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RESEARCH ARTICLE

The effect of surface water and wetting on gecko adhesion

Alyssa Y. Stark*, Timothy W. Sullivan and Peter H. Niewiarowski
The University of Akron, Integrated Bioscience Program, Akron, OH 44325-3908, USA
*Author for correspondence (ays3@zips.uakron.edu)

SUMMARY

Despite profound interest in the mechanics and performance of the gecko adhesive system, relatively few studies have focused on performance under conditions that are ecologically relevant to the natural habitats of geckos. Because geckos are likely to encounter surfaces that are wet, we used shear force adhesion measurements to examine the effect of surface water and toe pad wetting on the whole-animal performance of a tropical-dwelling gecko (*Gekko gecko*). To test the effect of surface wetting, we measured the shear adhesive force of geckos on three substrate conditions: dry glass, glass misted with water droplets and glass fully submerged in water. We also investigated the effect of wetting on the adhesive toe pad by soaking the toe pads prior to testing. Finally, we tested for repeatability of the adhesive system in each wetting condition by measuring shear adhesion after each step a gecko made under treatment conditions. Wetted toe pads had significantly lower shear adhesive force in all treatments (0.86±0.09 N) than the control (17.96±3.42 N), as did full immersion in water (0.44±0.03 N). Treatments with droplets of water distributed across the surface were more variable and did not differ from treatments where the surface was dry (4.72±1.59 N misted glass; 9.76±2.81 N dry glass), except after the gecko took multiple steps. These findings suggest that surface water and the wetting of a gecko's adhesive toe pads may have significant consequences for the ecology and behavior of geckos living in tropical environments.

Key words: gecko, adhesion, superhydrophobicity, wetting, van der Waals.

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INTRODUCTION

Geckos are common across a broad range of environments, including deserts and tropical forests. Though most species are suited for climbing on rocks and vegetation, many are also successful in urban environments, climbing smooth glass windows and rough walls of human dwellings (Niewiarowski et al., 2012). Although knowledge of the natural ecology and behavior of most gecko species is extremely limited, their geographic distribution suggests that adhesion can be maintained under a variety of conditions. Indeed, the diversity of environments that geckos can inhabit is likely due to the versatility of the gecko's van-der-Waals-based adhesive system, which takes advantage of weak intermolecular forces between two surfaces and is not surface specific (Autumn et al., 2002). Geckos achieve their strong attractive force to the substrate by utilizing small hair-like structures on their toes (Autumn et al., 2000). These structures, setae, are made primarily of β-keratin (Alibardi, 2003) and in many species the setae are a highly branched hierarchical structure (Maderson, 1964; Ruibal and Ernst, 1965; Williams and Peterson, 1982). Setae, whether branched or not, terminate in flexible flattened tips approximately 200 nm wide called spatulae (Ruibal and Ernst, 1965; Rizzo et al., 2006), which make intimate contact with rough and even dirty surfaces (Hansen and Autumn, 2005; Huber et al., 2007; Russell and Johnson, 2007). The van-der-Waals-based system is not without drawbacks, however; specifically, as a consequence of their intermolecular nature, van der Waals interactions are non-zero over only very small distances. Intimate contact between the spatulae and the substrate is required for van der Waals forces to be effective in the gecko adhesive system (Autumn et al., 2000; Gravish et al., 2008).

Although an extensive empirical and theory-based literature on the mechanics of gecko adhesion has developed over the last 10 years, much of the work has focused on idealized or highly controlled laboratory surfaces. Consequently, we know relatively little about how common environmental factors affect the adhesive capabilities of geckos moving in their natural habitats. Studies by Russell and Higham (Russell and Higham, 2009) and Russell and Johnson (Russell and Johnson, 2007) highlight this discrepancy, reporting that natural features such as substrate incline and surface roughness significantly impact the deployment and attachment of the gecko adhesive system. Furthermore, geckos that live in tropical environments may encounter additional variables such as wet surfaces and high or variable humidity, yet the way in which surface water and humidity affect the adhesion of geckos is still poorly understood. In the case of surface water, anecdotal observations suggest that geckos lose their grip when typical laboratory surfaces (e.g. glass or acrylic) are misted with water (Fig. 1). However, only three studies to date deal with surface water effects on gecko adhesion (Huber et al., 2005; Sun et al., 2005; Pesika et al., 2009), and they do not provide a simple explanation for this common observation. All three studies tested adhesive force normal to the substrate and found that adhesion significantly drops in water. However, Pesika et al. (Pesika et al., 2009) also tested the adhesive force of the system in the shear direction and found that frictional force was not affected by submersion in water across various loads. If normal adhesive force drops to almost zero when testing spatulae (Huber et al., 2005; Sun et al., 2005) and patches of the toe pad in water (Pesika et al., 2009), but frictional force is maintained at high loads in the setal patch (Pesika et al., 2009), then why do geckos lose their grip on wet surfaces (Fig. 1)? This question is not trivial when considering the natural habitat of many gecko species. Moreover, even when surfaces are not wet, the effect of variation in ambient humidity on gecko adhesion is incompletely understood. For example, as environmental humidity increases, whole-animal adhesion also increases, but the response is dependent on temperature (Niewiarowski et al., 2008). At relatively low temperatures, the response is strong and positive, but at high temperatures it is negligible. The effect of humidity on mechanical properties of individual setae may provide a partial answer: setae become softer in high humidity, which may provide more surface area for adhesive contact (Puthoff et al., 2010; Prowse et al., 2011). Although increased adhesive surface area may significantly contribute to increased adhesion in high humidity, it is still unclear why there is a complex interaction between temperature and humidity (Niewiarowski et al., 2008). Furthermore, unlike bird feather β-keratin (Taylor et al., 2004), gecko setae do not show distinguishable differences in stiffness until relative humidity surpasses 80% (Peattie et al., 2007; Huber et al., 2008; Prowse et al., 2011).

In an effort to expand our understanding of how water impacts gecko adhesion, we designed an experiment to test the shear adhesive strength of a tropical-dwelling gecko (*Gekko gecko*) on wet surfaces. Although geckos are a diverse group and can be found in multiple environments, those native to tropical environments likely encounter larger volumes of standing surface water and more frequent wetting of the substrates they utilize. Based on our observations shown in Fig. 1, we predict that at the whole-animal scale shear adhesion is compromised on wet surfaces, unlike results found using a setal patch at loads greater than 3 mN (Pesika et al., 2009). Although Pesika et al. (Pesika et al., 2009) tested frictional force under different applied loads, our intention here is to test performance of the system under the natural loading of the gecko. However, surface water itself is highly variable and we realize that in addition to the water drops shown in Fig. 1, geckos may also step on surfaces that are wetted enough to submerge an entire toe, leading to wetting of the adhesive pads themselves. This is shown in the inset of Fig. 1. Clear pools of water occur around and even under the toe pads, and the pads are wet to the touch. Thus our question becomes more complex; do geckos lose their adhesive grip in the shear direction in water and does wetting of the adhesive mats also have a negative impact on adhesion?

Our experiment tests multiple combinations of wetting on the adhesive system, keeping in mind natural environmental conditions and behavior of the geckos. Although it is unlikely that a gecko will fully submerge all four feet in water at the same time, we tested complete submersion in water to clarify how the system behaves when it is naturally loaded on a substrate that has water as an intervening medium. This treatment also directly tests the hypothesis that superhydrophobicity of the toe pads can sufficiently repel water at the interface [similar to results using a thin water layer (Hsu et al., 2012)] to allow for direct contact of the setae to the substrate, resulting in no difference in adhesive performance between wet and dry surfaces. We also tested wetted toe pads to measure the effect of wetting on adhesion. We know that the superhydrophobic nature of the toe pad can be lost after sustained contact with water (Pesika et al., 2009), but we do not know whether this change in wetting state is detrimental to adhesive performance. Wetting transition can be seen naturally on gecko toe pads, as shown in Fig. 1 (inset), where the gecko ran repeatedly up a wetted surface. We tested the hypothesis that toe pad wetting causes a drop in adhesion because infiltration of water into the mats affects multiple components of the system, such as surface chemistry, material property and van der Waals force. Finally, we tested the system in a more natural context by misting the substrate with water droplets, similar to wetting of natural substrates from rainfall. This treatment allowed us to test heterogeneous wetting of the surface, similar to what may actually occur under normal environmental conditions.

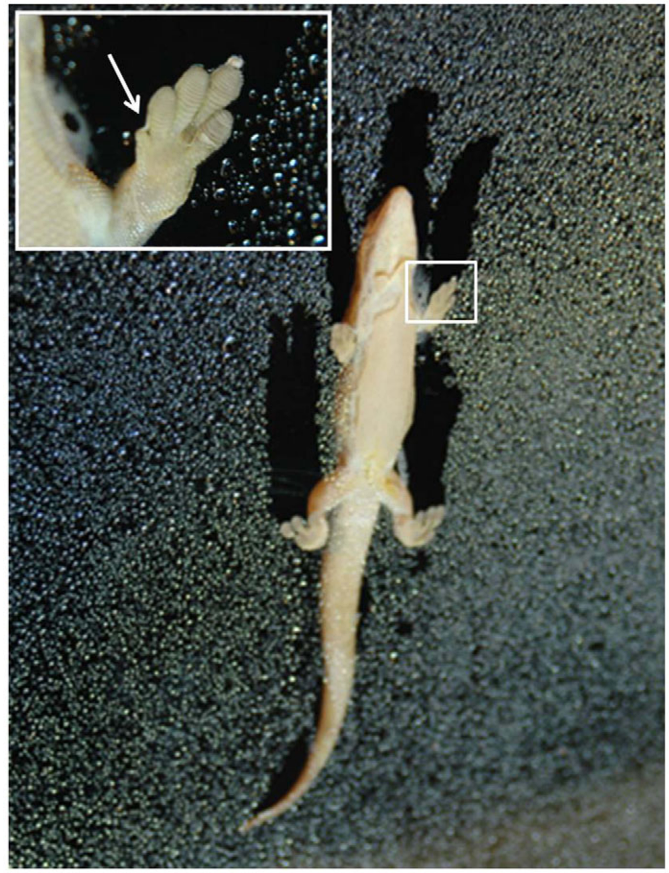

Fig. 1. A gecko (*Phelsuma dubia*) slides down a substrate misted with water in the shear direction. Inset is an image of the left forelimb and digits. Pools of water can be seen between the digits (arrow). Photo credit: E. A. Ramirez.

During these experiments we found that the pressure required to wet the toe pads could be achieved by the natural foot placement of a gecko and we began to consider stepping behavior as an important factor in wetting of the adhesive mats. One of the gecko's more touted achievements is its ability to self-clean dirt particles from its adhesive pads. This process occurs by repeatedly stepping on clean surfaces (Hansen and Autumn, 2005; Hu et al., 2012). We therefore controlled stepping for two reasons: first, to control for the level of wetting in each treatment such that all geckos were tested under the same conditions; and second, to test for a self-cleaning effect, which is supported by the low contact angle hysteresis of water droplets to the setal mat (Autumn and Hansen, 2006). Our experiment sheds light on a very important environmental condition that the gecko adhesive system encounters regularly yet has never been directly tested. Although little is known about the behavior of geckos in their natural environments, we would expect that if failure of the adhesive system is detrimental to the gecko, compensatory mechanisms should be favored by natural selection.

MATERIALS AND METHODS

Animals and experimental procedure

Seven adult Tokay geckos [*Gekko gecko* (Linnaeus 1758)] were used for trials. Geckos were housed individually in glass terrariums as outlined by Niewiarowski et al. (Niewiarowski et al., 2008). Geckos were fed crickets or cockroaches three times a week and misted with water three times a day. Temperature and relative humidity were maintained at 26.8°C and 40.0%, respectively. Toe pad area was measured using a flatbed scanner (Hewlett-Packard, Palo Alto, CA, USA) and images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Prior to experimentation, geckos were kept individually in plastic bins with screen lids inside a walk-in environmental chamber for at least 1 h to acclimate to experimental conditions. Temperature and relative humidity were maintained at 23.8±0.2°C and 35.7±0.4%, respectively, during the acclimation period and trials. Animals were weighed after experimental trials. All procedures involving live animals were consistent with guidelines published by the Society for the Study of Amphibians and Reptiles (SSAR 2004) and were approved by the University of Akron IACUC protocol 07-4G.

Shear forces were measured using a force rig similar to that described by Niewiarowski et al. (Niewiarowski et al., 2008), except in this experiment the rig was positioned horizontally. Shear adhesive force is defined here as the force generated by a gecko that has naturally loaded its adhesive system and is subsequently pulled across a substrate in the shear direction. A glass plate was used as a substrate and was mounted with Velcro (Manchester, NH, USA) inside a plastic Rubbermaid container (Atlanta, GA, USA). A small hole was drilled in one side of the container to allow small pulling harnesses to attach to the force rig and the gecko. After removing the gecko from the plastic container, harnesses were attached around the pelvis, ventrally and dorsally, so that the gecko could be pulled in a shear direction across the glass substrate. A third harness was placed around the thorax for positioning the gecko on the substrate. Geckos were lowered onto the glass suspended by the harnesses, which allowed for proper positioning of their feet. The gecko was then lightly restrained by hand to prevent it from taking any steps prior to data collection. After the initial value was measured, the gecko was made to move one foot at a time to measure individual step values. These methods were used for all treatment groups. Step values (0–4 steps) were collected randomly, as was application of treatment group.

Experimental treatments

Toe pad wetting condition

Shear forces were measured under two different toe pad wetting treatments: dry and soaked. Dry treatments did not involve wetting of the toe pads and geckos were placed directly onto the substrate after the acclimation period was complete. In soaked toe pad treatments, a cloth was first wetted with water inside the plastic bin. Each gecko was made to step and walk on the wetted cloth until the toe pads were visibly wetted (a change in lamellar color from white to gray; see Fig. 2). The cloth was then removed and the gecko was positioned in the bin and water was added until all toes were submerged. The head of each gecko was held out of the water using a foam neoprene pad and a wetted cloth was placed on top of the gecko to prevent excess movement during the soaking period. Geckos were maintained in this position for at least 90 min (92±1 min), consistent with the acclimation period used by Pesika et al. (Pesika et al., 2009) on setal patches. Water temperature was maintained at 25.0±0.3°C during the soak period.

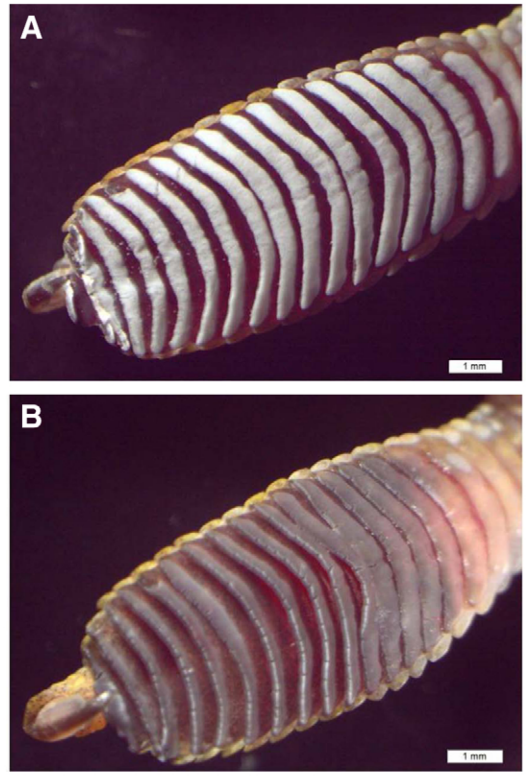

Fig. 2. Images of (A) dry and (B) wet setae using the same digit of an individual tokay gecko (*Gekko gecko*). The image in A was taken prior to exposure to water and the image in B was taken after 30 min of soaking in water.

Substrate wetting condition

Each toe pad treatment group (dry or soaked) was also tested on three different substrate conditions: submerged, misted and dry. For submerged trials, the container was filled with sufficient water to cover the glass plate, approximately 0.5 cm, fully submerging the gecko's feet. Shear force was then measured as described above. In misted trials, the glass was sprayed with a fine mist of water immediately before placing the gecko on the glass substrate and before each step. Dry trials were conducted in the same way as the other treatments, but no water was applied to the glass substrate. The glass plate was cleaned with ethanol between trials, except in the submerged treatments.

Each gecko was tested under six combinations of substrate and toe pad treatment. Treatments are described and abbreviated in Table 1. We also collected a complete data set of force values for each gecko in five step conditions. Step conditions ranged from initial or no steps (Step 0) to four steps, which was the complete replacement of all four feet by the gecko.

Table 1. Six treatment groups were tested that combined toe pad wetting condition and substrate wetting condition

Toe pad condition	Substrate condition	Abbreviation
Dry	Dry	DD (control)
Dry	Misted with water droplets	DM
Dry	Wet (fully submerged in water)	DW
Soaked (fully wetted)	Dry	SD
Soaked (fully wetted)	Misted with water droplets	SM
Soaked (fully wetted)	Wet (fully submerged in water)	SW

Table 2. Sex, mass and total toe pad area for each of seven Tokay geckos (*Gekko gecko*) used for experimental trials

Animal ID	Sex	Mass (g)	Toe pad area (cm^2)
T2	Male	78.6±1.7	5.57
T8	Male	117.9±0.5	4.94
T10	Male	103.9±1.0	5.98
T12	Female	89.5±0.4	5.11
T13	Female	72.5±0.6	5.50
T14	Female	78.0±0.8	5.13
T18	Male	109.9±2.0	7.45

Toe pad area was measured using scanned images of toes in contact with a surface. Mass is averaged over all trials (mean ±1 s.e.m.).

Statistical analysis

Three specific treatment groups of interest were compared with the control (DD; dry toe pads on dry glass). To test for the effect of surface water on shear adhesion we used a matched pairs test between the control and geckos with dry toe pads positioned onto glass that was fully submerged in water (DW). This comparison was made using force values from the fourth step, as shear force values from the fourth step best represent the condition where the gecko has voluntarily placed each of its feet on the substrate. Also using fourth step values we tested for an effect of soaking or wetting of the toe pads on shear adhesive force to a dry substrate using a matched pairs test between the control and the soaked toe pad treatment group tested on dry glass (SD). Finally, we compared our whole-animal results with the setal patch results by Pesika et al. (Pesika et al., 2009) using a matched pairs test between the control and the soaked toe pad tested on the fully wetted glass substrate (SW) in the initial cling, prior to the gecko taking full steps on the substrate. We used a matched pairs analysis to control for individual variations, such as toe pad area, because each individual serves as its own control across all treatments and steps. In other words, variation in toe pad size among individuals does not affect the statistical analysis of the treatment response because each gecko was exposed to all treatments. Individual variations in sex, toe pad area and body mass are reported in Table 2 for reference. The effect of step order on each treatment group was analyzed using a repeated-measures multivariate ANOVA (MANOVA). Data are reported as means ± 1 s.e.m.

RESULTS

The mean toe pad area for all seven individuals was 5.67±0.33 cm^2 and the mean mass over experimental trials ranged from 72.5±0.6 to 117.9±0.5 g depending on the animal (see Table 2). We found that the shear adhesive force generated by the DW treatment (0.40±0.04 N) was significantly lower (t=–5.15, d.f.=6, P=0.0021) than that of the control (DD; 17.96±3.42 N) after four steps had been taken. When comparing the shear adhesive force of the SD treatment (1.31±0.12 N) with that of the control, we found that the SD treatment was also significantly lower in the fourth step (t=–4.92, d.f.=6, P=0.0027). Finally, we found that the shear adhesive force generated by the SW treatment (0.43±0.07 N) was significantly lower than the force generated in the control treatment (9.76±2.81 N) under initial step conditions (t=–3.36, d.f.=6, P=0.0152).

When comparing across steps there was a significant difference in shear adhesive force across treatment ($F_{5,36}$=16.87, P<0.0001). This difference was driven by an interaction between step and treatment ($F_{20,110.4}$=2.37, P=0.0023; Table 3). We found that the shear adhesive force for the control treatment increased across Steps 0–4 whereas all other treatments did not differ significantly across steps. Although the control treatment produced higher force values than all other treatments, we chose to investigate the DM treatment further because this treatment was more variable and reached force values closer to the control than all others. Using a matched pairs analysis we found that the mean shear forces in the initial step of the control and DM treatments were not statistically different (t=–1.41, d.f.=6, P=0.2088). This was also true for the second step (t=–1.39, d.f.=6, P=0.2133) and the third step (t=–2.39, d.f.=6, P=0.0543), although for the third step the differences were nearly significant. In all other steps the DM treatment produced significantly lower shear adhesive forces when compared with the control (Fig. 3).

DISCUSSION

The current diversity of geckos is likely attributable to their ancient origins and multiple dispersal events that have occurred over millions of years (Gamble et al., 2011). Of the more than 1000 species of gecko (Han et al., 2004), many adhesive toe pad-bearing species live in tropical environments where surface water from rainfall and high levels of humidity are characteristic of the environmental conditions. Our results suggest that in at least one tropical-dwelling species (*G. gecko*), water can significantly impact the performance of the adhesive system. Although many species of adhesive pad-bearing geckos live in tropical environments that are periodically exposed to substantial rainfall, it is surprising that such a significant drop in adhesive performance was observed under our experimental conditions. We tested for an effect of water between the toe pads and the substrate by submerging the feet of live geckos underwater and measuring overall adhesion in the shear direction. We also measured shear adhesion after the toe pads had been wetted for 90 min, similar to previous experiments using a setal patch. Finally, we tested the system on misted glass, which mimics natural environmental conditions. In each treatment we controlled the stepping behavior of the gecko to minimize extraneous wetting and to test for changes in adhesive performance over steps.

We found that altering the wetting condition of the surface a gecko adheres to impacts the adhesive performance of the system. Trials where the surface was fully wetted and the toes were submerged underwater resulted in significantly lower shear adhesive force values (mean of DW and SW treatments 0.44±0.03 N) than those of the control (17.96±3.42 N) after the gecko took four complete steps. Our tests show that although a thin water layer can be expelled by the setal mat (Hsu et al., 2012), a thick layer of water (~0.5 cm) cannot be sufficiently displaced by toe pads and significantly compromises adhesive performance. Although the adhesive system was not

Table 3. Difference in shear adhesive force across treatments

	Wilks' lambda	Exact *F*	Numerator d.f.	Denominator d.f.	*P*
Treatment	2.343	16.868	5	36	<0.0001***
Steps	0.228	1.883	4	33	0.1368
Treatment × Steps	0.305	2.375	20	110.4	0.0023**

The MANOVA table shows a significant difference (***P*<0.01, ****P*<0.001) in shear adhesive force across treatments when comparing step value. This difference is due to the interaction between steps and treatment. The *F*-statistic is from the Wilks' lambda test.

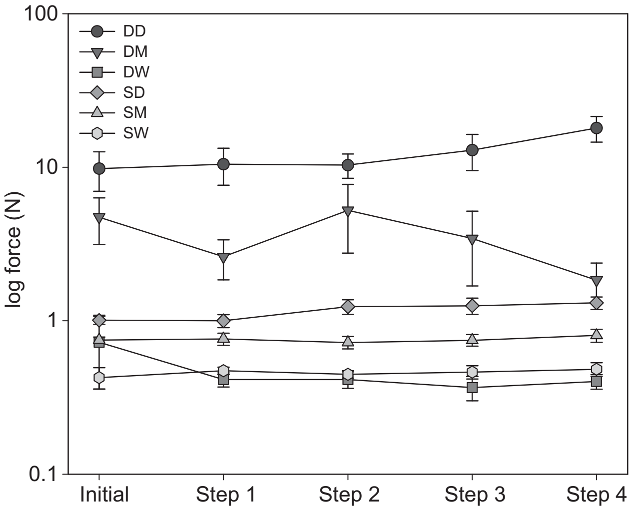

Fig. 3. Shear force of whole-animal (*Gekko gecko*) adhesion under six different treatment groups and across 0–4 steps. Treatment groups with dry, non-wetted toe pads include the control (DD; dry toe pads tested on a dry glass substrate), dry toe pads on a glass substrate misted with water (DM) and dry toe pads tested on a fully submerged glass substrate (DW). Three treatment groups with soaked or wetted toe pads are also shown. These include soaked toe pads tested on a dry glass substrate (SD), soaked toe pads on a misted glass substrate (SM) and soaked toe pads tested on a glass substrate fully submerged under water (SW). One step signifies the replacement of a foot by the animal where initial (Step 0) is the force measurement of a gecko without allowing it to take any natural steps and Step 4 is the force measurement of a gecko that has replaced all four of its feet using its natural stepping behavior. Symbols and error bars are means ±1 s.e.m.

maintained when submerged in water, we did observe maintenance of the superhydrophobic nature of the toe pads (Autumn and Hansen, 2006). When geckos with dry (superhydrophobic) toe pads were placed in water, cohesive surface tension caused the water to bend around the toes rather than rush over the top of the foot on initial contact. When the toe pads were fully wetted we did not observe this phenomenon and water tended to cover the feet immediately after being submerged. We also observed a clear silvery plastron when dry gecko toes were pressed into the water. This suggests that a wetting transition, known as a Cassie–Wenzel transition (Wenzel, 1936), had not occurred and the toe pads where still in the superhydrophobic Cassie regime (Cassie, 1948), even underwater (Lei et al., 2010; Poetes et al., 2010). Finally, in many cases, despite multiple steps underwater on the glass surface, parts of the overall system remained dry and superhydrophobic when the animal was examined after completing the trial. It is unclear what caused a wetting transition in particular feet, toes and even patches of the toe pad but not others. A clear example of this transition is shown in Fig. 4. In Fig. 4A the gecko toe pad is superhydrophobic and is in the Cassie wetting regime, evident by the high contact angle the water drop makes with the surface of the toe pad. In Fig. 4B, the toe pad is no longer superhydrophobic and has transitioned to the Wenzel wetting regime, as shown by the spreading behavior of a water droplet placed on the toe pad. Transition occurred after soaking the toe in water for 30 min, suggesting that prolonged exposure to water causes a wetting transition. Pesika et al. (Pesika et al., 2009) found similar results with the setal patch. Although superhydrophobic areas of the toe pad were maintained in many of our experimental trials, often after multiple steps, we found that excessive water around and under the toes compromises overall adhesive performance, likely because excess water cannot be sufficiently expelled to allow the close interfacial contact required for adhesion.

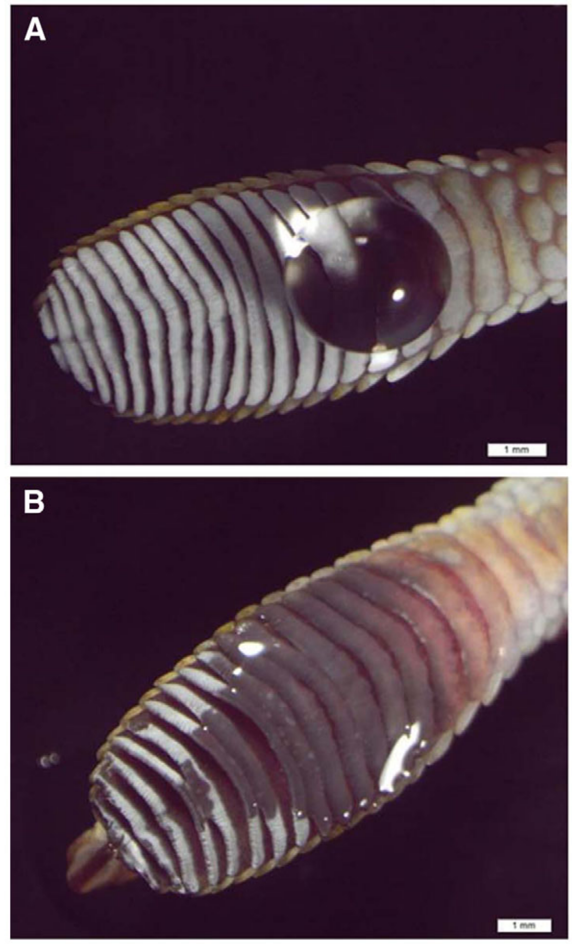

Fig. 4. Two wetting regimes of the gecko (*Gekko gecko*) toe pad. (A) The toe pad is in the Cassie, non-wetting regime and the toe pad is superhydrophobic, as shown by the spherical water droplet suspended on top of the setal mats. (B) Transition into the Wenzel wetting regime, where a droplet of water readily spreads across and into the setal mats. Transition was induced by soaking the toe pad in water for 30 min.

By forcing a wetting transition in the setal mats, we also observed a significant drop in adhesion across steps and treatments when compared with the control treatment carried out with non-wetted (Cassie regime) setal mats. In the fourth step, mean shear adhesive force of all three wetted treatments (SD, SM and SW) did not differ and was significantly lower (mean of three treatments 0.86±0.09 N) than the control (17.96±3.42 N). There may be several reasons for this finding. Wetting of the toe pads forced a transition that compromised the innate superhydrophobicity of the adhesive setal mats (Pesika et al., 2009), consequently allowing water to fill the mats. Although we let the toe pads drip-dry after the soaking treatment, we often observed small pools of water on the glass substrate after the gecko had taken a step. In many cases the small pools of water clearly represented the lamellar area by mimicking the macroscopic patterning of the toe pad. This is not surprising as the increased surface area of the hierarchically structured setal mats likely resulted in the entrapment of water in the lamellar structures. Although the forceful pressing of the natural foot step expelled some of the water held in the pads into the characteristic toe pad pattern on the glass, the pools of water created by this process likely disrupted the van-der-Waals-based adhesive system. Repetition of this observation suggests that although water is released from the toe pads by the natural loading of the system on a hydrophilic glass surface, it may take more than a few steps to fully expel all the water that has transitioned into the mat.

Another significant influence on adhesion in the soaking treatments (SD, SM and SW) is the softening of the setal material. Recent findings show that the modulus of a single seta decreases from 3.7±0.1 to 2.13±0.2 GPa when environmental humidity is increased from 30 to 80% relative humidity (Prowse et al., 2011). Setae that are hydrated in water are likely to also decrease in modulus, perhaps even more than in high humidity environments. The gecko adhesive system is also extremely directionally dependent: setae must be oriented and loaded correctly for successful adhesion (Autumn et al., 2000; Autumn and Hansen, 2006; Hill et al., 2011). A significant drop in setal modulus from extreme hydration likely increases disorder in the setal mat and causes setae to self-tangle and buckle more readily, thus lowering adhesive contact area and performance. Interestingly, experiments using a patch of the setal mat did not show an effect of wetting on adhesive performance. Pesika et al. (Pesika et al., 2009) held and tested patches of the setal mat in water and compared the frictional force of these treatments with those from a patch that was held and tested in dry N_2 atmosphere. Their results show that the frictional force of a setal patch is insensitive to environmental conditions when tested under high loads (>3 mN), which compress the setal mat to almost half its initial height. Although the natural loading of the gecko is not well understood, by replicating the wetting procedures of Pesika et al. (Pesika et al., 2009) in the 90 min SW treatment, we find that testing the system at the whole-organism scale provides significantly different results. This disparity further highlights the importance of assessing the system at the whole-animal level. Although it is difficult to clarify why the system behaves differently at each organizational scale, it is clear that the loading of the system by the animal does not sufficiently negate the effect of water on adhesive performance.

We hypothesized that the high contact angle hysteresis of the dry toe pad would expel droplets of water and allow a dry interface for adhesion, similar to results from experiments using a thin water layer (Hsu et al., 2012) and self-cleaning experiments (Hansen and Autumn, 2005; Hu et al., 2012). In support of this, force measurements from geckos with dry toe pads tested on misted glass (DM) were closer to the control treatment values than all other treatments. When investigating individual steps we found that there was not a significant difference in shear adhesive force of the DM group when compared with the control after initial placement onto the glass (4.72±1.59 N misted glass; 9.76±2.81 N dry glass). However, after four steps, force values were significantly lower than the control group (1.84±0.54 N misted glass; 17.96±3.42 N dry glass). This variability may be due to uncontrolled wetting of the toe pads and the surface. We observed heterogeneous wetting of the toe pads after the DM treatment, similar to the fully submerged treatment (DW). Likewise, small pools of water developed on the surface after the gecko stepped on misted glass, similar to the wetted treatment (SD). These observations suggest that the toe pads and the surface were likely wetting in the process of stepping on misted glass, finally resulting in lower overall shear adhesion after multiple steps. Heterogeneous wetting of the toe pads and the surface may also explain why the adhesion values in this treatment group were more variable than the other treatments. For instance, shear adhesive force after one step (2.60±0.76 N) was significantly lower than that in the control group (10.45±2.82 N; t=–2.81, d.f.=6, P=0.0309), whereas values for two steps were not (5.23±2.48 N treatment, 10.31±1.86 N control; P=0.2133). Interestingly, this treatment is most similar to what we would expect to occur naturally. Rainfall likely initially wets surfaces in the gecko's environment with droplets, and geckos, having not been exposed to water prior to the rainfall, have toe pads that are dry and superhydrophobic.

Depending on the frequency of rainfall events and the natural behavior of the gecko, our results suggest that the system has a limited capacity to withstand environmental water. For example, if we assume that a 100 g tokay gecko (approximately the average size used in this experiment) requires 1 N of force to support its body mass, then shear force measurements on misted glass are sufficient to support the gecko's mass for up to four steps (1.84±0.54 N). Force values for the soaked toe pad on dry glass (SD) were near this critical level; however, all other treatment groups fell below 1 N of shear force, providing insufficient force to support the gecko's body mass. Although the water treatments described here significantly impact adhesive performance when compared with the control, the over-built design of the gecko adhesive system may play a role in the maintenance of the system in water. Geckos use only approximately 0.04% of their theoretical adhesive capability (Autumn, 2006) and although a 100 g gecko produces ~20 N of shear adhesive force, much more than the 1 N required to maintain its body mass, when walking on misted glass with dry toe pads (DM) or with wet toe pads on dry glass (SD) shear adhesive force quickly drops into range with the minimum force required to support body mass. There are many explanations for the evolutionary selection of the over-built design of the gecko adhesive system and our results suggest that compromised adhesion, either as a result of surface water or wetting, may be another crucial selective factor in the evolution of the system.

When we looked at shear adhesive force across steps, we found that the control treatment positively increased from zero to four steps. This supports the directional dependence of the system, where geckos that were not allowed to naturally replace their feet had lower shear adhesive force than those that correctly aligned and placed each of their feet. In contrast we found that natural replacement did not significantly increase shear adhesive force in the treatment groups. These results contradict the innate dry self-cleaning property of the toe pads (Hansen and Autumn, 2005; Hu et al., 2012). The adhesive toe pads can effectively displace dry particulate simply by maintaining a lower attractive force than the surface (Hansen and Autumn, 2005), and this self-cleaning property is magnified when the geckos are allowed to perform their natural stick-peel behavior (Hu et al., 2012). Unlike adhesion tests where a dry fouling agent was used, we found that toe pads do not regain full adhesive performance after repeated use when water was used as the fouling agent.

Our results have important implications for our understanding of the behavior and ecology of tropical-dwelling geckos. For instance, we found that stepping or walking on a surface that has been misted with water (simulated rain droplets) eventually leads to wetting of the toe pads and a significant loss in adhesive performance. This finding suggests that during and even after a rainfall event, tropical-dwelling geckos should avoid walking on exposed surfaces. Few studies have reported the activity patterns of geckos in rainstorms or after significant rainfall events and, of these, the findings are not clear. Marcellini (Marcellini, 1971) found that significantly fewer geckos were active during rainfall events compared with dry conditions; however, Werner (Werner, 1990) found qualitatively no difference in gecko activity during rainstorms. These results may be confounded by other factors such as wind and temperature (Marcellini, 1971); however, the limitations of the system shown in the present study provides additional information. The loss in adhesion due to surface water and wetting may actually render geckos unable to move from a particular location during rain events or could elicit behaviors that have yet to be characterized. Although water significantly impacts the adhesive system, we found that the system was not compromised immediately, so perhaps distance from a dry shelter is a more important ecological requirement for geckos that live in the tropics than restriction of movement. Clearly, further investigation is necessary to understand how water from rainfall impacts gecko activity patterns and behavior. Another interesting consideration is the longevity of this effect. Pesika et al. (Pesika et al., 2009) found that after 20 min of sustained contact with water the setal patch was unable to regain its natural water repellency for more than 48 h. If this were the case with the whole animal, wetting of the toe pads could render the adhesive system useless for days. Preliminary observations from the whole animal suggest that this time scale is not consistent when compared with experiments using a small patch of the setal mat and that the whole-animal adhesive system recovers much faster (A.Y.S. and T.W.S., unpublished). Recovery of the adhesive system may also be dependent on the behavior of the animal. Although active grooming of the toe pads has never been documented, geckos may preferentially search out substrates that are anti-wetting or absorptive to either avoid toe pad wetting and surface water or actively recover their adhesive system after it is compromised. High levels of rainfall and humidity are environmental characteristics that describe tropical habitats and although water either on the surface or within the setal mat negatively impacts performance of the system, humidity has been shown to significantly increase performance under certain conditions (Huber et al., 2005; Sun et al., 2005; Niewiarowski et al., 2008; Pesika et al., 2009; Puthoff et al., 2010). Based on these results, it seems that a balance between environmental humidity and environmental water must be maintained by the adhesive system of geckos living in the tropics. Clearly, further investigation of the impact of water on the gecko adhesive system requires detailed observations of geckos in their natural environment. For example, geckos do not often cling to glass, as it rarely exists in their natural habitat, nor do they take the carefully placed steps we required in our study; rather, they run in short bursts across multiple surface types in a constantly changing environment. Further investigation into natural locomotor patterns, substrates and environmental conditions are necessary to begin to clarify the versatility of the gecko adhesive system as a whole.

The results of our experiment indicate that surface water and wetting of the adhesive toe pad significantly impacts the performance of the gecko adhesive system. Whole-animal measurements of adhesive force in the shear direction are significantly lower on wet surfaces and when the adhesive toe pads are wetted than control values. Our results do not support similar measurements using a setal patch. Unlike the dry self-cleaning properties of the adhesive system, we found that repeated use in water does not aid in the recovery of performance. Finally, although the system was compromised in all treatments, droplets of water did not immediately affect the adhesive system; this may provide insight into potential behavioral and ecological mechanisms which can circumvent complete loss of performance. Our findings also have particular relevance for bio-inspired design of synthetic dry adhesives that are not only easily reversible, like the gecko's foot, but also water resistant.

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