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Claw Morphology Impacts Clinging Performance on Rough Substrates

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Claw Morphology Impacts Clinging Performance on Rough Substrates

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Abstract

The digits of anoles and geckos have been extensively investigated for their adhesive pads, which allow them to adhere effectively to smooth surfaces. Many pad-bearing lizards also possess claws, which have been posited as the principal contributor to clinging on rough surfaces. Previous studies have found correlations between habitat use and claw morphology, but whether variation in claw morphology impacts function or not has remained relatively understudied. In addition, it is reasonable to suggest that abrasive wear of the claws may impact clinging ability. I examined both natural and induced variation in the claw morphology of Cuban knight anoles (*Anolis equestris*) to examine the effects of form and wear on clinging performance on several surfaces of various roughness profiles. I removed the claws of preserved specimens and modified claw morphology using a rotary tool. Claw morphology was quantified via univariate measures typically used in previous work as well as multivariate geometric morphometrics. Results show no effect of claw morphology on clinging on the two smoothest surfaces. However, on the two rougher surfaces, relatively more hooked and long claws induced the highest clinging forces. Additionally, claw wear did not affect induced maximum clinging force. These results suggest that the significance of claw morphology to the clinging ability of lizards may vary with substrate and habitat use. The relative insignificance of claw morphology to clinging on smooth surfaces supports the hypothesis that clinging in lizards with adhesive toe pads is achieved synergistically by both the claw and toe pad across substrates.

Introduction

Claws are one of the most common attachment structures among lizards, where they are found across almost all species regardless of habitat use (Zani 2000; Tulli et al. 2009; Crandell et al. 2014; D'Amore et al. 2018). Among lizards, claws are primarily used in locomotion and for clinging to substrates (Zani 2000; Naylor and Higham 2019). In some clades, including gekkotans and anoles, these structures are present in addition to adhesive subdigital pads (Ruibal and Ernst 1965). The presence of both claws and adhesive toe pads suggests some functional redundancy or synergism in relation to attachment (Naylor and Higham 2019).

While the claws and toe pads of lizards both contribute to attachment, they appear to function optimally under different conditions. The toe pads of geckos and anoles bear microscopic fibrils of β -keratin called setae, which arrange into expanded scales called scanzors (geckos) or lamellae (anoles) (Maderson 1964). Setae adhere by making close enough contact with substrates for van der Waals forces to become substantial (Autumn et al. 2006). Multiple studies have shown an interaction between the roughness of surfaces and the attachment forces of lizards, although this relationship is not straightforward (Huber et al. 2007; Naylor and Higham 2019). Some studies have suggested the relationship between adhesion and roughness is based on the morphology of the pad, with roughness amplitudes that correspond to lamellar length drastically decreasing adhesive capacity (Gillies et al. 2014). Others have suggested that the contact fraction of the setae is the most important factor for predicting adhesion, with more setal contact allowing for greater attachment; in this case, any amount of roughness would decrease adhesion (Russell and Johnson 2007). Analysis of setal morphology is difficult, however, and has rarely been conducted (Russell and Johnson 2007; Garner et al. 2019).

In contrast with toe pads, claws induce attachment forces primarily through mechanical interlocking with the substrate, though they are also capable of inducing frictional clinging forces (Dai et al. 2002). There must be some asperities present on the substrate for interlocking to occur and for claws to induce significant clinging forces. The claw's position is adjusted by the flexor muscles, which are responsible for producing the claw's clinging forces seen in lizards (Abdala et al. 2009).

The evidence suggests that claws and toe pads have evolved synergistically to function optimally on different substrates (Naylor and Higham 2019; Yuan et al. 2019). Despite this synergy, certain lineages of gekkotans have lost and regained toe pads over their evolutionary history, and claws have also been lost several times, though only in lineages with toe pads and often not on all digits (Gamble et al. 2012; Khannoon et al. 2015). These natural losses provide opportunities to study the relative functions of both components in the animal. Furthermore, selective environmental pressures likely relate to these gains and losses, as the presence of the structures may pose some risk from damage or locomotor impediment (Naylor and Higham 2019). However, the apparent synergy between digital morphology and local substrates is not always straightforward: clawless species of geckos are known to inhabit rough, rocky substrates (Russell and Johnson 2007), suggesting the relationship between substrate and digital morphology requires fine analysis of both the habitat and the morphology.

Several recent studies have correlated the forms of claws with certain aspects of habitat use and preference. For instance, Crandell and colleagues (2014), in investigating correlations between anoline digital morphology and habitat use, found that canopy-dwelling species tended to have taller and longer claws than other species. Another recent study of anoles found that claw height and curvature positively correlated with perch diameter and height, respectively,

suggesting that these traits are related to microhabitat (Yuan et al. 2019). Similar investigations of claw form and habitat preference have been conducted in other lizard clades as well. A study of *Varanus* (Varanidae) found that ground-dwelling species tended towards longer claws, and climbing and arboreal species tended towards shorter, taller, more pointed claws (D'Amore et al. 2018). A study of *Liolaemus* (Liolaemidae) also found arboreal and rock-dwelling species to have more highly arched claws, with terrestrial forms having longer and more smoothly curved claws (Tulli et al. 2009). These results suggest that lizard claw morphology correlates with microhabitat and points towards a potential functional relationship. Specifically, taller claws overall correlate with arboreal species, while longer claws predominate with ground-dwelling and trunk-dwelling species.

Fewer studies have directly investigated claw morphology in the context of performance on particular substrates. Zani (2000) measured clinging abilities and digital morphology across lizards and found significant relationships both between claw curvature (derived from the opening angle of the claw) and clinging force on the smooth substrate and between claw height and clinging on the rough substrates. These results do not match the functional expectations set forth by Zani (2000), which posited that claw height, curvature, and length would all positively correlate with enhanced clinging performance on rough surfaces (Zani 2000) (Zani 2000).

Additional studies have measured clinging capabilities of claws on various substrates either in the absence of examination of claw morphology (Dai et al. 2002) or only by recording the presence or absence of claws (Naylor and Higham 2019). Song and colleagues (2016) designed insect-like artificial attachment devices that incorporated a claw, toe pad, or both, but did not vary the morphology of either component, and found that the presence of a claw greatly increased clinging force on rougher substrates. Naylor and Higham (2019) measured the clinging

abilities of *Thecadactylus rapicauda* on various smooth and rough substrates before and after clipping the claws of the geckos and found again that claw removal impeded clinging force production by the animal. However, claw morphology is variable between species and individuals and is likely to impact clinging ability across substrates more finely than the presence or absence of the characteristic.

The claws of lizards, like those of other vertebrates, are composed of β -keratin (Alibardi 2009), rendering them prone to abrasive wear that may alter the morphology of the claw over time. This not only impacts the clinging ability of free-ranging lizards but also may influence the methodological integrity of studies if substrates significantly alter the shape of claws employed in clinging-focused experiments. Such concerns have been posited in past studies of claw form in anoles (Crandell et al. 2014). The claws of reptiles do grow continuously throughout life (Alibardi 2010), and it is therefore possible that there is a balance between daily wear from abrasion and regrowth of material. Thus, it is of interest to investigate the potential effects of wear on clinging performance, both to validate the present methodology and further inform potential difficulties related to clinging in natural settings.

My study examines variation in *Anolis equestris* claws and its impact on performance. The first part of the study compares the morphology of unmodified and reshaped claws in relation to performance on surfaces with differing roughness profiles. Based on Dai et al.'s mechanical interlocking hypothesis (2002), I predicted that claws that are more pointed and arched will induce the highest shear force measurements on rougher surfaces, whereas claws with greater tip diameter will induce greater friction and may have a slight advantage on smoother surfaces.

The second part of my study analyzes the effects of wear on clinging ability. Due to the concern that the claws may significantly wear down and change shape across samples, another set of claws was tested repeatedly on only two of the substrates. I expected that the tip of the claw may wear down over five trials, with greater wear on rougher surfaces, resulting in a larger tip diameter and reduced force induction.

Methods

Animals and Sample Preparation

Throughout the following procedures, five Cuban knight anole (*Anolis equestris*) specimens (35 total claws) from a previous study were used (Garner et al. 2019). The specimens were fixed in 10% neutral buffered formalin and subsequently stored in 70% ethanol. For testing, the claw was removed from the specimen at the distal end of the toe pad (**Figure 1**). This portion of the digit was then adhered via epoxy to a glass slide, with the claw inverted to approximate the angle of the claw at rest on the specimen (**Figure 1**). After the epoxy cured overnight, excess biological material at the proximal end of the sample was removed. To explore potential claw shapes not expressed in the sample, half of the claws were selected for shape adjustment. For these samples, a rotary tool (Dremel Moto-Tool Model 395 Type 3; Dremel, Racine, WI) was used to alter the shape of the claw. These adjustments involved removing claw material from any combination of the inner curve, outer curve, or tip of the claw, followed by some reshaping to maintain a pointed tip. Modifying shape in this way experimentally altered length and height of the claw directly, and likely influenced the overall shape as well.



Figure 1: Toe pad and claw of *Anolis equestris*. The red dashed line represents the plane where the claw was separated from the toe pad. After inverted attachment to the glass slide, more tissue was removed from the proximal end of the sample. Photo credit: Austin M. Garner.

Friction Cell Testing

To measure the shear forces each claw could induce, the claws were displaced in a friction cell along substrates of different roughnesses. The friction cell uses two picomotors to finely manipulate the relative positions of the substrate and the sample (Newport NewFocus Picomotors; Newport Corporation, Irvine, CA). When the substrate and sample come in contact, forces are exerted on connected springs that change the relative positions of capacitors. Measured changes in capacitance correspond to increases or decreases in applied normal and shear forces. For testing, each claw sample was brought into contact with the substrate with a preload of approximately 39.1 ± 4.8 mN. To determine the appropriate preload, the average mass of the *A. equestris* specimens was calculated and divided by 20, yielding the approximate loading on one of the animal's digits. The claw was brought into contact perpendicular to the substrate, which differs from how the claw would be placed by the animal, where it would come into contact at an angle. Then, the claw was displaced for 1 mm across the substrate, as if the animal were pulling its limb back towards its body. The shear and normal forces exerted on the claw were repeatedly measured throughout the displacement. From these data, maximum shear force,

average peak shear force, initial preload, and average normal force were each calculated in millinewtons. Typical curves for maximum force over time are shown in **Figure 2**.

This procedure was repeated once for each of twenty claws on each of the four substrates of interest, with the order randomized. These substrates include glass, 1500 grit sandpaper (average particle size 5 μm), 800 grit sandpaper (average particle size 15 μm), and 320 grit sandpaper (average particle size 40 μm ; 3M Wetordry Sandpaper; 3M, St. Paul, MN). Glass was included as a standard smooth surface for testing. For the study of claw wear, only the 1500 grit and 320 grit sandpapers were employed to test for differential wear effects, and fifteen samples were tested five times on the same substrate (eight on 1500 grit sandpaper; seven on 320 grit sandpaper).

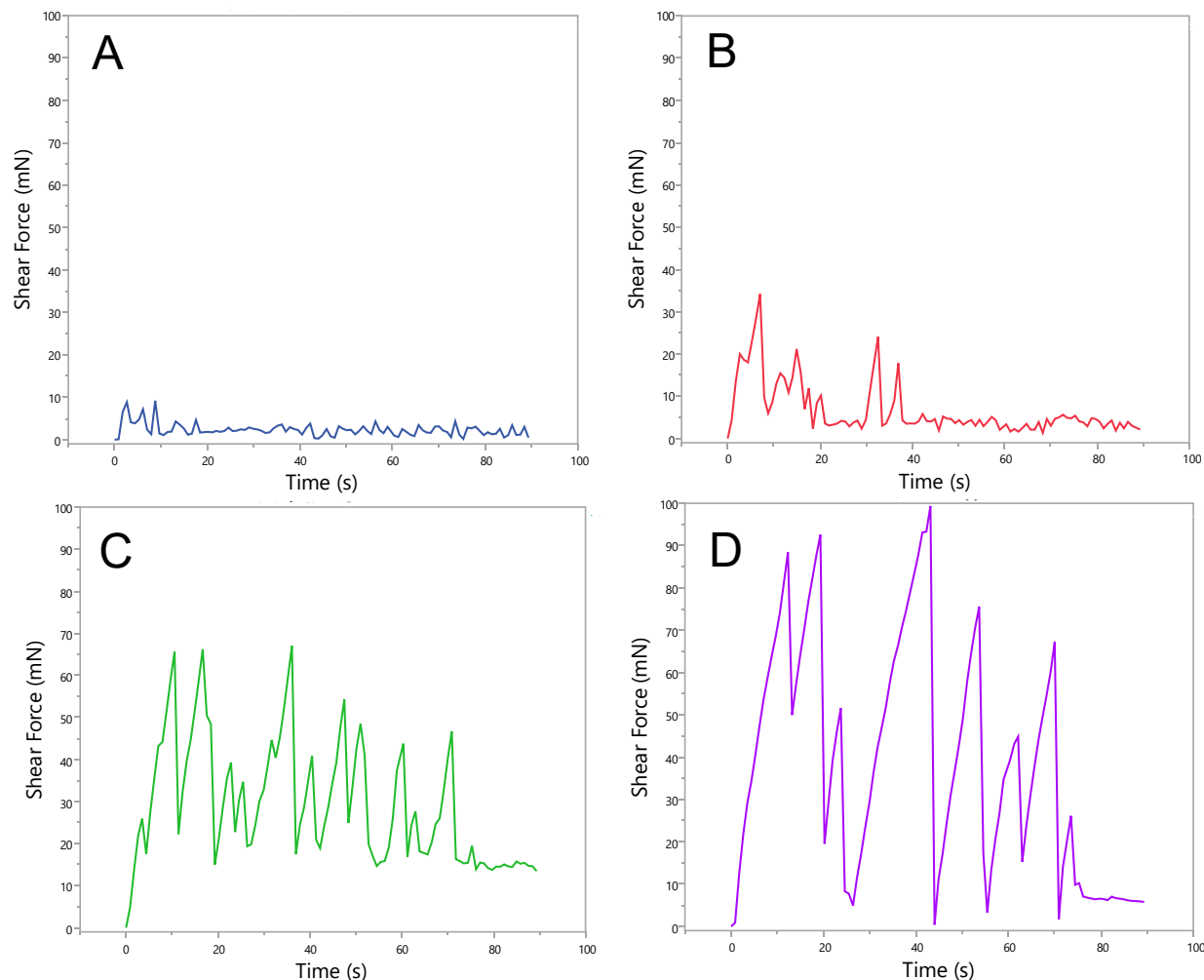


Figure 2: Curves showing the shear force produced over time during a typical trial on each of the four substrates: glass (a), 1500 grit (b), 800 grit (c), and 320 grit (d). Peaks in force may represent the claw interlocking with large asperities in the surface, though this observation could not be confirmed given the small size of the claws.

Morphometric Analyses

A lateral view image of each claw was taken prior to every trial via light microscope (Olympus SZX16 Stereomicroscope, Olympus Corporation, Japan). These images were paired with the corresponding trial. Pilot testing revealed no effects of wear on force induction, but wear led to an increase in tip diameter across trials. As a result, images were retaken before every trial to ensure that the referenced morphometric data were as accurate as possible.

Two different methods were used to analyze claw morphology. Traditionally, lizard claw morphology has been analyzed via comparison to a triangle, as originated by Zani (2000). The claws throughout this study were analyzed with this method to enable more direct comparison to past studies. More recently, Tinius and Russell (2017) originated the use of geometric morphometrics in lizard and bird claws. This method of shape analysis gives a more holistic view of claw shape and was therefore also used in this study. Both methods were applied to the study of claw form and performance, while only the Zani method was applied to the study of claw wear.

The procedure used for geometric morphometrics here is drawn primarily from both Tinius and Russell's work and that of Yuan and colleagues, who studied the ecomorphology of anole claws (Tinius and Russell 2017; Yuan et al. 2019). On each claw image, 60 semilandmarks were placed along the outline of the claw in *tpsDig2* (Rohlf 2018). 30 semilandmarks were placed along the outer curve and 30 were placed along the inner curve (Tinius and Russell 2017). During Procrustes superimposition, semilandmarks were allowed to slide along the curve to better approximate the shape of the claw (Gunz and Mitteroecker 2013). Sliding and subsequent shape analyses were achieved via the *geomorph* package for R (R Core Team 2017; Adams et al. 2017).

Zani's method of claw shape analysis fits the inner curve of the claw to a triangle, from which measurements of length and curvature are derived (Zani 2000). An illustration of this method's application is presented in **Figure 3**. Points are placed at the base of the claw, the tip of the claw, and the inflection point of the inner curve (Tinius and Russell 2017). Length A is the distance from base to inflection point, length B is the distance from inflection point to tip, and

length C is the distance from tip to base. Claw length is the sum of A and B, and curvature is calculated as:

$$Curvature = 57.296 * \frac{2 * \arcsine \sqrt{(2 * A^2 * B^2) + (2 * A^2 * B^2) + (2 * B^2 * C^2) - A^4 - B^4 - C^4}}{2AB}$$

In addition, claw height is measured as the distance from the base of the claw to the top of the claw, and tip diameter is measured as the smallest possible distance across the tip of the claw.

These measurements were taken in ImageJ (National Institutes of Health, Bethesda, MD).

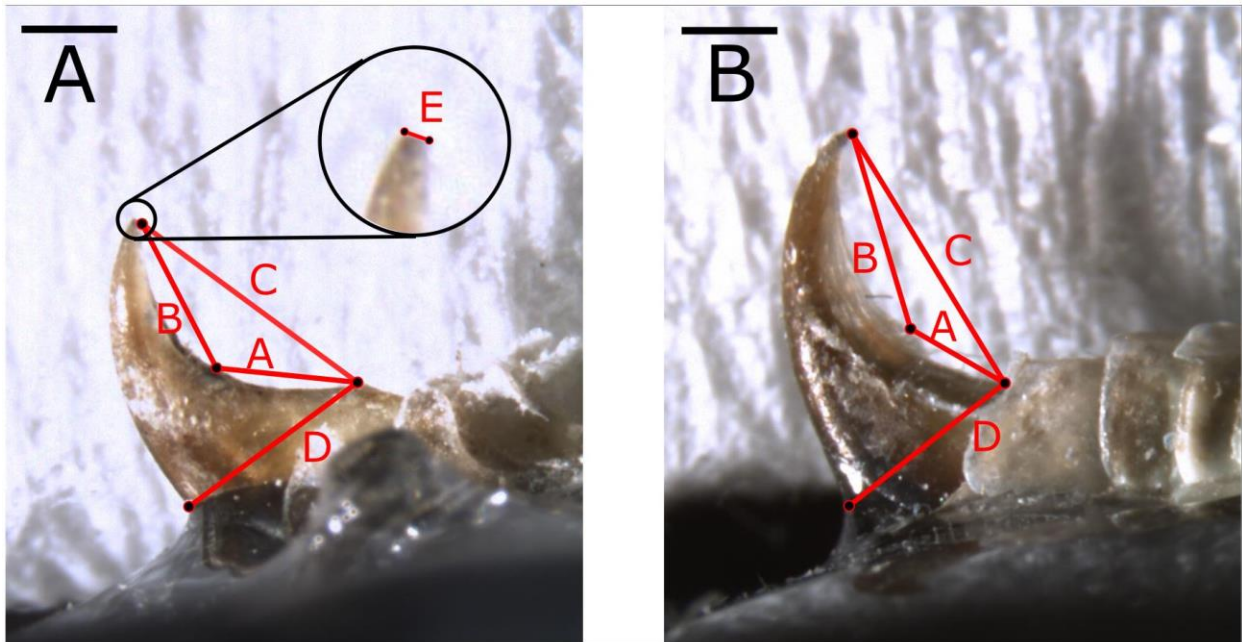


Figure 3: An example of the univariate measures taken in this study. The figure on the left (a) shows the claw from the second digit of the right hind limb of the specimen; the figure on the right (b) is from the same digit of the left hind limb of the same specimen; this sample was modified via rotary tool. Measurements are length A (distance from base to inflection point), length B (distance from inflection point to tip), length C (distance from tip to base), height (labeled D), and tip diameter (labeled E; Zani 2000). The scale bar represents 500 microns.

Statistical Analyses

A principal component analysis (PCA) was used to find mean shape values from the sliding semilandmarks, both for the samples collectively and the variation seen on each of the

significant principal component (PC) axes. The components that contributed significant variation as determined by the broken stick criterion were regressed against maximum shear force by substrate. While maximum shear force and average peak shear force were both collected, trends in the data were similar, so maximum shear force was used. To account for any confounding variables, the specimen, side of the body, and digit each sample came from, as well as the trial, were checked for relationships with maximum shear force. Differences between specimens, digits, and trial were each analyzed using one-way analyses of variance (ANOVAs); side of the body was analyzed via t-test. In addition, to check for an effect of claw size on shape, the Procrustes coordinates were regressed against centroid size with a one-way ANOVA. Whether modification of the claw had an effect was also analyzed via t-test. To analyze the effects of univariate claw characters on maximum shear force, bivariate regressions were used for curvature, length, height, and tip diameter. A repeated measures multivariate analysis of variance (MANOVA) was used to test for changes in morphological characters and maximum shear force induced over the five claw wear trials and between substrates.

Results

To determine whether variation in morphology influences clinging ability on different rough surfaces, geometric morphometric methods (GMM) of analysis were applied to the claw samples. The analyses returned seventy-nine principal components (PCs) that each explained some variation in shape. By applying the broken stick criterion, the first four PCs were selected for further analysis; each of these accounted for more than 5% of the overall variation (**Table 1**). The consensus shape with corresponding sample variation is shown in **Figure 4**. Full results for the effects of morphology on performance are shown in **Table 2**.

Principal Component	Variation Contributed	Cumulative Variation
PC1	0.5710	0.5710
PC2	0.15507	0.72609
PC3	0.10873	0.83482
PC4	0.05245	0.88727

Table 1: The first four principal components of the PCA measuring variation in claw shape. Collectively, these four PCs account for approximately 89% of the variation seen in claw shape, with each contributing more than 5% of the variation. Other principal components produced by the analysis were excluded from further analyses.

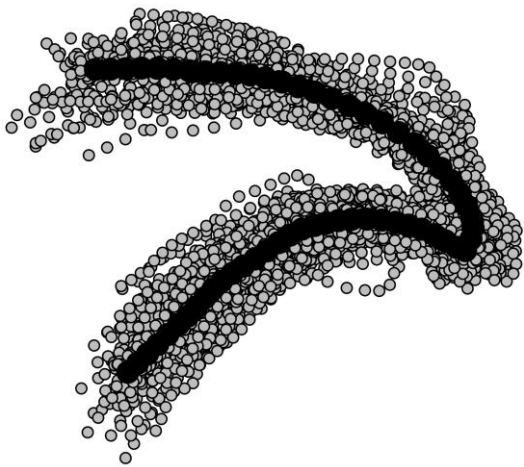


Figure 4: The consensus shape determined by the partial Procrustes superimposition while allowing semilandmarks to slide and indication of the variation around the shape. The consensus shape shows the average form taken by the samples and is formed by the black dots. Gray dots show the variation seen in samples around the consensus shape.

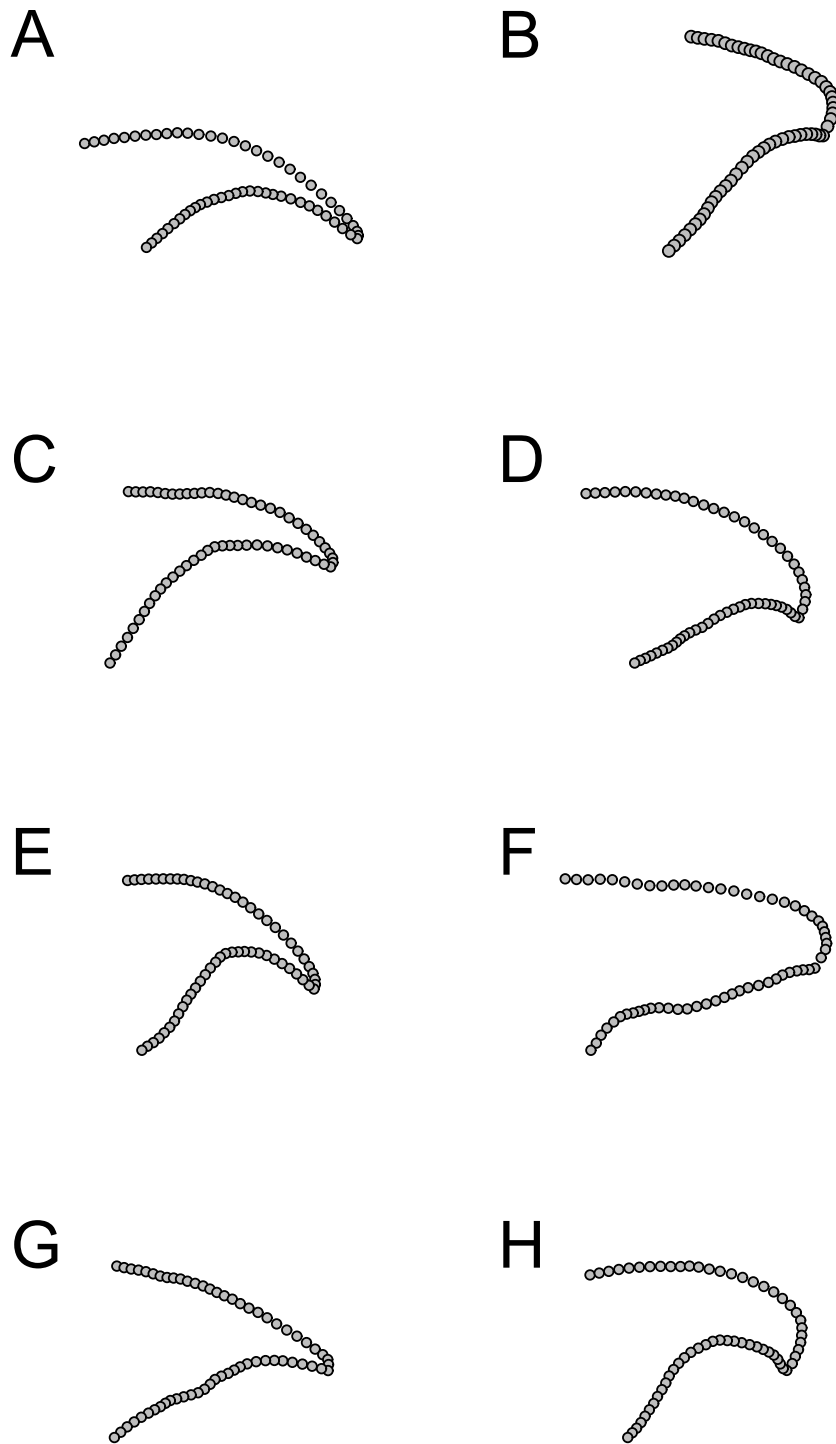


Figure 5: The minimum and maximum variants, respectively, for each of the principal component axes included in analyses: PC1 (a, b), PC2 (c, d), PC3 (e, f), and PC4 (g, h).

	Principal Component 1				Principal Component 2			
	<i>F</i>	d.f.	R ²	<i>p</i>	<i>F</i>	d.f.	R ²	<i>p</i>
Glass	0.0051	19	0.000284	0.9438	1.4154	19	0.0729	0.2496
1500 Grit	0.2545	19	0.0139	0.6201	0.9163	19	0.0484	0.3511
800 Grit	18.4013	18	0.520	0.0005	0.6031	18	0.0343	0.4481
320 Grit	0.8130	19	0.0432	0.3792	0.6374	19	0.0342	0.4351

	Principal Component 3				Principal Component 4			
	<i>F</i>	d.f.	R ²	<i>p</i>	<i>F</i>	d.f.	R ²	<i>p</i>
Glass	0.1047	19	0.00578	0.7500	2.0863	19	0.104	0.1658
1500 Grit	2.5148	19	0.123	0.1302	0.1895	19	0.0104	0.6685
800 Grit	0.6452	18	0.0366	0.4329	0.4470	18	0.0256	0.5128
320 Grit	24.0075	19	0.572	<0.0001	0.3553	19	0.0194	0.5586

Table 2: Full statistical results for the effect of each principal component on maximum shear force production on the four substrates used. Significant values are in bold.

Principal component 1 (PC1) primarily accounted for claw length and height, with smaller values representing relatively longer, less tall, more pointed claws and higher values representing relatively taller, shorter claws (**Figure 5a and 5b**). PC1 did not predict maximum shear force on glass ($p=0.9438$), 1500 grit sandpaper ($p=0.6201$), or 320 grit sandpaper ($p=0.3792$), but had an inverse relationship with maximum shear force on 800 grit sandpaper ($p=0.0005$; **Figure 6**). Claws that are long, thin, and pointed induced the highest forces on this substrate.

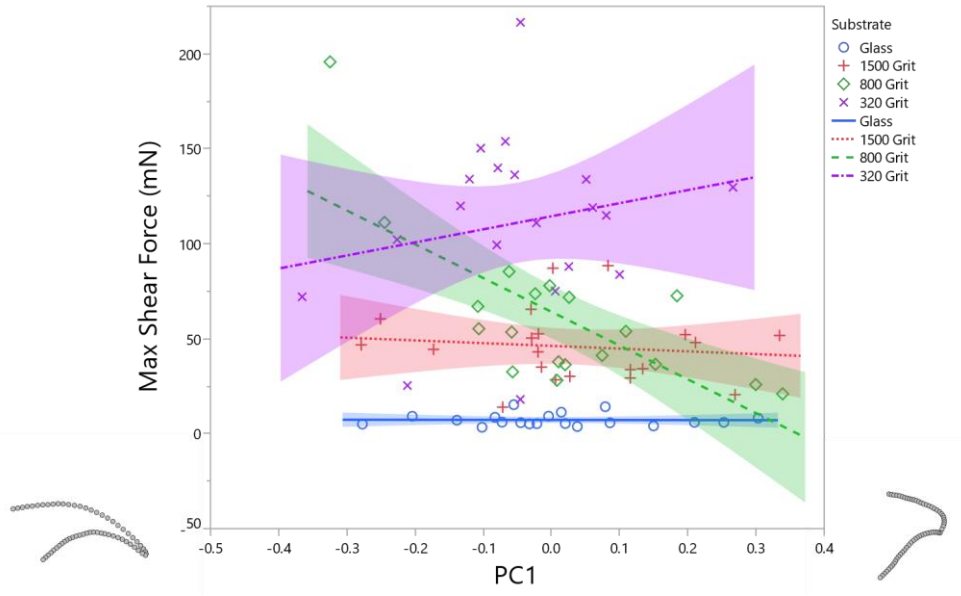


Figure 6: Maximum shear force induced on each of the four substrates by principal component axis 1. The x-axis represents the range of variation between the end figures, with the minimum representing longer, thinner, more pointed claws and the maximum representing taller, shorter claws. Shaded areas around the regression lines represent 95% confidence intervals. Only the regression line for 800 grit sandpaper (green diamonds) is significant ($p=0.0005$).

Principal component 2 (PC2) primarily revealed variation in claw length, with minimum values representing longer claws and maximum values representing shorter, almost hooked claws (**Figure 5c and 5d**). Unlike PC1, however, height was fairly constant. This principal component did not predict maximum shear force induction on any substrate ($p=0.2496$ for glass; $p=0.3511$ for 1500 grit sandpaper; $p=0.4481$ for 800 grit sandpaper; $p=0.4351$ for 320 grit sandpaper; **Figure 7**).

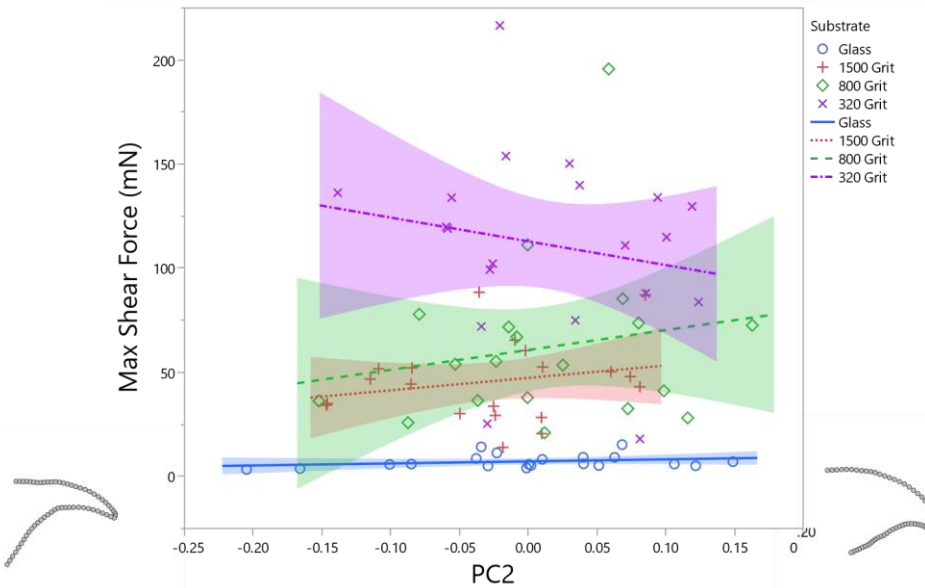


Figure 7: Maximum shear force induced on each of the four substrates along principal component axis 2. The x-axis represents the range of variation between the end figures, with the minimum representing thin, pointed claws and the maximum representing thick, slightly hooked claws. Shaded areas around the regression lines represent 95% confidence intervals. No regression lines are significantly different from the others.

Principal component 3 primarily indicated claws with a highly curved and pointed minimum and a relatively untapered, uncurved maximum (**Figure 5e and 5f**). While PC3 did not predict shear force on glass ($p=0.7500$), 1500 grit sandpaper ($p=0.1302$), or 800 grit sandpaper ($p=0.4329$), it did positively correlate with maximum shear force on 320 grit sandpaper ($p<0.0001$; **Figure 8**). Highly curved and pointed claws induced the highest force on this substrate, and unpointed, uncurved claws induced the lowest forces.

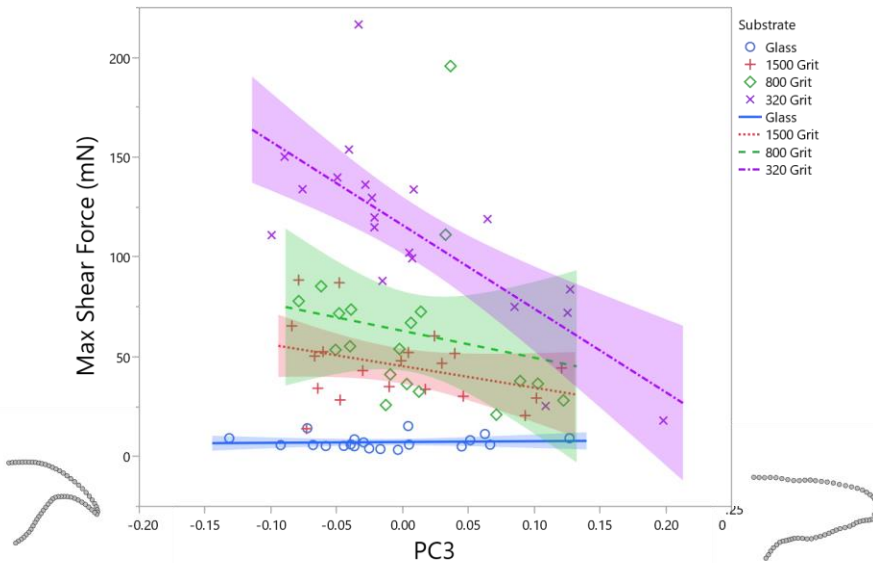


Figure 8: Maximum shear force induced on each of the four substrates along principal component axis 3. The x-axis represents the range of variation between the end figures, with the minimum representing tall, pointed claws and the maximum representing slightly longer claws with minimal taper. Shaded areas around the regression lines represent 95% confidence intervals. Only the regression line for 320 grit sandpaper (purple x's) is significant ($p < 0.0001$).

Principal component 4's minimum values represented uniformly tapered, pointed claws, and the maximum values represented highly arched and pointed claws (**Figure 5g and 5h**). Like PC2, this principal component did not significantly predict maximum shear force on any substrate ($p = 0.1658$ for glass; $p = 0.6685$ for 1500 grit sandpaper; $p = 0.5128$ for 800 grit sandpaper; $p = 0.5586$ for 320 grit sandpaper; **Figure 9**).

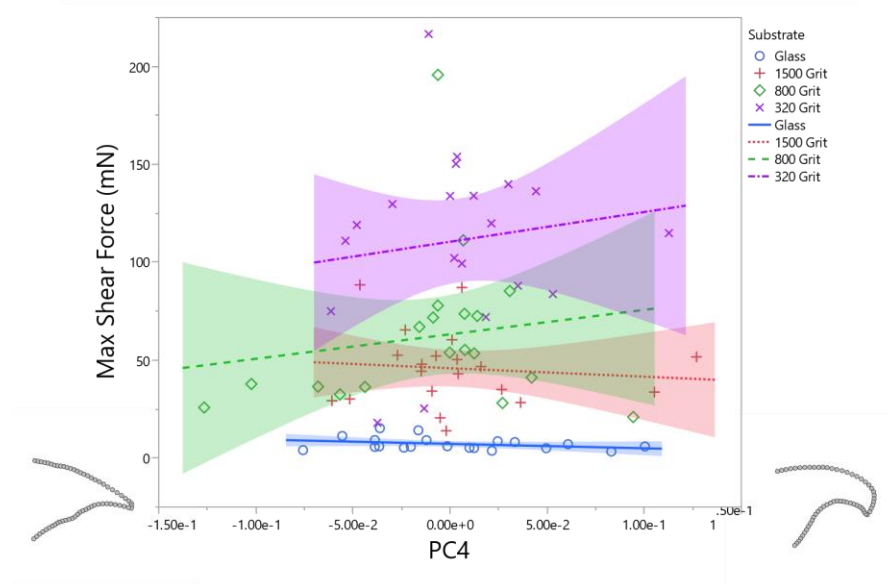


Figure 9: Maximum shear force induced on each of the four substrates along principal component axis 4. The x-axis represents the range of variation between the end figures, with the minimum representing triangular, pointed, uncurved claws and the maximum representing extremely hooked and pointed claws. Shaded areas around the regression lines represent 95% confidence intervals. No regression lines are significantly different from the others.

Claw morphology was also analyzed with the univariate characters curvature, height, length, and tip diameter; these results are summarized in **Table 3**. None of these traits were predictive of maximum shear force on glass ($p=0.1251$ for curvature; $p=0.4469$ for length, $p=0.3368$ for height; $p=0.4350$ for tip diameter), 1500 grit sandpaper ($p=0.6948$ for curvature; $p=0.8820$ for length; $p=0.8619$ for height; $p=0.2636$ for tip diameter), 800 grit sandpaper ($p=0.1308$ for curvature; $p=0.9875$ for length; $p=0.0852$ for height; $p=0.1785$ for tip diameter), or 320 grit sandpaper ($p=0.8913$ for curvature; $p=0.2136$ for length; $p=0.0585$ for height; $p=0.1960$ for tip diameter).

	Curvature				Length			
	<i>F</i>	d.f.	R ²	<i>p</i>	<i>F</i>	d.f.	R ²	<i>p</i>
Glass	2.5882	19	0.1257	0.1251	0.6046	19	0.0325	0.4469
1500 Grit	0.1590	19	0.00875	0.6948	0.0227	19	0.00126	0.8820
800 Grit	2.5206	18	0.129	0.1308	0.0003	18	0.0000148	0.9875
320 Grit	0.0192	19	0.00107	0.8913	1.6626	19	0.0846	0.2136

	Height				Tip Diameter			
	<i>F</i>	d.f.	R ²	<i>p</i>	<i>F</i>	d.f.	R ²	<i>p</i>
Glass	0.9737	19	0.0513	0.3368	0.6375	19	0.0342	0.4350
1500 Grit	0.0311	19	0.00173	0.8619	1.3315	19	0.0689	0.2636
800 Grit	3.3411	18	0.164	0.0852	1.9697	18	0.104	0.1785
320 Grit	4.0829	19	0.185	0.0585	1.8029	19	0.0910	0.1960

Table 3: Full statistical results for the effect of each univariate morphological characteristic on maximum shear force production on the four substrates used. Significant values are in bold.

To check for potential confounding variables, characteristics of the samples were analyzed for trends. Maximum shear force was not predicted by the individual the sample originated from ($F=0.6050$, d.f.=78, $p=0.6138$), the side of the body the sample originated from ($t=0.5479$, d.f.=74.41, $p=0.5854$), or the digit the sample originated from ($F=0.2756$, d.f.=78, $p=0.7599$). Trial did not significantly predict maximum shear force ($F=0.3527$, d.f.=78, $p=0.7873$). The size of the claw also did not have an impact on shape ($F=4.6807$, d.f.=78, $p=0.3333$). Interestingly, whether the claw's shape had been manipulated also did not significantly impact maximum shear force ($t=-1.4345$, d.f.=75.20, $p=0.1556$).

With regard to claw wear, samples were repeatedly displaced across either 320 grit or 1500 grit sandpaper. Across the five trials, claw tip diameter significantly increased ($F=5.6515$, $d.f.=4$, $p=0.0121$; **Figure 10**), but no other morphological characters were found to vary across trials ($F=0.6984$, $d.f.=4$, $p=0.6120$ for curvature; $F=2.6275$, $d.f.=4$, $p=0.0981$ for length; $F=3.4669$, $d.f.=4$, $p=0.0504$ for height). Despite the increase in tip diameter, maximum shear force induced did not change across trials ($F=0.0856$, $d.f.=4$, $p=0.9848$; **Figure 11**). Furthermore, trial did not interact with substrate ($F=1.0417$, $d.f.=4$, $p=0.4375$) Maximum shear force was, however, influenced by substrate ($F=16.7915$, $d.f.=1$, $p=0.0015$; **Figure 11**), with 320 grit sandpaper eliciting higher forces than 1500 grit sandpaper.

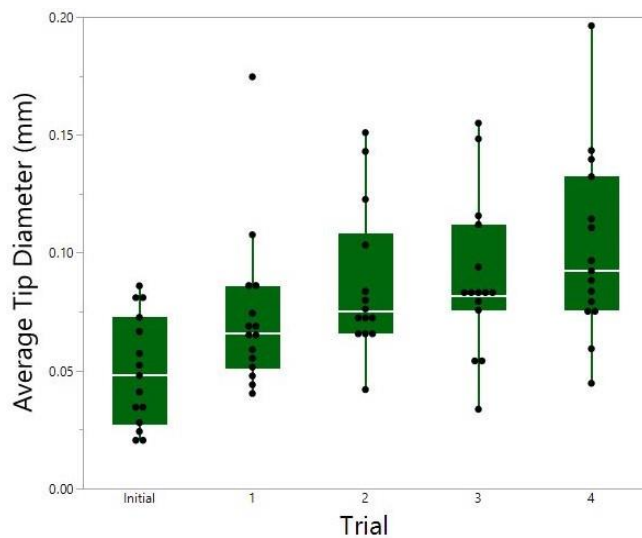


Figure 10: Trends in claw tip diameter (mm) over trials. Trends were similar across both 320 grit and 1500 grit sandpaper. Overall, tip diameter increases over the five trials. No other morphological characters were found to vary across trials.

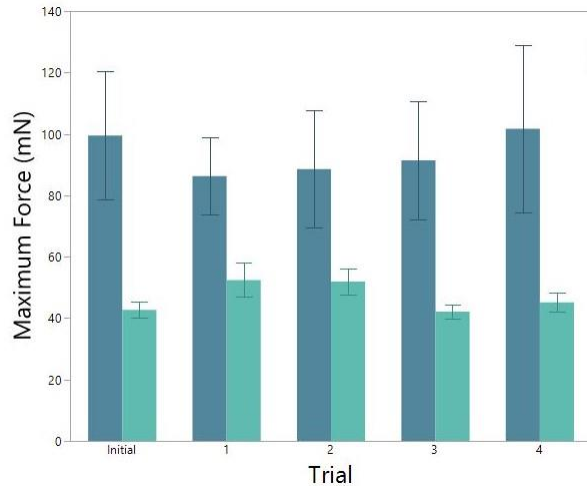


Figure 11: Maximum shear friction force (mN) induced across trials and substrates. Friction forces are higher for 320 grit sandpaper (dark blue bars) than 1500 grit sandpaper (light blue-green bars; $F=16.7915$, d.f.=1, $p=0.0015$). However, there are no trends across trials, despite changes in tip diameter ($F=0.0856$, d.f.=4, $p=0.9848$).

Discussion

Past studies of claw morphology have revealed correlations between claw characters and habitat use, such as canopy-dwelling species having taller and more arched claws and terrestrial species having longer, less arched claws (Tulli et al. 2009; D'Amore et al. 2018; Yuan et al. 2019). Potential functional relationships include claws with greater height being better for piercing or interlocking with the substrate and longer claws functioning as limb extensions for faster locomotion (Zani 2000; Dai et al. 2002; Higham 2015; Naylor and Higham 2019). These functional hypotheses are typically suggested from whole-animal studies, which correlate morphology with ecological variables, such as arboreality or habitat type. To more directly assess the relationship between claw form and performance, my study used claws from *Anolis equestris* specimens to examine the relationship between clinging performance and form on substrates of different roughness profiles.

When analyzed via geometric morphometric methods (GMM), claw form did not correlate with performance on the two smoothest substrates, glass and 1500 grit sandpaper. On 800 grit sandpaper, the next roughest surface, long, slightly arched, pointed claws with low height performed significantly better than taller, less elongated claws (**Figure 6**). On 320 grit sandpaper, highly arched, pointed claws performed significantly better than less curved, unpointed claws (**Figure 8**). Overall, more pointed claws appear to perform better on rougher substrates, with longer claws performing better on some rough substrates. Height may not have been a primary variant because epoxy coverage of some samples prevented measurement of the full height.

Despite these results, when claw shape was approximated with a triangle, no morphological characters were found to predict maximum clinging performance on any substrate. This stands in contrast to previous studies, particularly Zani's original study of claw form and performance, which found clinging to smooth surfaces to be highly correlated with claw curvature and clinging to rough surfaces to be highly correlated with claw height (Zani 2000). It is possible that our results vary because our data did not include other digital morphology characters, such as toe pad measurements, and the univariate data were not analyzed via principal components analysis. However, it has also been suggested that this measurement of curvature is not, in fact, truly measuring the curvature because it approximates the curve to a circle; because all circles have the same curvature, this approximation only shows differences in the diameter of the circle being approximated (Tinius and Russell 2017). A recent study has also suggested that the claw may interfere with placement of the toe pad, leading to more strongly altered performance on smooth substrates (Naylor and Higham 2019). Claw placement is

controlled by muscular input, so there may be some degree of control in the effectiveness of contact and interlocking (Abdala et al. 2009).

Using a separate sample of claws, changes in morphology resulting from wear were measured across repeated trials. While minor wear resulted in an increase in tip size (**Figure 10**), this wear did not affect clinging at these scales (**Figure 11**). This suggests that the biological specimens used during the study of morphology and performance were not significantly affected by wear across trials. Minor claw wear likely occurs for free-ranging lizards occupying roughened substrates. However, wear at these scales did not affect clinging, suggesting that natural wear only impacts clinging performance in free-ranging lizards when it occurs in greater amounts than observed in this study. The claws of lizards continue to grow throughout their lives (Alibardi 2010), and this growth may balance with wear over time, negating any effects of wear on claw form and effectiveness. Given that tip diameter does not seem to affect clinging, but the GMM results suggested that the pointedness of the tip is important for clinging on rough substrates, it seems that the minor changes induced in tip diameter by wear were not critical enough to impact clinging, though larger-scale changes in pointedness between samples observed with GMM did. It is possible that more intense wear may have an impact on clinging if it significantly changes the relative relationship between tip and asperity size.

Past studies of ecological morphology have used similar methods to examine correlations between habitat type and morphology. Species that primarily inhabit the upper canopies of trees tend to have the most highly arched and pointed claws (Tulli et al. 2009; D'Amore et al. 2018; Yuan et al. 2019). The results presented here suggest that such claws are optimal for performance on rough surfaces. Given the synergistic relationship between claws and toe pads, it is reasonable to suggest that on smoother leaves, toe pads are most responsible for clinging,

while claws may be maintained for locomotion and station-holding on bark and other rough substrates. Similarly, past studies of terrestrial lizards have found long, thin, pointed claws to be optimal for ground-dwelling species (Tulli et al. 2009; D'Amore et al. 2018; Yuan et al. 2019). My results suggest that this type of claw is also most effective on rough surfaces with moderate asperity sizes. This type of claw is also believed to increase locomotor capacity by acting as a limb extension (Tulli et al. 2009; Higham 2015). In the case of terrestrial lizards, it is possible that this form is dually optimized for both locomotion and clinging. However, within anoles, the twig and trunk-ground ecomorphs have relatively flatter and shorter claws (Yuan et al. 2019). Potential explanations for this trend include optimization for clinging the texture of the bark of their trees or a specific perch diameter, perhaps relative to body size (Yuan et al. 2019). It is unknown if the relatively convergent claw form of the twig and trunk-ground ecomorphs serves the same clinging function, or if instead, trunk-ground anoles use their claws primarily for locomotor benefit, while twig anoles use them for increased clinging capacity.

The mechanical interlocking hypothesis suggests that claws generate shear forces both by frictional forces and by mechanically interlocking with asperities in the substrate when applicable (Dai et al. 2002; Naylor and Higham 2019). My results suggest that frictional forces on smoother substrates are present but likely do not contribute largely to whole-body clinging. However, on rough substrates, the most pointed claws induced the highest forces, suggesting this pointedness is critical and optimal for clinging. Geometrically, more pointed structures are better able to interlock between asperities, but this depends on the precise orientation of the claw relative to the asperities. While some muscular input may optimize this interaction, the degree and effectiveness of this positioning is unknown.

Mathematical and physical models of claws may be able to further describe the relative contributions of different morphological characters to clinging ability. Fibrillar adhesion research has created synthetic adhesives to determine which components of setal morphology and composition are critical to adhesion (Spolenak et al. 2005). A similar approach to claw morphology, involving the construction of models that systematically vary in important characteristics, may impact the understanding of morphology on claw performance. Similarly, mathematical modeling may better explain why some morphological characters are associated with different levels of performance. Substrate characterization may also be critical to understanding the relationship between claw morphology and performance. Past studies of claw modelling in insects have suggested that the relationship between asperity size and claw size is critical to mechanical interlocking of the claw with the substrate (Song et al. 2016). While the grit of the sandpapers used in this study is known, more complete examinations of their roughness are required for comparison to the morphology of the claw, particularly across length scales. Furthermore, the microtopography of the substrates on which free-ranging anoles move has not been well characterized. Some recent studies have examined clinging and adhesion on natural surfaces of different roughnesses (Naylor and Higham 2019). However, finer characterization of these surfaces, such as that available through surface roughness power spectra (Higham et al. 2019; Niewiarowski et al. 2019), may yield a better understanding of the mechanical interaction of claws and toe pads with the substrate. While it is difficult to separate the influences of the claw and toe pad, studies that are able to examine one without the other, especially in living animals, may be able to examine the relative contributions of both structures and their synergy across various substrates.

Conclusions

Digital morphology is critical for understanding the adhesive ecology of anoles and geckos. These results suggest that while claw morphology does not affect clinging on smooth substrates, the pointedness and curvature of the claw is critical for achieving mechanical interlocking on rough substrates. This optimization for rough surfaces may interact synergistically in adhesive lizards that possess toe pads that are most effective for clinging on smooth substrates. Furthermore, minor claw wear does not impact clinging, suggesting that free-ranging lizards are able to cope with these stresses. A cohesive understanding of claw and toe pad morphology will inform the study of fibrillar adhesion and adhesion ecology in lizards.

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