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# The Effects Of Running Orientation on Gecko Locomotor Performance

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**The Effects of Running Orientation on Gecko Locomotor Performance**

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**Biology Department**

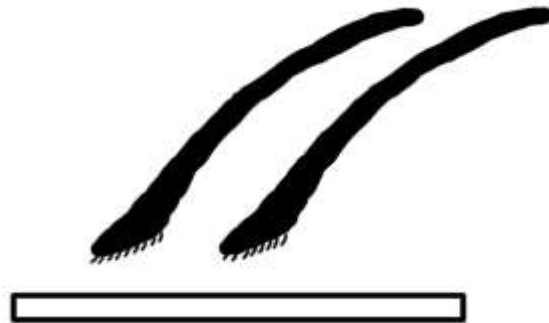
**Major: Biology**

**PI: Peter Niewiarowski**

## Introduction

Geckos reside in heterogenous environments, in which they encounter a multitude of substrates at various inclines and declines. Geckos thrive in habitats that require climbing and have developed a specialized adhesive system that facilitates locomotion within their habitat (Birn-Jeffery & Higham 2014). The gecko toepad features a hierarchical array of beta keratin fibrils (setae) that terminate into nanoscale contact points (spatulae) (Maderson, 1964; Ruibal & Ernst, 1965; Williams & Peterson, 1982; Alibardi 2003). This hierarchy allows for geckos to effectively adhere to surfaces by creating intimate contact with the surface, generating van der Waals intermolecular forces.

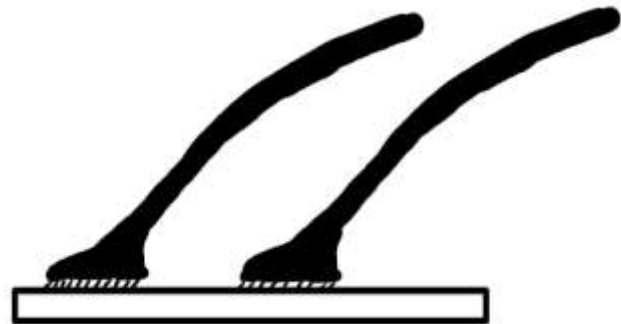
Interestingly, the gecko adhesive system is directional. Geckos apply a shear force in the distal to proximal direction to engage the system. The gecko's setae, as seen in Figure 1, are curved at the tips. This curvature is responsible for the system's directionality as the shear force allows the setae to make intimate contact with the surface. When a gecko is travelling upward on a surface they



**Figure 1.** Illustrates the setae curvature which causes the directionality of the system as it must be engaged through a shearing force that creates intimate contact with the setae and the surface for adhesion.

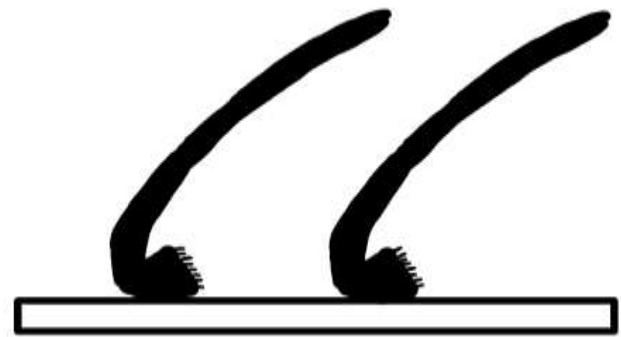
can engage the system simply by taking a normal stride, as the gecko naturally shears its toe pad with gravity in a head to tail fashion (Autumn et al. 2000). However, when travelling downward geckos must engage their system opposite the direction of gravity and the shear force that is applied. This proximal to distal fashion engagement of the system means that a gecko must also engage this system in the same fashion as they travel downward on a surface.

Travelling downward, should be more challenging as a gecko must engage its system opposite the direction of gravity and still in a proximal to distal fashion. Figure 2 illustrates the engagement of the setae being in intimate contact with the substrate. Geckos overcome this challenge by rotating their hind limbs posterior to their body to potentially engage this system (Birn-Jeffery & Higham 2014). By utilizing this rotation during downhill



**Figure 2.** Illustrates setae in intimate contact with the surface after a shear force was applied in distal to proximal fashion.

locomotion, it is believed they can shear their hindfeet in the proximal to distal fashion needed for adhesion. If there was no rotation of the hind limbs then the setae would not be aligned in such a way as to make intimate contact allowing for adhesion as shown in Figure 3. The discovery of this mechanism has raised many questions, including, whether this mechanism slows a gecko down while they are sprinting in a downward direction on a substrate.



**Figure 3.** Illustrates if the toe pad was sheared in a distal to proximal fashion the setae would fold under with no engagement.

Gecko adhesive locomotion has been heavily studied over the past few decades, but most studies investigate geckos sprinting upward on inclined or vertical substrates. While these studies have provided crucial knowledge regarding gecko adhesive locomotion, geckos likely move about in more than one orientation in their natural habitat. Since a gecko must engage its system opposite the direction of gravity and still in a proximal to distal fashion, many questions have been raised including: will this slow a gecko down while they are sprinting in a downward direction on a substrate?

Birn-Jeffery and Higham (2014) investigated a mechanism of adhesion for downhill adhesive locomotion. They discovered that geckos can rotate their hind limbs posteriorly to allow for adhesion as they travelled downward on a substrate. Birn-Jeffery and Higham (2016) further investigated downhill locomotion and found that sprint velocity was not affected by running orientation at substrate angles up to 45°. Wang et al. (2014) also investigated sprint velocity of geckos travelling at more extreme angles of 0° to 180°. They discovered a significant reduction in velocity at angles greater than 60°. Although Wang et al. (2014) investigated locomotion at extreme angles, the relationship between direction of travel at these more extreme angles was not investigated. These studies raise the question: Does running orientation affect the locomotor performance of geckos at greater vertical challenges (i.e. at substrate angles greater than 45°)?

The complex, heterogenous nature of a gecko's habitat may cause a gecko to be subjected to greater vertical challenges in multiple directions of travel than those previously tested. Given the directionality of the adhesive system and the nature of a gecko's natural habitat, we wanted to investigate the locomotor performance of geckos travelling at an upward and downward orientation at angles greater than those previously studied. In this study we sprinted *Gekko gecko* up and down at substrate angles of 60° and 90° while measuring their locomotor performance. This is different from Wang et al. as they only studied inclines and Birn-Jeffery and Higham as they only studied declines and inclines up to 45°. We hypothesized that, running in a downward orientation should decrease sprint velocity, geckos would spend more time moving as they travel up compared to down, and geckos would orient their hindlimbs more posteriorly when travelling downward. We expected these effects to be exacerbated as substrate angle increased.

## Materials and Methods

All experiments were approved by The University of Akron IACUC protocol 16-08-14-NGC. In this experiment six adult Tokay Geckos (*Gekko gecko*) were used for all trials. The geckos were housed in The University of Akron Research Vivarium in individual 10-gallon tanks. The room was maintained at 25°C with a relative humidity of 75-80%. The geckos were fed cockroaches and baby food three times a week with supplemental vitamin mix and calcium added to maintain proper nutrition. Each tank was also misted daily ensuring availability to water. Heating tape was placed on the underside of each tank allowing for the gecko to thermoregulate with the environment. Gecko health was assessed weekly to ensure adequate health for trials.

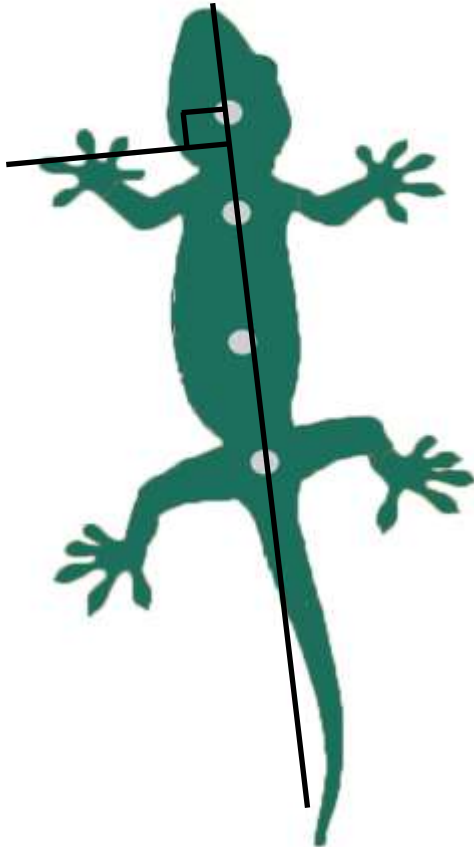
All trials were conducted in an environmental chamber. The chamber was set at a temperature of  $25.5^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  and a relative humidity of  $47 \pm 4\%$ . Prior to running trials, geckos were placed in a climate controlled chamber for one hour to acclimate at the experimental conditions. Geckos were sprinted on a custom acrylic racetrack that was capable of being positioned at 60° and 90°. The track's underside was painted black to assure that the geckos would willingly sprint along the surface. The track was cleaned using ethanol and reverse osmosis water prior to each trial. The acrylic substrate was dried with a Kimtech wipe after each solution. Prior to each trial, the geckos had a small strip of medical tape carefully placed around the mouth, avoiding the nostrils; and had four infrared (IR) reflective markers placed on the gecko's head, pectoral girdle, midbody, and pelvic girdle (Figure 4).

Geckos were sprinted  $1.37 \pm 0.27$  m at 60° up, 60° down, 90° up, and 90° down. Each gecko was sprinted a total of 3 times at each orientation and angle for a total of 12 trials per individual. After each trial, geckos were rested for one hour. Geckos were tested a maximum of three times before having (at least) a 24-hour rest period. A box with LED lights was placed below

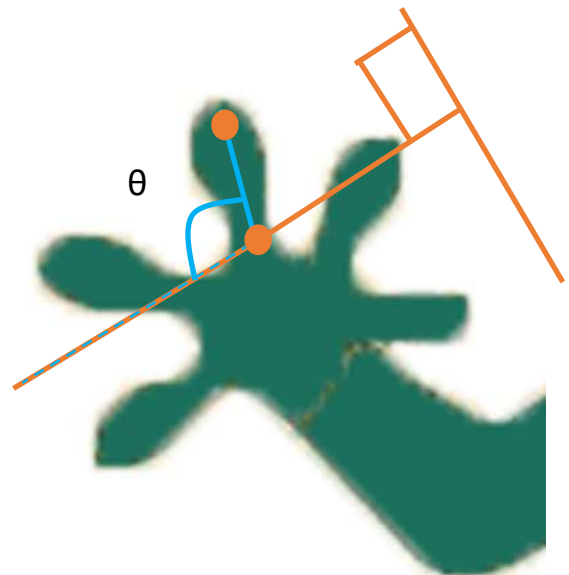
the gecko's direction of travel to encourage them to sprint away from the light. A black box with foam was placed at the opposite end of the track, to where the geckos could sprint, which simulated a hiding place for the gecko. Each gecko was placed on the track and chased by the handler's hand to simulate the gecko being chased by a predator in the wild, and encouraged the gecko to sprint. Each run was recorded using an OptiTrack Flex 13 motion capture system that consisted of four IR cameras with a frame rate of 120 frames per second. This system was linked to a computer which tracked the reflective markers and recorded the gecko's location in three-dimensional space as a function of time using Motive V.1.59 tracer by OptiTrack with a precision of <1 mm. A run was considered successful if a gecko sprinted more than half the distance of the track and all markers were in view for a majority of the run. The tracking of the IR marker on the head allowed for the collection of maximum and mean instantaneous velocity, ratio of time moving, and total distance travelled.

A DSLR camera, focused on the center of the track, was also utilized to record each trial to later be used for calculation of fore and hind foot orientation during each trial. The DSLR videos were then analyzed utilizing VLC Media Player and ImageJ. VLC was used to capture a screenshot of the video of each run when the left forefoot and left hind-foot were in complete contact with the surface. This created two images that could be imported into ImageJ for orientation analysis. Once the images were in ImageJ, orientation was calculated for each foot by

measuring the angle between the gecko's 3<sup>rd</sup> digit and an anatomical z-axis, that was created by drawing a line through the markers on the shoulder and pelvic girdle (Figure 4 and Figure 5).



**Figure 4.** A diagram showing the placement of IR markers and anatomical z-axis.



**Figure 5.** A diagram showing a close-up of the angle measured for foot orientation using the method as described.

## Statistical Analysis

The maximum and mean instantaneous velocities, ratio of time moving, and total distance travelled of each gecko per trial were calculated using a custom-written Python code. The code was able to smooth the data for analysis. Mean values of these parameters were calculated for the

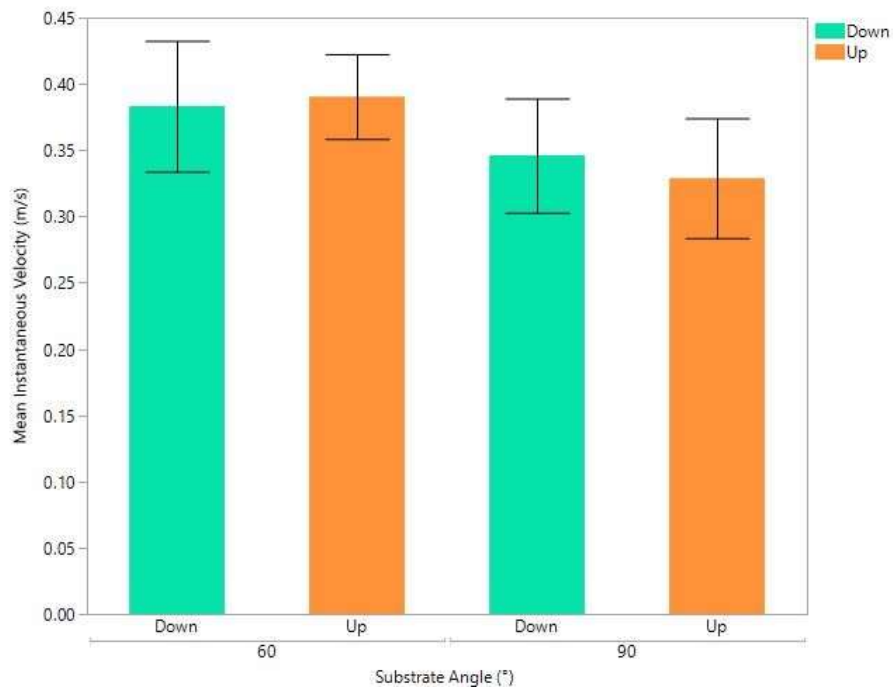


three trials per gecko per treatment. The effects of running orientation and substrate angle on maximum instantaneous velocity, mean instantaneous velocity (while moving), ratio of time moving, forefoot orientation, and hindfoot orientation were then investigated using mixed model analyses of variance (ANOVA). Maximum and mean instantaneous velocity, ratio of time moving, and fore and hindfoot orientation served as the dependent variables, while running orientation and substrate angle served as independent variables. Individual gecko was modeled as a random effect. All data met the assumptions of analysis of variance.

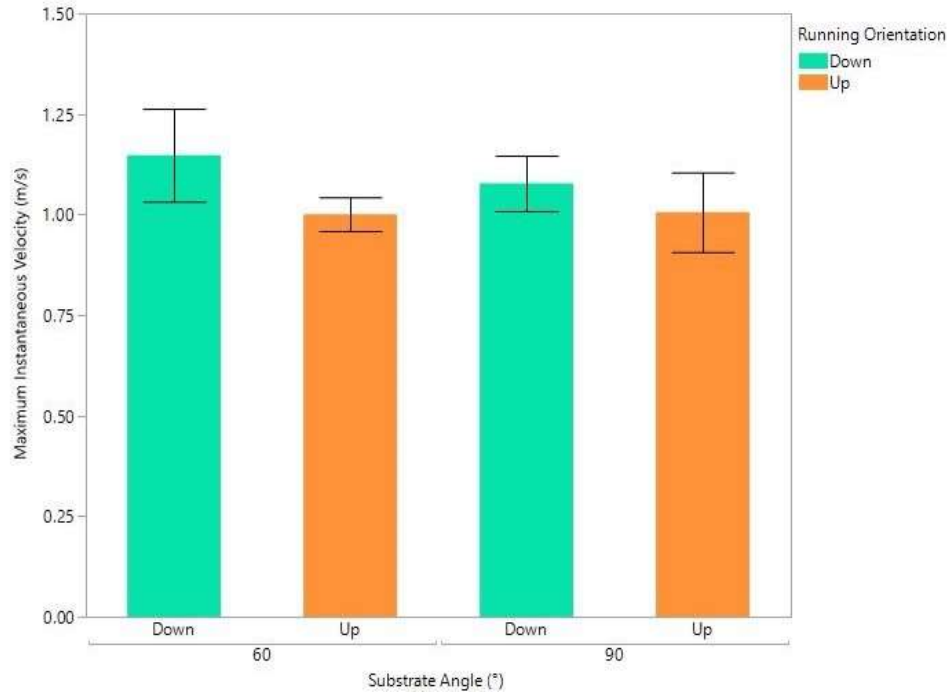
## Results

The mixed model ANOVA investigated the effects of running orientation and substrate angle on mean instantaneous velocities, maximum instantaneous velocities, ratio of time moving, and fore and hindfoot orientation (Figures 6-10). Mean instantaneous velocity was significantly affected by substrate angle with geckos having a decreased mean instantaneous velocity with increasing angle ( $F_{1,15} = 12.0$ ,  $P = 0.0035$ ) but was not significantly affected by running orientation ( $F_{1,15} = 0.122$ ,  $P = 0.731$ ). Maximum instantaneous velocity was not significantly affected by running orientation ( $F_{1,15} = 2.24$ ,  $P = 0.155$ ) or substrate angle ( $F_{1,15} = 0.199$ ,  $P = 0.662$ ). Ratio of time moving was significantly affected by running orientation, with geckos spending more time moving on inclines than declines ( $F_{1,15} = 35.4$ ,  $P = 0.001$ ), but was not significantly affected by substrate angle ( $F_{1,15} = 2.23$ ,  $P = 0.156$ ). Forefoot orientation was significantly affected by substrate angle with geckos rotating their forefeet more laterally with increasing angle ( $F_{1,15} = 8.00$ ,  $P = 0.0127$ ) but was not significantly affected by running orientation ( $F_{1,15} = 3.08$ ,  $P = 0.100$ ). Hindfoot orientation was significantly affected by running orientation with geckos rotating their hindfeet more posteriorly on declines ( $F_{1,15} = 107$ ,  $P = <0.0001$ ), but was not significantly affected by substrate angle ( $F_{1,15} = 0.957$ ,  $P = 0.034$ ). The interaction between substrate angle and running

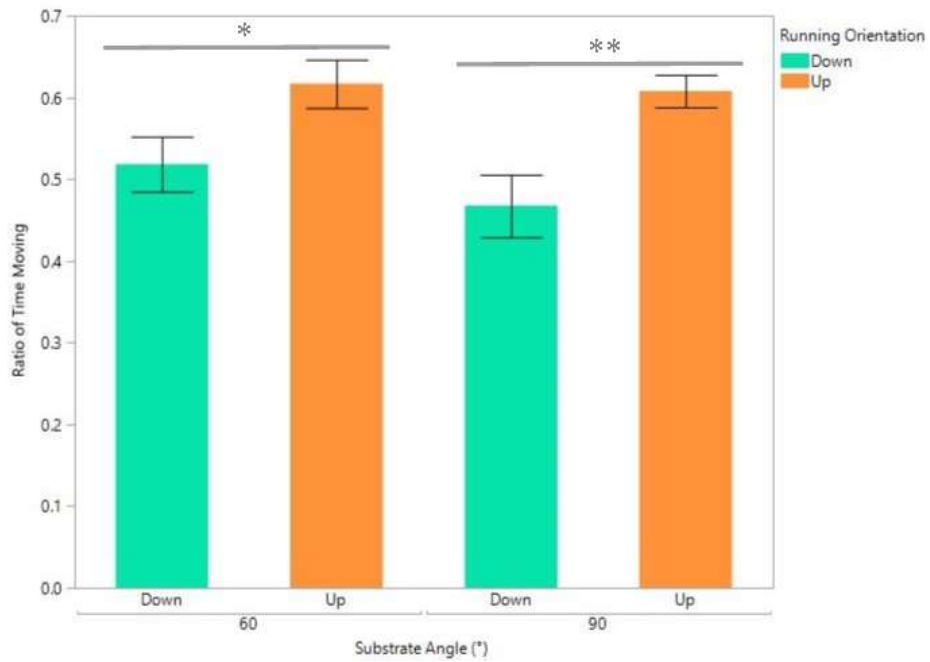
orientation had no significant effect on maximum instantaneous velocity ( $F_{1,15} = 0.265$ ,  $P = 0.614$ ), mean instantaneous velocity ( $F_{1,15} = 0.731$ ,  $P = 0.406$ ), ratio of time moving ( $F_{1,15} = 1.11$ ,  $P = 0.301$ ), forefoot orientation ( $F_{1,15} = 2.86$ ,  $P = 0.111$ ), or hindfoot orientation ( $F_{1,15} = 1.41$ ,  $P = 0.253$ ).



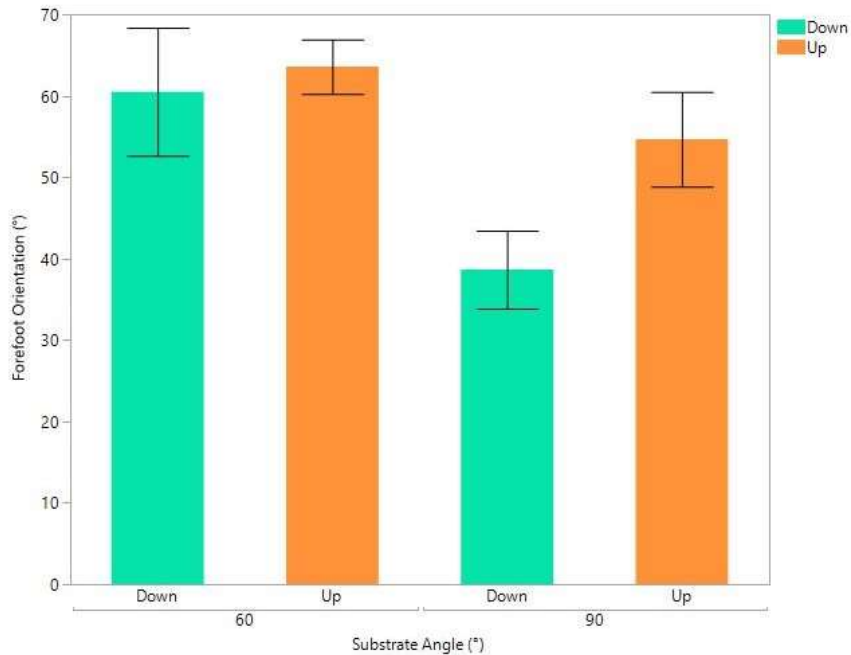
**Figure 6.** Mean instantaneous velocity is displayed as a function of substrate angle and running orientation. This graph shows that mean instantaneous velocity was affected by substrate angle ( $F_{1,15} = 12.0$ ,  $P = 0.0035$ ) but was not affected by running orientation ( $F_{1,15} = 0.122$ ,  $P = 0.731$ ). Error bars Represents  $\pm 1$  SE.



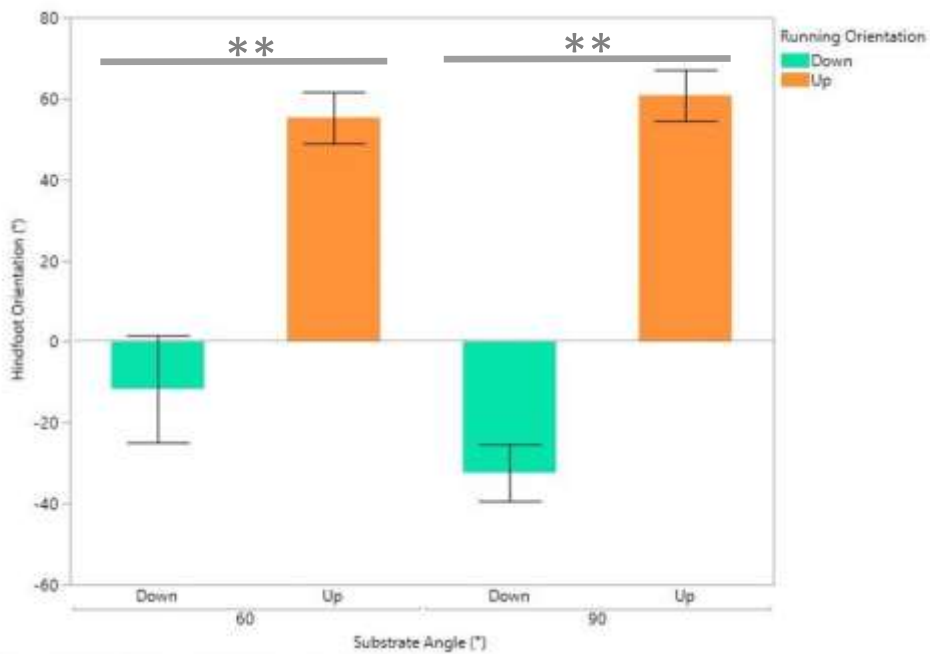
**Figure 7.** Maximum instantaneous velocity is displayed as a function of substrate angle and running orientation. This graph shows that maximum instantaneous velocity was not affected by either substrate angle ( $F_{1,15} = 0.199$ ,  $P = 0.662$ ) or running orientation ( $F_{1,15} = 2.24$ ,  $P = 0.155$ ). Error Bars Represents  $\pm 1$  SE.



**Figure 8.** Ratio of time moving is displayed as a function of substrate angle and running orientation. This graph shows that ratio of time moving was significantly affected by running orientation ( $F_{1,15} = 35.4$ ,  $P = 0.001$ ) but was not significantly affected by substrate angle ( $F_{1,15} = 2.23$ ,  $P = 0.156$ ). Error bars represent  $\pm 1$  SE. \* $P < 0.05$  \*\* $P < 0.001$ .



**Figure 9.** The mean forefoot orientation is displayed as a function of substrate angle and running orientation. This graph shows that forefoot orientation was significantly affected by substrate angle ( $F_{1,15} = 8.00$ ,  $P = 0.0127$ ) but not significantly affected by running orientation ( $F_{1,15} = 2.23$ ,  $P = 0.156$ ). Error bars represent  $\pm 1$  SE.



**Figure 10.** The mean hindfoot orientation is displayed as a function of substrate angle and running orientation. This graph shows that hindfoot orientation was significantly affected by running orientation ( $F_{1,15} = 107$ ,  $P < 0.0001$ ), but it was not significantly affected by substrate angle ( $F_{1,15} = 0.957$ ,  $P = 0.034$ ). Error bars represent  $\pm 1$  SE. \*\* $P < 0.001$ .

## Discussion

This experiment tested the locomotor performance of geckos sprinting in both upward and downward orientations. Geckos are likely to be subjected to a multitude of angles within their natural habitat and must also travel both up and down multiple substrates. Based off the directionality of the gecko adhesive system, travelling downward on substrates should decrease the gecko's velocity and it should be exacerbated by angle. This is because the gecko must be able to engage their adhesive system while travelling downward to ensure that they do not fall off the substrate which they are navigating.

We discovered that running orientation did not influence either the maximum or mean instantaneous velocity, meaning geckos, overall, were able to reach similar velocities in both running orientations, rejecting our hypothesis that running orientation would significantly reduce maximum and mean instantaneous velocity. However, running orientation had a significant effect on ratio of time moving, with a decrease in ratio of time moving when the gecko is sprinting in the downward direction. This is similar to snakes as they will stop to grip the surface on which they are descending to control their descent speed (Astley and Jayne 2007). This supports our second hypothesis as they spent a greater amount of time moving up compared to down. Although the mean and instantaneous velocity was not affected by running orientation, this measure of performance may be more biologically relevant for geckos in their natural habitat. For example, if it takes a gecko longer to traverse a 2-m declined substrate compared to an inclined substrate, this may be problematic during a predation event. Running orientation was also found to have a significant effect on hindfoot orientation. Geckos were found to rotate their hindfeet more posterior to their body when they were sprinted in a downward orientation when compared to upward. This supported our third hypothesis as they did orient their hindfeet more posteriorly when travelling

down as found in a previous study by Birn-Jeffery and Higham (2014). This rotation, potentially, allows for the geckos to engage their adhesive system opposite the direction of travel, allowing them to stick to the surface at either running orientation (Birn-Jeffery & Higham 2014). The above results suggest that there is a performance decrement when a gecko is descending a substrate, which may be a result of the time it takes to employ their adhesive system. Geckos were found to have a decreased mean instantaneous velocity with increasing substrate angle. This decrease in mean instantaneous velocity could be a result of the geckos having to utilize their adhesive system more at these challenging angles.

The posterior rotation of the geckos hindfeet was exacerbated by increasing substrate angle. This is likely due to the geckos needing to engage their system more for adhesion at greater declines. Ratio of time moving was also exacerbated by substrate angle as geckos spent more time moving while travelling upward compared to downward. This is likely a result of geckos being able to engage their system by taking a normal stride when travelling upward and it takes time for the gecko to posteriorly rotate their hindfeet when travelling downward. For example, Wang et al. 2014 showed that geckos' speed decreases as substrate angle increases, and at greater angles their speed decreased at a faster rate. The posterior rotation of the geckos hindfeet was exacerbated by increasing substrate angle. This is likely due to the geckos needing to engage their system more for adhesion at greater inclines or declines. The interaction between running orientation and substrate angle had no effect on any of the dependent variables.

The experiment revealed a lot about the difference in locomotor performance at different substrate angles. The mean instantaneous velocity was greater at 60° than at 90° which was expected as the lesser the angle the easier it should be for geckos to navigate. Maximum instantaneous velocity and ratio of time moving, however, were similar at both 60 and 90° angles.

This means that even though geckos can reach similar maximum instantaneous velocities they are able to maintain their velocity at the lesser angle when compared to the greater angles, meaning they are able to reach a greater velocity at lesser angles. The similar ratio of time moving demonstrated that when looking between substrate angles they spend a similar amount of time moving. Substrate angle was also found to affect forefoot orientation. It was found that as the substrate angle increased there was an increase in lateral rotation of the forefeet. However, substrate angle had no effect on the hindfoot orientation. This could be due to geckos attempting to overcome gravity by using their adhesive system to pull their weight toward their center of mass, preventing them from falling off the substrate they are navigating. This is shown in Wang et al. 2015 as they discovered geckos pull their limbs toward the center of their body to generate lateral and fore aft forces on the feet that act away from the body which help with adhesion to the substrate. By creating these forces geckos can overcome gravity and adhere to challenging angles by manipulating the angle of their forefeet.

Overall, this experiment illustrated that running orientation had no effect on either mean instantaneous velocity, maximum instantaneous velocity, or forefoot orientation, but, ratio of time moving and hindfoot orientation were found to be sensitive to direction. However, geckos were still able to sprint at similar speeds in both upward and downward directions, meaning they are able to overcome this posterior hindfoot rotation. Although this experiment looked at locomotor performance at more angles and orientations than previously studied it could still be expanded upon. This study did not analyze stride length or stride frequency, which are two more measurements of locomotor performance, that could add to the overall understanding of locomotor performance of the Tokay Gecko. This experiment also used only one species of gecko, and in the future, multiple species of geckos could be used to see if there is a difference in the locomotor

performances across species. This could lead to see if there is a morphological or mechanical reason for the difference across species. This experiment, overall, has relevance to both geckos in their natural habitat, as well as the design of gecko-inspired robots. This experiment allowed us to see how geckos might navigate their natural habitat in both upward and downward orientation and the mechanisms they utilize during adhesive locomotion.

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