THE EFFECT OF WATER ON THE GECKO ADHESIVE SYSTEM

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THE EFFECT OF WATER ON THE GECKO ADHESIVE SYSTEM

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The gecko adhesive system is a dry, reversible adhesive that is virtually surface-insensitive due to the utilization of intermolecular van der Waals forces. Remarkably, although detailed models of the adhesive mechanism exist and hundreds of gecko-inspired synthetics have been fabricated, our ability to fully replicate the system still falls short. One reason for this is our limited understanding of how the system performs in natural environments. To begin to resolve this I focused on one particular environmental parameter, water. Although thin layers of water can disrupt van der Waals forces, I hypothesized that geckos are able to retain or regain adhesive function on wet surfaces. I was motivated to investigate this hypothesis because many species of gecko are native to the tropics, a climate where we expect surface water to be prevalent, thus it is likely geckos have some mechanism to overcome the challenges associated with surface water and wetting.

Despite the challenge water should pose to adhesion, I found that when tested on hydrophobic substrates geckos cling equally well in air and water. Conversely, on wet hydrophilic substrates geckos cannot support their body weight. Investigating these results further, I found that the superhydrophobic nature of the adhesive toe pads allows geckos to form an air bubble around their foot, which when pressed into contact with a hydrophobic substrate likely removes water from the adhesive interface. When
the toe pads are no longer superhydrophobic however, geckos cannot support their body weight and fall from substrates. In order to regain adhesion geckos only need to take about ten steps on a dry substrate to self-dry their toe pads. Finally, when measuring a dynamic component of adhesion, running, we found that geckos are able to maintain speed on misted hydrophobic and hydrophilic substrates, contrary to what we would predict based on static shear adhesion measurements.

In conclusion, my research provides a detailed investigation of how water affects the gecko adhesive system and has applications for synthetic design of adhesives which retain or regain function in water and further motivates the study of this remarkable system in a more environmentally relevant context.
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CHAPTER I
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 in 2000 a landmark paper by Autumn and colleagues (Autumn et al., 2000) provided the next step in gecko research: the link between morphology and adhesive performance. In this paper Autumn et al. (2000) measured the forces produced by a single seta, a hair-like structure that is found on the bottom of the flattened toe pads and some distal tail regions of many species of gecko and other lizards (Autumn et al., 2000; Bauer, 1998; Williams and Peterson, 1982). Two years later Autumn and colleagues confirmed that adhesion was due to weak intermolecular van der Waals forces that occur between the tops of the setae, the spatula, and the substrate a gecko clings to (Autumn et al., 2002). Autumn and colleagues’ seminal work focused the attention of biologists and materials scientists on these small adhesive units, the latter mimicking their shape and hierarchical structure, modulus, dimensions and other morphological features in the quest for creating the next best synthetic gecko tape (Bartlett et al., 2012; Ge et al., 2007; Jeong et al., 2009; Kim et al., 2009b; Murphy et al., 2009b; Northen et al., 2008; Parness et al., 2009; Yurdumakan et al., 2005). While
much of this work has been highly successful, this one dimensional approach is not without limitations. For example, most of the 1,400+ species of gecko (Gamble et al., 2012; Han et al., 2004) have been neglected in the literature and rather only a few select species are studied for the purpose of understanding generalized gecko adhesion. The result of this approach is that important ecological parameters and evolutionary transitions are lost. Gecko habitat ranges from wet tropical environments to dry arid deserts. In fact, the gecko adhesive system has evolved multiple times (Gamble et al., 2012), likely in relation to environment and habitat use (Lamb and Bauer, 2006). Despite this wide diversity, very few studies focus on testing differences in adhesive performance across environment, phylogeny or morphological characteristics.

In my work I focus on one environmental parameter which may have a significant impact on adhesion: water. In species native to wet, tropical habitats surface water would likely be common. For instance, high levels of humidity and frequent rain will likely leave the substrates they utilize wet. For organisms that use capillary adhesion, like many insects and tree frogs, thin layers of water can actually aid adhesion. In geckos however, the dry van der Waals-based adhesive system should be significantly compromised in water because van der Waals is only effective when two surfaces come into very close contact. This close contact would almost certainly be interrupted even by thin water layers. So how do geckos from the tropics maintain functionality of their adhesive system in the presence of water? Because little work has been done to investigate gecko adhesion in environmentally relevant conditions, there are only a few interesting clues to follow. First, the gecko skin and moreover, the
adhesive toe pads, are hydrophobic. In fact, the adhesive toe pads are superhydrophobic, where a water droplet beads up on the toe pad at an angle (contact angle, $\theta$) above 150° (Autumn and Hansen, 2006). In addition, the superhydrophobic toe pads also have a very low contact angle hysteresis (2-3°), meaning water droplets roll off the toe pad at very low angles (Autumn and Hansen, 2006). Second, although we know that the toe pads are superhydrophobic, they can become wetted, a transition known as a Cassie-Baxter to Wenzel wetting transition (Cassie, 1948; Wenzel, 1936). This means that the superhydrophobic state is not thermodynamically the most favorable state, and instead the Wenzel state, where water floods the toe pad, is more favorable. This transition is not easy to achieve however, and can take several minutes or significant agitation to force the toe pad to wet. Third, using surface sensitive spectroscopy we found that thin water layers are not detected at the contact interface between a gecko toe and the substrate (Hsu et al., 2012). This would suggest that van der Waals adhesion can still be achieved, as there are no water layers to disrupt the close spatular-substrate contact interface.

Previous studies have had little success measuring adhesion on wet substrates (Huber et al., 2005b; Pesika et al., 2009; Sun et al., 2005), despite several clues that geckos may have the ability to remove water from the adhesive interface or keep from becoming wet. In an effort to understand adhesion on wet substrates we designed an experiment to broadly investigate adhesive performance in a variety of contexts. Chapter II investigates adhesion of Tokay geckos (Gekko gecko), a species native to the tropics, in a variety of conditions. First we tested surface water in three contexts: no
water (dry), misted water (water droplets simulating rain) and fully submerged. We also tested geckos with superhydrophobic toe pads (dry, in the Cassie-Baxter state) and those that were fully wetted (Wenzel state). Finally, using literature on the self-cleaning of dirt particles from gecko toe pads, we measured adhesion after each step a gecko took, from zero to four steps. This study was pivotal in that it provided results that were not expected for a tropical species and also provided several testable hypotheses. For instance, in Chapter III we investigate the effect of substrate wettability on underwater adhesion, a variable that is rarely considered in gecko adhesion studies but may be highly relevant in their natural environment. Next, in Chapter IV we directly test the role of superhydrophobicity on adhesion based on observations from Chapter III. Specifically, we measure adhesion on surfaces with different wettability in surfactant solutions, which should remove the ability of the adhesive toe pads to form a protective air layer (plastron). In addition, this study also begins to investigate the role of setal surface chemistry on adhesion in water (see Conclusion for more details). In Chapter V we investigate how geckos can regain adhesion when their superhydrophobicity is lost via a Cassie-Baxter to Wenzel wetting transition. A state we found in Chapter II and Chapter IV to virtually remove all adhesive function. Finally, in Chapter VI we take a broader approach to understanding how geckos use their adhesive system. In this study we measured locomotor performance (sprint speed), rather than adhesion, on wet and dry substrates, expecting geckos to reduce speed in contexts where we found reduced static shear adhesion. In addition to taking into account this more dynamic performance variable, we also measured several behavioral traits of running. This study also is the
first in this body of work to test multiple species which were chosen based on their native environment and phylgenetic relationships. By diversifying these parameters (environment and species) we hoped to identify trends that may be related to particular environmental adaptations. Chapter VI circles back to the motivation driving all the experiments, which is to investigate gecko adhesion in a more environmentally relevant context by exploring the variability of performance in the presence of surface water. Our results are relevant not only to biology but also to the quest to create a better, more robust adhesive, one which may be stable and reusable in wet conditions, a feat many of our synthetic products fail to achieve.
CHAPTER II
THE EFFECT OF SURFACE WATER AND WETTING ON GECKO ADHESION

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Summary

Despite profound interest in the mechanics and performance of the gecko adhesive system, relatively few studies focus 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 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.

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 200nm wide called spatula (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 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 developed over the last ten years, much of the work 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 (2009) and Russell and Johnson (2007) highlight this discrepancy, reporting that natural features like 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. 2.1). However, only three studies to date deal with surface water effects on gecko adhesion (Huber et al., 2005b; Sun et al., 2005; Pesika et al.,
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. (2009) also tested 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 spatula (Huber et al., 2005b; 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. 2.1)? This question is not trivial when considering the natural habitat of many gecko species. Moreover, even when surfaces are not wet, the effect on gecko adhesion due to variation in ambient humidity is also 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).

Figure 2.1. 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).

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. While 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 in Figure 2.1 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 3mN (Pesika et al., 2009). While 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. Surface water itself is highly
variable however and we realize that in addition to the water drops shown in Figure 2.1
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 Figure
2.1. Clear pools of water occur around and even under the toe pads and the pads are
wet to the touch. By observing this 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 system, keeping in
mind natural environmental conditions and behavior of the geckos. Although it is
unlikely 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 if this change in wetting state is detrimental to adhesive performance. Wetting transition can be seen naturally on gecko toe pads, as shown in Figure 2.1 (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.

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. Part 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

Seven adult Tokay geckos (*Gekko gecko* L.) were used for trials. Geckos were
housed individually in glass terrariums as outlined by 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 and images were
analyzed using ImageJ® software. Prior to experimentation geckos were kept
individually in plastic bins with screen lids inside a walk-in environmental chamber for at
least one hour to acclimate to experimental conditions. Temperature and relative
humidity were maintained at 23.8 ± 0.2°C and 35.7 ± 0.4% 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. (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 value (0-4 steps) was collected
randomly, as was application of treatment group.

Experimental Treatments

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. 2.2). 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 minutes (92 ± 1 min), consistent with the acclimation period used by Pesika et al. (2009) on setal patches. Water was 25.0 ± 0.3°C during the soak period.

Figure 2.2. Images of (A) dry and (B) wet setae using the same digit of one individual tokay gecko (Gekko gecko). (A) was taken prior to exposure to water and (B) was taken after 30 minutes of soaking in water. Scale bar is 1 mm.

Each toepad 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, about 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 however 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 2.1. 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 2.1. Six treatment groups were tested which combined toe pad
wetting condition and substrate wetting condition.

<table>
<thead>
<tr>
<th>Toe pad condition</th>
<th>Substrate condition</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>Dry</td>
<td>DD (control)</td>
</tr>
<tr>
<td>Dry</td>
<td>Misted with water droplets</td>
<td>DM</td>
</tr>
<tr>
<td>Dry</td>
<td>Wet (fully submerged in water)</td>
<td>DW</td>
</tr>
<tr>
<td>Soaked (fully wetted)</td>
<td>Dry</td>
<td>SD</td>
</tr>
<tr>
<td>Soaked (fully wetted)</td>
<td>Misted with water droplets</td>
<td>SM</td>
</tr>
<tr>
<td>Soaked (fully wetted)</td>
<td>Wet (fully submerged in water)</td>
<td>SW</td>
</tr>
</tbody>
</table>

Statistical Analysis

Three specific treatment groups of interest were compared to the control (DD).

To test for the effect of surface water on shear adhesion we used a matched pairs test
between the control (DD; dry toe pads on dry glass) 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 forth 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 (DD) and the soaked toe pad treatment group tested on dry glass (SD). Finally, we compared our whole-animal results to the setal patch results by Pesika et al. (2009) using a matched pairs test between the control (DD) 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 2.2 for reference. The effect of step order on each treatment group was analyzed using a repeated measures MANOVA. Sample means and error are reported as mean ± 1 s.e.m.

Table 2.2. Seven Tokay geckos (*Gekko gecko*) were used for experimental trials. Sex, weight and total toe pad area for each individual are reported. Toe pad area was measured using scanned images of toes in contact with a surface. Weight is averaged over all trials. Error is reported as mean ± 1 s.e.m.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Sex</th>
<th>Mass (g)</th>
<th>Toe pad area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>Male</td>
<td>78.6±1.7</td>
<td>5.57</td>
</tr>
<tr>
<td>T8</td>
<td>Male</td>
<td>117.9±0.5</td>
<td>4.94</td>
</tr>
<tr>
<td>T10</td>
<td>Male</td>
<td>103.9±1.0</td>
<td>5.98</td>
</tr>
<tr>
<td>T12</td>
<td>Female</td>
<td>69.5±0.4</td>
<td>5.11</td>
</tr>
<tr>
<td>T13</td>
<td>Female</td>
<td>72.5±0.6</td>
<td>5.50</td>
</tr>
<tr>
<td>T14</td>
<td>Female</td>
<td>78.0±0.8</td>
<td>5.13</td>
</tr>
<tr>
<td>T18</td>
<td>Male</td>
<td>109.9±2.0</td>
<td>7.45</td>
</tr>
</tbody>
</table>
Results

Average toe pad area for all seven individuals was 5.67 ± 0.33 cm² and average weight over experimental trials ranged from 72.5 ± 0.6 g to 117.9 ± 0.5 g depending on the animal (see Table 2.2). We found that the shear adhesive force generated by the DW treatment (0.40 ± 0.04 N) was significantly lower (t = -5.15, df = 6, p = 0.0021) than 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) to the control (DD) we found that the SD treatment was also significantly lower in the fourth step (t = -4.92, df = 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 (DD; 9.76 ± 2.81 N) under initial step conditions (t = -3.36, df = 6, p = 0.0152).

When comparing across steps there was a significant difference in shear force across treatment (F₅,₃₆ = 16.87, p < 0.0001). This difference was driven by an interaction between step and treatment (F₂₀,₁₁₀.₄ = 2.37, p = 0.0023; Table 2.3). We found that the shear adhesive force for the control treatment (DD) increased across steps 0-4 while 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 average shear force in the initial step of the DD and DM treatments were not statistically different (t = -1.41, df = 6, p = 0.2088). This was also true for the second step (t = -1.39, df = 6, p = 0.2133) and nearly true for the third step (t = -2.39, df
= 6, \( p = 0.0543 \)). In all other steps the DM treatment produced significantly lower shear adhesive forces when compared to the control (DD; Fig. 2.3).

Table 2.3. MANOVA table shows a significant difference 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.

<table>
<thead>
<tr>
<th></th>
<th>Wilks’ lambda</th>
<th>Exact F</th>
<th>Numerator d.f.</th>
<th>Denominator d.f.</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2.343</td>
<td>16.868</td>
<td>5</td>
<td>36</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Steps</td>
<td>0.228</td>
<td>1.883</td>
<td>4</td>
<td>33</td>
<td>0.1368</td>
</tr>
<tr>
<td>Treatment ( \times ) Steps</td>
<td>0.305</td>
<td>2.375</td>
<td>20</td>
<td>110.4</td>
<td>0.0023**</td>
</tr>
</tbody>
</table>

Figure 2.3. Shear force of whole-animal 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. Error bars are ± 1 s.e.m.
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 one thousand 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 native conditions. Our results suggest that in at least one tropical-dwelling species (*Gekko 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 minutes, similar to previous experiments using a setal patch. Finally we tested the system on misted glass which mimics native 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 adhesive performance of the system. Trials where the surface was fully wetted and the toes were submerged underwater were significantly lower (mean of DW and SW treatments 0.44 ± 0.03 N) than the control (DD; 17.96 ± 3.42 N) after full
replacement of the gecko’s feet. Our tests show that while 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 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 behavior 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 behavior 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 Figure 2.4. In Figure 2.4a the gecko toe pad is superhydrophobic and in the Cassie wetting regime, evident by the high contact angle the water drop makes with the surface of the toe pad. In Figure 2.4b, 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 minutes, suggesting that prolonged exposure to water causes a wetting transition. 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.

Figure 2.4. Two wetting regimes of the gecko toe pad (*Gekko gecko*). In 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. Transition into the Wenzel wetting regime is shown in B, 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 minutes. Scale bar is1 mm.
By forcing a wetting transition in the setal mats, we also observed a significant drop in adhesion across steps and treatments when compared to the control treatment carried out with non-wetted (Cassie regime) setal mats. In the fourth step, average 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 (DD; 17.96 ± 3.42 N). There may be several reasons for this finding. Wetting of the toe pads forced a transition which 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. While 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 while 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 GPa to 2.13 ± 0.2 GPa when environmental
humidity is increased from 30% to 80% RH (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. (2009) held and tested patches of the setal mat in water and compared the frictional force of these treatments to a patch that was held and tested in dry N\textsubscript{2} atmosphere. Their results show that 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. (2009) in the 90 minute 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. While 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 will expel droplets of water and allow a dry interface for adhesion, similar to the results
using a thin water layer (Hsu et al., 2012) and self-cleaning experiments (Hansen and Autumn, 2005; Hu, 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 (DD) 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 to the control (DD) after initial placement onto the glass (4.72 ± 1.59 N misted glass; 9.76 ± 2.81 N dry glass). After four steps however, 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 the DD control group (10.45 ± 2.82 N; t = -2.81, df = 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 a 100-gram tokay gecko (approximately the average size used in this experiment) required one Newton of force to support its body mass, then shear force measurements on misted glass are sufficient to support the gecko’s weight, up to four steps (1.84 ± 0.54 N). Force values for the soaked toe pad on dry glass treatment group (SD) were near this critical level however all other treatment groups fell below one Newton 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 to 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 about 0.04% of their theoretical adhesive capability (Autumn, 2006) and while a 100-gram 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 section of the over-built design of the gecko adhesive system and our results suggest that compromised adhesion, either due to surface water or wetting, may be another critical selective factor in the evolution of the system.
When we looked at shear adhesive force across steps, we found that the control treatment (DD) 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 gecko is 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 report the activity patters of geckos in rainstorms or after significant rainfall events and of those the findings are not clear. Marcellini (1971) found significantly fewer geckos active during rainfall events than compared to dry conditions however 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 we report here 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 which 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. (2009) found that after 20 minutes of sustained contact with water the setal patch was unable to regain its natural water repellency for more than 48 hours. 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 timescale is not consistent with the setal patch and that whole animal adhesive system recovers much faster (A. Y. Stark and T. W. Sullivan, 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 to actively recover their adhesive system after it is compromised. High levels of rainfall and humidity are environmental characteristics that describe tropical habitats and while 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., 2005b; Sun et al., 2005;
Niewiarowski et al., 2008; Pesika et al., 2009; Puthoff et al., 2010). Based on these
results it seems 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 water has on the gecko adhesive system requires
detailed observations of geckos in their native environment. For example, geckos don’t
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; but rather 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 and 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.

Acknowledgements

We thank Nancy Cross, Bo Yang, Joel Thorson, Briana Chambers and George Voros for assistance with trials and Edward A. Ramirez for the photographs in Figure 2.1.
CHAPTER III

SURFACE WETTABILITY PLAYS A SIGNIFICANT ROLE IN GECKO ADHESION UNDERWATER

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Summary

Although we now have thousands of studies focused on the nano, micro and whole animal mechanics of gecko adhesion on clean, dry substrates, we know relatively little about the effects of water on gecko adhesion. For many species of gecko however, rainfall frequently wets the natural surfaces they navigate. In an effort to begin closing this gap, we tested the adhesion of geckos on submerged substrates that vary in their wettability. When tested on a wet hydrophilic surface geckos produced a significantly lower shear adhesive force (5.4 ± 1.33N) when compared to a dry hydrophilic surface (17.1 ± 3.93N). When tested on an intermediate wetting surface and a hydrophobic surface, we found no difference in shear adhesion when comparing dry or wet contact. Finally, when tested on PTFE we found that geckos clung significantly better to wet PTFE (8.0 ± 1.09N) than dry PTFE (1.6 ± 0.66N). To help explain our results we developed models based on thermodynamic theory of adhesion for contacting surfaces in different
media and found that we are able to predict the ratio of shear adhesion in water to that in air. Our findings provide insight into how geckos may function in wet environments and also have significant implications for the development of a synthetic gecko mimic that retains adhesion in water.

Introduction

Over the last decade, researchers have made extraordinary progress in understanding how the gecko adhesive system works (Autumn et al., 2000; Autumn et al., 2002; Autumn and Peattie, 2002; Tian et al., 2006; Autumn et al., 2006a; Puthoff et al., 2010; Gravish et al., 2010; Gravish et al., 2008). Indeed, many laboratories have tested hundreds of synthetic mimics for potential use in robotics, medicine, space and everyday life (Yurdumakan et al., 2005; Ge et al., 2007; Bartlett et al., 2012; Lee et al., 2007; Menon et al., 2004; Murphy et al., 2009a; Murphy et al., 2009b; Aksak et al., 2008; Northen et al., 2008; Parness et al., 2009; Kim et al., 2009b; Jeong et al., 2009; Mahdavi et al., 2008). While the range and performance of synthetic “gecko-tapes” is impressive, there remain important gaps in our knowledge of the system and its capabilities in natural environments. Geckos are extremely diverse, constituting over 1,400 species world-wide (Han et al., 2004; Gamble et al., 2012). However, knowledge of the natural substrates and conditions geckos use is very limited. For example, it is likely that many species move across leaves and other plant structures which are not perfectly smooth and have variable surface chemistries (Koch et al., 2008; Jetter and Schaffer, 2001). In principle, the interaction of gecko feet with such surfaces can have a
significant effect on adhesion, yet gecko research has only just begun to tackle such questions (Niewiarowski et al., 2012; Russell and Johnson, 2007; Huber et al., 2007). Additionally, natural surfaces are also likely to become wet (especially in the tropics) and dirty, potentially reducing adhesion. Although research on the ability of geckos to remove dirt from their toes has received some attention (Hansen and Autumn, 2005; Hu et al., 2012), studies on wetting and the effect of water are limited, despite the well known anti-wetting properties of the toes which are both superhydrophobic and have a low contact angle hysteresis (Autumn and Hansen, 2006; Liu et al., 2012).

Somewhat surprisingly, geckos cannot stick to hydrophilic glass when it is covered with a layer of water (Stark et al., 2012). Anecdotally, this effect has been long and widely appreciated; nevertheless, the effect of water on gecko adhesion is complex. For example, a thin water layer on a hydrophilic sapphire substrate can be expelled at the adhesive interface between a gecko toe and the substrate (Hsu et al., 2012), likely due to the gecko’s superhydrophobic toe pads. Conversely, a thick water layer (~0.5 cm deep) on a hydrophilic glass surface cannot similarly be expelled. Moreover, a large drop in adhesive strength occurs when the adhesive system is submerged in water (Stark et al., 2012). At face value, this is perplexing: many geckos live in tropical habitats where surface wetting from rain and humidity is expected to be common. However, arboreal geckos likely utilize plant surfaces more than other substrates like dirt, sand or man-made glass and plastics, and many plant surfaces are hydrophobic (Koch et al., 2008). This begs the obvious question; can geckos stick to wet hydrophobic surfaces? Unfortunately, data necessary to answer this question are very limited. Early
experiments by Hiller tested for the effect of surface wettability on adhesion using surfaces whose water contact angle ranged from 62.6° to 92.7° (Hiller 1976). Although gecko adhesion was inversely related to water contact angle, adhesion when these surfaces were wetted with water was not tested. More recent work demonstrated that a submerged AFM probe contacting the tips (spatula) of gecko’s adhesive hairs showed a significant decrease in normal pull-off force when compared to a dry environment (Huber et al., 2005b). Similarly, Pesika et al. tested adhesion between a small patch of the gecko’s adhesive hairs (setae) and a silica surface in water under different loads and also found a significant drop in adhesive force (Pesika et al., 2009). Taken together however, these studies do not predict how the gecko adhesive system behaves under conditions that are likely quite common in their native environment.

In this study, we tested the effect of water on the gecko adhesive system using a range of surfaces with different wettability defined by water contact angle ($\theta_Y$). Since geckos probably encounter both hydrophilic (such as some flowers and roots) as well as hydrophobic (most plant leaves) surfaces (Koch et al., 2008; Feng et al., 2011), we tested surfaces that are hydrophilic ($\theta_Y = 50° \pm 1.4°$), intermediately wetting ($\theta_Y = 85° \pm 0.5°$) and hydrophobic ($\theta_Y = 94° \pm 0.5°$). We also tested the effect of water on adhesion to polytetrafluoroethylene (PTFE), a synthetic substrate geckos cannot adhere to in dry conditions (Hiller, 1976; Hiller 1968).

To explain our experimental results we developed a model in which we take into account adhesion between a “gecko hair-like” surface and surfaces with different wettability in different media (in air and in water). We used a classic thermodynamic
approach to calculate the work of adhesion between two separating surfaces: a “gecko hair-like” surface and each of the four surfaces we used in whole-animal experiments with different $\theta_y$. Further, we expected that the gecko’s setal morphology can have a significant effect on adhesion since the small adhesive hairs form a multi-contact interface rather than a flat, uniform contact. To take into account this geometric effect we calculated the thermodynamic work of adhesion between a structured “gecko hair-like” surface and the substrates with varying $\theta_y$. Our model allows us to predict adhesive forces across a range of surface wettability and environments that either the gecko or a synthetic mimic may encounter and thus, not only highlights a very important aspect of the gecko adhesive system but also crucial design parameters for mimetic systems.

Materials and Methods

Six Tokay geckos (*Gekko gecko*) with an average weight of 99.3g ± 2.25g were used during experimental trials. Detailed husbandry procedures are outlined by Niewiarowski et al. (2008). Prior to each experiment, geckos were allowed 30 minutes to acclimate to the testing environment which was maintained at 26°C ± 0.1°C and 33% ± 0.3% relative humidity through all experimental trials. Procedures involving live animals followed 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.

Four substrates: glass, polymethylmethacrylate (plexiglass; Philadelphia PA, USA), polytetrafluoroethylene (PTFE; Wilmington, DE, USA), and an
octadecyltrichlorosilane self-assembled monolayer (OTS-SAM) formed on the surface of glass (Appendix A) were used in this experiment. Surfaces were mounted securely with Velcro® (Manchester, NH, USA) to the bottom of a Rubbermaid® container (Atlanta, GA, USA) which was used to hold water during trials. Each gecko was fitted with two harnesses which were attached to a force sensor positioned horizontally on a motorized track; similar to the rig described by Niewiarowski et al. (2008) and Stark et al. (2012). Maximum shear adhesive force was defined as the point where all four feet had begun to slip along the surface. In the wet surface condition the substrate was fully submerged in water (24°C ± 0.2°C), so that approximately 1 cm of standing water covered the surface, completely submerging the gecko’s feet. Geckos were randomly tested on all surfaces except the OTS-SAM coated surface which was tested last. Each gecko was tested on all four surfaces and under both surface conditions (wet and dry) three times. Only the highest maximum shear force value collected from the three trials per individual was used in statistical analysis. The effect of surface type (glass, plexiglass, OTS-SAM coated glass or PTFE) on surface treatment (wet or dry) was tested using a repeated measures MANOVA. A matched pairs analysis was used to compare specific treatments of interest. All sample means are reported as mean ± 1 standard error. Adhesion Model

To explain adhesion between a gecko foot and the four different surfaces used in whole-animal adhesion experiments, a classical thermodynamic model of adhesion was used to predict the adhesive interaction between the two contacting surfaces in either air or water.
There are three main assumptions for the model calculations: first, the surface of the gecko foot at the contact interface is assumed to have surface properties similar to that of lipid-like n-hexadecane. This assumption is based on our previous experiments which indicated that gecko setae have phospholipids on their surface (Hsu et al., 2012). Second, the model assumes a normal direction of adhesion whereas the experimental trials were carried out in the shear direction. We know that typically normal and shear adhesion are proportional for hard surfaces (Carpick et al., 1996) whereas, for soft surfaces normal adhesion is only lower by the one half power (Chaudhury and Chung, 2007). Considering the proportionality between normal and shear force our assumption is reasonable. In addition, we only compared relative adhesion energies, rather than exact numbers, by calculating the ratio of the work of normal adhesion in water ($W_{\text{wet}}$) to the work of normal adhesion in air ($W_{\text{dry}}$) for each surface. Finally, our third assumption is that the contact interface is flat and contact between the two surfaces is chemically and structurally homogeneous. This includes the assumption that contact between the surfaces is dry and no intervening layer of water is present between the two surfaces during contact.

Smooth Surface

We used the Young-Dupré equation to calculate the work of adhesion between each of our four surfaces (glass, plexiglass, OTS-SAM coated glass and PTFE) and the “gecko hair-like” n-hexadecane surface (Chaudhury, 1996; Appendix B).

In the case of wet adhesion i.e. where water is the medium of contact, the work of adhesion ($W_{\text{wet}}$) is calculated using the following equation (derived in Appendix C):
\[ W_{\text{wet}} = A_c (\gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - \gamma_{water-air} \cos \theta_2) \]  

Here, \( W_{\text{wet}} \) is the work of adhesion between two surfaces contacting under water, \( A_c \) is the area of contact, \( \gamma_{h-water} \) is the “gecko hair-like” \( n \)-hexadecane-water interfacial energy, \( \gamma_{h-air} \) is the “gecko hair-like” \( n \)-hexadecane-air interfacial energy and \( \gamma_{water-air} \) is the interfacial energy at the water-air interface. To obtain the interfacial energies we measured the contact angle of \( n \)-hexadecane (\( \theta_1 \)) and water (\( \theta_2 \)) on all four surfaces used in experimental trials (Table 3.1; see Appendix D for procedure).

Table 3.1. Contact angles (\( \theta_x \)) of water and \( n \)-hexadecane on all four surfaces used in whole-animal experiments and modeling. Errors are means ± 1 SEM.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Water, °</th>
<th>( n )-Hexadecane, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>50 ± 1.4</td>
<td>13 ± 2.0</td>
</tr>
<tr>
<td>PMMA</td>
<td>85 ± 0.5</td>
<td>9 ± 2.3</td>
</tr>
<tr>
<td>OTS-SAM</td>
<td>94 ± 0.5</td>
<td>29 ± 2.9</td>
</tr>
<tr>
<td>PTFE</td>
<td>97 ± 0.3</td>
<td>30 ± 1.6</td>
</tr>
</tbody>
</table>

Patterned Surface

To take into account the surface morphology of gecko toes, we used a model unit cell. The unit cell consists of a tetrad pattern which is representative of the setal pattern on the surface of the Tokay gecko (\( Gekko gecko \)) toe (Figure 3.1a and 3.1b). The tetrad unit cell was made up of four square pillars each with a square side width of 4 \( \mu \)m and height of 60 \( \mu \)m. The distance between any two adjacent pillars in one tetrad was 1 \( \mu \)m and the separation between two adjacent tetrads was 2 \( \mu \)m (Figure 3.1c).
Figure 3.1. Scanning electron microscope images of (A) the tetrad patterning of the Tokay gecko (*Gekko gecko*) setal mats and (B) the four setae that are grouped together to form a single tetrad. To represent the tetrad morphology of the toe pad, a patterned surface was used to model the gecko setal mats (C) using similar dimensions as the gecko setal mat. Each column, representing one seta, was 60 \( \mu \text{m} \) high and 4 \( \mu \text{m} \) wide. The separation distance between columns (setae) was 1 \( \mu \text{m} \) and separation between tetrad unit cells was 2 \( \mu \text{m} \). The model unit cell used for calculations is boxed in C. Scale bars are 10 \( \mu \text{m} \).

When the tetrad structure of “gecko hair-like” n-hexadecane is in contact with either of the four flat surfaces (glass, plexiglass, OTS-SAM coated glass or PTFE), \( W_{\text{dry}} \) is estimated using the Young-Dupré equation (Appendix E). For \( W_{\text{wet}} \) of a patterned surface there are four different cases possible of “separated” and “in contact” states that are associated with air pockets between the square columns before or after contact with the flat test surface (see Table 3.2 for schematics of each case). In all cases the water surrounding the unit cell is also accounted for in \( W_{\text{wet}} \), whereas the adhesive interface is always assumed to be dry. In case 1, both, the “in contact” and the “separated” states maintain air pockets in the space separating the square columns. \( W_{\text{wet}} \) for case 1 can be calculated using the following equation (equation 2) where \( A_1 \) and \( A_2 \) correspond to total surface areas of the “gecko-hair-like” n-hexadecane tetrad.
patterned unit cell and the surface it is in contact with (glass, plexiglass, OTS-SAM coated glass or PTFE), respectively.

\[ W_{\text{wet}} = A_C(y_{h-water} + y_{h-air} \cos \theta_1) - A_2 y_{water-air} \cos \theta_2 \]  

(2)

In the “separated” state of case 2, water penetrates completely inside the tetrad asperities in such a way that the entire surface area of the tetrad unit cell is in contact with water. However, the “in contact” state expels all the water out and the asperities occupied with water initially are replaced by air pockets. The equation to calculate \( W_{\text{wet}} \) for case 2 is:

\[ W_{\text{wet}} = A_1(y_{h-water} - y_{h-air}) - A_2 y_{water-air} \cos \theta_2 + A_C y_{h-air} (1 + \cos \theta_1) \]  

(3)

Cases 3 and 4 represent the possibility that the gaps between the pillars in a tetrad are completely filled with water in the “in contact” state, however, in the “separated” state the gaps are either air pockets or filled with water for case 3 and case 4 respectively. Equation 4 is used to calculate \( W_{\text{wet}} \) for case 3 and equation 1 calculates \( W_{\text{wet}} \) for case 4 (since it is only \( A_c \) that is different for the patterned surface compared to the flat surface).

\[ W_{\text{wet}} = A_c[2y_{h-water} + y_{h-air}(\cos \theta_1 - 1) - y_{water-air} \cos \theta_2] + A_1(y_{h-air} - y_{h-water}) \]  

(4)

Derivations for each case are included in the supporting information (Appendix F), as are derivations for a non-tetrad patterned unit cell model (Appendix G).
Table 3.2. Ratios of wet to dry adhesion on all four surfaces used in whole-animal experiments and modeling. The $W_{\text{wet}} : W_{\text{dry}}$ ratio in the smooth and patterned surface models is calculated as the work of normal adhesion of two surfaces coming into contact in water ($W_{\text{wet}}$) and the work of normal adhesion of two surface coming into contact in air ($W_{\text{dry}}$). Models were calculated using a chemically similar “gecko hair-like” surface (n-hexadecane), represented as the yellow surface, and each of the four surfaces used for whole-animal experiments (glass, plexiglass, OTS-SAM coated glass and PTFE), represented as the grey surface. Ratios for normal adhesion of a patterned surface can be separated into four cases of pre-contact (“separated”) and “in contact” (shown by the arrow) with the “gecko hair-like” surface and the four experimental surfaces. When submerged in water (blue) the space between the patterned unit cell pillars is either filled with air or filled with water. These are shown schematically where case 1 shows a consistently dry inter-pillar region and case 4 shows one that is consistently wet. Case 2 represents the scenario where the inter-pillar region is first wet and then becomes dry on contact and case 3 represents the opposite, where the inter-pillar region is first dry and then becomes wet after contact. The wet to dry ratios for whole-animal shear adhesion on each of the four surfaces are shown in the last column.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Smooth Surface</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Gecko</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>0.56</td>
<td>-0.28</td>
<td>30.54</td>
<td>-30.27</td>
<td>0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>PMMA</td>
<td>1.38</td>
<td>1.27</td>
<td>31.9</td>
<td>-29.25</td>
<td>1.38</td>
<td>0.91</td>
</tr>
<tr>
<td>OTS-SAM</td>
<td>1.64</td>
<td>1.73</td>
<td>34.14</td>
<td>-30.76</td>
<td>1.64</td>
<td>0.89</td>
</tr>
<tr>
<td>PTFE</td>
<td>1.74</td>
<td>1.91</td>
<td>34.57</td>
<td>-30.92</td>
<td>1.74</td>
<td>5.19</td>
</tr>
</tbody>
</table>

Results

Gecko adhesion in the shear direction (frictional adhesion) was tested on four surfaces with different wetting properties: glass, plexiglass, OTS-SAM coated glass and PTFE. Water contact angles ranged from approximately 50° to 100° (Table 3.1). Whole-animal shear adhesion was measured on all four surfaces when submerged under water and when dry to test for a difference in adhesion when either air or water was the
medium of contact. When comparing surfaces of different wettability (glass, plexiglass, OTS-SAM and PTFE) and the medium of contact (wet or dry), we found a significant interaction between surface wettability and medium of contact ($F_{3,8} = 8.82, p = 0.0064$; Table A4). As reported previously (Stark et al., 2012), gecko adhesion on the glass surface was significantly higher ($t = -3.35, df = 5, p = 0.0204$) when the surface was dry ($17.1 \pm 3.93N$) than when the glass substrate was submerged in water ($5.4 \pm 1.33N$). When tested on the plexiglass and the OTS-SAM coated surfaces geckos did not show a significant difference ($t = -0.61, df = 5, p = 0.5691$ for plexiglass and $t = -0.80, df = 5, p = 0.4615$ for OTS-SAM) in wet ($24.0 \pm 3.92N$ plexiglass; $17.6 \pm 2.22N$ OTS-SAM) and dry surface conditions ($26.4 \pm 1.94N$ plexiglass; $20.0 \pm 1.71N$ OTS-SAM). Conversely, when tested on PTFE geckos produced a significantly higher ($t = 6.61, df = 5, p = 0.0012$) adhesive force underwater ($8.0 \pm 1.09N$) than on dry PTFE ($1.6 \pm 0.66N$), unlike the results on all other surfaces (Figure 3.2).
Figure 3.2. Whole-animal shear (frictional) adhesion values from Tokay geckos (*Gekko gecko*) tested on four surfaces in dry or wet contact. Each individual (N = 6) was tested three times on each surface (glass, plexiglass, OTS-SAM coated glass and PTFE) and the highest of the three tests were used for data analysis. Surfaces were either tested without water (dry) or fully submerged under water (wet). Error bars are means ± 1 SEM.

Similar to our previous report, geckos could not support their body weight (1N of force to support a 100g gecko) on a wet glass surface (Stark et al., 2012). These experiments however, were very controlled as we only allowed the gecko to take steps with one foot at a time. In the results reported in this study, we allowed each gecko to naturally place all four of its feet before beginning the experiment and we only measured one force reading per experimental trial. Consequently, our cling force values for geckos on a glass surface submerged in water were higher (5.4 ± 1.33N) than those reported previously (0.4 ± 0.04N; Stark et al., 2012). Because geckos were allowed to position all of their feet at one time, we noticed that five of the six geckos were successful in achieving sufficient contact with the glass surface and one of their feet.
This only occurred for these five geckos once out of three trials or about 28% of the total experiment time. When one foot was planted successfully on the hydrophilic glass surface, it was clear by visual inspection that this foot was the only foot contributing to overall whole-animal adhesion and these events all produced over 1N of force. If these data were removed from analysis our results on the submerged glass surface would be $0.7 \pm 0.07N$, similar to our previous findings. Using this value to calculate the wet to dry ratio of adhesion on a glass surface the ratio would be even lower (0.04) than that reported (0.32; Table 3.2).

Smooth Surface Adhesion Model

We tested the hypothesis that the ratio of shear adhesion values from the whole-animal experiments on wet and dry surfaces could be predicted from a classical thermodynamic model, based on the work of normal adhesion ($W$) between two flat surfaces in air and in water. Using the Young-Dupré equation and equation 1 we calculated the $W_{wet} : W_{dry}$ ratio for our model and compared this to the ratio of wet to dry shear adhesion values obtained experimentally from whole-animal adhesion measurements on each of the four surfaces (Table 3.2). The model calculations show that relative normal adhesion on any given test surface when tested in air and under water is a function of surface wettability. As the hydrophobicity of the surface increases (water contact angle increases), the value of $W_{wet} : W_{dry}$ increases. The experimental values from whole-animal adhesion experiments also show a similar trend (Table 3.2), supporting our hypothesis that surface hydrophobicity can have a significant thermodynamic impact on gecko adhesion when tested underwater.
Patterned Surface Adhesion Model

To consider the effect of patterning of the setal mats we took into account the gecko toe surface morphology by modeling a tetrad-patterned “gecko hair-like” surface. Using our calculations of the $W_{wet} : W_{dry}$ ratios of each surface in various conditions we qualitatively compared our model with experimental results. The $W_{wet} : W_{dry}$ ratios for smooth surfaces and ratios for case 4 of the patterned surface are the same (Table 3.2). This is due to the fact that the common multiplication factor corresponding to the total surface area for $W_{wet}$ and $W_{dry}$ cancels out as the ratio of the two is taken. Similar to the smooth surface model, hydrophobicity of the surface has a significant impact on the $W_{wet} : W_{dry}$ ratio however each case presents different results for a given surface, suggesting that the presence of water between or around the hairs also plays an important role in adhesion underwater. Based on our results we can clearly rule out case 2 and case 3 as possible conditions of pre- and post-contact between the patterned “gecko hair-like” surface and the flat test surfaces; as ratio values are either extremely high, favoring wet adhesion over dry (case 2) or extremely low, favoring dry adhesion over wet (case 3), neither of which occur in the whole-animal system. $W_{wet} : W_{dry}$ ratios for case 1 and case 4 are most similar to those from whole-animal experiments, where glass is the least favorable in wet conditions and ratios on the hydrophobic surfaces are near one, supporting equal adhesion in wet and dry conditions (Table 3.2). Modeled ratios involving PTFE do not explain our experimental results (the experimental ratio is much higher than all modeled ratios) and will be discussed later.
To further investigate case 1 and case 4 we imaged the gecko foot contacting glass and the OTS-SAM coated surfaces under water after the gecko took four steps, similar to experimental trials (Figure 3.3). The hydrophobic OTS-SAM coated surface (Figure 3.3b) shows typical characteristics of a dry contact (Figure 3.3a) such as a white rather than a gray appearance of the adhesive toe pads and their tendency to remain dry when taken out of the water. This suggests that our results are most similar to case 1, a constantly dry contact under water with air pockets between the surface asperities in both the “separated” and “in contact” states. On the hydrophilic glass surface however, we see one of three scenarios taking place typically, either a consistently dry contact (case 1), an entrapped air bubble between the toe and the glass surface (shown as a silvery layer on the toe; Figure 3.3c) or one where water wets the toes (case 3) and the wet toes appear gray when removed from the test surface.

Figure 3.3. Images of a gecko foot (A) in dry contact with glass, (B) in wet contact with a hydrophobic surface (OTS-SAM coated glass) and (C) in wet contact with a hydrophilic surface (glass). Geckos were allowed to take four natural steps to ensure adhesive contact with the surface. Areas where the toe is in dry contact with the surface appear white in color, similar to the dry contact in A, and areas where the toe appears grey have been wetted with water. Silvery air bubbles can be seen between wet lamellae in B and on most of the toe pad in C. Scale bars are 0.5 cm.
Discussion

Despite substantial interest in the field, little work has been done to investigate performance of the gecko adhesive system under conditions similar to the gecko's native environment. While gecko species are widely distributed, many are native to tropical environments where surfaces are likely to be dirty or wet. In this study we took into account the effect of surface wettability on adhesion to wet and dry surfaces by testing geckos on four different surfaces ranging from hydrophilic (glass), intermediately wetting (plexiglass), hydrophobic (OTS-SAM coated glass) and finally PTFE a hydrophobic surface geckos fail to cling to in dry conditions. To explain our findings from whole-animal experiments we modeled adhesion between two surfaces, a “gecko hair-like” surface (n-hexadecane) and each of the four surfaces tested in whole-animal experiments using both air and water as the medium of contact. Results from whole-animal experiments and surface modeling suggest that gecko adhesion is highly dependent on surface wettability and the presence of water or air between the toe pad and contact surface.

Similar to our previous report (Stark et al., 2012), shear adhesion to the hydrophilic glass surface was much lower in water than in air experimentally (Figure 3.2) and was consistently lower in our model calculations when compared to the other three surfaces (Table 3.2). The anomalous behavior in the case of the glass surface is not surprising given that the surface is hydrophilic and thus shows higher affinity to water compared to other test surfaces. For example, three scenarios may occur when two surfaces contact underwater (Chaudhury and Whitesides, 1991). First, a layer of water
between the surfaces acts as a barrier to the establishment of contact between the two. Second, roughness of the contact surface may change the contact area and may also leave small pools of water behind. Thirdly, water may be fully excluded from the two surfaces and completely dry contact occurs. Depending on the gecko’s foot placement, we found that either a dry contact formed by squeezing water out completely (case 1) or an air bubble formed (Figure 3.3c) which acted like a lubricating layer causing the gecko to slip. There is also another scenario that may occur in whole-animal gecko adhesion on wet surfaces where a wetting transition occurs and the toe wets completely, causing a substantial drop in adhesion experimentally (Stark et al., 2012) and theoretically (case 3). These observations suggest that natural placement of the foot on the wet surface is important and adhesion can be highly variable when the surface is hydrophilic, potentially limiting a gecko’s movement on wet hydrophilic surfaces. Interestingly not all feet, and possibly toes, show the same scenario in any single trial; rather it is always a combination of two or more behaviors happening at the same time which complicates the use of models, like ours, that assume homologous contact.

Our results on plexiglass and OTS-SAM coated glass are consistent with our hypothesis that adhesion will not be affected by water on surfaces that are more hydrophobic in nature than glass. This suggests that gecko adhesion is not impaired by surface wetting in their natural environments, assuming their native substrates are at least moderately hydrophobic (perhaps $\theta_Y \geq 85^\circ$). One of the main surfaces we expect geckos to be walking on are plant surfaces, such as leaves, which are primarily
hydrophobic due to their waxy cuticle (Koch et al., 2008). By imaging the foot in contact with the hydrophobic OTS-SAM coated glass it is clear that water is excluded from the majority of the adhesive toe pad and the contact made underwater is dry (Figure 3.3b). These results are similar to the contact made by a terrestrial beetle underwater, where trapped air bubbles actually allow dry contact to occur on hydrophobic surfaces and traction force of the beetle walking underwater does not differ from forces collected when the beetle was walking in air (Hosoda and Gorb, 2012). Our experimental results are also supported by our model and ratios of wet to dry adhesion are near one (wet adhesion is not different from dry adhesion) in case 1 where the “gecko hair-like” surface remains dry prior to and during contact. The maintenance of dry contact is interesting because when two surfaces become separated water is likely to penetrate the separation crack forming between the two surfaces and even propagate further growth (Haidara et al., 1995). This may be what occurs when testing normal and frictional (shear) adhesion of a patch of setae, where normal adhesion in water is much lower, due to water penetration, than frictional adhesion in water under high loads which presumably keep water from separating the two surfaces (Pesika et al., 2009).

The dominant mechanism behind the gecko’s ability to stick is van der Waals forces which in dry contact should be relatively insensitive to surface chemistry (Autumn et al., 2002). Of the three surfaces with similar Hamaker constants (see derivations in Appendix H): glass ($6.5 \times 10^{-20}$J), plexiglass ($6.2 \times 10^{-20}$J) and a glass plate coated with OTS-SAM ($6.5 \times 10^{-20}$J, assuming the OTS coating has no effect), dry adhesion on the plexiglass surface was almost significantly higher than both the dry glass ($t = 2.33, df = 5$,
p = 0.0672) and the dry glass coated with OTS-SAM (t = -2.52, df = 5, p = 0.0535). The plexiglass surface had a contact angle of 85° ± 0.5° and a slightly lower Hamaker constant. Although it is not entirely clear why dry plexiglass was marginally better than the other two surfaces in our whole-animal experiments, it is interesting to consider an optimal surface for the gecko adhesive system and how such a surface may correlate to the natural surfaces of their environment.

In contrast, the Hamaker constants calculated in water are lower than in air for all the substrates studied here (Appendix H). This is expected based on van der Waals interactions, and may indicate that the adhesion forces to separate non-polar surfaces in water should be lower in water. However, this is misleading because Hamaker constants do not accurately predict the interfacial energies of non-polar materials in water and requires the addition of hydrogen bonding (Table 11.4 in Israelachvili, 1991). A simpler explanation for higher shear adhesion forces for non-polar surfaces in water is the higher interfacial energies of non-polar materials in water than in air as calculated by our model (Chaudhury and Chung, 2007).

Our final surface, PTFE, provided surprising results. We quantitatively confirmed previous observations (Hiller, 1976; Hiller, 1968) that geckos do not stick well to dry PTFE (1.6 ± 0.66N). When tested on PTFE submerged under water however, the geckos clung to the fully wetted surface significantly better than the dry (8.0 ± 1.09N), contrary to our findings on all other tested surfaces. Unlike our previous results on each of the other surfaces, our model does not predict the whole-animal experimental results. Our experimental ratio is fivefold higher than our theoretical ratio and this discrepancy could
be due to the low adhesion of geckos to dry PTFE or the comparably high adhesion geckos have on wet PTFE. Additionally, the experimentally measured shear adhesion to dry PTFE is 10-15 times lower than shear adhesion to a surface with similar water contact angle (OTS-SAM coated glass). This low adhesion on PTFE cannot be explained by the small difference in the Hamaker constants (4.6 x 10^{-20}J for PTFE compared to 6.5 x 10^{-20}J for OTS-SAM coated glass). We also do not believe that static charging is playing a role here because the adhesion values for PTFE are lower, rather than higher, than those predicted by contact angles or Hamaker constants. In addition, surface charges will be neutralized under water and cannot influence the shear adhesion values measured in water. One reason the experimental values of dry shear adhesion on PTFE are lower than the expected values from our theoretical models, or compared to a surface with similar water contact angle, may be related to the abnormally low coefficient of friction of PTFE. Interestingly, underwater shear adhesion values for PTFE are vastly improved and closer to our expected values for hydrophobic surfaces. We believe that the adhesion in air is anomalous for PTFE, resulting in much larger ratios for wet versus dry shear adhesion forces. Additionally, we hypothesize that the roughness of PTFE may also play an important role. Although the roughness of the PTFE surface does not change when wet or dry, when PTFE is submerged under water the roughness may be less important to adhesion because water is able penetrate between the rough surface asperities. However when dry, the roughness of PTFE may cause air gaps and reduced contact area, lowering adhesion values. It is clear that further work is necessary to clarify the effect of roughness on adhesion to wet and dry surfaces. Interestingly,
synthetic gecko-like PTFE pillars tested underwater with a silica probe were also successful in achieving adhesion; however adhesion values underwater were not five times higher than dry as measured in our experiments (Izadi et al., 2012).

Our main goal was to answer a puzzling question: can geckos living in tropical environments maintain adhesion on wet hydrophobic surfaces? Using our whole-animal adhesion results we found that wet surfaces that are even weakly hydrophobic allow the gecko adhesive system to remain functional for clinging and likely locomotion as well. Our findings suggest a level of versatility in the gecko adhesive system that was previously not accounted for and calls into question interesting evolutionary, ecological and behavioral predictions. For example, maintenance of the superhydrophobic toe pads is likely critical for geckos living in the tropics. The ability of the toes to shed water droplets relies on the wettability of the toe pad and as such a surface chemistry conducive to water shedding should be conserved in species native to wet environments. Our recent finding of lipid-like molecules at the contact interface (Hsu et al., 2012) may help to maintain or prolong this anti-wetting property however further experiments are needed to confirm this. Evolutionarily geckos are an interesting group in that adhesive toe pads have evolved multiple times (Gamble et al., 2012) and in at least one group digital toe pads appear to be strongly correlated with ecological factors such as substrate utilization (Lamb and Bauer, 2006). While our experiment focuses on one tropical species of gecko (Gekko gecko), it is not unreasonable to consider potential variation in surface chemistry, toe pad roughness or other anti-wetting mechanisms that are dictated by environment. Although this study highlights yet another remarkable
property of the gecko adhesive system, it is important to remember that the system remains limited on hydrophilic surfaces and loses functionality when the toes become wet (Stark et al., 2012). It is unclear how these limitations affect adhesive performance in natural conditions however it could be related to compensatory behaviors or ecological constraints which have yet to be evaluated. Our findings highlight the importance of considering the natural environments geckos utilize their adhesive system in as well as how the chemical composition and patterning of their adhesive structures can play a role in the success of the system in challenging environments, such as those that frequently become wet. Our study also provides important information for the design of synthetic mimics that can attach equally well in water as in air, or in the case of PTFE better in water than air, similar to what the gecko can achieve.

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CHAPTER IV

REDUCTION OF WATER SURFACE TENSION SIGNIFICANTLY IMPACTS GECKO ADHESION UNDERWATER

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Summary

The gecko adhesive system is dependent on weak van der Waals interactions that are multiplied across thousands of fine hair-like structures (setae) on geckos’ toe pads. Due to the requirements of van der Waals forces, we expect that any interruption between the setae and substrate, such as a water layer, will compromise adhesion. Our recent results suggest, however, that the air layer (plastron) surrounding the superhydrophobic toe pads aid in expelling water at the contact interface and create strong shear adhesion in water when in contact with hydrophobic surfaces. To test the function of the air plastron, we reduced the surface tension of water using two surfactants, a charged anionic surfactant and a neutral non-ionic surfactant. We tested geckos on three substrates: hydrophilic glass and two hydrophobic surfaces, glass with a
octadecyltrichlorosilane self-assembled monolayer (OTS-SAM) and polytetrafluoroethylene (PTFE). We found that the anionic surfactant inhibited the formation of the air plastron layer and significantly reduced shear adhesion to all three substrates. Interestingly, the air plastron was more stable in the non-ionic surfactant treatments than the anionic surfactant treatments and we found that geckos adhered better in the non-ionic surfactant than in the anionic surfactant on OTS-SAM and PTFE but not on glass. Our results have implications for the evolution of a superhydrophobic toe pad and highlight some of the challenges faced in designing synthetic adhesives that mimic geckos’ toes.

Introduction

Recently we discovered that geckos are able to adhere to wet substrates that have an intermediate or hydrophobic water contact angle ($\theta_y$) (Stark et al., 2013). This result was somewhat surprising given that the gecko adhesive system is van der Waals-based (Autumn et al., 2002), and even thin layers of water should disrupt the weak intermolecular forces between the setae (hair-like adhesive structures) and substrate (Hosoda and Gorb, 2012; Lee et al., 2007), causing geckos to fall from wet surfaces. Interestingly this behavior has been observed on substrates with low water contact angles, such as glass. When directly tested on submerged glass ($\theta_y = 50 \pm 1.4^\circ$), geckos cannot reliably support their body weight (Stark et al., 2013; Stark et al., 2012). On the contrary, when making contact to more hydrophobic substrates such as polymethylmethacrylate (PMMA; $\theta_y = 85 \pm 0.5^\circ$) and an octadecyltrichlorosilane self-
assembled monolayer formed on the surface of glass (OTS-SAM; $\theta_y = 94 \pm 0.5^\circ$), geckos adhered equally well when submerged in water as when in air (Stark et al., 2013).

Finally, when tested on polytetrafluoroethylene (PTFE; $\theta_y = 97 \pm 0.3^\circ$), a substrate to which geckos cannot adhere in air, we found that shear adhesion was significantly better in water than in air (Stark et al., 2013). Taken together, these results suggest that substrates with higher contact angles are better for wet shear adhesion than are substrates with lower water contact angles. While it is unlikely that a gecko will encounter a completely submerged surface in their natural habitat, nor is it likely they will regularly move across glass or Teflon surfaces, it is entirely reasonable to expect natural substrates to be periodically wet from rainfall and humidity, especially in the tropics where many species of gecko are native. Furthermore, substrate wettability can vary significantly in natural surfaces, e.g., wax on leaf cuticles, dirt and water deposits, making our findings relevant for understanding how geckos utilize their adhesive under free-ranging conditions.

We hypothesized that the key to adhesion underwater is the gecko's ability to remove water from the contact interface (Stark et al., 2013). One way geckos’ toe pads may achieve dry contact with the substrate is by utilizing its innate superhydrophobicity ($\theta_y = \sim 150^\circ$) and very low contact angle hysteresis, which causes water to roll off the toe pad at very small tilt angles (only 2-3°) (Autumn and Hansen, 2006; Liu et al., 2012). Despite the superhydrophobicity of geckos’ toe pads, they can be wetted, a phenomenon known as a Cassie-Baxter to Wenzel transition, after repeated or prolonged exposure to water (Pesika et al., 2009; Stark et al., 2012). In the Cassie-Baxter
wetting state the water droplet is held above the setal mat (Fig. 4.1a), resulting in a liquid-air-solid interface (Cassie and Baxter, 1944). In the Wenzel state the water droplet penetrates into the setal mats and the toe wets, resulting in a liquid-solid interface without air pockets (Fig. 4.1b) (Wenzel, 1936). When the wetting transition occurs, whole-animal shear adhesion is significantly decreased in air and in water (Stark et al., 2012). Thus, it is clear that the maintenance of dry toe pads is also key to adhesion in any condition (air or water). Although it has never been directly tested, we believe that geckos are able to keep their toe pads dry by forming an air plastron (Poetes et al., 2010), or bubble, around their toes, as shown schematically in Figure 4.2a. In addition, it is also likely that this plastron works in tandem with hydrophobic substrates to push water out of the adhesive interface (Hosoda and Gorb, 2012; Stark et al., 2013). This behavior is shown schematically in Figure 4.2b. The formation of the air plastron is related to the high surface tension of water (72 mN/m) and also the superhydrophobicity of the toe. Therefore, similar to a study with aquatic beetles walking on submerged substrates (Hosoda and Gorb, 2012), we hypothesize that when geckos are forced to adhere in a liquid with surface tension lower than pure water, adhesion will be significantly reduced due to the loss of the air plastron.
Figure 4.1. A gecko’s toe in the Cassie-Baxter wetting state, in which the water droplet is clearly suspended above the adhesive toe pad and easily rolls off the toe at low angles (A). The inset shows the side configuration of the water droplet (blue) on the setal surface (yellow). In contrast, a gecko’s toe in the Wenzel wetting state is completely wet and water penetrates into the setal mat, fully wetting the toe (B). The inset shows the side configuration of water (blue) on the setal surface (yellow).

Figure 4.2. Schematic of a gecko’s foot prior to contact with a submerged substrate, where water (dark grey) forms an air bubble (plastron; shown in white) around the adhesive setae (light grey) (A). When making contact, we hypothesize the plastron helps to expel water out of the contact interface while keeping the setae dry (white air pockets are retained) (B).

A relatively easy way to decrease the surface tension of water is to introduce a surfactant that adsorbs at the air-water surface. In geckos’ adhesive system we expect the surfactant to affect adhesion in two ways. First, the decrease in surface tension should cause the air plastron to be unstable or fail to form, which would cause the superhydrophobic toe pads (Cassie-Baxter state) to wet (Wenzel state) (Chang et al., 2007; Ferrari et al., 2006; Mohammadi et al., 2004) and likely be non-adhesive and
unable to aid the expulsion of water from the interface. Second, surfactant can also alter the surface properties of both the substrate and the gecko’s surface (setae). We expect surfactants to adsorb on the setae and the substrate according to their chemistry (Briscoe et al., 2006; Chang et al., 2007; Ferrari et al., 2006; Haidara et al., 1995; Mohammadi et al., 2004). For some substrates this is relatively straightforward to predict, such as substrates like OTS-SAM formed on glass. On this substrate, polymer tail groups point away from the glass surface and we would expect the surfactant to align itself so that the head groups face outward (Fig. 4.3), thus reducing the interfacial tension. On other substrates, such as glass or PTFE, the ordering, if any, is more difficult to predict. Likewise, the alignment of surfactant on geckos’ setae that have become wet is also hard to predict, due to our limited knowledge of the surface chemistry of the setae. Specifically, there is increasing support that the surface material of the setae actually changes confirmation when exposed to water, in some mechanism that is yet to be identified (Hsu et al., 2012; Pesika et al., 2009). Finally, it is possible that surfactant aligns on both surfaces and that the interaction between these two similarly charged surfaces can also disrupt adhesion due to opposing electrostatic forces, or double-layer forces that can cause water to become trapped (Hsu and Dhinojwala, 2012).
Figure 4.3. Schematic of surfactant alignment on an OTS-SAM coated glass substrate. When submerged in the anionic surfactant, polymer chains associate with the surfactant tails, allowing for the negatively charged head groups to face outward, toward the gecko’s surface (light grey). When submerged in the non-ionic surfactant, the head groups face the gecko’s surface but are not charged.

In response to these hypotheses we have designed an experiment to first, directly test the importance of the air plastron to wet adhesion on three different substrate types that range in surface wettability; and second, to probe the aqueous setal-substrate interaction. We hypothesize that the reduction of the surface tension of water by the surfactant will cause the plastron to be unstable and significantly reduce adhesion. Based on our previous results we chose glass ($\theta_y = 50 \pm 1.4^\circ$), OTS-SAM coated glass ($\theta_y = 94 \pm 0.5^\circ$), and PTFE ($\theta_y = 97 \pm 0.3^\circ$), because each of these substrates have different wet and dry adhesion ratios (Stark et al., 2013). To investigate the surface dynamics of geckos’ setae and the substrate, we used a negatively charged anionic surfactant (sodium dodecyl sulfate; SDS) and a non-ionic neutral surfactant (polyoxyethylene [20] oleyl ether; POE). Although it is difficult to predict adhesion of the surfactant on some of the surfaces (gecko’s toe or substrate), we do expect the
surfactant to adsorb on the ordered OTS-SAM substrate with the preferential alignment of the charged or polar head groups in contact with water, causing the surface to be either negatively charged (SDS) or neutral (POE) (Fig. 4.3). If the plastron is unstable or fails to form and the setae become wet, surfactant can also align on the setae themselves in relation to the unknown and likely dynamic chemical groups at the surface of the gecko’s setae. By testing each of these treatment pairs we hope to shed light on what occurs at the adhesive interface when a gecko’s toe contacts a substrate in water. Specifically, our results will help to inform the setal-substrate interaction and the importance of the air plastron in keeping the toe pads dry and able to expel water from the contact interface.

Materials and Methods

Seven adult tokay geckos (Gekko gecko) were used in experimental trials. Geckos were weighed at the completion of each experimental trial and this weight is reported as an average weight over the duration of the experiments. Outside of experimental trials, animals were housed individually and given cockroaches three times a week. Glass terraria were misted twice a day with water. Geckos were acclimated to the experimental conditions for half an hour prior to testing. Ambient temperature, water temperature, and humidity were maintained at 24.4±0.1°C, 23.7±0.3°C, and 32.6±0.33% respectively over the acclimation period and during experimental trials. 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).

Experimental Treatments

Two surfactants were used in experimental trials: negatively charged anionic sodium dodecyl sulfate (SDS) and nonionic polyoxyethylene [20] oleyl ether (POE). Surfactants were mixed into solution well above their critical micelle concentration (cmc). A 0.01M solution was used for the SDS treatment (cmc = 8mM) and a 100µM solution was used for the POE treatment (cmc = 30µM). The surface tensions of the two solutions above cmc are similar (γ = 37 mN/m for SDS and γ = 39.4 mN/m for POE) and much lower than that of water (γ = 72mN/m). Geckos were tested on three different substrates: glass (θ_y = 50 ± 1.4°), OTS-SAM coated glass (θ_y = 94 ± 0.5°), and PTFE (θ_y = 97 ± 0.3°). The procedure for preparing the OTS-SAM substrate is described elsewhere, as is the method for measuring θ_y for each substrate (Stark et al., 2013).

Experimental Procedure: A force-sensing apparatus, similar to the one used by Niewiarowski et al. (2008), was used to measure frictional or shear adhesive force of geckos positioned horizontally in the surfactant bath. Geckos were chosen at random, as were the treatment conditions (substrate and surfactant). Prior to experimentation, two small harnesses were secured around the gecko’s pelvis and attached to a force sensor. Geckos were then placed onto the substrate and allowed to naturally position themselves on the substrate by taking at least four steps, one step per foot. After taking all four steps, geckos were pulled backwards along the substrate by a custom-designed force apparatus positioned on a motorized track. The two pelvic harnesses attached to
the moving force apparatus allowed us to measure the force geckos produce while clinging to a substrate. Maximum shear adhesion was defined as the force reading at the point all four of the gecko’s feet began to slip along the substrate. Test substrates were attached to the force apparatus as described by Stark et al. (2012, 2013), in which a large Rubbermaid container held the surfactant solution and allowed the gecko’s feet to be completely submerged (~1cm in height). During the course of the experiment, the presence or absence of dry patches on the adhesive toe pads after the completion of each trial was noted as a way to measure the maintenance of an air plastron. Dry patches appear white and are dry to the touch, whereas wet toe pads become grey (see Fig. 2 in Stark et al., 2012). This was logged in a sub-set of trials and thus percentages of wet or dry toe pads are calculated based on this sub-set of trials rather than on the entire set of experimental trials.

Substrates and gecko feet were washed thoroughly and immediately with water at the completion of each set of trials. Geckos were never tested more than once a day and all geckos were given at least a one-day rest between trials. Substrates were washed with alcohol and water prior to experimental trials.

Statistical Analysis

The effect of substrate type (glass, OTS-SAM coated glass, or PTFE) and surfactant treatment (anionic or nonionic) on shear adhesion was tested using a repeated-measures MANOVA. Each gecko was tested in all combinations of treatments, which effectively removed the need to account for individual differences in such features as area of the toe pads. A matched-pairs analysis was used to compare specific
treatments of interest, such as the comparison between the two surfactant types on each of the three substrates. Means are reported as mean ± 1 s.e.m.

Results

The average weight of the seven Tokay geckos (*Gekko gecko*) used during experimental trials was 93.7 ± 2.3g. When testing the effect of substrate (glass, OTS-SAM coated glass, or PTFE) and surfactant treatment (anionic or non-ionic) on shear adhesion, we found that shear adhesion was significantly affected by substrate, treatment, and the interaction of substrate and treatment ($F_{2,11}=5.098$, $p=0.0271$; Table 4.1). Specifically, we found that there was no significant difference in shear adhesion between the two surfactant types when tested on glass (0.32 ± 0.02N anionic, 0.35 ± 0.03N non-ionic; $t = -1.00$, d.f. = 6, $p = 0.3547$) but there was a significant difference in shear adhesion when tested on the OTS-SAM substrate (0.32 ± 0.05N anionic, 0.51 ± 0.06N non-ionic; $t = -4.87$, d.f. = 6, $p = 0.0028$) and the PTFE substrate (0.27 ± 0.02N anionic, 1.20 ± 0.27N non-ionic; $t = -3.50$, d.f. = 6, $p = 0.0129$). On both of the latter substrates (OTS-SAM and PTFE) the non-ionic surfactant allowed for significantly higher shear adhesion than did the anionic surfactant. Values of shear adhesion from animals tested when each of the three substrates was fully submerged in water or was tested in air were adapted from Stark et al. (2013) and are included in Figure 4.4 for reference but were not quantitatively compared due to differences in experimental subjects.
Table 4.1. The MANOVA table shows a significant difference in shear adhesive force across treatment (non-ionic and anionic soap), substrate (glass, OTS-SAM coated glass, and PTFE) and the interaction between substrate and treatment. The F-statistic is from the Wilks’ lambda test. Asterisks denote significance values where $p < 0.05$.

<table>
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<th>Denominator df</th>
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<tr>
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<td>5.098</td>
<td>2</td>
<td>11</td>
<td>0.0271*</td>
</tr>
</tbody>
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Figure 4.4. Bars represent maximum shear adhesion when tested in an anionic surfactant (black bars) and a non-ionic surfactant (grey bars) on three substrates (glass, OTS-SAM coated glass, and PTFE). Points represent maximum values of shear adhesion from a previous study in which geckos were tested in air (black) and in water (white) on each of the three substrates; values for air and water are adapted from Stark et al. (2013). Error bars are mean ± 1 s.e.m.

We inferred whether an air plastron formed or not by noting the presence or absence of dry setae at the completion of each test. When geckos were tested in the anionic surfactant all toes wetted immediately and the plastron failed to form (except for one instance on glass). When tested in the non-ionic surfactant, geckos tested on
glass and OTS-SAM coated glass finished the adhesion tests with partially dry toes about 60% of the time (60% for glass; 58.8% for OTS-SAM). Conversely, when tested in the non-ionic surfactant on PTFE we observed full or partially dry toes in all of the trials measured (100%).

Discussion

Compared to studies in laboratory environments, published observations of geckos in their natural environment are rare. Even fewer reports document how geckos and other pad-bearing lizards behave under wet conditions, such as rain, high humidity, or fog (Lopez-Darias et al., 2012; Marcelli, 1971; Werner, 1990), conditions that should be challenging for adhesive pad-bearing species that rely on a van der Waals-based adhesive system (Autumn et al., 2002). We hypothesized that the dry contact necessary for adhesion in water is facilitated by an air plastron that keeps the toes dry by expelling water from the adhesive interface (Fig. 4.2). Because a surfactant lowers the surface tension of water, we predicted that the plastron would fail to form and adhesion would be significantly impaired. We also hypothesized that variation in the amount of surfactant absorbed on the gecko’s toe and in the wettability of different substrates would have a significant effect on adhesion. To investigate these hypotheses we tested geckos on three substrates with different $\theta_y$ in two different surfactants (anionic and non-ionic). We found that shear adhesion in surfactant solutions is significantly lower than in water on all substrates (Fig. 4.4). This was especially true for the anionic surfactant, for which the air plastron failed to form in virtually all trials. Conversely,
when testing shear adhesion in the non-ionic surfactant, geckos generated significantly higher shear adhesion on the OTS-SAM and PTFE substrates than when tested in the anionic surfactant, and in most cases the plastron was sustained in the non-ionic treatment.

When observing the toe pads after experimental trials in an anionic surfactant, we found that the toes were immediately and completely wetted (Wenzel wetting state; Fig. 4.1b). Because the plastron did not form, it is likely that water was not excluded from the adhesive interface (Fig. 4.2b); however, it is possible that water could be expelled on a microscopic scale during shear-sliding along the substrate resulting in direct contact between the setae and substrate. To investigate the interaction of the two surfaces in the surfactant solution when water is completely expelled at the interface, we used a generalized work of adhesion model that can predict the relative work of adhesion in a surfactant-water solution ($W_{wet}$) when two surfaces are separated in the direction normal to each other. Model calculations were made in the normal geometry, rather than shear, due to the lack of a clear approach to modeling shear adhesion. There is a correlation between normal and shear adhesion, however (Israelachvili et al., 1994), and thus our model allows us to predict adhesion in the shear direction. We consider one surface to be a gecko’s hair-like surface ($h'$), which can either be coated with surfactant or not, and the other surface to be one of the three substrates used in experimental trials ($s'$), which may also have a surfactant coating. We can then estimate the $W_{wet}$ using the following equation (Equation 1) where $A_c$ is the contact area, $\gamma_{s'-sw}$ is the interfacial energy of the substrate and the surfactant-water
solution, $\gamma_{h'\text{-sw}}$ is the interfacial energy of the gecko’s hair-like surface and the surfactant-water solution, and $\gamma_{s'-h'}$ is the interfacial energy of the substrate and the gecko’s hair-like surface. This equation assumes that the surfaces in contact are dry and when separated, the surfaces are in contact with the surfactant-water solution.

$$W_{\text{wet}'} = A \cdot (\gamma_{s'-\text{sw}} + \gamma_{h'-\text{sw}} - \gamma_{s'-h'}) \quad (1)$$

Considering the OTS-SAM substrate first, we know that $\gamma_{s'-\text{sw}}$ will be lower than pure water because the anionic surfactant adsorbs with the charged head groups facing the surfactant-water solution (Fig. 4.3), causing the surface to become negatively charged and lowering the interfacial energy. Because the plastron failed to form it is also likely that the surfactant molecules have adsorbed with the hydrophobic tail facing the surface of the gecko’s toe (Hsu et al., 2012), leading to wetting of the toe pads. Thus $\gamma_{h'-\text{sw}}$ should also decrease when compared to that of the gecko’s surface in contact with pure water. Finally, we expect that the paired negative charges on the toe and the substrate will cause electrostatic repulsion and thus increase the interfacial energy ($\gamma_{s'-h'}$). Incidentally, these negatively charged surfaces can also cause water to become trapped (Hsu and Dhinojwala, 2012), which would further reduce adhesion. As a result of all these effects, we predict that the value of $W_{\text{wet}'}$ is lower on OTS-SAM in anionic surfactant solution than the work of adhesion in water alone ($W_{\text{wet}}$). This matches our results (Fig. 4.4).

A similar argument can be used to predict the trend of $W_{\text{wet}'}$ for the glass and PTFE substrates, in which the $\gamma_{h'-\text{sw}}$ term is consistently lower in the anionic surfactant solution than when tested in pure water due to surfactant adsorption on the toe. On the
glass and PTFE substrates it is possible that the surfactant does not adsorb on the surface; however, again, the overall net $W_{\text{wet}}$ should still be lower in the anionic surfactant than in water, simply based on $\gamma_{h'-\text{sw}}$ alone. On the PTFE substrate the effect on $\gamma_{s'-h'}$ is unclear as the interaction of the surfactant-covered toe and fluorinated groups at the surface of PTFE may cause $\gamma_{s'-h'}$ to increase; however, this would still cause an overall net decrease of $W_{\text{wet}}$. In conclusion, we can expect that the gecko’s adhesion should be lower in the anionic surfactant solution than in water on all three substrates. This prediction is based on three possible scenarios: (1) the toes become wet and shear adhesion is reduced as it is in pure water (Stark et al., 2012), (2) the plastron is unstable and therefore cannot help to create a dry contact interface, and (3) if a dry contact interface does occur, $W_{\text{wet}}$ should still be lower based on changes in interfacial energy due to the adsorption of surfactant molecules.

In contrast to the anionic solution, which was completely wetting, we found that in about 60 - 100% of the observed experimental trials, parts or all of the toe remained dry when tested in the non-ionic surfactant. Interestingly, this only resulted in improved shear adhesion (when compared to the anionic treatment) on the OTS-SAM and PTFE substrates. Using Equation 1 we can make predictions about the work of adhesion in the non-ionic surfactant solution, assuming that dry contact occurs. Unlike the anionic surfactant, the plastron is maintained in 60-100% of the non-ionic surfactant treatments; thus, the non-ionic surfactants are not adsorbed, or are only partially adsorbed, on the toe pads. Furthermore, the non-ionic surfactant is not charged. Taken together, we would expect $\gamma_{h'-\text{sw}}$ to be lower for the non-ionic surfactant treatment than
for a pure water treatment, but not as low as $\gamma_{h'-sw}$ for the charged and fully wetting anionic treatment. This should be relatively consistent across all three substrate treatments, given the percentage of dry toe pads observed.

When investigating the surface term, $\gamma_{s'-sw}$, in the non-ionic surfactant treatment on OTS-SAM we know that $\gamma_{s'-sw}$ is reduced because the surfactant is expected to adsorb with the polar head group facing the surfactant-water solution (Fig. 4.3); however, we expect that $\gamma_{s'-sw}$ for the non-ionic surfactant is not as low as $\gamma_{s'-sw}$ for the anionic surfactant. Thus, we would expect $\gamma_{s'-sw}$ to be lower than for pure water but not as low as $\gamma_{s'-sw}$ in presence of anionic surfactants. For both the glass and PTFE substrates it is possible that $\gamma_{s'-sw}$ does not differ from $\gamma_{s-water}$ as surfactant may not be adsorbing on either of these substrates, similar to our predictions with the anionic surfactants. Therefore, the surface term ($\gamma_{s'-sw}$) suggests that glass and PTFE have higher predicted adhesion values than does OTS-SAM when making dry contact, which is not entirely the case here (Fig. 4.4).

Finally, due to plastron formation on the toes and the alignment of surfactant on the substrates, it is difficult to predict $\gamma_{s':h'}$ for the non-ionic surfactant treatments. Overall, however, the partial reduction of $\gamma_{h'-sw}$ should lower $W_{wet'}$ when compared to water but not as low as $W_{wet'}$ for the anionic surfactant solution that completely wets and coats the toe pads. Again, this equation is assuming dry contact between the two surfaces, which may also be more likely to occur in these treatments due to the maintenance of the plastron. Interestingly, however, the plastron does not always aid in making dry contact, as we found with geckos tested in water on hydrophilic glass (Stark
et al., 2013). In this context we found that the air plastron, in coordination with the hydrophilic glass substrate, caused a water layer to become trapped between the foot and the glass substrate, disallowing it from making adhesive contact. This behavior may be occurring here, causing our values of shear adhesion on glass to be lowered further.

Using our observations of the toe pads, our results on whole-animal shear adhesion, and our estimates from simplified models, we can begin to describe how surface tension and surface energy relates to wet adhesion in the gecko adhesive system. Our results are relevant for two reasons. First, although it is unlikely that a gecko would encounter fluids with low surface tension in their natural environment, our results with surfactant highlight the importance of the air plastron and its ability to keep the toes dry and help expel water at the contact interface. Interestingly, a plastron is required for wet adhesion (in water or surfactant); however, it does not guarantee adhesion, as pointed out in the case of hydrophilic substrates where water can become trapped. Secondly, the adsorption of anionic surfactant molecules, which was confirmed by the complete and immediate wetting of the toe pads, suggests that the toes themselves were hydrophobic prior to the adsorption of surfactant. Interestingly, the uncharged surfactant did not cause the toes to fully wet. This difference should not relate to the difference in surface tension of the surfactant solutions, which is similar (γ = 37 mN/m for SDS and γ = 39.4 mN/m for POE), but rather relates to the adsorption of the anionic surfactant molecules on hydrophobic toe pads. Our results show that the interaction of the superhydrophobic toe pad, the plastron it creates, the substrate it
contacts, and the interfacial energies of each of the surfaces and interfaces are all essential for adhesion to occur on wet surfaces.

The retention of an air plastron is directly related to the superhydrophobicity of the geckos’ toe pad (Poetes et al., 2010). With the recent discovery of lipids on geckos’ setae (Alibardi et al., 2011; Hsu et al., 2012), we hypothesize that lipids aid in the formation of a superhydrophobic surface and the resulting air plastron. Cutaneous lipids in the reptilian epidermis are mainly associated with reducing water loss (Alibardi et al., 2011; Lillywhite, 2006); however, we believe that the evolution of the lipid-keratin association in the setae may also help create this highly important property for wet adhesion. Our results provide a first step in exploring how setal surface chemistry and the charge on the substrate can affect adhesion underwater. Additionally, our results also have significant implications for the design of a synthetic adhesive that can be reused repeatedly in air and water (Kizilkan et al., 2013) and perhaps even in fluids with lower surface tensions, like blood ($\gamma = 58\text{mN/m}$), for the potential development of a more versatile medical bandage.

Acknowledgements

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CHAPTER V

SELF-DRYING: A GECKO'S INNATE ABILITY TO REMOVE WATER FROM WET TOE PADS

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Summary

When the adhesive toe pads of geckos become wet, they become ineffective in enabling geckos to stick to substrates. This result is puzzling given that many species of gecko are endemic to tropical environments where water covered surfaces are ubiquitous. We hypothesized that geckos can recover adhesive capabilities following exposure of their toe pads to water by walking on a dry surface, similar to the active self-cleaning of dirt particles. We measured the time it took to recover maximum shear adhesion after toe pads had become wet in two groups, those that were allowed to actively walk and those that were not. Keeping in mind the importance of substrate wettability to adhesion on wet surfaces, we also tested geckos on hydrophilic glass and an intermediately wetting substrate (polymethylmethacrylate; PMMA). We found that time to maximum shear adhesion recovery did not differ in the walking groups based on substrate wettability (22.7 ± 5.1min on glass and 15.4 ± 0.3min on PMMA) but did have
a significant effect in the non-walking groups (54.3 ± 3.9min on glass and 27.8 ± 2.5min on PMMA). Overall, we found that by actively walking, geckos were able to self-dry their wet toe pads and regain maximum shear adhesion significantly faster than those that did not walk. Our results highlight a unexpected property of the gecko adhesive system, the ability to actively self-dry and recover adhesive performance after being rendered dysfunctional by water.

Introduction

The self-cleaning property of the adhesive toe pads of geckos has inspired and challenged material design of synthetics that are both adhesive and self-cleaning (Sethi et al., 2008; Lee and Fearing, 2008; Kim et al., 2009a; Lee and Fearing, 2012). The benefit to having a self-cleaning or an anti-fouling adhesive is clear. An adhesive that can clean itself, or avoid fouling all together, is likely one that can also be used multiple times and can be used on non-pristine surfaces such as those covered with dirt or dust. The self-cleaning behavior of the gecko’s toes has two components. First, toes are cleaned by a passive self-cleaning method where dirt particles are more attracted to the surface a gecko walks on than the adhesive hairs, setae, which make up the small adhesive units of the toe pad (Autumn et al., 2000; Hansen and Autumn, 2005). By lightly touching a dirty gecko toe to a clean surface dirt is removed and adhesion is recovered by 35.7% after eight simulated steps (Hansen and Autumn, 2005). Recently however an active self-cleaning mechanism was also confirmed (Hu et al., 2012). In active self-cleaning the peeling nature of the gecko toe via digital hyperextension helps
expel dirt particles from the toe pads, significantly improving shear adhesion to nearly 80% of their original grip in only four steps (Hu et al., 2012). The application of these findings are highly relevant to bio-inspired materials design, showing that after repeated use the fouled adhesive actually regains its adhesion rather than loses it. This recovery property is certainly not applicable for most pressure sensitive adhesives or commercially available adhesive tapes that can be easily contaminated (Lee and Fearing, 2008; Lee and Fearing, 2012; Persson, 2007a).

Another innate and not entirely independent property of the gecko toe pad is its anti-wetting behavior. Similar to the self-cleaning property, gecko toes do not foul easily with water and although many synthetic adhesives either fail when used in water or after being exposed to moisture, the gecko toe pad is superhydrophobic and has a low contact angle hysteresis which causes water drops to bead up on a gecko toe and easily roll off without penetrating into the adhesive pad (Autumn and Hansen, 2006; Liu et al., 2012). In addition to cleaning dirt and water from the toe, we hypothesized that the anti-wetting toe pads should also allow the gecko to use its adhesive system in wet environments (Stark et al., 2012; Stark et al., 2013). As we found recently however, this is only partially true. In some instances a gecko toe can expel water trapped between the toe and a surface, but this is dependent on the thickness of the water layer (Stark et al., 2012; Hsu et al., 2012) and the wettability of the substrate the gecko clings to (Stark et al., 2013). In fact, under certain conditions the toes can even lose their anti-wetting property (Stark et al., 2012; Stark et al., 2013; Pesika et al., 2009). For instance, we observed that geckos climbing surfaces wet with water droplets began to slip after
running multiple times along that surface (Stark et al., 2012). After inspecting their toes it was clear that the toe pads had become wet with water. We tested shear adhesion of wet toes to a dry glass substrate and found that even after taking four complete steps (involving digital hyperextension), shear adhesion was significantly lower (1.31 ± 0.12N) than geckos tested with dry toes (17.96 ± 3.42N) (Stark et al., 2012). The results from this experiment show that even with the peeling action of four steps on a dry surface, similar to the active self-cleaning of dirt, geckos were only just able to support their body weight on a smooth glass substrate (~1N of force for a 100g gecko), providing no safety factor for adhesion to the highly variable surfaces in their natural environment.

Previous studies highlight the gecko's retention of a high safety factor on smooth surfaces, approximately 20 times their body weight or more for a 100g Tokay gecko (Gekko gecko) (Stark et al., 2012; Stark et al., 2013; Niewiarowski et al., 2008; Irschick et al., 1996; Autumn, 2006). While there may be many reasons for the disparity between the necessary force to support body weight and the actual force available to geckos, wet toe pads may be one such factor. Many gecko species are native to tropical environments that experience high levels of atmospheric humidity and rainfall, likely wetting the surfaces a gecko moves across. While much effort has been focused on measuring the maximum adhesion geckos can obtain using dry toe pads on dry surfaces (Niewiarowski et al., 2008; Irschick et al., 1996; Losos, 1990b; Bergmann and Irschick, 2005; Niewiarowski et al., 2012), an important question remains: how do non-functional toes that have become wet, perhaps after moving repeatedly across wet, rain soaked surfaces become dry and functional again? Is there some mechanism to enhance the
removal of water and speed up the time it takes to regain adhesion? Contrary to the findings of self-cleaning dirt particles, our previous results do not show strong evidence for enhanced self-drying of toe pads that have become wet after four steps on hydrophilic glass (recovering only about 7% of their shear adhesion) (Stark et al., 2012). Yet we expect geckos to encounter wet surfaces in many of their native habitats and to need to move successfully across them, which includes regaining adhesion after being fouled with water.

Although many studies focus on testing gecko adhesion on hydrophilic glass (Stark et al., 2012; Niewiarowski et al., 2008), in their native environments geckos likely move across a diversity of surfaces, including those that are hydrophobic, like many plant leaves. The effect of wet toe pads on adhesion to a hydrophobic substrate has yet to be investigated but could help explain how geckos regain or even maintain functionality of their adhesive system in tropical environments where their toes can wet with water. Using the self-cleaning of dirt particles as an example, we hypothesize that active walking or stepping, using the gecko’s unique stick-peel mechanism (digital hyperextension), will help to expel water from the toe pads and recover shear adhesion at a faster rate when compared to treatments when individuals are not allowed to step. Because the adhesive system is van der Waals-based (Autumn et al., 2002), separation of the toe from the surface by a water layer can interrupt van der Waals forces and therefore we expect a hydrophilic glass substrate to be the least effective substrate for initial adhesion, as layers of water are more likely to remain trapped between the toe and the hydrophilic surface than mutually expelled by two hydrophobic surfaces (see
Stark et al., 2013). As the gecko steps however, the hydrophilicity of the glass may help to pull water from the toe pads, recovering adhesion at a faster rate than a hydrophobic surface. To investigate both the effect of active self-drying (stepping) and substrate wettability on the recovery of shear adhesion after toe pads become wet, we tested geckos on a hydrophilic substrate (glass) and an intermediately wetting substrate (polymethylmethacrylate; PMMA) that we know geckos can adhere to underwater (Stark et al., 2013). Geckos were either allowed to actively walk across the substrate prior to adhesion measurements or they were confined for a similar time period and not allowed to actively move across the testing substrate. Our results have significant implications for an improved understanding of gecko ecology, behavior and toe pad evolution, as well as for the novel design of a synthetic gecko-like adhesive that can recover functionality after becoming wet.

Materials and Methods

Six adult Tokay geckos (Gekko gecko) were used for experimental trials. Geckos were individually housed as described in Niewiarowski et al. (2008) and fed cockroaches three times a week and misted twice a day with water. Prior to experiments geckos were introduced to a walk-in environmentally controlled chamber that was kept at 24.2 ± 0.1°C and 31.4 ± 0.1% relative humidity for all experiments. After acclimating for at least 10 min, geckos were then acclimated to a foot soaking treatment. Foot soaking treatments were carried out similar to Stark et al. (2012) where geckos were placed on a wet cloth and their toes were agitated to induce wetting of their toe pads for 11.0 ±
0.3min. Toe pads were visually inspected and confirmed to be completely wet (toe pads appear grey in color and are no longer superhydrophobic; see Figure 5.1A verses Figure 5.1B) prior to placing the gecko in standing water for at least 20min. The 20min time interval was chosen because a wetting transition in gecko setal mats appears to occur after 20min of exposure to water (Pesika et al., 2009). After the agitation period, all geckos were placed in plastic tubs that had ~0.5cm deep water, enough to fully submerge their feet, and were soaked for 24.8 ± 1.7min. Water was kept at 21.9 ± 0.1°C. After the 30min total acclimation time (10min pre-soak and 20min soak with feet held underwater), geckos were then removed and their bodies were towel dried to remove excess water. Their toes were not touched but drip dried until water droplets stopped falling from their toe pads.

Figure 5.1. Wet and dry Tokay gecko (*Gekko gecko*) toe pads. (A) Dry foot in contact with a glass substrate where the setal mats appear white in color and (B) a wet foot in contact with a glass substrate where the setal mats appear grey in color. When wet the toe pads are no longer superhydrophobic and water droplets fall into the setal mat, completely wetting it.
After soaking treatments geckos were either tested immediately or were induced to walk at least 10 steps with the front and at least 10 steps with the back feet on either an inclined glass or inclined polymethylmethacrylate (PMMA) substrate. Inclined substrates were used to induce digital hyperextension (toe peeling) (Russell and Higham, 2009). Average number of steps for each treatment is reported below. When testing shear adhesion on a glass substrate, geckos walked along a hydrophilic glass substrate to self-dry. The same was true for the PMMA substrate. After testing shear adhesion the geckos were introduced to a small dry plastic box to prevent further stepping. Each gecko was tested at 0min, 15min, 30min, 45min and 60min post-soak in succession, confined in the small box between timed adhesion tests. All six geckos were randomly tested in all experimental conditions which included adhesion tests on both glass (hydrophilic) and PMMA (intermediate wetting), with and without 20 steps prior to being tested at each time interval. Stepping substrates and adhesion testing substrates were cleaned first with ethyl alcohol and then water between each gecko. At the completion of each experimental trial geckos were weighed.

Shear adhesion was measured vertically using a custom rig as outlined by Niewiarowski et al. (2008). Geckos were outfitted with two pelvic harnesses and induced to take about four vertical positioning steps on the test substrate. Once all four feet had taken a step we moved a motorized force sensor at a controlled rate which pulled the gecko down the substrate via the harnesses that were attached to both the gecko and the motile force sensor. Maximum adhesion was measured as the point where all four feet begin to slide along the substrate. In some cases we found that
damage occurred before all four feet began to slide, where strips of lamellae detached from the sliding toes. Because we were interested in the recovery time of shear adhesion, rather than maximum force, we outlined an experimental threshold for what we considered "time to maximum shear adhesion". First, force values near or above 20N of force were considered "maximum" based on our previous average forces on glass and PMMA (Stark et al., 2012; Stark et al., 2013). Once a force reading of near or above 20N was recorded we stopped testing that individual and no further timed adhesion tests were completed, therefore in this scenario time to maximum shear adhesion was recorded for the trial where ~20N was reached. Second, we considered time to maximum shear adhesion to also be instances where damage to the toes occurred, even if this was below the 20N threshold. When this occurred we also discontinued further timed testing and recorded the time where damage occurred as the maximum force the animal could sustain (to the point of damage). Finally, in some treatments, specifically the non-stepping glass treatment, neither "maximum" force (~20N) nor toe damage occurred after 60min of testing, making this last time interval (60min) our final cut-off for repeated timed testing. 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).

We used a repeated measures MANOVA to test for an effect of substrate type (glass or PMMA) and self-drying treatment (stepping or no stepping) on time to maximum shear adhesion. Each gecko is tested under all combinations of treatment effects, removing the need to account for differences in toe pad area. To investigate
time to maximum shear adhesion values of each treatment group we used a matched pairs analysis. Means are reported as mean ± 1 s.e.m.

Results

The average weight of the six Tokay geckos (*Gekko gecko*) during the experimental trial period was 102.87 ± 3.30g. In the self-drying stepping trials geckos stepped an average of 72 ± 10 steps on the glass substrate and 67 ± 4 steps on the PMMA substrate before the time to maximum shear adhesion threshold occurred, this includes approximately four steps to position themselves on the experimental apparatus. In the non-stepping group, where active self-drying was prevented, geckos were only allowed approximately four steps to position themselves on the substrate prior to adhesion testing. Geckos stepped an average of 18 ± 1 positioning steps on the glass substrate and 11 ± 1 positioning steps on the PMMA substrate in the non-stepping treatment groups before time to maximum shear adhesion was reached (Figure 5.2).
When testing for the effect of substrate (glass or PMMA) and self-drying treatment (stepping or non-stepping) on time to maximum shear adhesion we found a significant interaction between substrate and self-drying treatment \( (F_{1,10} = 9.54, p = 0.0115; \text{Table 5.1}) \). When geckos were not allowed to self-dry (non-stepping) and were tested on the glass substrate it took significantly longer to regain shear adhesion \( (54.3 \pm 3.9 \text{ min}) \) when compared to when stepping on glass \( (22.7 \pm 5.1 \text{ min}; t = -7.98, \text{df} = 5, p = 0.0005) \) and stepping on PMMA \( (15.4 \pm 0.3 \text{ min}; t = -10.30, \text{df} = 5, p = 0.0001) \). The non-stepping glass treatment also took longer to achieve time to maximum shear adhesion
than the PMMA non-stepping treatment (27.8 ± 2.5min; t = -7.28, df = 5, p = 0.0008).

When we compared stepping on glass with the remaining groups we found that time to maximum shear adhesion did not differ from either the PMMA stepping treatment or the PMMA non-stepping treatment (t = -1.44, df = 5, p = 0.2094 and t = 1.03, df = 5, p = 0.3496, respectively). Finally, when tested on PMMA we found a significant difference in time to maximum shear adhesion between stepping and non-stepping treatments (t = -5.00, df = 5, p = 0.0041)(Figure 5.2).

Table 5.1. Repeated measures MANOVA shows a significant difference in time to regain maximum shear adhesion based on substrate (glass or PMMA), treatment (stepping or no stepping) and their interaction.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Wilks' lambda</th>
<th>Exact F</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3.42</td>
<td>34.23</td>
<td>1</td>
<td>10</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Substrate</td>
<td>2.94</td>
<td>29.43</td>
<td>1</td>
<td>10</td>
<td>0.0003*</td>
</tr>
<tr>
<td>Substrate X Treatment</td>
<td>0.95</td>
<td>9.54</td>
<td>1</td>
<td>10</td>
<td>0.0115*</td>
</tr>
</tbody>
</table>

Discussion

Many previous studies have focused on the remarkable properties of the gecko adhesive system. It is self-cleaning, superhydrophobic, functional in water under specific circumstances, strong yet reversibly and directionally adhesive, reusable and virtually surface-insensitive (Autumn et al., 2000; Hansen and Autumn, 2005; Autumn and Hansen, 2006; Stark et al., 2013; Autumn et al., 2002; Tian et al., 2006; Gravish et al., 2008; Autumn and Peattie, 2002). In this study we tested a new hypothesis: gecko toe pads are self-drying. It is counterintuitive that geckos from tropical environments routinely encounter wet surfaces which make their toes dysfunctional (Stark et al.,
yet have no way to regain their adhesion quickly. In response to this we found that active self-drying of toe pads occurs in Tokay geckos (*Gekko gecko*), and that substrate wettability does not have an effect on time to recovery.

When comparing stepping, or active self-drying, recovery times on either substrate (glass or PMMA) we found that stepping significantly quickened the time to regain maximum shear adhesion when compared to not stepping, allowing passive evaporation to dry the toe pads. This occurred on both substrates but the overall difference in time (non-stepping verses stepping) was larger when using glass as a substrate (difference of 31.7 ± 4.0min on glass and 12.4 ± 2.5min on PMMA). This supports our hypothesis that hydrophilic glass helps to wick away water more efficiently than a more intermediately wetting substrate like PMMA. Interestingly, while stepping on PMMA had the fastest recovery time (15.4 ± 0.3min), this did not significantly differ from stepping on glass (22.7 ± 5.1min). Therefore the time to maximum shear adhesion in active self-drying is not dependent on substrate wettability. When geckos were not allowed to step however, substrate had a significant impact on time to maximum shear adhesion, as geckos were able to regain adhesion through passive drying on PMMA faster than glass. This difference was large (a difference of 26.5 ± 3.6min) and it is important to note that in fact, half of the glass non-stepping group never reached the maximum force threshold (~20N or material failure) during our experimental trials. If we use the linear regression of force across time in the glass non-stepping group we can estimate that full adhesive recovery, assuming recovery rate is linear, will occur around 99min, as shown as a "*" in Figure 5.2. This difference, of about 71min, when compared
to passive drying on PMMA is striking and shows that substrate wettability when passively drying (not stepping) has a strong effect on how quickly a gecko regains function of its adhesive system. It is not clear why active self-drying (stepping) is substrate insensitive and passive self-drying (non-stepping) is so clearly substrate sensitive, especially since we do not expect there to be a significant difference in surface roughness or amount of water initially held within the toe pad, which could contribute to differences in time to maximum shear adhesion. Using our previous work (Stark et al., 2013), we can explore this observation by considering the work of adhesion (W) to separate two surfaces (gecko setae and the substrate) in a direction normal to the surfaces when water is trapped between the setae prior to and during contact with either the glass or the PMMA substrates in air (Figure 5.3). We model the gecko setae as an oil-like surface (n-hexadecane) which is patterned in the shape of a tetrad (four setae) (see Stark et al., 2013). When this surface (h) makes contact with either substrate (s; glass or PMMA) water is held in the setal mat and does not interfere with the dry contact interface (an assumption of the model). Using Equation 1 we can predict adhesion between the two surfaces (h and s) where $A_c$ is the total contact area ($64\mu m^2$) and $A_s$ is the area of the substrate ($121\mu m^2$). Contact angles for the substrate (glass or PMMA) with n-hexadecane ($\theta_1$) and water ($\theta_2$) where measured elsewhere (Stark et al., 2013). Finally, the surface energy of the gecko surface (h) in air ($\gamma_{h\text{-}air} = 25mJ/m^2$) and the surface tension of water ($\gamma_{air\text{-}water} = 72mJ/m^2$) are used.

\[
W = A_c \gamma_{h\text{-}air}(1 + \cos \theta_1) + (A_s - A_c)\gamma_{air\text{-}water}(1 + \cos \theta_2)
\]

(1)
Using Equation 1 and reporting the work of adhesion as a ratio ($W_{\text{PMMA}}:W_{\text{glass}}$), we see that the work of adhesion ratio is 0.77, favoring adhesion on glass. Thus our thermodynamic model used here and previously (Stark et al., 2013), does not explain why geckos passively recover adhesion on dry PMMA faster than dry glass when water is trapped between the adhesive mats. This suggests that there are factors other than surface energies, such as water at the adhesive interface between the hairs and substrate, which causes passive adhesive recovery on glass to be much slower than PMMA.

![Figure 5.3](image)

**Figure 5.3.** Schematic of the work of adhesion model geometry. Schematic depicts a patterned gecko surface (pattern of four setae represented as yellow pillars) filled with water (blue) both prior to and during contact with the substrate (either glass or PMMA) in air (white space).

Contrary to self-cleaning studies in beetles and passive self-cleaning in geckos (Hansen and Autumn, 2005; Clemente et al., 2010), our self-drying results show that over time and across steps adhesion is fully recovered. This is likely because water eventually evaporates in addition to being actively removed from the toes during active self-drying, whereas dirt particles can be trapped within the adhesive pads (Hansen and Autumn, 2005; Hu et al., 2012; Clemente et al., 2010; Orchard et al., 2012). Unlike self-cleaning of well defined dirt particles in laboratory studies, it is difficult to partition the
contributions of evaporative drying and active removal of water via stepping. Clearly our results show active removal of water helps regain adhesion faster, but how? To observe differences in toe drying between the stepping (active drying) and non-stepping (passive evaporative drying) groups we imaged the toe pads at each of the early wetting intervals used for experiments. Initially toes in all groups were grey in color and clearly wet (Figure 5.1B) however after our treatment (stepping or non-stepping) we find distinguishable drying patterns at the next timed interval (15min) (Figure 5.4). Here we see that geckos who were not allowed to take self-drying steps had variable drying patterns on their toes, where some toes remained wet and others became partially dry, shown by a patchy gray and white (wet and dry) appearance, often producing a clear evaporation line within a single toe (Figure 5.4A). Conversely in active self-drying by stepping we see well defined wet and dry regions of each toe where the perimeter of the toe dries first, leaving a wet patch in the center of all the toes on the foot (Figure 5.4B). This observation was made regularly in experimental trials. At the 30min interval all toes appeared to be dry or nearly dry, where little grey color was observed and toes appeared qualitatively similar to a dry toe (Figure 5.1A). The difference in drying patterning is interesting because all groups were kept in small confinement boxes while waiting for the next timed testing interval, all being exposed to ambient evaporation, therefore the striking pattern difference in the self-drying group is clearly due to the active peeling mechanism of the toe.
Figure 5.4. Tokay gecko (*Gekko gecko*) active and passive toe pad drying patterns. Appearance of toe pads at 15min post-soak in non-stepping (A) and stepping (B) groups. Areas that are grey in color are wet and areas that are white in color are dry. Without stepping toes heterogeneously dry, where some toes are wet and others show an irregular evaporation line (A). Conversely, when allowed to actively step toes dry in a more homogenous fashion, where the outside of the toe dries first, leaving a wet patch (grey in color) in the center of each of the toes (B).

In self-cleaning models we know that detachment of seta may help to actively and rapidly expel dirt particles from the toe (Hu et al., 2012), so perhaps a similar mechanism is occurring in self-drying. When dynamically self-cleaning dirt the toes are peeled distally, the setae separate in a fan-like manner and dirt particles jump off the setae (Hu et al., 2012). If water droplets behaved similarly we would expect the toes to dry uniformly, however this is not the case. Likewise, if the fanning of the setae caused air to penetrate deeper into the setal mat for evaporation we would again expect no clear drying pattern. Instead we see an outer to inner medial pattern in the stepping groups. If we more broadly investigate the effect of morphological structuring on removal or transport of water however, there are several examples of structures in
other organisms. Perhaps the more notable of these systems is the tree frog adhesive
system. Tree frogs have a wet adhesive system where patterned microchannels hold
fluid for use in capillary adhesion (Persson, 2007b). To retain the fluid, the channels use
pressure differences to either move water out for adhesion or draw water back in for
rapid removal of the toe and conservation of the adhesive liquid for the next step
(Persson, 2007b). If we consider the wet gecko toe pad and the ordered array of setae,
we can make the comparison to the tree frog toe pad where water is held between the
setae of a wet toe and can be actively moved within the inner-setae and even inner-
lamellar channels. Unlike the tree frog, we would expect movement of water out of the
channels to be more highly emphasized than movement back into the gecko toe pad.
This may be why frog micro- and macrochannels are hexagonally packed, which helps to
move water in and out of the channels without removing it from the overall system
(Persson, 2007b), and why the gecko toe pad has channels that are linear, perhaps being
used to direct water out of the toe permanently.

Using model predictions based on tree frog toe pads, we can roughly calculate
the behavior of water in a wet gecko toe pad. Using the Tokay gecko (*Gekko gecko*) as
our morphological model and only focusing on one level of hierarchy, the inner-setal
regions, we estimate the spacing between the tetrad units to be about 2µm (Stark et al.,
2013). If we consider this distance to represent the width of the microchannels (W) and
h the height of the water layer between the toe and substrate, we find that W > h when
the toe approaches close contact with the substrate (i.e. where h \( \rightarrow 0 \)). Estimation of
the local pressure in both the fluid film and the inner-setal channels shows \( p_1 \approx -\gamma/r (r = \)
h/2) for the film and \( p_0 \approx -\gamma/r^* \) \((r^* = W/2)\) for the channel where the surface tension of water is \( \gamma \approx 0.07 \text{ N/m} \) (Persson, 2007b). The difference in pressure of the channels and thin film is thus \( p_1 - p_0 < 0 \) because \( W > h \), therefore fluid flows from the channeled setal mats to the space between the toe and substrate. This pressure difference drives the movement of water out of the setal mats. To detach their adhesive toe pads both geckos and tree frogs use a peeling step which changes the pressure difference \((p_1 - p_0 > 0)\) at the point where \( h \) is greater than \( W \). At this point \((h > W)\) the difference in \( p_1 \) and \( p_0 \) causes water to be drawn back into the toe. For tree frogs conservation of the water-lipid solution for the next adhesive step is advantageous, but for geckos it is clearly not and this could be why we see very defined patches of water in the toes pads after taking peeling steps, where water that was not completely removed from the toe-substrate interface is drawn back up into the setal channels at some critical peel angle where \( h > W \). In groups that were not allowed to peel (step), drying patterns are more heterogeneous (Figure 5.4A) and thus are likely due to passive evaporation rather than active transport of water out of the toe pad by changes in pressure from the pressing and peeling of the toes in the stepping groups. Although it deserves further investigation, we hypothesize that when wet with water the inner-setal and even lamellar channels can act like the channeled treds on tires to help expel excess water from wet toe pads so that adhesion to the substrate can be regained more quickly than in a non-patterned surface.

Although the discovery of the self-drying mechanism and the observations we made have direct relevance for application to synthetics, the biological relevance of
such a finding is also noteworthy. It is not entirely clear why adhesion is compromised when the toe pads of a gecko become wet but there are four possible explanations that may not be mutually exclusive. Generally, as expected, water layers between the setae and the substrate may cause the gecko to slip due to inadequate surface contact for van der Waals forces to occur. This occurs at the level of the toe with thick layers of water (~0.5cm) on glass (Stark et al., 2012; Stark et al., 2013) and this may also occur between the setal tips, the spatula, and the substrate when the setal mats are permeated with water. We also know that at high levels of relative humidity (> 80%) the setal modulus lowers (Prowse et al., 2011; Puthoff et al., 2010), which may impair the ability of the setae to orient and attach, especially when soaked in water for 30min as was done here. Seta can also become self-matted when the modulus lowers or capillary forces draw them together, again limiting attachment and adhesion. Finally, two studies have reported that surface chemistry of the setal mat changes in some way that has yet to be fully understood when in the presence of water (Hsu et al., 2012; Pesika et al., 2009). Our results here suggest that self-matting can be reduced or even eliminated by standing on the toes, as there is no clear evidence of matting in the toe pads when in contact with a substrate (Figure 4) and that any changes in surface chemistry or modulus of the material is reversible over a relatively short timeframe (~15min for adhesive recovery). Therefore it is most likely that actual removal of water is most critical for regaining adhesion after toes have become wet.

To our knowledge there exists little evidence of self-drying in the natural world. Insects, including beetles, and tree frogs, use capillary adhesion and thus self-drying
would be detrimental. For the dry adhesive system of the gecko however this appears to be imperative. But how can we relate these controlled laboratory tests to how geckos may utilize this unique property in their natural environments? First, we found that actively stepping significantly reduces the time it takes to regain maximum shear adhesion on either of the two substrates used. But how much distance is necessary to regain adhesion? If we estimate that Tokay geckos (Gekko gecko) can run 1m in 3-4 strides, where one stride is two steps (Autumn et al., 2006b) the self-drying groups took 30-40 strides on average, a distance of about 10m. Conversely the non-stepping groups took 5-10 strides, a distance of only 1-2m. While it is difficult to predict what geckos do in their native environments, it seems unlikely that geckos would move 10m at any one time. What is interesting here however is that we did not test geckos running, but rather taking controlled steps on an inclined surface. First, the distance covered by walking is likely much shorter than that by running, in fact we estimate that controlled stepping reduces the estimated running distance by at least half and second, the dynamic process of running may enhance self-drying and further lower the distance needed to regain maximum adhesion. In addition to the dynamics of running verses walking, our previous results where geckos took four steps on a dry horizontally mounted glass substrate with wet toes (Stark et al., 2012) are not comparable to those in this study where geckos took four steps on dry vertically mounted glass with wet toes, suggesting orientation may also have a significant effect on self-drying. The difference in initial cling forces on vertical and horizontally mounted glass (0N and 1.31 ± 0.12N respectively) suggests a gecko can cling better when sitting horizontally, likely due to the pressure of their body
weight and gravity helping to expel water, than those attempting to cling vertically.

Although it is difficult to observe how geckos behave in their native environments, our laboratory-based studies suggest hypotheses of potential behaviors geckos may utilize when exploiting their natural habitat.

While it is interesting to consider a gecko making behavioral choices about where to walk or run, for how long and in what direction, it is important to be reminded of the complexity of the system as a whole. Specifically, the impressive safety factor that geckos utilize will likely allow for negligible effects on overall adhesion when only one or two toes are wet and all others are dry. While the total number of wet toes can certainly vary in their natural habitats and change based on how they utilize their adhesive system (walk verses run), the high safety factor geckos use may allow them to self-dry one or two wet toes while using the others to sufficiently maintain adhesion.

Interestingly, we have observed that once wet, gecko toes are much more likely to become wet again over some time period. This was also suggested by Pesika et al. (2009) when observing setal patches. So while it is unlikely all the toes of a gecko get wet all at the same time, repeated wetting may be a significant problem for geckos. Thus active self-drying may be important not just for drying a newly wetted toe, but also to help remove water from the toe pad after repeated exposure. It is also important to note that ambient temperature and humidity can also play significant role in self-drying and this may be highly relevant to geckos living in the tropics where temperature and humidity levels are high and wet surfaces are more prevalent. Further studies should
investigate self-drying in different temperature and humidity regimes, pairing species-specific environmental values to rate of self-drying.

In this study we tested if an active self-drying mechanism, similar to the active self-cleaning mechanism, can help geckos recover the adhesive function of their toe pads. Our results reveal a surprising new property of the gecko adhesive system, the ability to self-dry and regain adhesion after being fouled by water. To our knowledge there are few, if any, instances were an adhesive, especially a non-permanent reusable adhesive, can regain adhesion after becoming wet. While this finding can be used to help improve synthetic adhesives, it is also relevant to gecko biology and helps to provide testable predictions about how geckos utilize their adhesive system in their natural environments. Clearly the natural habitat of geckos poses a variety of challenges, and as such we highlight here yet another new property of the gecko adhesive system, the ability to completely repair functionality after being rendered useless by water, the ability to actively self-dry.

Acknowledgements

The authors thank Scott Thomas for help with preliminary trials.
CHAPTER VI

RUN DON'T WALK: LOCOMOTOR PERFORMANCE OF GECKOS ON WET SUBSTRATES

In Review:

Stark, A. Y., Ohlemacher, J., Knight, A. and Niewiarowski, P. H. In Review. Run don’t walk: Locomotor performance of geckos on wet substrates.

Summary

The gecko adhesive system has been under considerable scrutiny for over a decade however little is known about how the 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 rain-soaked 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 hydrophilic or intermediately wetting and oriented either vertically or horizontally. Surprisingly we found no indication that wet substrates impact average sprint velocity over one meter, and rather, in some species sprint
velocity was increased on wet substrates rather than reduced. When investigating behaviors 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.

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; Irshick 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 (Losos, 1990b; Irshick et al., 1996; Bergmann and Irshick, 2005; Niewiarowski et al., 2008; Niewiarowski et al., 2012; Stark et al., 2012; Stark et al., 2013), yet in their native environments the substrates a gecko moves across are likely 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., 2006b; Dai et al., 2011). To add further complexity, natural environments are also unpredictable and 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; Autumn et al., 2002; Pesika et al., 2009; Stark et al., 2012; Stark et al., 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 gecko adhesion, we tested maximum shear adhesion on a glass substrate misted with water (Stark et al., 2012). We found that shear adhesion 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 (1.84 ± 0.54N misted; 17.96 ± 3.42N dry). This suggests that walking on wet hydrophilic glass negatively impacts shear adhesion, even after only four steps. This result is perplexing given that this species is endemic to tropical environments that commonly experience rainfall. Considering the diversity of substrates available to geckos in their environment, we also tested geckos on substrates that vary in wettability. We found that when geckos walk on wet hydrophobic substrates they cling as well as when the substrate is dry (Stark et al., 2013). Thus our results suggest that a gecko’s ability to cling or walk on natural substrates that have become wet, may not be impaired on hydrophobic substrates.
Since geckos are equally likely to walk or run between retreat and foraging sites as they are to cling to substrates while waiting for prey (Aowphol et al., 2006), quantifying the effects of water on clinging and clinging subsequent to walking may not capture the impact of wet surfaces on free ranging gecko locomotion. There is a broad literature on lizard locomotor biomechanics (Russell and Bels, 2001), however there exists 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., 2006b; Dai et al., 2011). Furthermore, although the mechanisms behind gecko adhesion have been studied across multiple scales (Irschick et al., 1996; Autumn et al., 2000; Autumn et al., 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 and horizontally, we would expect that a 100g Tokay gecko (Gekko gecko) requires a force equivalent to two times its body weight, 2N of force, to run one step (Autumn et al., 2006b). Using our previous shear adhesion results (Stark et al., 2012), we found that after four steps on a misted hydrophilic substrate geckos produced 1.84 ± 0.54N of force, suggesting that their ability to run either vertically or horizontally (assuming constant force requirements) could be compromised. 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 be likely 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 effects 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 (θ \textasciitilde 50°), and acrylic as a less hydrophilic substrate (θ \textasciitilde 85°). We also chose to test for an effect of running orientation (vertical or horizontal) for three reasons. First, our prior studies did not investigate shear adhesion on a vertical substrate (Stark et al., 2012; Stark et al., 2013). Second, geckos apply different forces when running vertically and running horizontally (Autumn et al., 2006b) 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 our previous work (Stark et al., 2012; Stark et al., 2013) and force values from geckos sprinting along a force plate (Autumn et al., 2006b), 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 run. Conversely, when tested on the acrylic substrate we hypothesized that the adhesive system would remain functional, as suggested by the static shear adhesion trials (Stark et al., 2013),
and geckos would be able to run equally fast on wet and dry acrylic in either orientation.

There are more than 1,400 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 have investigated species-level performance differences in the gecko adhesive system (Irschick et al., 1996; Niewiarowski et al., 2008; Russell and Higham, 2009), even though there appears to be both significant variation and also conservation of particular anatomical structures related to adhesion (Williams and Peterson, 1982; Peattie and Full, 2007; Gamble et al., 2012). To understand how water affects performance in multiple species of gecko we tested five species from tropical, sub-tropical and arid habitats (Table 6.1). In addition to measuring sprint velocity, we also recorded frequency of three specific observations which may affect 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 questioned if these were significantly related to running on a wet substrate (glass or acrylic), in any particular orientation (vertical or horizontal) and by any specific species. One major challenge to sprint performance may also be the physical change in the 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) and
therefore should negatively impact locomotor performance, however it is unclear if toe wetting occurs more often in particular orientations (vertical or horizontal) or on specific substrates (hydrophilic or intermediately wetting). Our primary goal with this study is to investigate the interaction between water and the gecko adhesive system in a more ecologically, behaviorally and evolutionarily relevant context.

Table 6.1. Species were chosen based on habitat and phylogenetic pairings where *P. dubia* and *P. bibronii* (Group 1) are more closely related to each other than they are to the other three species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample Size</th>
<th>Phylogenetic Group</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phelsuma dubia</em></td>
<td>4</td>
<td>1</td>
<td>Tropical</td>
</tr>
<tr>
<td><em>Pachydactylus bibronii</em></td>
<td>4</td>
<td>1</td>
<td>Arid</td>
</tr>
<tr>
<td><em>Gekko gecko</em></td>
<td>5</td>
<td>2</td>
<td>Tropical</td>
</tr>
<tr>
<td><em>Tarentola mauritanica</em></td>
<td>3</td>
<td>2</td>
<td>Sub-tropical</td>
</tr>
<tr>
<td><em>Rhacodactylus auriculatus</em></td>
<td>3</td>
<td>3</td>
<td>Sub-tropical</td>
</tr>
</tbody>
</table>

**Materials and Methods**

Geckos native to arid, sub-tropical and tropical environments were used: including, four *Pachydactylus bibronii* (Smith 1846), five *Gekko gecko* (Linnaeus 1758), three *Tarentola mauritanica* (Linnaeus 1758), three *Rhacodactylus auriculatus* (Bavay 1869) and four *Phelsuma dubia* (Boettger 1881) (Table 6.1). 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 in pairs (Table 6.1) as suggested by Garland and Adolph (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). As a result, we expect that differences between these two species will be due to an effect of treatment conditions on divergent characteristics rather than ancestry. We chose G. gecko to represent the Gekkonidae and T. mauritanica to represent the Phyllodactylidae phylogenetic branches where G. gecko is found in the tropics of Southeast Asia and T. mauritanica is found in subtropical regions of the Mediterranean (Zaaf and Van Damme, 2001; Gamble et al., 2011). Finally, R. auriculatus is a representative of 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 one hour to equilibrate to test temperature (26.2 ± 0.04°C) and humidity (61.4 ± 0.13%), and then chased by hand up or along a one meter race track (Huey et al., 1989). The race track was equipped with four sensors, placed at 25cm intervals, yielding three split time measures in cm/sec. 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 indentifying all toes that had become grey 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.

Geckos were allowed at least one hour rest between sprints and race order and treatment type were randomly assigned. Geckos were raced no more than three times per day with an hour break between races. After running on a misted substrate geckos were not allowed to run any additional races for at least a 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. gecko 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 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 is more relevant for geckos running across wet substrates. Differences in SV on dry and misted substrates were collapsed into a single dependent variable (SV<sub>D-M</sub>) 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<sub>D-M</sub> we used an ANOVA where SV<sub>D-M</sub> 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<sub>D-M</sub> as the response variable and substrate as the only independent variable. SV<sub>D-M</sub> 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 or toe pad area, as each individual contributed equally to all treatments (matched pairs analysis), therefore effectively serving as their own control across treatments.

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.

Results

We found no effect of substrate, running orientation or species on $SV_{D-M}$ ($F = 1.0555, \text{d.f.} = 15, p = 0.4199$). 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 thus they were analyzed separately. There was also no effect of substrate on $SV_{D-M}$ in trials where *R. auriculatus* was tested horizontally ($F = 0.3744, \text{d.f.} = 1, p = 0.5737$) (Fig. 6.1).
Figure 6.1. The difference of average sprint velocity on a dry substrate versus misted substrate across species in each substrate and orientation treatment group. Difference values (displayed on the y-axis) that are positive indicate higher velocity on dry compared to 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. Error bars are means ± 1 s.e.m.

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. 6.2). 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 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. 6.3), 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. 6.4).

![Figure 6.2. Total number of stops during all treatments separated by species. Bars with the same letter indicate no statistical significance between species. Error bars are means ± 1 s.e.m.](image-url)
Figure 6.3. 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. Error bars are means ± 1 s.e.m.

Figure 6.4. Total number of wet toes at the completion of all misted substrate treatments separated by orientation and substrate (HA = horizontal acrylic, HG = horizontal glass, VA = vertical acrylic, VG = vertical glass). After controlling for multiple tests, none of orientation and substrate treatments were significantly different from one another although the interaction was in significant in the model (F = 6.5515, d.f. = 1, p = 0.0129). Error bars are means ± 1 s.e.m.
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; Puthoff et al., 2010; Prowse et al., 2011; Stark et al., 2012; Stark et al., 2013), although to date all have focused on static adhesive performance. 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 we discovered that after four steps on a misted hydrophilic glass substrate geckos may not be able to generate the static shear adhesion required to counter their body weight during locomotion (Autumn et al., 2006b; 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 due to reduced adhesive traction. Our results do not support this hypothesis and we found that geckos run equally fast, on average, whether on misted or dry hydrophilic glass, regardless of orientation (Fig. 6.1). 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. 6.1). Our results suggest that although static adhesive performance can be significantly affected by water (Stark et al., 2012; Stark et al., 2013), sprint velocity, at least over a one meter distance, is not affected by wet substrates.
The lack of impact of water on sprint velocity over one meter is especially interesting considering our previous work which shows 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. 6.1). 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. Interestingly these species stopped more often than the other species and are the only representative species from the tropics (Fig. 6.2 and Table 6.1). Thus this increase in velocity is clearly independent of what we would typically characterize as a velocity reducing behavior (stopping) and rather, may be related to particular adaptive traits which allow these species to thrive in their native habitat. 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., 2006b) 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. In general we would expect geckos from the tropics to outperform species from other habitats on wet substrates and while more work needs to be done to fully investigate this, our results lead us to the hypothesis that stopping more often increases speed on wet substrates, perhaps by paring stops with sprinting bursts, increasing overall average sprint velocity.

Although velocity was well maintained on misted substrates when compared to dry, even improved in some treatments, 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 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. We know that when the toe pads are wet geckos cannot generate enough force to support their body weight (Stark et al., 2012) and it is easy to observe slipping due to wet toe pads (see Fig. 1 in Stark et al., 2012), however over a one meter 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 but not wet the toe pad. Further investigation is needed to fully clarify the interaction of water and the toe pad prior to and during contact with a surface, particularly in this species and other desert-dwelling species. 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. 6.3). We noticed that this species tended to jump more frequently than all other species which may have caused the increase in toe wetting due to a pressure change and/or increased agitation which we know can cause toe pad wetting (Stark et al., 2012). This species is also native to sub-tropical regions and are not particularly high climbers (even being observed to move terrestrially (Bauer et al., 2012)), thus higher prevalence
of wet toe pads could be of little to concern to low climbing species which do not rely on their adhesive system for high vertical climbs. 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 (Fig. 6.4).

Our results suggest a remarkable level of resiliency of gecko toe pads to wet substrates, over short distances. Our static shear adhesion tests (Stark et al., 2012), which only permitted geckos to take four steps, suggested that a one meter distance would be sufficient to detect measurable differences in performance based on walking. When running we see that there is no direct effect of water on performance over one meter, 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 one meter is likely better than walking a distance of a meter on a misted substrate, leading us to make the hypothesis that geckos run in wet conditions rather than walk. 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.

Our experiment brings up an important relationship in the field of ecological morphology: 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 all these components in relation to the gecko adhesive system by considering morphology (frequency of toe pad wetting), performance (sprint velocity), behavior (stopping and slipping) and native habitat (arid, sub-tropical and tropical species) as it relates to wet substrates. Performance has been a primary focus of the field as of late and while important, it is difficult to fully understand and appreciate the system as it works in an ecologically realistic and evolutionary 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, 1990a; Vitt et al., 2003). We found that performance on wet substrates is comparable among five species sampled widely phylogenetically. We did notice interesting ecology-related differences in stopping, slipping and toe wetting where geckos from the tropics stopped more than other species, geckos from arid habitats slipped when running on misted vertical glass and species that are active close to the ground tended to have more wet toes after moving across misted horizontal substrates. It is unclear if the variation in stopping behavior, slipping and toe pad wetting among species is related to specific adaptations to wet or challenging conditions or a particular morphological feature, but the differences related to native habitat are intriguing.

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 field-based. 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. Furthermore,
what types of behavioral choices do they employ when faced with substrates that are
wet? And finally, can these ecological conditions shape the morphology, behavior and
performance of geckos who routinely navigate wet substrates? 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 during running on misted
substrates, showing that a simple transition from walking to running may make all the
difference on wet substrates.
CHAPTER VII

CONCLUSION

Our work shows that gecko adhesion on wet surfaces depends on several factors. First, although thin water layers can be expelled at the contact interface (Hsu et al., 2012), a thick water layer (~0.5 cm) can only reliably be expelled on intermediately wetting and hydrophobic substrates (Chapter III). For example, glass, a hydrophilic substrate, is particularly challenging for adhesion when fully submerged underwater due to a lubricating water layer that causes geckos to slip (Chapter II and Chapter III). This layer is held between the hydrophilic substrate and the superhydrophobic toe by an air plastron (Chapter III and Chapter IV). On substrates with higher water contact angles we see that this mutual affinity to remove water aids in gecko adhesion underwater. Furthermore, when the air plastron is not allowed to form, adhesion is weak, if present at all (Chapter IV). Second, although very little is known about the surface chemistry of the adhesive setae, using charged surfactants we were able to begin to understand the surface of the setae. Particularly, when tested in a negatively charged surfactant, the toe pads immediately wetted (transitioned to the Wenzel wetting state), suggesting that the setal material is hydrophobic. This did not occur as frequently or completely when using a neutral surfactant. Thirdly, when testing adhesion after the toe pads had become wet (Wenzel state), adhesion is greatly reduced (Chapter II and Chapter V). This
poses a significant limitation to gecko adhesion, as we have observed wet toe pads during the course of experimental observations (Chapter II) and measured frequency of toe pad wetting in the natural system under semi-natural conditions (Chapter VI). Interestingly this transition state can be quickly recovered in 15 minutes or less simply by walking about ten steps, a feat traditional pressure sensitive adhesives (PSAs) cannot achieve. Finally, we found that static adhesion on wet surfaces, even after walking, does not relate to the dynamics of running on wet surfaces (Chapter VI). Specifically, when geckos run, rather than walk, on misted substrates they suffer no significant loss in sprint speed and in some species speed even increases on wet surfaces. Performance and behavior measurements from this study suggest that there could be environment-related species differences in conditions we expect to be challenging to the adhesive system.

This work points to several interesting directions for future work. First, we believe that understanding the surface chemistry and behavior of surface groups at the adhesive interface is critical to understanding the gecko adhesive system as a whole, especially in water. Our recent discovery of lipids (Alibardi et al., 2011; Hsu et al., 2012) supports this and several of the results here provide testable hypotheses. For instance, are the adhesive setae hydrophobic as suggested by Chapter IV? Is this why adhesion to wet surfaces is sensitive to substrate wettability in water? Furthermore, is the ability to self-clean and remove water from the toe due to setal hydrophobicity? Up until recently gecko setae have been described as hydrophilic due to their composition (keratin). We are currently investigating this line of research by using a novel technique: modification
of skin sheds or natural molts from the gecko. By using plasma enhanced chemical vapor deposition (PECVD) we have been able to alter the surface chemistry of gecko setae without significantly changing the geometry. Our results from this study have now been able to answer some of our previous questions. Specifically, we found using thermodynamic modeling that the contact angle of the adhesive setae is between 70-90°, neither strongly hydrophobic nor hydrophilic. By altering the surface chemistry of the gecko setae we found no clear effect of surface chemistry on adhesion in air or water on a hydrophobic substrate but a significant drop in adhesion in water on a hydrophilic substrate, regardless of setal surface chemistry. We also found a significant effect of setal surface chemistry on the ability to self-clean water from the toe pad, where sheds that were coated with a hydrophilic polymer wet completely (no Cassie-Baxter state) and those that were coated with a hydrophobic polymer did not. Although these results begin to shed light on the importance of surface chemistry, a property virtually neglected by those fabricating gecko-like adhesives, there is also a level of complexity we cannot easily recreate in these experiments. Specifically, we found using sum frequency generation (SFG) spectroscopy that setal surface chemistry is dynamic, where surface methyl groups change to methylene groups in the presence of water. We are currently investigating the dynamic conformation changes of surface groups on the setae when making contact to different substrates in water and surfactant solutions.

Second, one particular substrate continually gives us surprising results: polytetrafluoroethylene (PTFE), commonly known as Teflon (Chapter III and Chapter IV). Why does adhesion improve in water when compared to air on PTFE? This question is
also currently being investigated and recent results suggest that in general, the interaction of a gecko-like surface and two different types of fluorinated substrates do not follow the basic modeling of Hamaker coefficients, where water should reduce van der Waals interactions. Finally, there are two major components of this work that need further attention from our group and the field as a whole. First, the application of our findings to bio-inspired design and second, the departure from a few representative species to a more ecologically aware investigative approach. Specifically, our findings here would not have been possible without taking into account a very relevant ecological variable: water. Likewise, without considering adaptation to surface water, synthetic design of underwater adhesives inspired by the gecko adhesive system may not have been obvious. Further investigation of other ecological variables and design of synthetics for wet applications are currently being investigated however much more work and focus is necessary to fully uncover all of the properties of the gecko adhesive system.


APPENDICES
To form the OTS-SAM, a glass plate (25 cm x 15 cm) was rinsed with de-ionized water to remove water soluble contaminants and dried with N\textsubscript{2}. Following this, the plate was rinsed with isopropyl alcohol (IPA), dried with N\textsubscript{2} and put in a base bath for about 3 hours. After being removed from the base bath, the plate was rinsed thoroughly with de-ionized water and blow dried with N\textsubscript{2}. The cleaned glass plate was dried further at 120°C for about 3 hours to ensure complete drying. After drying the glass plate was immersed in a freshly prepared OTS solution (1mM solution of OTS in toluene) and allowed to stay immersed for 30 minutes. The glass container containing the solution was sealed to minimize contact with atmospheric air and avoid possible degradation of OTS. After 30 minutes, the plate was taken out of the solution and was rinsed successively with toluene, acetone, chloroform and IPA, blow drying with N\textsubscript{2} between. The SAM formed on the surface of the glass plate was annealed in a vacuum oven at approximately 150°C overnight.
APPENDIX B

DRY CONTACT OF SMOOTH SURFACES

The Dupré equation for calculating the work of adhesion between two surfaces, 1 and 2, in its general form is written as follows:

\[ W_{\text{dry}} = A_c(\gamma_1 + \gamma_2 - \gamma_{1-2}) \]  

(1)

Here, \( W_{\text{dry}} \) is the work of adhesion between the surfaces 1 and 2, \( A_c \) is the area of contact, \( \gamma_1, \gamma_2, \) and \( \gamma_{1-2} \) are the surface energies of components 1 and 2 and interfacial energy of the contact between 1 and 2 respectively.

We used equation 1 to calculate the work of adhesion between each of our four surfaces (glass, plexiglass (PMMA), OTS-SAM coated glass and PTFE) and the “gecko hair-like” n-hexadecane surface, assuming that the contact interface formed as a result of contact between the two is flat (Table 3.2). The Young-Dupré equation for the dry contact between the two surfaces when air is the medium of contact can be written as follows (Chaudhury, 1996):

\[ W_{\text{dry}} = A_c(\gamma_{s-\text{air}} + \gamma_{h-\text{air}} - \gamma_{s-h}) \]  

(2)

Where, \( \gamma_{s-h} \) is the interfacial energy at the contact interface between the “gecko hair-like” surface and the contact surface (glass, plexiglass (PMMA), OTS-SAM coated glass or PTFE), \( \gamma_{h-\text{air}} \) is the surface energy of “gecko hair-like” n-hexadecane surface and \( \gamma_{s-\text{air}} \) is the surface energy of the contact surface.
Young’s equation for the contact angle ($\theta_1$) that n-hexadecane makes on a given contact surface is:

$$\gamma_{s-h} = \gamma_{s-air} - \gamma_{h-air} \cos \theta_1 \quad (3)$$

Substituting equation 3 in equation 2 for $\gamma_{s-h}$ we get:

$$W_{dry} = A_c \gamma_{h-air} (1 + \cos \theta_1) \quad (4)$$

We measured the contact angle of n-hexadecane on all four surfaces that we used for the gecko trials to obtain the value of $\theta_1$ (see second column of Table 3.1). The value of $\gamma_{h-air}$ is known to be 25 mJ/m$^2$. Substituting all the known values in equation 4 gives the work of dry adhesion ($W_{dry}$).
APPENDIX C

WET CONTACT OF SMOOTH SURFACES

In the case of wet adhesion i.e. the case where water is the medium of contact, the work of adhesion \( W_{\text{wet}} \) is calculated using the following equation:

\[
W_{\text{wet}} = A_c (\gamma_{s-water} + \gamma_{h-water} - \gamma_{s-h}) \tag{5}
\]

Here, \( W_{\text{wet}} \) is the work of adhesion between two surfaces contacting under water. Similar to dry contact, \( \gamma_{s-water} \) denotes the interfacial energy at the contact surface-water interface (contact surface is glass, plexiglass (PMMA), OTS-SAM coated glass or PTFE), \( \gamma_{h-water} \) is the n-hexadecane-water interfacial energy and \( \gamma_{s-h} \) is the interfacial energy at the surface-n-hexadecane contact interface.

The contact angle of water \( \theta_2 \) was also measured on all four surfaces (first column of Table 3.1). It gives the following relationship:

\[
\gamma_{s-water} = \gamma_{s-air} - \gamma_{water-air} \cos \theta_2 \tag{6}
\]

Where \( \gamma_{water-air} \) is the surface tension of water. Substituting equations 3 and 6 in 5 for \( \gamma_{s-h} \) and \( \gamma_{s-water} \) gives the following equation:

\[
W_{\text{wet}} = A_c (\gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - \gamma_{water-air} \cos \theta_2) \tag{7}
\]

The values of \( \gamma_{h-water} \) and \( \gamma_{h-air} \) are known to be 50 mJ/m\(^2\) and 25 mJ/m\(^2\) respectively. \( \theta_1 \) and \( \theta_2 \) were determined experimentally as discussed in Appendix D. Table 3.1 summarizes the contact angles of water and n-hexadecane on different test
surfaces. Substituting all the values in equation 7 gives the value of wet adhesion. Thus, knowing all the parameters, we can estimate $W_{wet} : W_{dry}$ using equation 8 below (derived from equations 4 and 7):

$$\frac{W_{wet}}{W_{dry}} = \frac{\gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - \gamma_{water-air} \cos \theta_2}{\gamma_{h-air} (1 + \cos \theta_1)}$$

(8)

Ratios are reported in Table 3.2.
Contact Angle Measurements

Samples of about 1 cm x 1 cm in size were cut from the actual test surfaces used for whole-animal adhesion trials. Surfaces were rinsed with ethyl alcohol, since ethyl alcohol is the only cleaning step between trials, followed by blow drying. The contact angle of de-ionized water and n-hexadecane was measured using Ramé-Hart Instruments Advanced Goniometer 500 F1 with Drop Image Advanced software. A droplet of 10 - 12 μL of the given test liquid was deposited on the surface and the contact angle was measured. At least 3 measurements were taken for each sample and the average and standard error was calculated to estimate the deviations in the measurements.
APPENDIX E

DRY CONTACT FOR A TETRAD-PATTERNED SURFACE

In the case of tetrad-patterned surface in dry contact with different surfaces, equation 4 can be used to calculate $W_{dry}$. $A_c$ in this case is only a fraction of total surface area that forms a contact interface.
APPENDIX F

WET CONTACT FOR A TETRAD-PATTERED SURFACE

In the case of contact between the tetrad-patterned “gecko hair-like” surface and the contacting surfaces (glass, plexiglass (PMMA), OTS-SAM coated glass or PTFE), there are four possible cases as shown schematically in Table 3.2. The ratio $W_{wet} : W_{dry}$ for all the cases can be estimated as follows (final ratios are reported in Table 3.2).

Case 1:

$$W_{wet} = A_1 \gamma_{h-water} + A_2 \gamma_{s-water} - A_1 \gamma_{s-h} - (A_2 - A_1) \gamma_{s-air}$$  \hspace{1cm} (9)

$A_1$ and $A_2$ in the case of patterned surfaces correspond to total surface areas of the “gecko-hair-like” n-hexadecane tetrad patterned unit cell and the surface it is in contact with (glass, plexiglass (PMMA), OTS-SAM coated glass or PTFE), respectively.

Further simplification of equation 9 and appropriate substitutions give an equation to calculate $W_{wet}$ for case 1 (below).

$$W_{wet} = A_1 \gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - A_1 \gamma_{w_{water-air}} \cos \theta_2$$ \hspace{1cm} (10)

$W_{wet} : W_{dry}$ is thus calculated using equations 4 and 10:

$$\frac{W_{wet}}{W_{dry}} = \frac{\gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - (A_2/A_1) \gamma_{w_{water-air}} \cos \theta_2}{\gamma_{h-air} (1 + \cos \theta_1)}$$ \hspace{1cm} (11)

Case 2:
Similar to case 1, the equation for $W_{wet}$ for case 2 is derived as:

$$W_{wet} = A_1 \gamma_{h-water} + A_2 \gamma_{s-water} - A_C \gamma_{h-air} - (A_1 - A_C) \gamma_{h-water} - (A_2 - A_C) \gamma_{s-air}$$

(12)

Further simplification gives equation 12 in terms of parameters measurable experimentally.

$$W_{wet} = A_1 (\gamma_{h-water} - \gamma_{h-air}) - A_2 \gamma_{water-air} \cos \theta_2 + A_C \gamma_{h-air} (1 + \cos \theta_1)$$

(13)

$W_{wet}$ : $W_{dry}$ for this case is derived using equations 4 and 13:

$$\frac{W_{wet}}{W_{dry}} = \frac{(A_1/A_C) (\gamma_{h-water} - \gamma_{h-air}) + \gamma_{h-air} (1 + \cos \theta_1) - (A_2/A_C) \gamma_{water-air} \cos \theta_2}{\gamma_{h-air} (1 + \cos \theta_1)}$$

(14)

Case 3:

$W_{wet}$ for case 3 is derived in a similar way as cases 1 and 2 above.

$$W_{wet} = (2A_C - A_1) \gamma_{h-water} + (A_1 - A_C) \gamma_{h-air} + A_C (\gamma_{s-water} - \gamma_{s-h})$$

(15)

Simplification and substitution reduces equation 15 in the following form:

$$W_{wet} = A_C [2 \gamma_{h-water} + \gamma_{h-air} (\cos \theta_1 - 1) - \gamma_{water-air} \cos \theta_2] + A_1 (\gamma_{h-air} - \gamma_{h-water})$$

(16)

Using equations 4 and 16, the ratio $W_{wet}: W_{dry}$ can be calculated as shown below.

$$\frac{W_{wet}}{W_{dry}} = \frac{2 \gamma_{h-water} + \gamma_{h-air} (\cos \theta_1 - 1) - \gamma_{water-air} \cos \theta_2 + (A_1/A_C) (\gamma_{h-air} - \gamma_{h-water})}{\gamma_{h-air} (1 + \cos \theta_1)}$$

(17)

Case 4:

The equation for $W_{wet}$ in case 4 is calculated as follows:

$$W_{wet} = A_C (\gamma_{h-water} + \gamma_{s-water} - \gamma_{s-h})$$

(18)

Simplification on substitution gives equation 18 in terms of $\theta_1$ and $\theta_2$.

$$W_{wet} = A_C (\gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - \gamma_{water-air} \cos \theta_2)$$

(19)
The equation for the ratio $W_{wet} : W_{dry}$ is derived below:

\[
\frac{W_{wet}}{W_{dry}} = \frac{\gamma_{h-water} + \gamma_{h-air} \cos \theta_1 - \gamma_{water-air} \cos \theta_2}{\gamma_{h-air} (1 + \cos \theta_1)}
\]  

\[\text{(20)}\]

$A_1$, $A_2$ and $A_c$ here represent total surface area of tetrad unit cell, total surface area of the test surface and the contact area respectively. We calculated the areas using the dimensions of a unit cell we estimated from SEM images. For the tetrad-patterned unit cell, $A_1 = 3961 \mu \text{m}^2$, $A_2 = 121 \mu \text{m}^2$ and $A_c = 64 \mu \text{m}^2$. The respective ratios of areas for $W_{wet} : W_{dry}$ were calculated based on these values and substituted.
A schematic of a non-tetrad patterned surface is shown below (Figure A.1). One unit cell is boxed. Similar to a tetrad-patterned unit cell, dimensions of this type of gecko toe morphology were estimated using SEM imaging. For this type of unit cell, $A_1 = 996 \, \mu m^2$, $A_2 = 36 \, \mu m^2$ and $A_c = 16 \, \mu m^2$. $W_{wet} : W_{dry}$ ratios were thus calculated using these values and equations derived above for four different cases of wet contact. The results are tabulated below (Table A.1).

Figure A.1. A schematic representation of the non-tetrad patterned unit cell morphology. One unit cell is boxed in blue. Columns are 60µm tall and 4µm wide. Each column is separated by 1µm.
Table A.1. Table of $W_{\text{wet}} : W_{\text{dry}}$ ratios for the non-tetrad patterned surface in each of the four wetting cases and on each test surface.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>-0.62</td>
<td>30.39</td>
<td>-30.45</td>
<td>0.56</td>
</tr>
<tr>
<td>PMMA</td>
<td>1.23</td>
<td>32.04</td>
<td>-25.43</td>
<td>1.38</td>
</tr>
<tr>
<td>OTS-SAM</td>
<td>1.78</td>
<td>34.37</td>
<td>-30.95</td>
<td>1.64</td>
</tr>
<tr>
<td>PTFE</td>
<td>1.98</td>
<td>34.83</td>
<td>-31.12</td>
<td>1.74</td>
</tr>
</tbody>
</table>
APPENDIX H

HAMAKER CONSTANT CALCULATIONS

The parameters used for Hamaker constant calculations and the values obtained for different substrates are tabulated in Table A.2 and Table A.3 respectively.

Table A.2. The parameters used to calculate Hamaker constants for the absorption frequency (νa) of \(3 \times 10^{15} \text{ s}^{-1}\).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Dielectric constant, ( \varepsilon )</th>
<th>Refractive index, ( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>80</td>
<td>1.33</td>
</tr>
<tr>
<td>\textit{n}-Hexadecane</td>
<td>2.05</td>
<td>1.42</td>
</tr>
<tr>
<td>Glass</td>
<td>3.7</td>
<td>1.54</td>
</tr>
<tr>
<td>PMMA</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>PTFE</td>
<td>2.1</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Table A.3. Hamaker constant values calculated for different contact surfaces; the subscripts 1, 2 and 3 correspond to “gecko hair-like” \textit{n}-hexadecane, substrate (glass, plexiglass (PMMA) and PTFE) and air (or water) respectively.

<table>
<thead>
<tr>
<th>Surface</th>
<th>( A_{132(\text{air})} ) ( \times 10^{-20} \text{J} )</th>
<th>( A_{132(\text{water})} ) ( \times 10^{-20} \text{J} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>6.53</td>
<td>0.75</td>
</tr>
<tr>
<td>PMMA</td>
<td>6.16</td>
<td>0.68</td>
</tr>
<tr>
<td>PTFE</td>
<td>4.59</td>
<td>0.34</td>
</tr>
</tbody>
</table>
APPENDIX I

MANOVA TABLE

Table A.4. The MANOVA table shows a significant difference (p < 0.0001*) in shear adhesive force across surfaces (glass, plexiglass (PMMA), OTS-SAM coated glass and PTFE) and this difference is due to the interaction between surface and treatment (wet or dry; p < 0.0064*). The F-statistic is from the Wilks’ lambda test.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Wilks’ lambda</th>
<th>Exact F</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0.163</td>
<td>1.628</td>
<td>1</td>
<td>10</td>
<td>0.2309</td>
</tr>
<tr>
<td>Surface</td>
<td>19.896</td>
<td>53.057</td>
<td>3</td>
<td>8</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Surface × treatment</td>
<td>3.308</td>
<td>8.821</td>
<td>3</td>
<td>8</td>
<td>0.0064**</td>
</tr>
</tbody>
</table>