Superhydrophobicity of the gecko toe pad: biological optimization versus laboratory maximization

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While many gecko-inspired hierarchically structured surfaces perform as well as or better than the natural adhesive system, these designs often fail to function across a variety of contexts. For example, the gecko can adhere to rough, wet and dirty surfaces; however, most synthetic mimics cannot maintain function when faced with a similar situation. The solution to this problem lies in a more thorough investigation of the natural system. Here, we review the adhesive system of the gecko toe pad, as well as the far less-well-studied anti-adhesive system that results from the chemistry and structure of the toe pad (superhydrophobicity). This paradoxical relationship serves as motivation to study functional optimization at the system level. As an example, we experimentally investigate the role of surface lipids in adhesion and anti-adhesion, and find a clear performance trade-off related to shear adhesion in air on a hydrophilic surface. This represents the first direct investigation of the role of surface lipids in gecko adhesion and anti-adhesion, and supports the argument that a system-level approach is necessary to elucidate optimization in biological systems. Without such an approach, bioinspired designs will be limited in functionality and context, especially compared to the natural systems they mimic.

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1. Introduction

Despite their complexity, hierarchically structured surfaces are surprisingly prevalent in the natural world [1]. Examples of hierarchical surfaces span taxonomic phyla (broad groupings of biological organisms), suggesting that these structures have a specific functional role for the organism. These functions include the production of colour in the butterfly wing and bird feather [2], the self-cleaning of dirt from the lotus leaf [3], or even the layering of filaments and fibrils in mechanically resilient biological materials like bone, nacre and chitin [4]. While the structure–function relationships of these common examples appear to be well resolved, most are only studied with one specific relationship in mind, rather than taking into account a system-level series of relationships and possible trade-offs (though see [5] for a recent example of a system-level approach).

The adhesive gecko toe pad is another well-known example of a biological hierarchically structured surface. The gecko toe pad consists of thousands of fine, hair-like structures which often branch and terminate into flattened tips that make millions of contact points with the surface a gecko clings to [6]. Over the past 15 years, the hierarchical gecko toe pad has been intensely studied, and the adhesive mechanism, via this complex hierarchy, has been well resolved [7]. Interestingly, in addition to being adhesive, the structured surface of the gecko toe is also superhydrophobic and has a low contact angle hysteresis [8,9]. Superhydrophobicity requires a structured surface and appropriate surface chemistry to take advantage of air gaps between surface asperities, which suspend water above the surface at a high contact angle (greater than or equal to 150°) [10]. The superhydrophobicity of the gecko toe pad leads to an interesting paradox for the gecko, however. The complex hierarchy of the toe pad creates both an adhesive and an anti-adhesive surface. While the adhesive nature of the gecko toe pad has been studied extensively, the anti-adhesive nature has received far less attention. Specifically, we do not fully understand if the anti-adhesive behaviour of the toe pad is (i) relevant for adhesion or some other function, or (ii) if it is a non-functional remnant of the development of an adhesive made out of the materials available to the gecko (i.e. proteins and lipids) and its requirements (i.e. multiple contact points). We believe the answer to this question can only be resolved by investigating the complex interaction between adhesion and anti-adhesion jointly, and in an ecological context.

The gecko paradox represents a broader challenge we all face when studying biological systems. The structure and function of biological systems are shaped by the materials that are available, multiple and often competing functional requirements and environmental challenges, as well architectures and processes that have provided solutions over the history of a lineage [11]. It is tempting to assume that the gecko has had millions of years to perfect its adhesive system independently [12]; however, by only looking at a single function—adhesion—we miss a larger, system-level perspective. For instance, we recently found that the anti-adhesion property of the gecko toe pad (superhydrophobicity) is highly relevant for adhesion to wet substrates, which may be critical to the survival of geckos native to the tropics where surfaces are often wet [13,14]. These results show that the adhesive and anti-adhesive systems interact, begging the question: is there a functional trade-off due to this interaction in one or both systems? Since our discovery of invisible lipid footprints left behind by geckos as they walk [15], we have questioned what the role of lipids is in adhesion, if any role exists, and if they relate to a higher level interaction between adhesion and anti-adhesion. While it appears lipids likely contribute to anti-adhesion [9], if and how they relate to adhesion is unknown. It is tempting to focus on one structure–function relationship, such as the hierarchal structure of the gecko toe pad and adhesion in dry environments, without taking into account the larger system-level requirements or limitations. This narrow approach, however, limits our perspective both scientifically, and in terms of the application-based knowledge that these complex biological hierarchal systems can supply for bioinspired design.

This short review, combined with original work, will explore biological systems at the system level by using the gecko adhesive/anti-adhesive paradox as an example. Specifically, we will review the adhesive system of the hierarchical gecko toe pad, as well as its anti-adhesive system (superhydrophobicity). Because the latter is much less well studied, we will discuss the potential...
roles anti-adhesion may contribute to the overall gecko system. Then, we will explore the question of optimization, or the balance between adhesion and anti-adhesion, using a targeted experimental approach to investigate the role of surface lipids in adhesion in air, underwater and on rough substrates. Finally, we conclude with a brief discussion of how this example sheds light on a common, yet often ignored challenge with exploring biological systems for their use in synthetic design. We believe that by taking a system-level approach to studying biological systems, such as hierarchically structured surfaces, we will significantly improve our understanding of the structure–function relationships of that system, as well as provide important parameters to consider when pursuing bioinspired design.

2. The gecko toe pad

(a) Adhesion

The gecko adhesive system is composed of several levels of hierarchy. First, the adhesive gecko toe is composed of muscular and vascular tissue along with connective tissue, such as tendons, which interact with the unique skeletal system of the toe [16–19]. On the underside of the toe, folds of skin, known as lamellae, transect the toe perpendicularly (figure 1a) [16,19]. These folds are covered in small micrometre-scale hairs, called setae, which vary in size across species (figure 1b) [20–23]. In the Tokay gecko (Gekko gecko), the setae are about 100 μm long and 5 μm wide (figure 1c), though this varies across the lamella and the toe itself [24,25]. Approximately the last 20% of a seta splits into finer structures which eventually terminate in a flattened pad known as a spatula (figure 1d) [23]. In the Tokay gecko (G. gecko), the spatulae are about 200 nm wide and only about 5 nm thick [24]. There are approximately 6.5 million setae on the Tokay gecko (G. gecko), making millions of tiny contacts with the surface a gecko adheres to [6]. It is these small contacts that allow geckos to take advantage of weakly attractive intermolecular van der Waals forces [26].

The use of van der Waals forces as the primary mechanism of adhesion provides the gecko with several remarkable properties. First, van der Waals forces are virtually surface insensitive. This allows geckos to climb across any surface they encounter in their environment, as well as many artificial surfaces (e.g. [13,26–29]). Second, van der Waals forces only occur at small distances. This means that the setae are only sticky when small directional loading forces are applied [30]. Likewise, only small angles of peel are required for detachment [31,32]. Combined, this allows a gecko to attach and detach easily and rapidly from almost any surface it is likely to encounter. Because the gecko does not use a glue, there appears to be little wear, degradation or residue left behind by this reusable system (though see our discussion of lipid residue below [15]). Finally, the adhesive system is highly versatile. Not only can it attach to many different types of substrates, it can attach to rough, dirty and wet substrates in a variety of environments (i.e. varying temperature and humidity) [13,33–36]. Collectively, these and other remarkable properties of the gecko adhesive system have attracted the attention of engineers and material scientists in search of inspiration to design a better performing, more versatile pressure sensitive adhesive.

(b) Anti-adhesion

While the adhesive system of the gecko is relatively well resolved, the anti-adhesive system, used here to describe the remarkable ability of the gecko to remove water from the toe pad, has received far less attention. As expected, both the hierarchical nature of the gecko toe and the surface chemistry of the setae contribute to the superhydrophobicity of the gecko toe pad [9]. The gecko toe pad has a water contact angle of approximately 150° and a contact angle hysteresis of 2°–3° (figure 2a) [8,9]. There is some indication that the directionality of the setal mats and spatula create an anisotropic rolling direction for water droplets [37], though this is hard to observe by eye, because droplets of water suspended on the surface are so easily perturbed (A.Y.S. 2014,
personal observation). This even occurs on the less structured skin [38,39]. To approximate the water contact angle of the gecko setae, Autumn & Hansen [8] used the smooth scale of the gecko eye, which is approximately $90^\circ$. Later, theoretical calculations supported this approximation, reporting that the setae have a water contact angle between $70^\circ$ and $90^\circ$ [9]. This means that the setae are neither strongly hydrophobic nor hydrophilic. The composition of the gecko setae is not fully resolved, but they appear to consist of $\beta$-keratin and other proteins, as well as several types of lipids [15,40–42]. Interestingly, proteins specific to the adhesive setae have been found [43], and lipid mobility differs in the setae compared to the skin [40]. These chemical and structural differences specific to the adhesive setae suggest that proteins and lipids, and their arrangement, have a functional role, though it is not clear if this role is specific to the adhesive system, anti-adhesive system, both or something entirely different.

The anti-adhesive, superhydrophobic gecko toe pad is also dynamic. The surface groups of the gecko setae rearrange in water [44], changing from methyl to methylene [15]. It is likely that these groups are associated with unbound lipids on the surface of the setae [15,40]. The implication for this rearrangement is unknown, though it may significantly hinder the anti-adhesion property of the gecko toe [44]. Specifically, though the gecko toe is highly superhydrophobic and stable, even at pressures more than 12 kPa [9], agitation can change the wetting state from the Cassie–Baxter (heterogeneous solid–liquid and air–liquid interface) to the Wenzel (homogeneous solid–liquid interface; figure 2) [9,14,45–47]. In fact, the Wenzel state is the thermodynamically stable wetting state, as shown by wetting of the toe pad by water after being kept in 100% RH for 3–4 days and by thermodynamic modelling [9]. When the gecko toe pad transitions into the Wenzel state, the toe is no longer adhesive [14,45,48], though the gecko is capable of active self-drying by walking [45]. Although the Cassie–Baxter state is the non-thermodynamically stable state, the toe’s ability to retain this property, even underwater, allows geckos to adhere to wet surfaces [13,48].

(c) Potential roles for anti-adhesion

The role of the anti-adhesive nature of the gecko toe pad, if there is one, is not well resolved. Clearly, this property is critical for adhesion on wet surfaces [13,48], but why the Cassie–Baxter, non-wetting state is not the stable state, why surface groups are dynamic, and why the protein

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**Figure 1.** The hierarchical gecko adhesive system. The gecko toe consists of flattened pads (a) with folds of skin called lamellae (b), which are covered with fine-hair-like structures known as setae (c). The tips of the setae branch and terminate into flattened pads called spatulae (d). (Online version in colour.)
Figure 2. The hierarchical gecko anti-adhesive system. The gecko toe’s hierarchical nature, paired with surface chemistry, cause the gecko toe pad to be anti-adhesive to water or superhydrophobic (a). The gecko toe pad is primarily in the anti-adhesive, non-wetting Cassie–Baxter state (schematically represented in b); however the thermodynamically stable state is the Wenzel wetting state (schematically represented in d) where the toe is wet (c). (Online version in colour.)

and lipid arrangements in the setae seem to be specific to these structures is unclear. Some of these observations do suggest some functional role to the anti-adhesive, superhydrophobic nature to the gecko toe pad (i.e. adhesion to wet surfaces), but others seem illogical and suggest there may be some trade-off between adhesion and anti-adhesion (i.e. wetting transition from Cassie–Baxter to Wenzel). Because the hydrophobic lipid component of the adhesive setae seems to be the obvious contributor to superhydrophobicity [9], we will focus on the surface lipids of setae to speculate on potential functions for the anti-adhesive property of the gecko toe.

Narrowing our focus to surface lipids on the adhesive setae of the gecko toe, the most obvious role these lipids may play is in adhesion directly. It is unclear how a soft, wearable, fat-like layer helps adhesion, and rather, it intuitively seems like it would impede it. As emphasized in the Introduction, however, it is our common misconception to assume that all adaptations in biological systems like the gecko are related to a single function, without considering system-level concerns and trade-offs [49]. For instance, if the setae were completely hydrophilic and lacked this lipid layer they may be more adhesive, increasing the likelihood of self-adhesion and fouling. Directionality of the setae and the intimate contact made by spatular tips have been shown to be imperative for successful attachment [30,31,50]; thus, reducing self-adhesion or adhesion of dirt by adding a non-sticky lipid surface layer could benefit the gecko at a system level. Furthermore, consider the repeated use of the setae. Geckos shed their skin and replace the adhesive setae every few months. Until then, the setae are used over and over on a variety of surfaces. Of all the microscopic images of gecko setae in the literature, none document obvious evidence of wear. In fact, the only wear appears to be in the form of the lipid gecko footprint [15]. Perhaps, it is the wear of this fine lipid layer that protects the larger setal structure against constant abrasion in the gecko’s natural environment. This again points to a system-level requirement. With regards to skin, lipids have been shown to help other reptiles, like snakes, avoid water loss [51]. The lipids act like a protective barrier in the skin to prevent desiccation, which is imperative to the survival
of reptiles. In this instance, lipids associated with reptile skin may be a by-product of a separate system-level requirement. It is important to note however that desiccation preventative lipids are located in the mesos layer of the epidermis, rather than the surface, and skin surfaces like the gecko tail do not leave behind detectable lipid residue [40].

Finally, consider that many species of gecko live in the tropics, which can have significant environmental implications for the setal material, and ultimately the lipid layer that coats the surface of the adhesive setae. Specifically, Young’s modulus of bird feathers (made of β-keratin) significantly decreases as humidity increases [52], even at moderate levels (i.e. approx. 4 GPa at 0% RH to approx. 2.5 GPa at 50% RH); however, gecko setae resist this reduction up to 80% RH [53–56]. In this case, perhaps having a hydrophobic lipid layer keeps water from softening the structural keratin fibrils within the setae, allowing the setae to maintain integrity in tropical environments. Lastly, it has become clear that adhesion to wet surfaces benefits from the superhydrophobic toe pad; however, the role of lipids in this function is difficult to determine since adhesion on wet surfaces is also highly substrate dependent [9,13]. It does appear that in whole-animal adhesion tests, an air-filled plastron resulting from the superhydrophobic toe pads is required for successful attachment on wet surfaces, a surface condition that could be common in the tropics [13,48]. We believe that resolving the role of lipids, if one exists, in the gecko adhesive and anti-adhesive systems will significantly contribute to the design of gecko-inspired synthetics which are able to capture more of the remarkable properties of the natural system.

3. The role of lipids in adhesion: a case study for understanding system-level requirements

(a) Background

To resolve the question about the role of lipids in the adhesive and anti-adhesive systems of the gecko, we specifically focused our attention on the non-covalently bonded lipid layer that likely coats the surface of gecko setae. As mentioned previously, the material composition and structure of the gecko setae are not fully clarified; however, here we will use the model proposed by Jain et al. [40] to direct our study. Jain et al. used solid and solution-state nuclear magnetic resonance spectroscopy (NMR), thin layer chromatography (TLC) and several published models and observations of gecko setae to develop a structural model for gecko setae that consists of β-keratin filaments surrounded by covalently bound lipids and ‘coated’, or covered by a non-covalently lipid layer on the outside of the gecko setae (see figure 3 for a model schematic) [40]. In this experiment, we directly target the non-covalently bound surface lipid layer, as it is likely to be relevant to both the adhesive and anti-adhesive systems. Variation in adhesive performance in several key scenarios that are relevant to gecko adhesion in their natural environment will be explored, as well as the role of this lipid layer in anti-adhesion.

The gecko adhesive system must function in a variety of contexts. In this experiment, we will focus on three specific environmental challenges that are relevant to both the adhesive and anti-adhesive systems of the gecko. First, to investigate the role of the surface lipid layer in the adhesive system of the gecko, we will test adhesion of gecko setae on hydrophilic glass and on glass coated with a hydrophobic octadecyltrichlorosilane self-assembled monolayer (OTS-SAM). Adhesion to these two substrates will be tested with surface lipids intact and when removed using a chemical treatment. If adhesion between untreated and treated samples differs, this would be the first example of a functional role for lipids in gecko adhesion. Additionally, Persson proposed that a compliant, soft, liquid-like material layer on the surface of setae may be used to conform to small, nanometre-scale surface asperities, improving adhesion on these challenging substrates [57]. We could not neglect the relevance of this assertion to the discovery of the presumably soft lipid coating that wears from gecko feet when they walk [15]. To investigate the role of surface lipids in adhesion to fine surface roughness, we tested adhesion of treated and
untreated gecko setae to fine grit sandpaper in air. If the hypothesis by Persson is supported, the anti-adhesive nature of the gecko toe may be a by-product of adhesion to rough surfaces in air, rather than have a direct function specific to anti-adhesion.

To investigate the role of surface lipids in the anti-adhesive system of the gecko, we will measure water contact angle on treated and untreated setae. If the anti-adhesive system of the setae is dependent on surface lipids, the treated setae will wet, transitioning from the Cassie–Baxter non-wetting state to the Wenzel wetting state. Lastly, to investigate the interaction of the adhesive and anti-adhesive gecko systems, adhesion to the hydrophobic OTS-SAM-coated glass will be tested in water with and without surface lipids. We have found that adhesion underwater requires the maintenance of both the anti-adhesive and the adhesive systems, as well as contact with a hydrophobic or intermediately wetting surface. Thus by comparing this result to the results of the experiments aimed at the adhesive and anti-adhesive systems independently, we will be able to understand the system-level requirements of geckos. These four tests are far from exhaustive, but represent a first look into the role of surface lipids in the hierarchically structured gecko toe pad with a focus on teasing apart the influence of the adhesive and anti-adhesive systems.

4. Material and methods

(a) Sample preparation and characterization

Adhesive setae were collected in the form of a toe pad skin shed, which is epidermis that geckos shed at approximately monthly intervals. Shed skin from six moulting Tokay geckos (G. gecko) was stored at $-20^\circ$C (see [9,40] for more details on sample collection). Adhesion of single toe sheds was tested in air on three substrates: glass, OTS-SAM-coated glass and sandpaper. The OTS-SAM-coated glass surface has been described previously [9] and has a water contact angle of $95 \pm 2^\circ$. The water contact angle on the glass surface was fully wetting at the beginning of all experiments. The sandpaper surface (2500A, 3M Wet/Dry sandpaper, Lee Valley Tools, Canada) has a root mean square roughness of approximately $500 \text{ nm } (50 \times 50 \mu \text{m sample area), as determined using atomic force microscopy (Veeco Dimension Icon AFM), and a water contact angle of } 56 \pm 4^\circ$. This fine microstructure was used to specifically target scale of the
spatulae, where it is likely incomplete contact will occur due to the similarity in dimensions of the sandpaper asperities and the spatulae (see [58] for a curve of optimal gecko adhesion at fine-scale roughness). We also measured adhesion of toe pad sheds in water using the OTS-SAM-coated glass substrate. Each of the substrates (glass, OTS-SAM-coated glass and sandpaper) and environments (air or water) were tested with sheds that had either been left untreated or were chemically treated to remove surface lipids from the setae. There were eight total pairings of surface, environment and treatment (lipids intact or removed), where six toe pad sheds were used per pairing, totalling 48 toe pad sheds across all adhesion experiments. Each of the six geckos contributed one toe pad shed per pairing, which allowed us to control for individual differences across all treatments.

We used a chloroform–methanol treatment, outlined first by Swartzendruber et al. [59] and adapted by Jain et al. to remove surface lipids from the toe pad sheds [40]. Briefly, the toe pad sheds were immersed in three solutions of chloroform–methanol with concentration ratios of $2:1$, $1:1$, $1:2$ for 2 h each and then for 1 h each. We confirmed removal of surface lipids using TLC and solution-state NMR, as detailed elsewhere [40]. To investigate the effect of chemical treatment, we tested adhesion of toe pad sheds that had been treated with acetone on glass (an additional six sheds per treatment). Acetone was used because it does not significantly remove the lipids of interest [60,61], but reflects chemical treatment and handling that the experimental groups received.

The treated toe pad sheds were air dried for 7 days post-treatment and vacuum dried for 45 min after the drying period. Treated toe pad sheds appeared both mechanically and visually intact [40]. Adhesive area of the sheds was measured after experiments using a dissecting microscope with a mounted camera and IMAGEJ software (National Institutes of Health, Bethesda, MD, USA).

(b) Adhesion testing

Adhesion measurements were collected using a motorized force testing apparatus [9,33] in an environmentally controlled chamber that was maintained at $23.8 \pm 0.1^\circ C$ and $32.6 \pm 0.2\%$ RH. Samples were mounted onto a glass slide using double-sided copper tape and positioned in a testing arena described elsewhere [9]. A weighted glass slide, a weighted OTS-SAM-coated glass slide and a weighted glass slide with sandpaper attached to the back (all around 46 g) were used to test for the effect of surface lipids on adhesion to hydrophilic, hydrophobic and rough surfaces, respectively. Slides were cleaned with ethanol and water before each sample test (except the sandpaper slide). The glass slide was also cleaned in base bath and oven dried at $120^\circ C$ prior to experiments. The test substrate (weighted slide) was attached to a motorized force sensor using a nylon string. A motorized force sensor then slid the test substrate across the setal samples at a controlled rate, recording force as a function of time. The same procedures were followed to test samples on the OTS-SAM-coated glass substrate in water, except that after the sample was mounted in the test arena, the arena was filled with enough water to cover the sample. The acetone experiment followed the same procedure as all other treatments performed in air. Maximum shear adhesion was determined as the highest force reading during the approximately 4 cm slide of the substrate across the shed sample. Samples were tested randomly within treatment groups and test order of treatment groups was also randomized.

(c) Anti-adhesion testing

To investigate the effect of surface lipids in the anti-adhesive system of the gecko, we compared water contact angle of untreated and treated samples using methods from Badge et al. [9]. We used three toe pad sheds per treatment, and measured water contact angle at three different locations per sample. None of the samples used in adhesion trials were used for water contact angle measurements.
(d) Statistical analysis

To investigate the role of surface lipids in the gecko adhesive system, we used an analysis of covariance (ANCOVA) to test for differences in maximum shear adhesion of toe pad sheds, where treatment (lipid removal), substrate (glass, OTS-SAM-coated glass and sandpaper) were the main effects and toe pad area was a covariate. To investigate the role of surface lipids in the gecko anti-adhesive system, we used a Student’s t-test to compare average water contact angle of treated and untreated samples. Next, to investigate the interaction of the adhesive and anti-adhesive gecko systems jointly, we used an ANCOVA which tested for a difference in maximum shear adhesion on the hydrophobic OTS-SAM-coated glass substrate where treatment (lipid removal), environment (air or water) were the main effects and area was the covariate. Finally, to test shear adhesion of toe pad sheds that were treated with acetone or left untreated, we used an ANCOVA where treatment (acetone treatment or untreated) was the main effect and area was the covariate. Maximum shear adhesion was log transformed in all adhesion tests to conform to the assumptions of the models used. To test for differences within groups, we used a Tukey HSD test to control for multiple comparisons. Means are reported as mean ± 1 s.e.m.

5. Results and discussion

Our first goal was to investigate the role of surface lipids in the gecko adhesive system by measuring shear adhesion of gecko toe pad sheds on hydrophilic glass, hydrophobic OTS-SAM-coated glass and fine sandpaper in air. We found that lipid treatment, substrate and their interaction all had a significant effect on shear adhesion of gecko toe pad sheds \( (F_{6,29} = 50.43, p < 0.0001; \) electronic supplementary material, table S1). Specifically, we found that samples that had their surface lipids removed had significantly higher adhesion to glass than untreated samples, and that there was no difference in adhesion across treatment in the other two substrates (figure 4). This result suggests that surface lipids impair adhesion on hydrophilic surfaces but not hydrophobic or rough surfaces. This could be due to increased polar interactions between the treated setae (i.e. hydrophobic surface lipids removed) and glass. Overall, we also found that adhesion to glass was higher than the other two substrates. We have observed this difference in adhesion between glass and OTS-SAM-coated glass previously in toe pad sheds, and thermodynamic models support this trend, but these models do not explain the magnitude of this difference [9]. Interestingly, the difference in adhesion to glass and OTS-SAM-coated glass is not observed at the whole-animal level [13]. Finally, our results also lead us to reject the hypothesis by Persson that the surface lipid layer conforms to rough asperities on the surface to improve adhesion [57]. However, this was only tested at one scale and at ambient temperature and humidity. Although the results were consistent with the expected reduced adhesion to the fine-scale sandpaper as shown by Huber et al. [58], it is unclear why adhesion to the OTS-SAM-coated glass is even lower than the sandpaper surface.

Our second goal was to investigate the role of surface lipids in the gecko anti-adhesive system by measuring water contact angle. Unlike oxygen plasma treatment of gecko setae, which changed the elemental chemical signatures at the setal surface and caused structural deformity [9], the static water contact angle measurements of setae with surface lipids removed was no different than pristine setae \( (142.2 ± 1.83°; t = -1.81, \text{d.f.} = 15.56, p = 0.0897)\). This suggests that the surface lipids removed using this treatment do not significantly contribute to the anti-adhesive behaviour (i.e. superhydrophobicity) of the gecko toe, as measured by static water contact angle. Our third and final goal was to investigate the role of surface lipids in the adhesive and anti-adhesive systems jointly. Here, we saw no difference in adhesion between treated and untreated samples. Specifically, we found that differences in shear adhesion on OTS-SAM-coated glass were driven by environment (air or water) only, and that treatment had no effect \( (F_{4,19} = 7.93, p = 0.0006; \) electronic supplementary material, table S2). In this study, adhesion in water was higher than in air (with treatments pooled across environment; figure 5). Improved adhesion in water compared to air has been reported previously for other substrates...
Figure 4. Adhesion of gecko toe pad sheds in air on hydrophilic glass, hydrophobic OTS-SAM-coated glass and fine sandpaper (root mean square roughness approx. 500 nm for a 50 × 50 μm sample area) after treatment to remove the setal surface lipids. There was a significant difference in maximum shear adhesion between the treated and untreated toe pad sheds on hydrophilic glass, but not on the other two surfaces. There was also a significant difference across substrates, where glass produced the highest values followed by sandpaper and finally OTS-SAM-coated glass. Letters above the bars indicate statistical significance, where bars with different letters are significantly different from one another. Error bars are mean ± 1 s.e.m.

Figure 5. Adhesion of gecko toe pad sheds in air and water on hydrophobic OTS-SAM-coated glass after treatment to remove the setal surface lipids. There was no significant difference between treatment in air or in water, but adhesion was overall higher in water than air. Error bars are mean ± 1 s.e.m.

such as polytetrafluoroethylene, fluorinated ethylene propylene, ethylene tetrafluoroethylene and polydimethylsiloxane, and this trend was nearly significant in the similar study by Badge et al. [9,13,29,62]. The reason for these differences remains unclear. We found no difference in adhesion between the acetone treated and untreated shed samples ($F_{2,9} = 0.14, p = 0.8680$; electronic supplementary material, figure S1); however, observationally the acetone treated sheds appear to produce similar adhesion values on glass as the lipid-treated sheds (these could not be compared statistically). Previous work has shown successful removal of lipids in the integuments of geckos and other vertebrates using the methods described [40,59,63], and limited removal of these lipids using acetone [60,61]; thus we believe that the lipid removing treatment did not significantly alter the setae. However, the similarity between the two on glass warrants further investigation.

The results of these experiments suggest that the gecko adhesive system is negatively impacted by surface lipids, at least when adhering to hydrophilic surfaces, and that adhesion to a rough
surface at the scale tested here is not aided by surface lipids either. Because surface lipids do not affect the anti-adhesive property, nor do they affect the adhesive performance on hydrophobic OTS-SAM-coated glass, there is no performance trade-off related to surface lipids when the two systems are considered jointly in water given the experimental context (i.e. static water contact angle in the Cassie regime and shear adhesion). This work represents the first investigation of the role of surface lipids in the adhesive and anti-adhesive systems since the finding of lipid footprints left behind geckos when they walk [15], and contrary to the common expectation that lipids contribute to some positive performance enhancement, they do not, and rather they hinder adhesion in one specific context. Future work should focus on other system requirements such as self-cleaning and detachment. Another important step in exploring the interaction of the adhesive and anti-adhesive systems of the gecko and how surface lipids contribute to, hinder, or provide no functional benefit to the system, is to consider the ecology of the gecko. Natural history studies investigating the substrates geckos use in their native environment are lacking [25], thus it is difficult to predict how the reduction of adhesion on hydrophilic substrates due to surface lipids impacts geckos. If geckos encounter few hydrophilic surfaces in their environment, the role of surface lipids in adhesion and anti-adhesion is inconsequential; however, if geckos encounter hydrophilic surfaces regularly, we may then question if the reduction in adhesion due to surface lipids is relevant to the functional needs of the gecko. For instance, perhaps adhesion to hydrophilic surfaces is strong enough to support a gecko, or in contrast, perhaps adhesive toe pads without surface lipids are too sticky to quickly and reliably detach from these surfaces. The answers to these questions will provide the next steps in understanding the evolution of the adhesive and anti-adhesive systems of the gecko, and accelerate functional improvements of the synthetic designs that mimic them.

6. Implications of using a system-level approach to investigating natural models for synthetic design inspiration

The results of the study above serve as an important example to all who study hierarchical biological surfaces, and to those who wish to mimic them. In these experiments we found that the gecko adhesive system does not perform ‘maximally’ in all conditions (i.e. hydrophilic glass), and rather, reduction of adhesion due to surface lipids is likely related to broader, system-level requirements that may be a result of any number of sources. It is also important to remember that only three types of surfaces were tested in air, and one in water in these experiments; imagine all the surface conditions and substrates a gecko encounters during its normal activities and what the results of those experiments may yield [25].

The majority of gecko-inspired synthetic adhesives focus completely on adhesion, rather than anti-adhesion (though see [64]) and other properties. In fact, most gecko-inspired synthetics only mimic the fibrillar setal geometry, paying little attention to chemistry, material properties or even context (i.e. the adhesive environment), all of which appear to be important parameters in the natural system. Remarkably, many of these synthetics perform well in laboratory conditions, some of which even outperform the natural system in terms of adhesive performance (e.g. [65]). Yet, why do we not have a gecko-inspired adhesive that can resist high loads, attach and detach with ease, self-clean, remove water, maintain material properties in variable environments, and resist wear and deformation simultaneously? The answer to this question is likely our narrow focus on singular structure–function relationships which do not incorporate system-level requirements. Optimization is not a foreign concept to engineers and designers [66], yet it is surprising that we do not appreciate the need for optimization in nature as well. A ‘perfect’ hierarchically structured gecko foot likely does not exist, rather a gecko foot that functions well enough to support the survival of a particular species of gecko, specific to a particular region or habitat, does. There are over 1400 species of gecko [67], many of which possess hierarchically structured toe pads, yet current work focuses on only a small handful of species, with a strong bias towards the Tokay gecko (G. gecko) [25], a species native to tropical forests in southeast
Asia. Without broadening our approach, both to system-level properties and trade-offs, and to species-level evolution and ecology, our pursuit of well performing, optimized gecko-inspired adhesives will never leave the laboratory.

The argument that system-level investigation and design is necessary to make the next steps in gecko-inspired adhesives is not limited to this system. Consider our other examples of biological hierarchically structured surfaces such as the butterfly wing or bird feather, the lotus leaf or bone. The highly structured bird feather certainly has functions other than colour production, principally flight. How does the hierarchical surface of the feather impact air flow and is there a trade-off in colour production, a trait that is generally tied to successful breeding in birds? Furthermore, how do colour, flight and other system-level requirements interact with the environmental conditions a bird lives in? For example, Eliason & Shawkey [68] found that duck feather structural iridescence decreases hydrophobicity, which means that shiny feathers are less effective at lotus leaf-like self-cleaning. For a bird that lives most of its life in and around water, this represents a significant system-level trade-off. Similar to the gecko adhesion/anti-adhesion paradox, the answers to the bird feather questions, and others related to biological hierarchically structured surfaces, require a series of investigations that target independent and interactive properties. Only this system-level approach will highlight where important trade-offs exist, allowing biomimics and bioinspired designers to make optimization choices when creating these surfaces synthetically.

7. Conclusion

We believe that the hierarchically structured gecko toe pad provides a relevant and current example of how system-level investigation will facilitate improved understanding of both the biological system and design parameters that can be used in synthetic mimics. Although the hierarchical structuring of the gecko toe has been investigated extensively, previous work has principally been focused on the adhesive system. Geckos however also have a very sophisticated anti-adhesive system. This system has been studied far less often, and together these seemingly opposing systems must work together to support a gecko’s survival in its natural environment. As a result, it is perhaps not surprising that we find a clear trade-off in adhesion related to the surface lipids that cover the adhesive/anti-adhesive setae. Our results represent the first study to directly investigate the role of surface lipids in gecko adhesion and anti-adhesion, and highlight the need to consider system-level interactions in the investigation of natural structured surfaces for improved design of synthetic mimics.

Ethics. All procedures involving live animals were approved by the University of Akron IACUC protocol 07-4G and are consistent with the guidelines published by the Society for the Study of Amphibians and Reptiles (SSAR 2004).

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors’ contributions. A.Y.S. and S.S. performed the adhesion experiments. D.J. performed the anti-adhesion experiments and confirmed the lipid treatment methods. A.Y.S., P.H.N. and A.D. analysed the data. All authors discussed the results, wrote and commented on the manuscript.

Competing interests. We declare we have no competing interests.

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References


