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The Effects of Substrate Roughness in Air and Water on the Gecko Adhesive System

Abstract

In an effort to better understand the fascinating gecko adhesive system, scientists have long tested the abilities of the gecko in controlled conditions that mimic the gecko's native environment. The effects of humidity, environmental temperature, and exposure to water have all been tested. Roughness, however has not been studied in great detail. Surfaces of varying roughnesses are all over the gecko's natural environment. We tested geckos on hydrophilic silicon carbide sandpapers of varying roughnesses in both air and water to attempt to better study the effects of roughness on gecko adhesion. When tested on the rougher (1 μm) surface, the geckos shear adhesive pull-off force was significantly larger than on the less rough (0.5 μm) surface. Finally, we tested the effect of treatment (air or water) on the rough surfaces and found that there was no significant effect on adhesion when being exposed to water or air on a rough surface. There was a non-significant trend for the difference between the two roughnesses to be larger in water than in air, but sample sizes and statistical power to test this effect were low.

Introduction

In recent years, the gecko adhesive system and its limitations have fascinated us and thus been a part of many studies. On man-made substrates, the animals demonstrate amazing adhesion that can support several times their own body weight (Autumn & Peattie 2002, Irschick *et. Al.* 1996, Autumn 2006). The conditions that a gecko faces in the wild, however, are often quite variable. Humidity, environmental temperature, substrate surface chemistry, and substrate roughness all contribute to the gecko's ability to adhere, among a number of other factors. It is suggested that the "overbuilt" nature of the gecko adhesive system is the evolutionary result of the hardships faced in the wild (Persson & Gorb 2003, Russell & Johnson 2013). Substrate roughness is a particularly interesting factor as a wild gecko is likely to encounter a variety of different rough surfaces throughout its life. Additionally, realizing how roughness affects the system has benefits in industrial development in adhesive synthetics for use on rough surfaces. Indeed, previous studies have suggested that several species of gecko struggle to adhere fully to rough substrates (Russell & Johnson 2013, Gorb *et. al.* 2007). It is suggested that at the whole animal level, a minimum frictional force can be found on surfaces with roughness height profiles that match the size of the gecko's spatula (Gorb *et. Al.* 2007). It is this same study that suggests that the adhesion of the animal increases on surfaces with roughnesses that are both smaller than the spatula and roughnesses that are larger than the spatula. The softness of the gecko's keratinous adhesive system allows for maximal surface area contact. However, with substrates featuring intermediary asperity sizes, the system may not be soft enough to fully contact the substrate. This experiment will measure an exact pull-off force of geckos on controlled rough substrates and attempt to explain the performance of the animal based on the surface asperity size.

It is interesting to note that the presence of water is likely something that a gecko would encounter in its natural environment, especially amongst tropical-dwelling species. The gecko's setae are superhydrophobic (Autumn 2006). The exposure to water on a substrate can significantly drop a tropical-dwelling gecko's adhesive performance (Stark *et. Al.* 2012, Stark *et. Al.* 2013) on relatively hydrophilic substrates. Wet hydrophobic substrates allow for significantly greater adhesive performance from the gecko than when dry. These previous studies about the effects of water have only been performed on relatively smooth surfaces. Thus, the interaction (and overall adhesive performance) between water, the rough surface, and the gecko foot is unknown. This experiment addresses the effects of water on gecko adhesive performance on rough substrates.

Expected Outcome

According to a study by Gorb (above), the size of the gecko setae directly influences the gecko's performance on rough surfaces. It is likely that roughnesses which are smaller than the setae, or larger than the setae, allow for strong adhesion. Intermediate roughnesses which are approximately the size of the setae interfere with setal adhesive abilities. To test this hypothesis, we used substrate roughness sizes of 0.5µm and 1µm, and expected stronger adhesion to the 0.5µm substrate than the 1µm, which is approximately the size of the seta (Gorb et. Al. 2007, Rizzo et. Al. 2006). This should hold true in both air and water. If the adhesive abilities are significantly different between air and water performances, the water molecules are likely to be filling the spaces in between the gaps of the particles on the sandpaper.

Materials and Methods

Animal Husbandry and Handling

Seven healthy adult tokay geckos (*Gecko gekko*) were used throughout the experiment. Each gecko was housed individually in separate glass terraria and exposed to a continuous cycle of 12 hours of light followed by 12 hours dark. A diet of cockroaches was fed three times per week to ensure proper nutrition and a healthy weight. Cages were misted twice each day to keep experimental animals hydrated. Before each trial, animals were acclimated to the experimental conditions for thirty minutes. An ambient temperature of 25˚C ± 2˚C, humidity at 35% ± 5%, and water temperature (if applicable) of 25˚C ± 2˚C was maintained during acclimation period and throughout experimental trials. The University of Akron IACUC protocol 07-4G approved all of the procedures used on the animals, and the procedures were consistent with the guidelines provided by the Society for the Study of Amphibians and Reptiles (SSAR 2004).

Experimental Procedures

A force sensing apparatus similar to that as described (Niewiarowski et al. 2008) was used in the horizontal position. Geckos were placed on a substrate and pulled with the force sensing apparatus via two harnesses attached at the pelvis. The force at which all four feet slipped on the substrate was measured and recorded.

Each of the seven geckos was tested three times on each substrate, both underwater and in air. Geckos had to take a step on the substrate with all four feet before the pull was started to ensure that the adhesive system was engaged. They were only tested horizontally. If tested in water, the geckos were placed in just enough water (~1 cm) to completely cover the foot and ankle. The highest force produced from each gecko from each of the treatments was used in data analysis. Geckos had at least one day of rest between each set of trials, totaling no more than three pulls in a day. Geckos that were tested in water were not tested again until having at least one day of rest to allow their feet to dry and prevent water from interfering with subsequent trials.

Substrates Tested

Two substrates were used throughout the experiment. Both substrates were 9” by 11” 3M Wet/ Dry sandpapers provided by Lee Valley Tools. One sandpaper was 2000A (approx 1µ particle size) and the other was 2500A (approx 0.5µ particle size.) Surface chemistry remained the same for both surfaces as both were silicon carbide surfaces

Statistical Analysis

We tested for differences in whole species performance between the two roughness’s, 2500A and 2000A in air and water. The maximum adhesion force for each gecko was recorded on each substrate under each treatment. A normal distribution of these forces was made using the natural log of the given force. A fixed effect ANOVA was used to determine if the role of surface roughness significantly affected the performance under different combinations of roughness and medium (air and water).

Results

Gecko gekko performed significantly different between our rough surfaces. On the 2000A sandpaper substrate, geckos adhered with significantly greater forces than on the 2500A sandpaper substrate, with (ANOVA $P= 0.0134$, $DF= 1$, and $F= 7.5192$). The average force needed to overcome the gecko adhesive system on 2000A, regardless of treatment, was 3.7178 N. The average force needed to overcome the gecko adhesive system on the 2500A surface, regardless of treatment, was only 2.2129 N. The treatment (air or water treatments) that the animals were exposed to during shear adhesion testing did not significantly affect the way that they performed; $P= 0.5537$, $DF= 1$, $F= 0.3641$. The average max force to overcome adhesion in air was 2.6378 N whereas the max force to overcome adhesion in water was 2.9840 N. The interaction of surface (2500A or 2000A sandpapers vs. Air treatment or Water treatment) was not significantly different; P= 0.1867, DF= 1, F= 1.8843. There was no control substrate as there is no known substrate with the same surface chemistry as the sandpapers but features an average asperity size of ~0 µm

Comment [pn1]: Ok, this is a good first pass, but modify in the following way:
Make an ANOVA table. You can copy the form and details of the one I sent you.
Make a table of the averages as reported here. The table can show air and water as first two rows of first column, and the top two columns can be the two sandpaper values. Put mean + 2 se which is on the output I sent you. You will find the table labeled surface x treatment has the values you are looking for.

Average Pull-off Forces (N)

	Air	H2O	Treatment x Substrate
2000A Sandpaper	3.0343± 2*(0.2713)	4.5553± 2*(0.2713)	3.7178± 2*(0.2291)
2500A Sandpaper	2.2896± 2*(0.2713)	1.9547± 2*(0.2713)	2.1155± 2*(0.2291)
2000A Sandpaper and 2500A Sandpaper	2.6358± 2*(0.2291)	2.9840± 2*(0.2291)	X

Fixed Effect ANOVA

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Surface	1	1	18	7.5192	0.0134*
Treatment	1	1	18	0.3641	0.5537
Surface* Treatment	1	1	18	1.8843	0.1867

Discussion

In previous studies regarding whole animal adhesion of the *Gecko gekko*, minimal adhesion was found on substrates with asperity sized between ~.1 µm and ~.3 µm. It was suggested that geckos perform most poorly (with the lowest in shear adhesion pull-off force) with surfaces with asperity sizes with are similarly sized with the gecko's setae. If this is true, the 0.5 µm (which is most similar to the minimum in the Gorb 2007 experiment) should yield the minimum pull-off force. However, our results suggest that the 2000A sandpaper (~0.5 µm asperity size) yielded the highest pull-off force in shear adhesion. According to the previous experiments, the 1 µm asperity size substrate should yield a high pull-off force, as the asperity size is so much different than the size of the gecko's setae. However, the average performance of the cohort on the 1 µm sandpaper substrate (compared to the 0.5 µm sandpaper), was significantly lower in pull-off force.

It is interesting to note that the presence or absence of water did not significantly affect the gecko's ability to cling to the sandpaper surface. The sandpapers, made of silicon carbide, did not yield similar results in previous experiments which used glass, a silicon dioxide smooth substrate. In Stark 2013 (PNAS), geckos had a significantly higher adhesive pull-off force on dry glass (air) compared to the pull-off forces of geckos on glass in water. In this study, hydrophobic surfaces yielded a higher pull-off force when the gecko was under water. Because our analysis revealed no significant difference between treatment (air and water) and substrate, this may suggest that roughness on a substrate could allow water to fill into the crevices in a way which allows the gecko to be unaffected by the presence of water. Normally a hydrophilic surface such as a SiO_2 surface would have a significantly lower performance in water than air, but this was not the case in our trials with a SiC. Further experimentation would use a hydrophobic surface to determine if roughness indeed negates the effects of treatment (exposure to either air or water). Additionally, a smooth (~0 µm asperity) control (SiC) surface would also be tested in order to

Comment [pn2]: Is this the right name? it is silicon dioxide, right?

The sandpaper is SiC whereas the glass is SiO2. I corrected this.- amp better analyze the effects of substrate roughness on gecko adhesion. In our experiment, we focused on roughnesses in size that would challenge the setae.

It would be interesting to see roughnesses in sizes that would challenge the other hierarchical structures of the gecko foot, such as the lamellae. Despite the current interest in the field about the gecko adhesive system, little has been done to study the effects of potentially rough surfaces on performance. It is likely that in the wild, geckos would be exposed to a variety of roughnesses throughout their lives. It is just as likely that tropical species such as *Gecko gekko* will also encounter water on these rough surfaces. It is important to study how these animals adapt to their natural environment. Not only does this provide important ecological and evolutionary information, but it could also help to uncover the information that we need to create biomimetic adhesives that may adhere to substrates of varying roughnesses.

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