the start of a trial and at 0.8 meters without an obstacle was quantified for *A. sexlineata* (Figs 3C, 3D). In trials without an obstacle present, there was no difference between the use of the four categories of forelimb position either at the start, or at 0.8 meters for *A. sexlineata* ($p = 0.2450$, $\chi^2 = 1.352$, df = 1, n = 29). During bipedal locomotion at the start of the trial, limbs were caudally extended in 93.3% of the trials while gait cycle was used in 6.7% of the trials. These behavior were used significantly more than cranial extension or flexion adduction, which was not observed ($p < 0.0001$, df = 3, n = 15). While running bipedally at 0.8 meters, caudal extension was used 100% of the time compared to the other three positions ($p < 0.0001$, df = 3, n = 12).

In trials with an obstacle, there was no difference between the use of the four categories of forelimb position either at the start, or at 0.8 meters for *A. sexlineata* ($p = 0.2721$, $\chi^2 = 12.206$, df = 1, n = 27). When running bipedally at the start of a sprint trial, caudal extension 100% of the time compared to the other three forelimb positions ($p < 0.0001$, df = 3, n = 14). Only 1 out of 17 *A. sexlineata* touched the obstacle while sprinting bipedally, and this individual immediately transitioned to a quadrupedal posture after contact.

DISCUSSION

The goal of this study was to understand the mechanisms and tradeoffs associated with facultative bipedal locomotion. It is clearly established that bipedalism involves a shift in the BCoM (Van Wassenbergh and Aerts, 2013; Aerts et al., 2003; Clemente, 2014; Clemente and Wu, 2018), and that the presence of an obstacle often elicits the facultative use of bipedal posture in lizards (Parker and McBrayer, 2016; Tucker and McBrayer, 2012). Here, we placed the obstacles beyond a lizard's acceleration threshold, but this had little effect of the frequency of bipedal posture overall. Furthermore, the forelimbs had predictable patterns of use that aided the posterior movement of the BCoM during bipedalism. *Sceloporus woodi* rarely maintained a bipedal posture during a sprint, despite using it often for short periods (Parker and McBrayer, 2016). Regardless of obstacle presence, the stockier *S. woodi* infrequently used bipedal posture compared to the streamlined *A. sexlineata*. When running bipedally, the forelimbs of *S. woodi* were generally flexed and adducted. This position

does not significantly shift the BCoM posteriorly. Thus, using flexion and adduction only provides clearance over an obstacle, but does not aid in shifting to, or maintaining, a bipedal posture. *Aspidoscelis sexlineata*, which ran bipedally in 89% of all trials, primarily used caudal extension both when crossing the obstacle and at the start of a trial. Extending the forelimbs caudally causes a significant posterior shift in the BCoM (Fig. 4). The posterior shift in BCoM from caudal extension, plus having a very long tail relative to the trunk, is likely beneficial as *A. sexlineata* frequently maintains a bipedal posture over long distances (Olberding et al., 2012; Clemente and Wu 2018). Given that the degree of facultative bipedalism is variable among taxa, highly contrasting body forms are expected to employ a range of strategies (e.g. forelimb positions) when using bipedal posture. In the species examined here, the frequency of bipedalism differs regardless of obstacle presence. Forelimb position during bipedal locomotion is variable in *S. woodi,* and the use of a bipedal posture is infrequent. Yet forelimb position is stereotyped in *A. sexlineata*, bipedalism is used often, and thereby suggests that forelimb position plays a role in shifting the BCoM posteriorly during bipedal locomotion.

Locomotor frequency with and without an obstacle

Sceloporus woodi exhibits facultative bipedalism (Tucker and McBrayer, 2012). The use of a bipedal posture increases when an obstacle is placed within the acceleration threshold of 0.4 - 0.5 m (Parker and McBrayer, 2016). However, an obstacle placed beyond this (e.g. 0.8 meters) had little effect on the frequency of bipedal posture (Fig 2). *Sceloporus woodi* has a short tail relative to their trunk which makes sustained bipedalism over long distances difficult. Furthermore, the lack of bipedalism in *S. woodi* during the strides crossing an obstacle suggests that bipedalism is primarily an effect of initial acceleration (Wassenbergh and Aerts, 2013). However, for those *S. woodi* which ran bipedally over an obstacle, they had higher velocities than those which ran quadrupedally (Table 2). Hip height is greater during bipedal locomotion than quadrupedal locomotion (Parker and McBrayer, 2016). Increasing the distance between the lizard and the obstacle decreases potential pausing related to obstacle negotiation (Kohlsdorf and Biewener, 2006). While bipedal posture may occur more frequently at the start of a sprint in *S. woodi*, we provide strong evidence that bipedal posture facilitates obstacle negotiation via sustained velocity when an obstacle is beyond the acceleration threshold (roughly 0.4-0.5m for small lizards; McElroy and McBrayer, 2010).

Aspidoscelis sexlineata has a long tail relative to the trunk and can maintain a bipedal posture over long distances (Olberding et al., 2012). Regardless of obstacle placement, *A. sexlineata* primarily ran bipedally (Fig 2). Furthermore, velocities were higher during obstacle navigation when using a bipedal posture. Continual bipedal locomotion with and without an obstacle suggests that that bipedalism is a common form of locomotion in this species. Additionally, high velocities during

bipedal locomotion may provide a benefit by decreasing pausing and stumbling relating to crossing an obstacle (Kohlsdorf and Biewener, 2006).

Contingency of Forelimb Position based on Body Plan

      Aspidoscelis sexlineata has a long trunk and can reach maximum forward speed around 4 m/s when navigating obstacles (Olberding et al., 2012). The BCoM of *A. sexlineata* is shifted posteriorly by their long tail and vertically elevated trunk during bipedalism (Aerts et al, 2003; Clemente, 2014). In conjunction with tail and trunk elevation, *A. sexlineata* uses caudal extension during bipedal locomotion (Figs 3C, 3D). This forelimb position aids in further shifting the body center of mass posteriorly when maintaining a bipedal posture over long distances. *Aspidoscelis sexlineata* do not modify their hindlimb kinematics when approaching an obstacle (Olberding et al, 2012), but instead make small adjustments during obstacle negotiation. Likewise, caudal extension of the forelimbs was used both at the start of the trial and when crossing an obstacle (Figs 3C, 3D). This suggests that forelimb position may not only be a behavioral adjustment for navigating obstacles, but also a mechanism to adjust BCoM, affecting pitch. Indeed, trunk angle increased significantly in *A. sexlineata* when the forelimbs were caudally extended (Fig. 6b). Shifting the BCoM posteriorly aids in maintaining bipedal postures over long distances (Aerts et al, 2003; Clemente and Wu, 2018). Alternatively, forelimbs support the body mass during quadrupedal locomotion (Snyder, 1952). However, *A. sexlineata* touched the obstacle with their forelimbs only three out of 18 trials when sprinting bipedally and immediately reverted to a quadrupedal posture when they did (Fig 5). Extending the forelimb toward the obstacle leads to a forward shift in the BCoM and decreased trunk angle, likely facilitating quadrupedal locomotion. Maintaining a bipedal posture likely aids obstacles negotiation viale maintaining forward velocity (this study; Self, 2012; Olberding et al., 2012).

      When sprinting bipedally at the start of a trial, *S. woodi* showed behavioral adjustments of the forelimbs which did not posteriorly shift the BCoM (Figs 3A, 3D). The continuing gait cycle of the forelimbs at the start of a trial, little change in trunk angle, and lack of sustained bipedalism suggests that bipedalism is a result of high acceleration in this species (Van Wassenbergh and Aerts, 2013). Yet, the forelimbs are primarily flexed and adducted when bipedally crossing an obstacle (Figs 3A, 3D), which likely facilitates an increase in trunk angle (Fig. 6a). To avoid collision with an obstacle, lizards must raise both hip height and the forelimbs to avoid touching the obstacle (Irschick and Jayne, 1991). The hips and forelimbs are raised as a product of bipedalism, which enhances obstacle avoidance (Van Wassenbergh and Aerts, 2013). As bipedalism is less frequent in *S. woodi*, keeping the forelimbs flexed and adducted allows obstacle clearance without shifting the BCoM. *Sceloporus woodi* has a short tail relative to their trunk and reach velocities around 2.4 m/s when crossing an obstacle (Parker and McBrayer, 2016). *Sceloporus woodi* did not touch the obstacle with their forelimbs in 100% of the bipedal trials (Fig 5). If bipedalism is not a posture for sustained locomotion

in *S. woodi,* then they need only hold the forelimbs up against the trunk to avoid contacting the obstacle so that forward speed is not disrupted (Self, 2012; Kohlsdorf and Biewener, 2006).

Conclusion

Aspidoscelis sexlineata, which has a long tail relative to the trunk, and *S. woodi*, which has a short tail relative to the trunk, were used to understand how bipedal posture and forelimb position varies when faced with a distantly placed obstacle. An obstacle placed beyond the acceleration threshold had no significant effect on the frequency of bipedal locomotion. Forelimb position was stereotyped in *A. sexlineata*, which primarily uses a bipedal posture, and variable in *S. woodi*, which primarily uses a quadrupedal posture. While bipedalism aids in obstacle negotiation, its occurrence is often an effect of a high starting acceleration in *S woodi*. However, *A. sexlineata* frequently uses a bipedal posture, and this species positions it forelimbs such that the BCoM is shifted posteriorly, and thereby aids the pitching motion necessary to maintain bipedal posture. Thus, species whose body plans are suited for more sustained bipedal locomotion are likely to employ behavioral adjustments that aid in the maintenance bipedalism, regardless of obstacle presence. Future studies should model the changes in forelimb position in tandem with ground force reactions and joint kinematics to understand the range of effects that shifts in forelimbs position may have on bipedalism. Furthermore, future work should include additional bipedal species with a range of body forms so that phylogenetic inferences can be made regarding how this trait evolves.

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TABLES

Table 1. Summary statistics of locomotor behavior in sprint trials with and without an obstacle.
Numbers are the frequency of occurrence for each behavior among species and trials. Posture at the
start of a trial was quantified by assigning the first four strides of a sprint as either bipedal or
quadrupedal. Bipedalism at 0.8 meters was quantified as four strides preceding the obstacle at 0.8
meters. Pauses before and after an obstacle were quantified in the four preceding strides of the
obstacle. (*n* = number observed).

Frequency of locomotor behaviors in *Sceloporus woodi* and *Aspidoscelis sexlineata*

	S. woodi		*A. sexlineata*	
	Obstacle Presence		Obstacle Presence	
Variable	Obstacle	No Obstacle	Obstacle	No Obstacle
(Sample Size)	*(n = 51)*	*(n = 49)*	*(n = 18)*	*(n = 17)*
Number of bipedal runs	20	17	16	15
Number of quadrupedal runs	31	32	2	2
Bipedal at start of trial	16	17	12	15
Bipedal at 0.8 meters	11	6	14	14
Forelimbs touch obstacle	19	---	3	---
Pause on obstacle	6	---	1	---
Pause before obstacle	10	---	0	---
Pause after obstacle	26	---	2	---

Table 2. Velocity during locomotion over an obstacle at 0.8 meters. Average velocities and standard error were calculated from the first two strides before and after obstacle navigation. Average velocities (V) were regressed on snout vent length (SVL) to account for variation due to size and analyzed using a one-way ANOVA. Average velocity between bipedal and quadrupedal locomotion was significantly different in *S. woodi* (n = number of individuals observed; significant differences are noted in bold with an asterisk)

Average velocities for *Sceloporus woodi* and *Aspidoscelis sexlineata*

Variable	*S. woodi*		*A. sexlineata*	
	Bipedal	Quadrupedal	Bipedal	Quadrupedal
Mean Velocity	**2.57 ± 0.12***	**2.01 ± 0.09***	3.03 ± 0.24	1.75 ± 0.45
Residuals (V/ SVL)	**0.39 ± 0.11***	**-0.24 ± 0.08***	0.25 ± 0.34	-0.95 ± 0.53
Sample Size	7 / 22 trials	15 / 22 trials	5 / 7 trials	2 / 7 trials

Figure 1. Ethogram of common forelimb positions observed during bipedalism in lizards. A) The more streamlined *Aspidoscelis sexlineata* (right) and stockier *Sceloporus woodi* (left) have differing body plans. In both frames, forelimbs are in a gait cycle **B)** A diagram showing the anterior - posterior and abduction - adduction axis. **C)** Four commonly used forelimb positions used during bipedal locomotion. Lateral and dorsal views are shown.
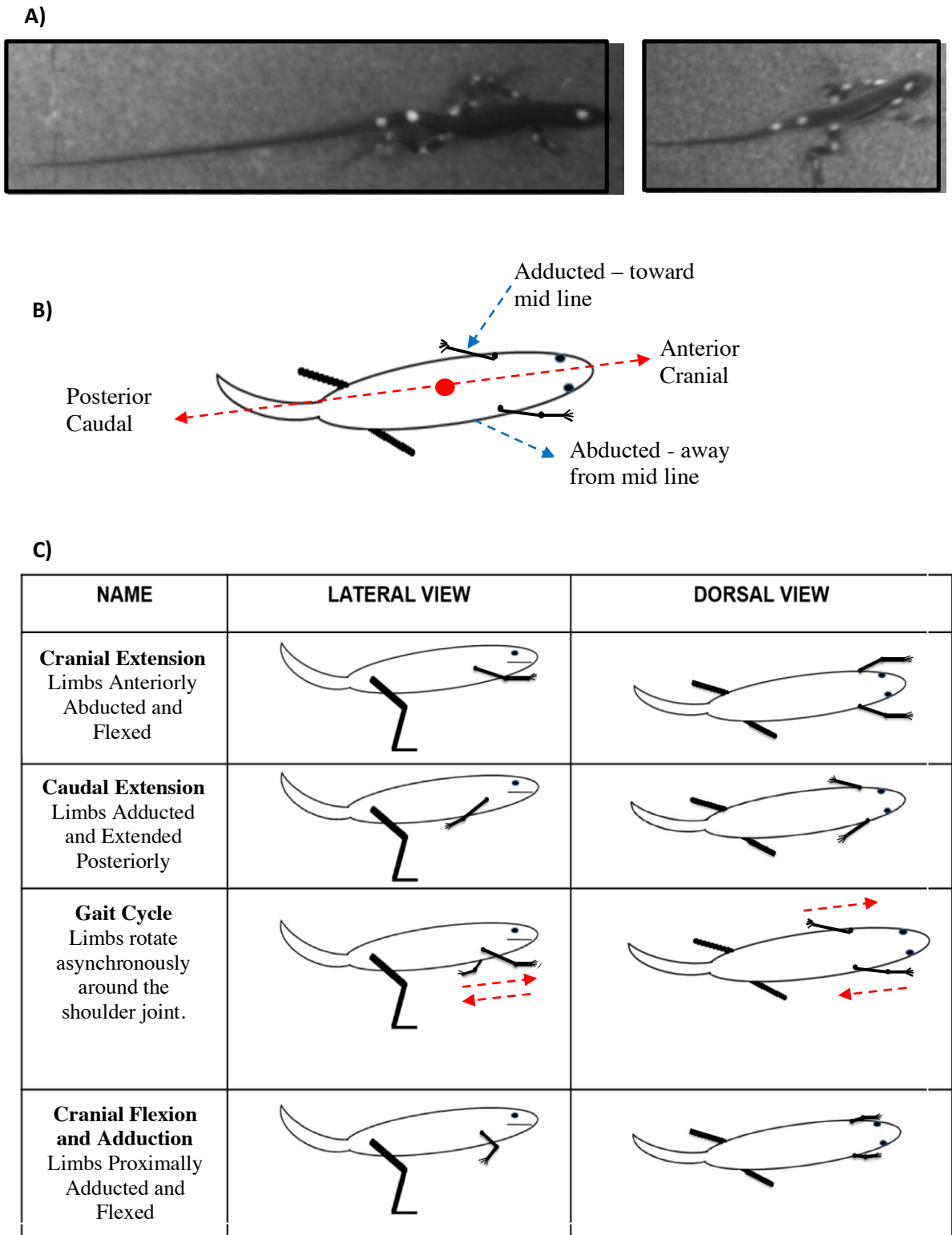
A)

B)

C)

Figure 2. Frequency of bipedal posture with vs. without an obstacle. A) Bipedal posture was used significantly more than quadrupedal posture with and without an obstacle for *A. sexlineata*. **B)** Quadrupedal posture was used significantly more than bipedal posture without an obstacle for *S. woodi*. Different letters indicate p-values are ≤ 0.05 from χ^2 analysis.
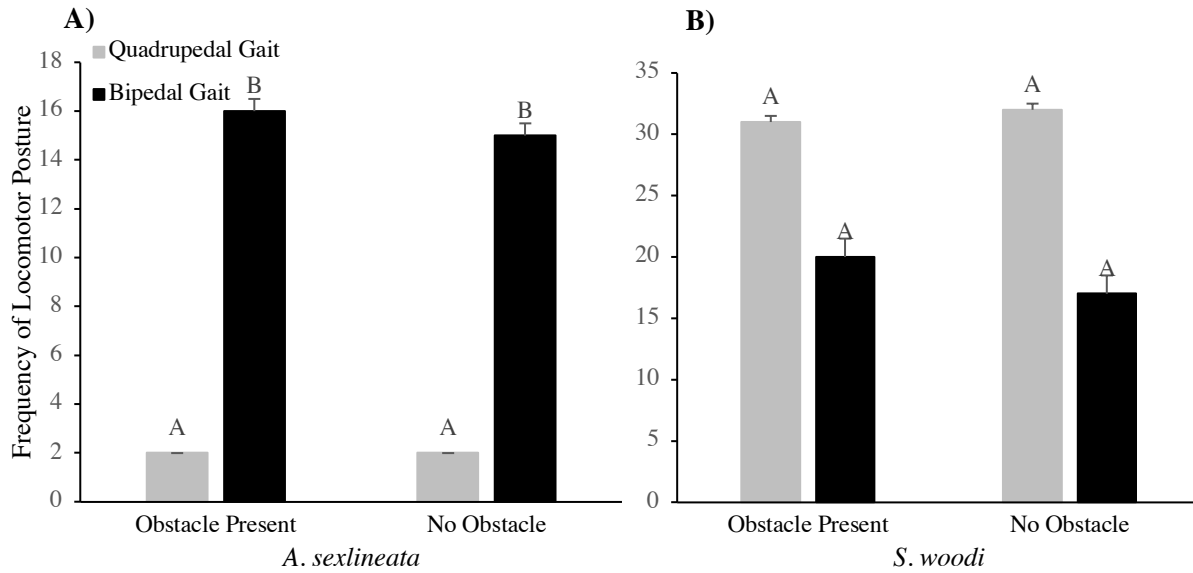
Figure 3. The frequency of forelimb positions during bipedal locomotion at the start of a sprint trial and at 0.8 meters with and without an obstacle for *S. woodi* and *A. sexlineata*. (A) Without an obstacle, *S. woodi* used flexion adduction and gait cycle significantly more than other forelimb positions at both the start of the sprint and 0.8 meters. **(B)** In trials with an obstacle *S. woodi* used both flexion adduction and gait cycle at the start of the sprint trial, but used flexion addduction when crossing an obstacle. In trials without an obstacle **(C)** and trials with an obstacle **(D)** *A. sexlineata* used caudal extension when running bipedally. A χ_2 analysis was used to compare the percentage of different forelimb positions used within two separate trials. Comparisons are not made across species or trials. Astrisks represent p-values ≤ 0.05.
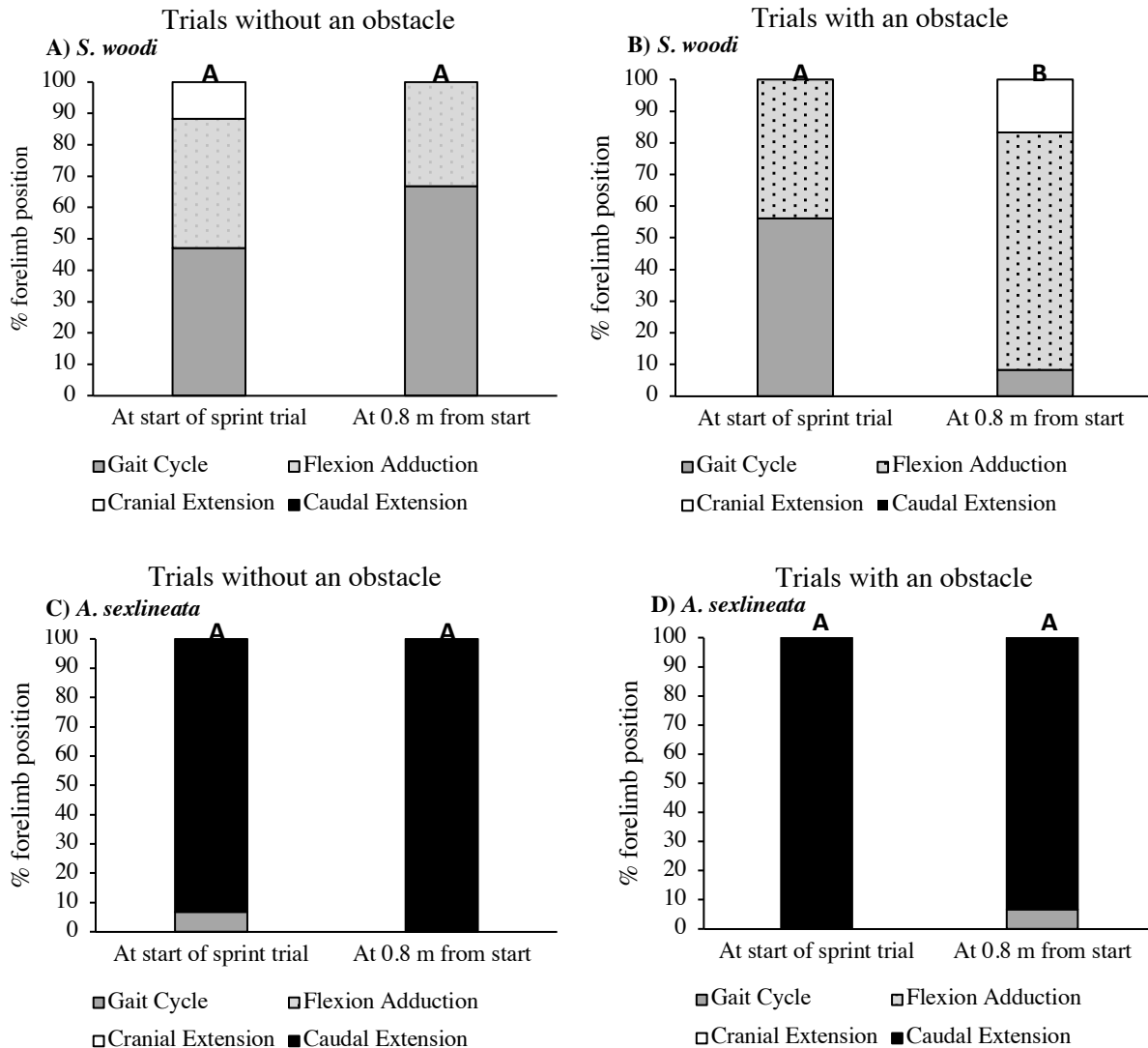
Figure 4. The body center of mass (BCoM) of three forelimb positions. The BCoM with forelimbs in caudal extension (8.47 ± 2.50 mm) was significantly different from cranial extension (9.8 ± 2.25 mm), but not gait cycle in *A. sexlineata*. In *S. woodi* cranial extension shifted the BCoM anteriorly (13.51 ± 0.56 mm) while caudal extension moved the BCoM posterior (12.25 ± 0.56 mm). Data were analyzed using a one-way ANOVA. Different letters indicate p-values are ≤ 0.05. Mean +/- 1 S.E. is shown.
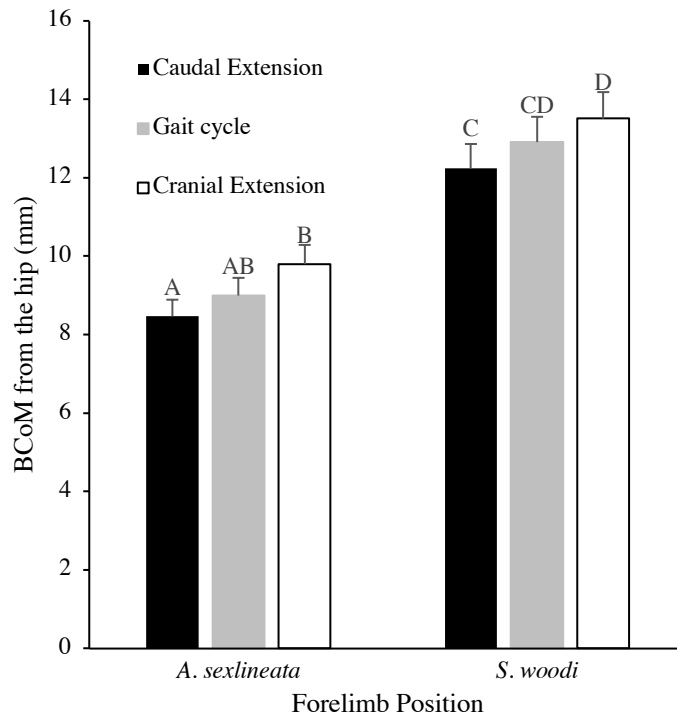
Figure 5. Frequency of forelimbs which touch, versus do not touch, an obstacle during bipedal locomotion. When crossing an obstacle, *S. woodi* touched the obstacle in 37% of the trials. In contrast, *A. sexlineata* touched the obstacle in 18% of the trials. Overall, *S. woodi* are more likely to touch the obstacle than *A. sexlineata*. Asterisks represent p-values ≤ 0.05 via χ² analysis.
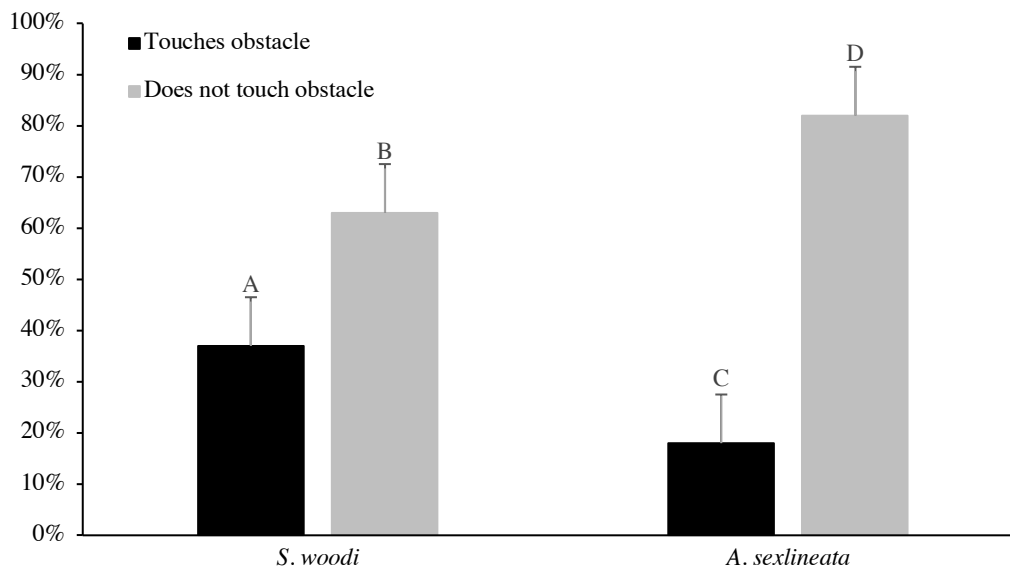
Figure 6. Average trunk angle for each forelimb position. A) In *S. woodi,* trunk angle was significantly greater with forelimbs flexed and adducted than with limbs cranially extended, or in a gait cycle (n = 23, df = 2, F-ratio = 12.77, p = 0.0003). **B)** In *A. sexlineata*, trunk angle was significantly greater with forelimbs caudally extended versus in a gait cycle (n = 12, df = 1, F-ratio = 19.68, p = 0.0013). Cranial extension was not observed.