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Passively stuck: death does not affect gecko adhesion strength

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Many geckos use adhesive toe pads on the bottom of their digits to attach to surfaces with remarkable strength. Although gecko adhesion has been studied for hundreds of years, gaps exist in our understanding at the whole-animal level. It remains unclear whether the strength and maintenance of adhesion are determined by the animal or are passively intrinsic to the system. Here we show, for the first time, that strong adhesion is produced passively at the whole-animal level. Experiments on both live and recently euthanized tokay geckos (*Gekko gecko*) revealed that death does not affect the dynamic adhesive force or motion of a gecko foot when pulled along a vertical surface. Using a novel device that applied repeatable and steady-increasing pulling forces to the foot in shear, we found that the adhesive force was similarly high and variable when the animal was alive (mean $\pm$ s.d. = 5.4 $\pm$ 1.7 N) and within 30 min after death (5.4 $\pm$ 2.1 N). However, kinematic analyses showed that live geckos are able to control the degree of toe pad engagement and can rapidly stop strong adhesion by hyperextending the toes. This study offers the first assessment of whole-animal adhesive force under extremely controlled conditions. Our findings reveal that dead geckos maintain the ability to adhere with the same force as living animals, disproving that strong adhesion requires active control.

1. Introduction

A gecko's remarkable ability to climb on vertical and inverted surfaces has captivated scientists for hundreds of years [1]. Investigations have revealed that the underside of gecko feet exhibit toe pads containing tiny hair-like structures, called setae [2], which adhere to contacted surfaces through intermolecular (van der Waals) [3] and frictional [4,5] forces. The adhesive strength of a single seta is less than a millinewton [3], but the millions of setae on gecko feet can support 20 times the animal's body weight [6]. The structure of setae [7] and micro-mechanics of setal adhesion [3,4] have been well described, but our understanding of how geckos control their clinging ability at the whole-animal level is limited.

Does a gecko control the maintenance and strength of adhesion, or are these factors intrinsic to the system and, hence, fundamentally passive? In order for an isolated seta to adhere, it must be appropriately oriented and preloaded with subtle forces directed normal and in parallel to the surface [3]. While this suggests that geckos may control adhesion with limb and digit movements used to preload the many setae [8], preload forces have not been detected when a gecko deploys its adhesive system [3] and the source of this preload has been elusive [9]. It has also been proposed that geckos may finely control adhesive strength with muscles that depress the setae-bearing toe pads towards the surface [2,8]. A complex tendon system connecting limb muscles with the toe pads [2] potentially allows the gecko to modulate how the toe pads interact with the surface and, in turn, control the attachment and loading of setae [9]. However, limb muscle activity has not been linked to adhesion strength. Therefore, it remains unclear if actions by the animal, such as specific movements or muscle activity, influence the strength of adhesion.

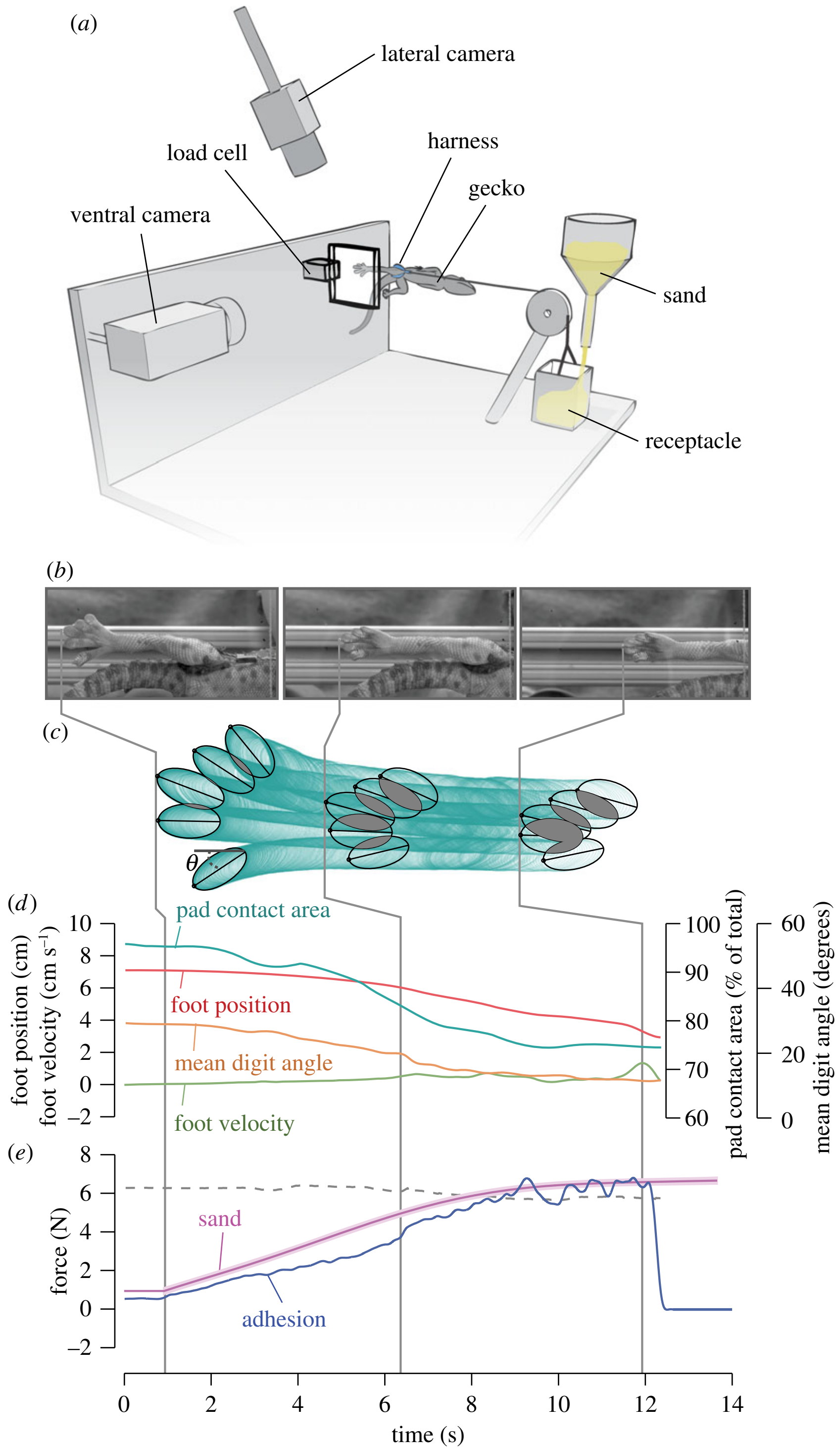

Figure 1. Measuring adhesion by a gecko foot. (*a*) Illustration of the custom pulling device. (*b*) Video images of the ventral surface of the clinging gecko foot. (*c*) The positions of the adhesive toe pads were modelled with ellipses (teal) throughout the cling, with black ellipses indicating the toe pad positions corresponding to the images in (*b*). Areas of toe pad overlap are shown in grey. θ, digit angle. (*d,e*) The kinematics and force production of the gecko foot during the cling, respectively. The dashed grey line shows our predictions for maximum adhesion based on digit posture and contact (see §2). The purple-shaded region in (*e*) indicates the 95% prediction intervals for the sand force, measured prior to experiments.

Here we examined the passive versus active nature of whole-animal adhesion in geckos by comparing clings before and immediately after death. The goals of this study were as follows: (i) determine how death affects whole-animal adhesive strength, and (ii) determine how death affects the posture and motion of clinging gecko feet. This was achieved with a novel device that measured whole-animal clinging ability with unprecedented standardization, thus permitting controlled comparisons between gecko clings.

2. Material and methods

(a) Measuring gecko clings

We developed a novel pulling device (figure 1*a*) that measured the clinging ability of five tokay geckos (*Gekko gecko*, mean body mass = 46.1 ± 10.0 g) before and immediately after death. This device measured clings by pulling a gecko foot in a highly controlled manner along a vertical 10 × 10 cm acrylic sheet (thickness = 0.6 cm; cleaned with 95% ethanol) while simultaneously recording shear adhesion with a precision load

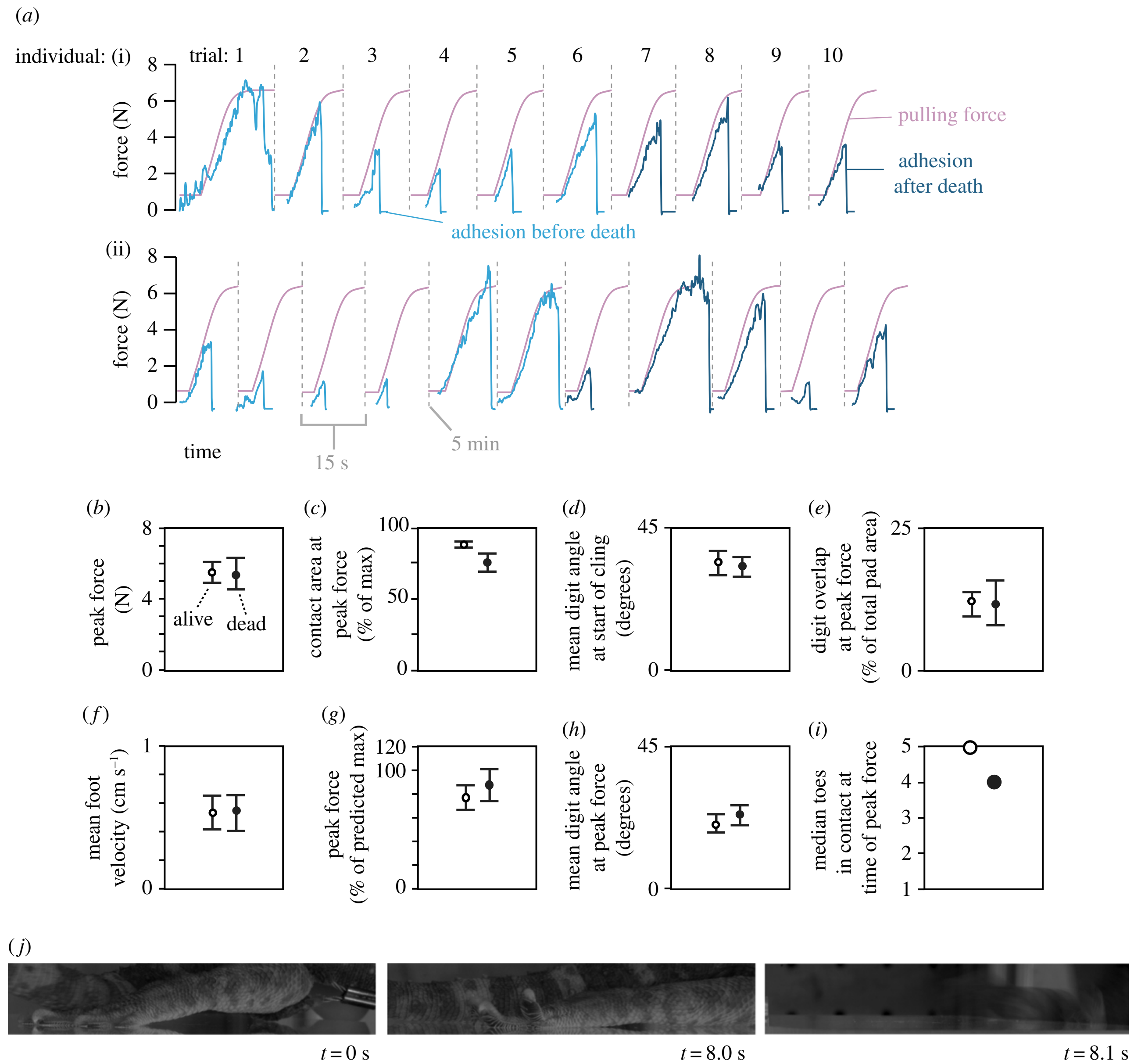

Figure 2. Gecko adhesion before and after death. (*a*) Rows show the adhesive force over time for two representative geckos during subsequent trials. Trials conducted when the animal was alive (light blue) and during the 30 min after death (dark blue). Trials in sequence are distinguished by vertical dashed lines and indicate a rest period of at least 5 min. (*b*)–(*i*) Measurements of adhesion before death (open circles) and after death (closed circles). Data have been pooled for all individuals. Error bars indicate the 95% CIs of the estimate for the mean. (*j*) Video stills of a gecko rapidly detaching from the surface by hyperextending its toes.

cell (model LSB200, FUTEK, Inc., Irvine, CA, USA) along with foot position and toe attachment with two video cameras. The pulling device exerted its increasing force by way of a 600 cm^3 volume of sand that poured from a holding container into a suspended receptacle at a constant rate. The increasing weight of sand transmitted highly repeatable and linearly increasing pulling forces to the gecko foot, allowing us to assess whole-animal adhesion under extremely controlled conditions for the first time. Other approaches where geckos are pulled by hand [10,11] or with speed-driven motors [12,13] do not standardize the dynamic pulling force.

To investigate adhesion after death, geckos were euthanized with an intracardiac injection of 2 µl EUTHASOL (31% Sodium Pentobarbital, Virbac AH, Inc., Fort Worth, TX, USA) per gram of body weight. Death was confirmed at 7 min post injection and clinging ability was measured, in sequential trials, over the subsequent 30 min period. To ensure that our method of contacting the dead foot with the surface did not affect adhesion, we alternated our procedure for initiating foot contact (manually depressing the toes with approximately 0.5 N normal force versus no depression), which did not affect adhesion strength after death (paired *t*-test, $p = 0.16$, d.f. $= 4$). By contrast, live animals voluntarily attached the foot to the surface.

(b) Measuring foot kinematics during clings

We measured the position, axis and angle of each toe during clings using video images of the foot and custom software in Matlab (v. R2012a, The MathWorks, Natick, MA, USA). We also measured the area of toe pad contact and the degree that neighbouring digits overlapped using five ellipses that modelled the shape, position and overlap of each toe pad through time (figure 1*c*). We predicted maximum toe adhesion (F_{toe}) during trials using previous estimates of single-seta strength ($F_{seta} = 14\ \mu N$) [14] and density ($\rho = 5000$ setae mm^{-2}) [6], combined with our measurements of toe pad contact area (A) and digit angle (θ)

$$F_{toe} = F_{seta} \times \rho \times A \times \cos|\theta|. \qquad (2.1)$$

3. Results

The clings of gecko feet were strong but variable, despite our standardized pulling force (figure 2). For all trials, the adhesive shear force increased with the pulling force, with the peak force being both large (mean = 12.0 times body weight) and highly variable (1 s.d. = 4.4 times body weight). Death surprisingly did not affect the strength of shear adhesion. We found no difference in the peak adhesive force produced by animals while living and during the 30 min period following euthanasia (paired *t*-test, $p > 0.99$, d.f. = 4; figure 2). The variation in peak adhesive force was similarly high for trials conducted before and after death, with one standard deviation in peak force being 33 and 36% of the mean, respectively (paired *t*-test, $p = 0.80$, d.f. = 4). We considered how quickly shear adhesion increased during each trial and found that death also did not affect the rate that the adhesive force increased when pulled (paired *t*-test, $p = 0.33$, d.f. = 4).

Death did not affect the motion or posture of clinging gecko feet (electronic supplementary material, Movie S1, Movie S2). In both live and dead animals, digit angle decreased as the foot slid while adhering, causing the digits to increasingly overlap, which reduced the pad area available to contact the surface (figure 1*c*,*d*). The foot's sliding speed, mean digit angle and the degree of digit overlap during clings were unaffected by death (figure 2). However, we detected the ability of live geckos to completely detach from the surface while clinging by hyperextending the toes (figure 2*j*).

Dead gecko feet contacted a smaller region of the surface than live gecko feet. Although live geckos attached all five toes to the surface when clinging, 57% of trials on dead geckos exhibited one or more toes detaching before peak adhesion was generated (figure 2*i*). The detachment of entire toes reduced toe pad contact area in dead geckos (figure 2*c*), but surprisingly did not affect the adhesive strength when comparing clings where three, four or five toes were attached (ANOVA, $p = 0.38$, d.f. = 59). The reduced contact area measured in dead geckos results in a given adhesive force generated by fewer, more heavily loaded setae.

4. Discussion

We found no difference in the adhesive force or the motion of clinging digits between before and after death treatments (figure 2*b*,*f*). Although previous research and anecdotes have shown that some adhesion can still occur after death in geckos [15–17], our controlled experiments are the first to show that dead animals maintain the ability to adhere with the same force as living animals. These results refute the hypothesis that actions by a living animal, such as muscle recruitment or neural activity, are required for gecko feet to generate our high measured forces.

The high variation in adhesive force among trials on both living and dead geckos is surprising. Despite the controlled pulling force applied by our pulling device, peak adhesive force spanned a range that was 19.9 times the mean body weight. When averaged among individuals, the mean coefficients of variation for adhesive force before and after death were 0.33 and 0.36, respectively. After pooling alive and dead trials, a multiple linear regression (*F*-stat = 2.14, $p = 0.09$, $r^2 = 0.13$) indicated that peak adhesive force was unaffected by digit contact area ($t = 1.00$, $p = 0.28$), degree of digit overlap ($t = 0.93$, $p = 0.35$) and the average digit angle (θ) both at the start of each pull ($t = 1.28$, $p = 0.26$) and at the time of peak force ($t = 1.16$, $p = 0.25$). Previous studies where geckos were pulled by hand also reported high variation in adhesive strength [10,18], but it is unclear whether this variation was due to the animal or the method of force measurement. Our controlled method allows us to conclude that high variation in adhesive force results from the animal as opposed to the observer. While the exact mechanisms underlying this high variation cannot be presently identified, it is possible that live geckos produced weaker clings with muscle activity that actively reduced the loading of attached setae. With regard to dead geckos, it is possible that passive changes in the posture of the dead feet produced variation in adhesion among trials.

Our results indicate that geckos do not actively increase adhesive force, highlighted by the lack of differences between our treatments, and the main regulatory mechanism for reducing force is by active hyperextension. That said, active hyperextension does not exist in other pad-bearing lizards, such as anoles, and the adhesive forces are lower in those groups [6]. We suggest that reduction of adhesive force via active hyperextension may be necessary for a system that exhibits such extremely high force capacity. Through evolution, active hyperextension may have been co-opted as a 'shut-off' mechanism during locomotion, allowing geckos to keep their digits hyperextended when running fast on level surfaces [19].

Ethics statement. Experimental procedures were carried out according to The UC Riverside Institutional Animal Care and Use Committee guidelines, AUP A-20110038.

Data accessibility. Data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.8hk5r.

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