

RESEARCH ARTICLE

Run don't walk: locomotor performance of geckos on wet substrates

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ABSTRACT

The gecko adhesive system has been under particular scrutiny for over a decade, as the field has recently attracted attention for its application to bio-inspired design. However, little is known about how the adhesive system behaves in ecologically relevant conditions. Geckos inhabit a variety of environments, many of which are characterized by high temperature, humidity and rain. The van der Waals-based gecko adhesive system should be particularly challenged by wet substrates because water can disrupt the intimate contact necessary for adhesion. While a few previous studies have focused on the clinging ability of geckos on wet substrates, we tested a dynamic performance characteristic, sprint velocity. To better understand how substrate wettability and running orientation affect locomotor performance of multiple species on wet substrates, we measured average sprint velocity of five species of gecko on substrates that were either hydrophilic or intermediately wetting and oriented either vertically or horizontally. Surprisingly, we found no indication that wet substrates impact average sprint velocity over 1 m, and rather, in some species, sprint velocity was increased on wet substrates rather than reduced. When investigating physical characteristics and behavior that may be associated with running on wet substrates, such as total number of stops, slips and wet toes at the completion of a race, we found that there may be habitat-related differences between some species. Our results show that in general, unlike clinging and walking, geckos running along wet substrates suffer no significant loss in locomotor performance over short distances.

KEY WORDS: Gecko, Adhesion, van der Waals, Wetting, Locomotion

INTRODUCTION

The gecko adhesive system has been a topic of interest for decades, if not centuries (Home, 1816; Maderson, 1964; Ruibal and Ernst, 1965; Stewart and Daniel, 1972; Russell, 1975; Williams and Peterson, 1982; Irschick et al., 1996; Autumn et al., 2000; Autumn, 2006). However, relatively little is known about how geckos take advantage of their adhesive capabilities in natural environments. In laboratory settings geckos perform remarkably well on smooth, clean substrates with uniform surface chemistry (Autumn et al., 1999; Chen et al., 2000; Losos, 1990a; Irschick et al., 1996; Bergmann and Irschick, 2005; Niewiarowski et al., 2008, 2012; Stark et al., 2012, 2013), yet in their native environments the substrates a gecko moves across are likely to be substantially different from those used in a laboratory setting. Additionally, the

way a gecko utilizes its adhesive system can be quite variable. Geckos cling, walk and run across substrates, changing the loading force and mechanical requirements of the system with each step (Autumn et al., 2006; Dai et al., 2011). To add further complexity, natural environments are also unpredictable (Russell and Johnson, 2007), as substrates can become wet from rainfall, humidity or even fog and these conditions can occur suddenly and persist for extended periods of time. Water should be particularly challenging because it can disrupt the close, intimate contact required for the van der Waals-based adhesive system (Autumn et al., 2000, 2002; Pesika et al., 2009; Stark et al., 2012, 2013). As such, we would expect pad-bearing geckos to have ways to maintain function of their adhesive system when clinging, walking and running on wet substrates.

In an effort to understand how water could affect the gecko adhesive system, maximum shear adhesion on a glass substrate misted with water was measured (Stark et al., 2012). The forces generated by Tokay geckos (*Gekko gecko*) immediately subsequent to their coming into contact with the substrate did not differ between dry or misted glass. However, after allowing geckos to take four steps on the misted glass substrate, they produced significantly lower force values than on a dry glass substrate (misted, 1.84 ± 0.54 N; dry, 17.96 ± 3.42 N). This suggests that walking on wet hydrophilic glass negatively impacts the adhesive system, even after only four steps. This result is perplexing given that this species is endemic to tropical environments that commonly experience rainfall and high humidity. Considering the diversity of substrates available to geckos in their environment, geckos were also tested on substrates that vary in wettability. Contrary to the results on hydrophilic glass, when geckos walk on wet hydrophobic substrates, maximum shear adhesion is not reduced in water compared with air (Stark et al., 2013). These results suggest that the ability of a gecko to cling to or walk on natural substrates that have become wet may not be impaired on hydrophobic substrates.

Because geckos are likely to walk or run between retreat and foraging sites, as well as cling to substrates while waiting for prey (Aowphol et al., 2006), quantifying the effects of water on clinging alone may not fully capture the impact of surface water on free-ranging gecko locomotion as a whole. There is a broad literature on lizard locomotor biomechanics (Russell and Bels, 2001); however, there are relatively few studies that have investigated the relationship between locomotion and adhesion in pad-bearing species (Zaaf et al., 2001; Irschick et al., 2003; Vanhooydonck et al., 2005; Autumn et al., 2006; Dai et al., 2011). Furthermore, although the mechanisms behind gecko adhesion have been studied across multiple length scales (Irschick et al., 1996; Autumn et al., 2000, 2002; Huber et al., 2005a; Tian et al., 2006; Zhao et al., 2008), no studies directly address the effect of wet substrates on dynamic locomotor performance. If we consider the dynamics of a gecko running either vertically or horizontally, we would expect that a 100 g Tokay gecko (*Gekko gecko*) requires a force equivalent to two

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times its body weight, 2 N of force, to run one step (Autumn et al., 2006). Using previous static adhesion results (Stark et al., 2012), after four steps on a misted hydrophilic substrate geckos produced 1.84 ± 0.54 N of force, suggesting that their ability to run either vertically or horizontally (assuming constant force requirements) could be compromised, and this may be especially true when countering the overturning force in the vertical direction. Based on these estimates, a gecko running on a substrate misted with water droplets should begin to lose traction and thus speed, perhaps even slip and fall, after only four steps, yet this behavior has never been documented in field observations and would probably be detrimental to the gecko. Furthermore, we would expect this to only hold true for hydrophilic substrates, which are a clear challenge for the adhesive system when they become wet (Stark et al., 2013).

In an effort to quantify the effect of water on locomotion across wet substrates, we compared sprint performance of geckos on two substrates that vary in their wettability as measured by their water contact angle, θ . Glass served as a hydrophilic substrate ($\theta \sim 50$ deg), and acrylic as a less-hydrophilic substrate ($\theta \sim 85$ deg). We also chose to test for an effect of running orientation (vertical or horizontal) for three reasons. First, prior studies did not investigate shear adhesion on a vertical substrate (Stark et al., 2012, 2013). Second, geckos apply different forces when running vertically and running horizontally (Autumn et al., 2006) and these forces may have an impact on the likelihood of water disrupting adhesive contact at the surface. Third, we know that in at least one species of pad-bearing gecko the adhesive system is not deployed until a critical incline angle is reached (Russell and Higham, 2009) and thus geckos running in the horizontal orientation may not utilize their adhesive system. Based on previous work (Stark et al., 2012, 2013) and force values from geckos sprinting along a force plate (Autumn et al., 2006), we hypothesized that geckos running on the misted hydrophilic glass substrate would have a lower average sprint velocity than when running on a dry glass substrate, and this should be exacerbated in the vertical orientation where geckos must use their adhesive system to not only provide propulsion upward but also counter overturning. Furthermore, in this treatment, geckos cannot hold their toes above water (via digital hyperextension); thus, during a vertical run, water can disrupt the adhesive system by forming an intervening layer (see Stark et al., 2013) and also through toe wetting, which can further reduce adhesion of the toe pads to the substrate (Stark et al., 2012, 2014a). Conversely, when tested on the acrylic substrate, we hypothesized that the adhesive system would remain functional, as suggested by the shear adhesion results (Stark et al., 2013) and geckos would be able to run equally fast on wet and dry acrylic in either orientation.

In addition to measuring sprint velocity, we also recorded frequency of three specific observations which may be related to sprint velocity: first, the total number of stops an individual made during a race, second, the total number of foot slips that occurred during a race and finally, the total number of wet toes measured after a race. Clearly, behaviors such as stopping and slipping can

significantly impact sprint velocity and we tested whether these were significantly related to running on a wet substrate (glass or acrylic), in either orientation (vertical or horizontal). One major challenge to sprint performance may also be the physical change in ‘anti-wetting’ behavior (superhydrophobicity) of the toe pads (Autumn and Hansen, 2006; Pesika et al., 2009). Toes that become wet are no longer adhesive (Stark et al., 2012, 2014a) and therefore should negatively impact locomotor performance; however, it is unclear whether toe wetting occurs more often in particular orientations (vertical or horizontal) or on specific substrates (hydrophilic or intermediately wetting). Finally, as a way of sampling variation in how water affects performance among different species of gecko, we tested five species (Table 1). There are more than 1400 species of gecko, inhabiting many ecological niches and the gecko adhesive system has evolved multiple times (Gamble et al., 2012), with gains and losses correlated with habitat preference in at least one group (Lamb and Bauer, 2006). Despite this diversity, very few studies of adhesion have investigated species other than the Tokay gecko (Irschick et al., 1996; Niewiarowski et al., 2008; Russell and Higham, 2009), even though species of gecko do vary in several conspicuous ways related to adhesion, including morphology and behavior (Williams and Peterson, 1982; Peattie and Full, 2007; Gamble et al., 2012). Our primary goal with this study is to investigate the interaction between water and the gecko adhesive system under conditions that relax some of the standardized conditions typical of gecko laboratory performance studies.

RESULTS

We found no effect of substrate, running orientation or species on differences in sprint velocity between dry and misted surfaces (SV_{D-M}) ($F=1.0555$, d.f.=15, $P=0.4199$). In the course of our experiments, we found that *Rhacodactylus auriculatus* was unable to reliably cling to smooth surfaces (glass or acrylic). We were therefore unable to measure sprint speed for *R. auriculatus* in trials with vertical orientation and they were analyzed separately. There was also no effect of substrate on SV_{D-M} in trials where *R. auriculatus* was tested horizontally ($F=0.3744$, d.f.=1, $P=0.5737$) (Fig. 1).

In addition to sprint velocity, we also measured the frequency of stops, slips and wet toes during experimental trials. Frequency of stops varied significantly among different treatment groups ($F=3.7127$, d.f.=11, $P<0.0001$). Species had a strong impact on frequency of stops ($F=8.3122$, d.f.=4, $P<0.0001$), but orientation and substrate as simple effects did not ($F=0.8012$, d.f.=1, $P=0.3724$ for orientation; $F=0.5422$, d.f.=1, $P=0.4629$ for substrate). We found that *Phelsuma dubia* stopped significantly more than *Pachydactylus bibronii*, *Tarentola mauritanica* and *R. auriculatus* but not more than *Gekko gecko* (Fig. 2). Although interaction terms between orientation and treatment ($F=3.0981$, d.f.=1, $P=0.0808$) and substrate and treatment ($F=3.1726$, d.f.=1, $P=0.0773$) suggested there may be complex effects on stopping frequency, they were not significant. When investigating slipping, we found

Table 1. Measurements of the five gecko species tested

Species	N	Mass (g)	SVL (cm)	Toe pad area (cm ²)	Habitat
<i>Phelsuma dubia</i>	4	7.7±0.1	6.2±0.1	0.74±0.05	Tropical
<i>Pachydactylus bibronii</i>	4	32.1±0.5	8.1±0.2	1.37±0.16	Arid
<i>Gekko gecko</i>	5	59.5±0.4	12.5±0.2	5.01±0.15	Tropical
<i>Tarentola mauritanica</i>	3	7.9±0.2	6.2±0.4	1.06±0.10	Sub-tropical
<i>Rhacodactylus auriculatus</i>	3	14.2±0.3	8.6 ±0.2	1.37±0.13	Sub-tropical

Data are means±s.e.m.

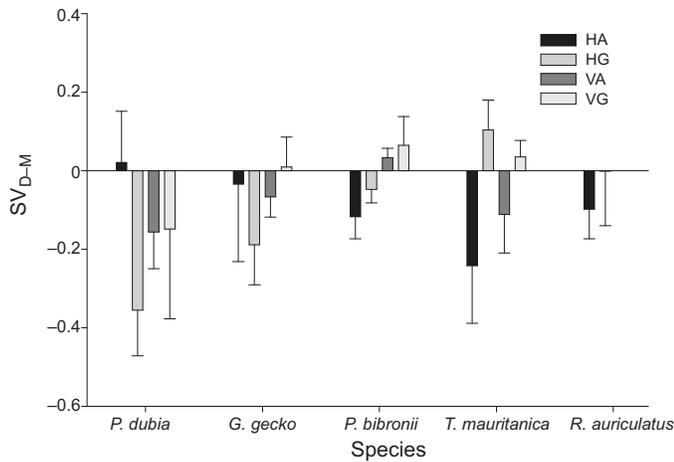


Fig. 1. The difference of average sprint velocity on dry versus misted substrates across gecko species. Difference values (displayed on the y-axis) that are positive indicate higher velocity on dry compared with the misted surfaces. Values near zero suggest similar performance on misted and dry substrates. Each species is reported across the x-axis and pairs of running orientation (H, horizontal; V, vertical) and substrate type (G, glass; A, acrylic) are separated as indicated in the legend. *R. auriculatus* was unable to run vertically, thus there are no values reported for vertical acrylic (VA) and vertical glass (VG) in this species. Data are means \pm s.e.m.

that the majority of recorded slips were from *P. bibronii* running on misted vertically oriented glass. The total number of wet toes at the completion of each trial on misted substrates varied significantly ($F=3.1353$, d.f.=7, $P=0.0067$) and was driven by differences among species ($F=3.7838$, d.f.=4, $P=0.0081$; Fig. 3) and the interaction of orientation and substrate ($F=6.5515$, d.f.=1, $P=0.0129$); however, significance tests between orientation and substrate pairings were not significant when controlling for multiple tests (Fig. 4).

DISCUSSION

Studies of the potential effects of water on gecko adhesion are increasing (Huber et al., 2005b; Sun et al., 2005; Niewiarowski et al., 2008; Pesika et al., 2009; Prowse et al., 2011; Stark et al., 2012, 2013, 2014a,b), although, to date, all have focused on static adhesive performance. In contrast, work on dynamic performance seems to be restricted to investigations related to atmospheric humidity rather than surface water and has not been investigated in whole animal experiments (Puthoff et al., 2010, 2013; Gravish et al.,

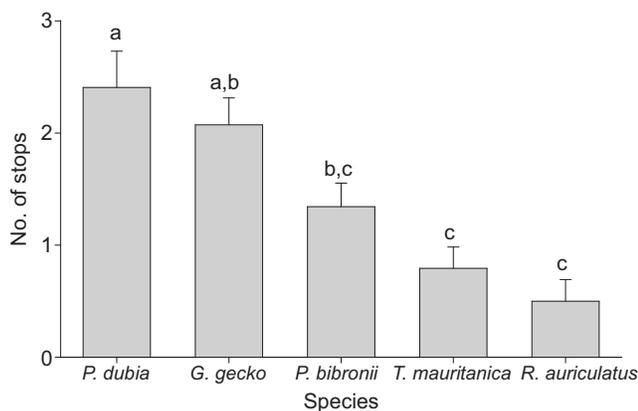


Fig. 2. Total number of stops during all treatments across gecko species. Bars with the same letter indicate no statistical significance between species. Data are means \pm s.e.m.

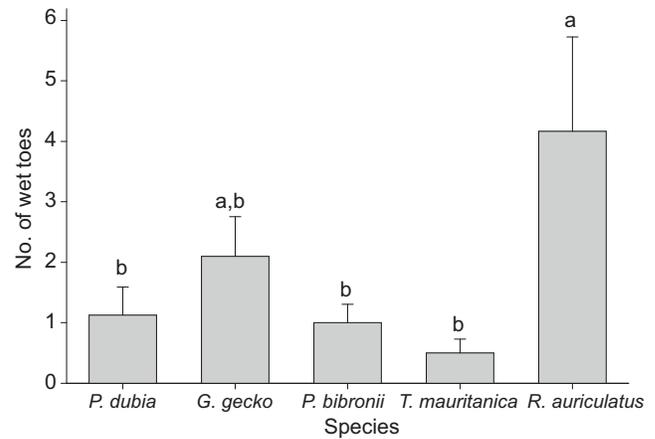


Fig. 3. Total number of wet toes at the completion of all misted substrate treatments separated by species. Bars with the same letter indicate no statistical significance between species. Data are means \pm s.e.m.

2010). In this study, we focused on sprint velocity to understand how a more dynamic utilization of the adhesive system can be affected by wet substrates. Recently, it was found that after four steps on a misted hydrophilic glass substrate, geckos may not be able to generate the static adhesive force required to counter their body weight during vertical locomotion (Autumn et al., 2006; Stark et al., 2012). We hypothesized that geckos running on misted hydrophilic glass substrates, especially when running vertically, would have lower sprint velocity than when running on a dry glass substrate because of reduced adhesive traction. However, our results do not support this hypothesis, as we found that geckos run equally fast on average, whether on misted or dry hydrophilic glass, regardless of orientation (Fig. 1). Our second hypothesis was that geckos would suffer no loss in adhesive traction, and thus sprint velocity, when running on a misted intermediately wetting substrate. This hypothesis was supported and average sprint velocity did not differ between misted and dry treatments on the acrylic substrate (Fig. 1). Our results suggest that although static adhesive performance can be significantly affected by water (Stark et al., 2012, 2013), sprint velocity, at least over a 1 m distance, is not affected by wet substrates.

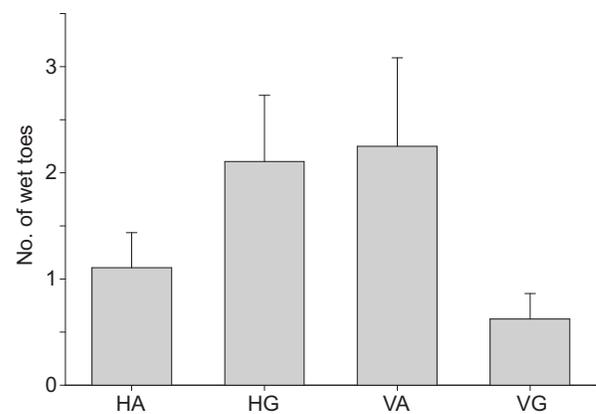


Fig. 4. Total number of wet toes at the completion of all misted substrate treatments. Counts are separated by orientation and substrate (HA, horizontal acrylic; HG, horizontal glass; VA, vertical acrylic; VG, vertical glass). After controlling for multiple tests, none of the orientation and substrate treatments were significantly different, although the interaction was significant in the model ($F=6.5515$, d.f.=1, $P=0.0129$). Data are means \pm s.e.m.

The lack of impact of water on sprint velocity over 1 m is especially interesting considering previous work showing that taking steps on misted hydrophilic glass gradually decreases adhesion (Stark et al., 2012). In fact, surprisingly, SV_{D-M} was often negative, meaning that sprint velocity was faster on the misted substrate than the dry and in many cases this difference was large (Fig. 1). This observation is especially clear in *Phelsuma dubia* and *Gekko gecko*, where under most conditions these species ran equally fast or faster on misted substrates than dry, despite having stopped more often than the other species (Fig. 2). Initially, we hypothesized that stopping would either reduce sprint velocity directly by disrupting the run, or indirectly by pushing water into the toe pads, causing them to wet and reduce adhesion and therefore velocity. An increase in average velocity despite an increase in stops during the trials is counterintuitive, but locomotion is a complex performance trait comprising interacting morphological, behavioral and physiological components. It is not uncommon to change stride frequency or stride length to increase velocity (Zaaf et al., 2001; Irschick et al., 2003; Autumn et al., 2006) nor is it uncommon to change stopping frequency based on running orientation (Higham et al., 2011), so it is possible that these species are altering sprint behavior to improve velocity on wet substrates while keeping their toes relatively dry. To what extent this result might be related to the current habitat association of these species and their evolutionary history (Table 1) is an interesting but open question that can only be reliably addressed with a phylogenetically based comparative study (e.g. Irschick et al., 1996). Our results led us to the hypothesis that stopping more often increases average velocity on wet substrates, perhaps by paring stops with sprinting bursts. In this scenario we may expect velocity to reduce to zero during a stop but then increase quickly because of a running burst, thereby maintaining the overall average sprint velocity across a substrate. This of course is contingent on the duration of the stop and the speed of the burst after a stop. It is also important to remember here that these species only stopped about twice on average while running the 1 m track. Thus, further studies should focus directly on stop and start velocity and frequency when running on substrates that are typically considered challenging to the gecko adhesive system.

Although velocity was well maintained on misted substrates when compared with dry, even appearing to be improved in some species, the condition we expected to be the most challenging – misted vertical glass – did produce the only obvious slipping events. Slipping on a misted substrate occurred exclusively in *Pachydactylus bibronii*, a species that happens to be native to arid regions in southern Africa (Zaaf and Van Damme, 2001). Intuitively, we predicted that slipping would lower sprint velocity; however, when slipping occurred in this species, average velocity was maintained. Slipping may occur by two mechanisms: first, by toe pad wetting (Stark et al., 2014a) and second, by an intervening water layer (Stark et al., 2013). We know that when all the toe pads are wet geckos cannot generate enough force to support their body weight (Stark et al., 2012, 2014a) and it is easy to observe slipping due to wet toe pads (see fig. 1 in Stark et al., 2012); however, over a 1 m distance this species did not have significantly more wet toes than any of the other species. Because we did not observe a significant increase in wet toes, the slipping behavior we observed on vertical glass by *P. bibronii* may instead be related to a lubricating water layer that is held between a hydrophilic substrate (glass) and the superhydrophobic toe pad (Stark et al., 2013), causing the gecko to slip on water without wetting the toe pad. Additionally, it is also possible that adhesive setae themselves are affected differently by water than those of other species. This may

include reduction of the setal modulus due to hydration (Prowse et al., 2011; Puthoff et al., 2010). In this context, Puthoff and colleagues questioned whether species from dry climates would suffer impaired adhesion due to the low humidity of their environment (Puthoff et al., 2010). Our results provide motivation for further study of variation among species in adhesive locomotion under a variety of conditions such that robust ecological and evolutionary hypotheses can be tested. Results from such work would further our understanding of the evolution of the gecko adhesive system and provide insight into potential biomimetic applications.

Interestingly, the only species which could not run vertically (*Rhacodactylus auriculatus*) had the highest number of wet toes at the completion of runs on misted substrates (Fig. 3). We noticed that this species tended to jump more frequently than all other species, which may have caused the increase in toe wetting because of a pressure change and/or increased agitation, which we know can cause toe pad wetting (Stark et al., 2012). *Rhacodactylus auriculatus* is native to sub-tropical regions and are not particularly high climbers (and have even been observed to move terrestrially; Bauer et al., 2012), thus higher prevalence of wet toe pads could be of little concern since they do not rely on their adhesive system for high vertical climbs. In general, toe pad wetting has significant consequences for adhesion and thus velocity; however, it is unclear how or when the transition from wet to dry occurs during more natural, dynamic motion. Interestingly, we found that substrate type and running orientation had a weakly significant effect on toe pad wetting across all species. Further data are necessary to clarify this relationship, but our work here shows that running on misted horizontal hydrophilic glass and on vertical intermediately wetting acrylic may increase the likelihood of toe pad wetting through some mechanism that has yet to be fully clarified (Fig. 4).

Our results suggest a remarkable level of resiliency of gecko toe pad performance on wet substrates over short distances. Tests measuring shear adhesion (Stark et al., 2012), which only permitted geckos to take four steps, suggest that a 1 m distance would be sufficient to detect measurable differences in performance based on walking. When geckos are running, we see that there is no direct effect of water on performance over 1 m; however, it is possible this does not hold true over larger distances. For instance, over a longer distance, would the total number of wet toes reach a critical threshold? Would significant, even catastrophic slipping occur? The answers to these questions are the subject of future study. Our experiment does point out that sprinting a distance of 1 m is likely to be better than walking a distance of 1 m on a misted substrate. The dynamics of water movement and perhaps drainage during dynamic running rather than walking is interesting and may have significant application to synthetics, which can be used in wet conditions. Furthermore, we believe that our results represent the first data showing rate-dependent friction aiding whole animal performance in semi-natural conditions. During dynamic running, the adhesive setae probably slide at a higher velocity than they do in static tests where geckos are pulled along a substrate. Thus, as slide velocity of the setae increases in dynamic running, so does adhesion (Gravish et al., 2010; Puthoff et al., 2010, 2013). Hence, geckos may maintain or even improve sprint velocity on wet surfaces as a result of increased adhesion resulting from the rate-dependency of the adhesive system. Further work is required to clarify how movement of water under the toe (to avoid the lubricating layer and wet toe pads) and the sliding velocity of the setae couple to maintain performance on wet surfaces.

Our experiment serves as a reminder about the relationship between morphology, performance and behavior, ecology and ultimately, fitness (Arnold, 1983; Garland and Losos, 1994; Wainwright and Reilly, 1994; Aerts et al., 2000). While we focus primarily on performance here, our study highlights the importance of investigating variation in all these components in relation to the gecko adhesive system by considering morphology (frequency of toe pad wetting), performance (sprint velocity) and behavior (stopping and slipping) across multiple species as it relates to wet substrates. Performance has been a primary focus of the field recently and although important, it is difficult to fully understand and appreciate the system in an ecologically realistic and evolutionary relevant context. Geckos have adapted their adhesive morphology in order to exploit various niches, perhaps not unlike *Anolis*, another pad-bearing group with clearly defined ecomorphological variation (Losos, 1990b; Vitt et al., 2003). Unlike *Anolis*, however, very few studies have focused on the relationship between gecko morphology and ecological niche, despite significant variation in digital and setal morphology (Gamble et al., 2012; Peattie and Full, 2007), both of which may be significant to the gecko's interaction with surface water. For instance, in this study we found that sprint performance on wet substrates is comparable among five taxonomically distinct species. However, sources of variation in stopping behavior, slipping and toe pad wetting among species are unclear, but intriguing, because they suggest hypotheses about morphological, ecological and evolutionary covariation with adhesive system performance that could be tested with phylogenetically controlled comparisons of performance across multiple species endemic to a variety of environments.

While future studies are crucial to understanding how our observations and measurements of morphology, behavior and performance relate to the ecology and natural history of geckos, effort should be focused not only on laboratory-based studies but also in the field. For instance, our treatment groups focus on several combinations of substrate orientation and surface wettability; however, we do not know what kinds of substrates geckos utilize in their natural habitats. Specifically, it is likely that geckos cling and locomote across rough, variable substrates, unlike the controlled man-made surfaces used here. Furthermore, what types of behavioral choices do they employ when faced with substrates that are wet? Perhaps geckos do not move when surfaces are wet. Additionally, we expect sprinting to be associated with only a small percentage of the gecko's total movement and may specifically be used only in prey capture, predator avoidance and conspecific interactions - all of which may only occur over a very short distance. Resounding interest in the gecko adhesive system over the last decade has pushed our knowledge of the system in terms of static adhesive performance. However, to clearly understand and utilize the system for bio-inspired design, specifically in designing a dynamic reusable underwater adhesive, we need to continue to investigate how the system is utilized under natural conditions, such as dynamic locomotion on various substrates and orientations in a variety of species that depend on the reliability of their adhesive system even in the most challenging of environments. Our work here highlights the astonishing resiliency of the gecko adhesive system when running on misted substrates, showing that a simple transition from walking to running may make all the difference on wet substrates.

MATERIALS AND METHODS

Animals

Geckos native to arid, sub-tropical and tropical environments were used: including, four *Pachydactylus bibronii* Smith 1846, five *Gekko gekko*

Linnaeus 1758, three *Tarentola mauritanica* Linnaeus 1758, three *Rhacodactylus auriculatus* Bavay 1869 and four *Phelsuma dubia* Boettger 1881 (Table 1). We chose these species because they are endemic to different environments where surface water may or may not be regularly encountered. We also attempted to sample across the phylogeny (Table 1) as suggested by Garland and Adolph (1994) for the consideration of small sample sizes. For instance, *P. dubia* and *P. bibronii* are closely related (Gamble et al., 2011), yet inhabit different environments, broadly ranging along the coast and northwest areas of Madagascar and also surrounding tropical islands for *P. dubia* and arid Southern Africa for *P. bibronii* (Zaaf and Van Damme, 2001; Van Heygen, 2004). We chose *G. gekko* to represent the Gekkonidae and *T. mauritanica* to represent the Phyllodactylidae phylogenetic branches. *G. gekko* is found in the tropics of Southeast Asia and *T. mauritanica* is found in sub-tropical regions of the Mediterranean (Zaaf and Van Damme, 2001; Gamble et al., 2011). Finally, *R. auriculatus* is a representative of the Diplodactylidae (Gamble et al., 2011) and resides in sub-tropical forests in New Caledonia (Bauer et al., 2012).

Experimental procedure

Geckos were housed individually and misted twice a day with water and fed cockroaches three times a week (Niewiarowski et al., 2008). A fruit supplement was also provided for *P. dubia* and *R. auriculatus*. Prior to experimentation, geckos were allowed at least 1 h to equilibrate to test temperature ($26.2 \pm 0.04^\circ\text{C}$) and humidity ($61.4 \pm 0.13\%$), and then chased by hand along a 1 m race track (Huey et al., 1989). Temperature and humidity were held constant across species to control for a complicated interaction between these parameters and adhesion (Niewiarowski et al., 2008). In addition, the response variable SV_{D-M} is independent of temperature and humidity when they are held constant as each gecko is compared against itself (i.e. difference of dry and misted runs). The race track was equipped with four sensors, placed at 25 cm intervals, yielding three split time measures in cm s^{-1} . The length of the track allowed us to test our hypothesis that after four steps, geckos would lose traction because all species needed more than four steps to complete the race and all races that were not completed were removed from analysis. The sprints were done in two orientations, vertical and horizontal, and on two substrates, acrylic (intermediately wetting) and glass (hydrophilic). For each orientation and substrate combination there were two assigned treatments. First, geckos were tested on dry substrates and second, the substrates were misted with a uniform mist of water prior to sprinting. We counted the number of wet toe pads at the completion of each race on misted substrates by immediately identifying all toes that had become gray in color and wet to the touch (see Stark et al., 2012). Total number of slips and stops along the race track were counted if they occurred when the gecko was running between the first and last sensor. A stop was defined as a loss of motion in all four feet, and a slip was defined as a failed step where the step was not fully weight bearing. To control for observer bias, we only had one behavior observer and we make the assumption that behaviors seen visually are obvious and have the same observer error across all treatments.

Geckos were raced no more than three times per day and were allowed at least 1 h rest between sprints. Race order and treatment type were randomly assigned. After running on a misted substrate geckos were not allowed to run any additional races for at least 1 day to ensure their toes were no longer wet and had regained their natural superhydrophobicity. Test surfaces were cleaned first with ethyl alcohol and then with water after each race. Species were tested at times appropriate to their natural behavior, where the diurnal day geckos (*P. dubia*) were tested during the day and all other geckos were tested at night using only a red light for researchers to observe the sprint. To induce the running response in *G. gekko*, we used a thin piece of medical tape to tape their mouths closed, increasing their likelihood of running. All procedures using live animals were approved by the University of Akron IACUC protocol 07-4G and are consistent with guidelines published by the Society for the Study of Amphibians and Reptiles (SSAR, 2004).

Statistical analysis

Each gecko was raced three times on each substrate (glass or acrylic), orientation (vertical or horizontal), and surface treatment (dry or misted with water). We used overall average sprint velocity (SV) for each

individual (averaged across three runs) in each treatment to estimate effects of the independent variables. Average sprint velocity was used, rather than maximum, because maximum sprint velocity estimates peak performance rather than overall performance, which we believe is more relevant for geckos running across wet substrates. Specifically, we expect particular parameters associated with water, especially toe pad wetting, to become more significant as the race progresses, similar to the finding that geckos initially cling equally well to dry and misted surfaces but after four steps the difference in adhesion is quite significant (Stark et al., 2012). This would not be captured in maximum sprint velocity measurements. In addition, average sprint velocity across a 1 m track directly relates to our observations of total number of stops, slips and wet toes after running the complete 1 m track. Differences in SV on dry and misted substrates were collapsed into a single dependent variable (SV_{D-M}) by subtracting the SV on dry substrate from SV on misted substrate for each individual in each treatment group.

To analyze the effect of substrate, orientation and species on SV_{D-M} for each individual, we used an ANOVA where SV_{D-M} was the response variable and substrate, orientation and species were the independent variables. Because *R. auriculatus* could not run vertically, they were removed and analyzed separately with SV_{D-M} as the response variable and substrate as the only independent variable. SV_{D-M} on dry and misted substrates were independently log transformed prior to statistical testing to meet the assumptions of the ANOVA. We did not control for body size, mass, toe pad area or individual, as each individual contributed equally to all treatments (matched-pairs analysis), therefore effectively serving as their own control across treatments. Furthermore, our response variable SV_{D-M} is the difference in performance on dry and misted surfaces, thus individual variation is accounted for in this variable (i.e. body mass, toe pad area etc. does not change across dry and misted surfaces).

When investigating frequency of stopping, we used an ANOVA to test the effect of running orientation (horizontal or vertical), substrate (glass or acrylic), treatment (dry or misted) and species on total number of stops. To investigate species-level differences in stops we used a Tukey HSD test to investigate all pairings. An ANOVA for slips was not necessary since almost all observed slips occurred in one treatment and in one species (see Results). We also tested for the effect of running orientation (horizontal or vertical) and substrate (glass or acrylic) on total number of wet toes after running across a misted substrate using an ANOVA. To investigate orientation and substrate differences in wet toes we used a Tukey HSD test to investigate all pairings. Total number of stops, slips and wet toes was calculated by summing all three runs for each individual.

Competing interests

The authors declare no competing or financial interests.

Author contributions

A.Y.S., J.O. and P.H.N. conceived and designed the study. A.Y.S., J.O. and A.K. performed the experiments. A.Y.S. and P.H.N. analyzed the data. A.Y.S., J.O. and P.H.N. wrote the manuscript.

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