

RESEARCH ARTICLE

Self-cleaning in tree frog toe pads; a mechanism for recovering from contamination without the need for grooming

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SUMMARY

Tree frogs use adhesive toe pads for climbing on a variety of surfaces. They rely on wet adhesion, which is aided by the secretion of mucus. In nature, the pads will undoubtedly get contaminated regularly through usage, but appear to maintain their stickiness over time. Here, we show in two experiments that the toe pads of White's tree frogs (*Litoria caerulea*) quickly recover from contamination through a self-cleaning mechanism. We compared adhesive forces prior to and after contamination of (1) the whole animal on a rotatable platform and (2) individual toe pads in restrained frogs mimicking individual steps using a motorised stage. In both cases, the adhesive forces recovered after a few steps but this took significantly longer in single toe pad experiments from restrained frogs, showing that use of the pads increases recovery. We propose that both shear movements and a 'flushing' effect of the secreted mucus play an important role in shedding particles/contaminants.

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INTRODUCTION

The ability to climb is a significant advantage for animals, as it makes available habitats not accessible to non-climbers or non-flyers. Indeed, many animal phyla and groups are represented in arboreal habitats. These animals are characterised by mechanisms that facilitate climbing and reduce the risk of falling. On rough surfaces, friction pads and claws can be effective, but on smooth surfaces and significant overhangs, some mechanism of adhesion is essential (Peattie, 2009). Adhesion allows an organism to remain attached to an inclined, vertical or even an upside down surface whilst resisting falling or slipping. Animals have evolved two different mechanisms for adhesion – dry and wet. Dry adhesion, typified by geckos, involves toe pads covered in very large numbers of finely branching setae, each ending in a flattened spatula. These spatulae make such close contact with the surface that van der Waals forces, which hold molecules together in solids, form the adhesive bonds (Autumn et al., 2002). In contrast, tree frogs adhere by wet adhesion. Mucus, secreted by glands ending on the surface of the toe pads, makes an adhesive bond by a combination of capillarity and viscous forces (Emerson and Diehl, 1980; Hanna and Barnes, 1991; Federle et al., 2006). Tree frog adhesive pads (and those of insects such as crickets) have a hexagonally patterned surface (Green, 1979; Emerson and Diehl, 1980; Smith et al., 2006), the pad epithelial cells being separated by mucus-filled channels that spread the mucus over the pad surface. Effective adhesion occurs when there is a thin layer of fluid between the pad and substrate (Barnes et al., 2006b). As this paper shows, the toe pad secretion is multifunctional as it additionally plays a role in self-cleaning.

An important aspect of any climbing organism that uses adhesive pads is keeping these pads clean and therefore functional. In the case of tree frogs, it is easy to imagine that the presence of a secretion causes contaminants to stick to the pads and become wedged in the

narrow channels that separate the epithelial cells. Contamination can be caused by dirt and particles of plant material (e.g. pollen, and sometimes waxy crystals produced by the plant to deter insects) (e.g. Edwards, 1982; Eigenbrode, 2004). Many organisms, such as insects, will typically clean by grooming (Orchard et al., 2012), and will do so when their adhesive pads prove ineffective regardless of contamination (Hosoda and Gorb, 2011). However, for many climbing animals, grooming is not a complete solution as the adhesive surface of a toe pad cannot be groomed when it is in use, providing adhesive and frictional forces to maintain the animal on, for instance, a vertical surface. Although insects, geckos and frogs can moult, and thus shed any contaminated skin, this does not happen frequently enough to act as an effective cleaning mechanism for locomoting animals. Therefore, many organisms have evolved self-cleaning mechanisms. Self-cleaning usually refers to a natural property of the body of the organism, meaning that the animals will self clean passively whilst climbing (Hansen and Autumn, 2005).

Previous studies on geckos (Hansen and Autumn, 2005; Hu et al., 2012) and insects (Clemente et al., 2010; Orchard et al., 2012) have shown that self-cleaning occurs in both dry and wet adhesive pads. In the case of geckos, Hu and colleagues hypothesise that geckos clean their feet through a unique dynamic self-cleaning mechanism *via* digital hyperextension (Hu et al., 2012), though, as claimed by Hansen and Autumn, it is likely that particles are also shed because they have a greater attraction to the substrate than the animals' skin, which is ultrahydrophobic (Hansen and Autumn, 2005). Recovery of forces happens quickly within a few simulated steps, e.g. an 80% recovery after four steps (Hu et al., 2012). Studies on insects displayed 50% recovery of adhesive forces by the eighth step in both beetles and stick insects (Clemente et al., 2010). To date, there have been no studies of self-cleaning in tree frogs, and so it is unclear whether they show similar levels of recovery to those

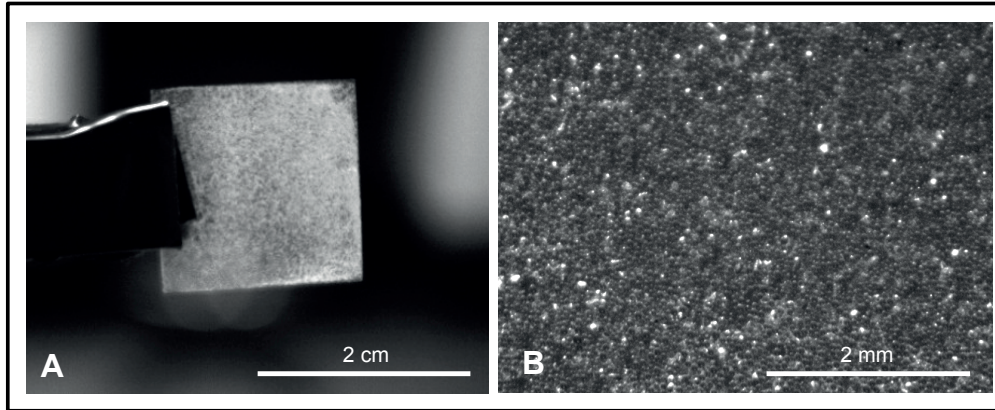


Fig. 1. Images of the beads used for contamination in the single toe pad experiment. (A) The 50 μm glass beads arranged as a monolayer on a glass coverslip. (B) The bead layer from underneath, viewed through a binocular microscope.

found in insects such as ants and stick insects, with which they share a similar adhesive structure (smooth adhesive pads) and method of adhesion (wet adhesion).

In this study, we demonstrate that the toe pads of tree frogs facilitate self-cleaning by means of force measurements on both unrestrained free-walking frogs and individual toe pads. Our hypothesis – that the pads become cleaner with repeated use, as has been seen in both geckos (Hansen and Autumn, 2005) and insects (Clemente et al., 2010) – was verified using glass beads as a contaminant and our findings provide new insights into the actual mechanics of the self-cleaning process.

MATERIALS AND METHODS

Study animals

Australian green tree frogs (*Litoria caerulea* White, Family Hylidae), also known as White's tree frogs, were kept at a temperature of 28°C (day) and 24°C (night) in vivaria (30×45×76 cm l×w×h) containing plants on which the frogs could climb. The frogs were fed live house crickets dusted with a calcium balancer and multivitamin supplement (Nutrobal, purchased from Peregrine Live Foods, Ongar, Essex, UK) thrice weekly. Cu-free fresh water was used to maintain a high humidity and to provide water for the frogs to submerge themselves in. Five frogs were used in the experiments (mass 29.095±9.635 g and snout–vent length 69.5±9.5 mm).

Prior to experiments, the frogs were washed to remove any contaminants possibly present on their bodies, and carefully blotted to prevent the water from affecting toe pad adhesion and friction forces.

Contamination

Glass spheres (Ballotini beads, Jencons, VWR International, Lutterworth, Leics, UK; diameter 50±15 μm) were used to contaminate the frog's toe pads. For the single toe pad experiments, the beads were arranged as a monolayer (Fig. 1) on a 60×20 mm glass coverslip, by statically charging the coverslip and dipping it into a jar of the beads. For the whole-animal experiments, the beads were arranged as a thin layer in a Petri dish (9 cm diameter) so that the frogs pads could be contaminated by stepping each pad onto them once.

Force measurements on unrestrained frogs

In order to obtain evidence of self-cleaning in tree frogs, an initial study was conducted on whole unrestrained tree frogs using a previously developed procedure (Emerson and Diehl, 1980; Hanna and Barnes, 1991). The frogs were placed in a head-up orientation on a glass platform, which could rotate through 360 deg (0 deg

being horizontal, 90 deg being vertical and 180 deg being completely upside down). The frogs were rotated ($\sim 4 \text{ deg s}^{-1}$) and observed to see at what angle they began to slip, and when they fell from the platform, with a gentle movement of a hand around (but not touching) the frog discouraging them from jumping (otherwise the frog would frequently jump to avoid a fall). These two angles, the slip angle (between 0 and 90 deg) and fall angle (between 90 and 180 deg), were recorded each time the experiment was run. The slip angle was used to calculate the shear (friction) force, and the fall angle was used to calculate the normal (adhesive) force. By knowing the body weight of the frog and the angle of slip or fall, the two force components (friction and adhesion) could be calculated using simple trigonometry (Barnes et al., 2006b), namely:

$$F_{\text{friction}} = \cos(90 - \theta) \times Mg \quad (\text{where } 0 \text{ deg} < \theta < 90 \text{ deg}), \quad (1)$$

$$F_{\text{adhesion}} = \cos(180 - \theta) \times Mg \quad (\text{where } 90 \text{ deg} < \theta < 180 \text{ deg}), \quad (2)$$

where θ is the angle of tilt, M is the mass of the frog and g is the force of gravity. As maximum friction and adhesion forces in this experiment (which may not represent the maximum forces that the frog can produce) are produced at 90 and 180 deg, respectively, slip angles >90 deg were scored as 90 deg, and fall angles <90 deg or >180 deg were scored as 90 and 180 deg, respectively.

For each frog, a control measurement (i.e. uncontaminated pads) was taken before the frog's pads were contaminated with the 50 μm glass beads as described above. Slip and fall angles were measured immediately afterwards. The frog was then either kept still for a 1 min rest or encouraged to take 1–3 steps with each foot, giving a total of 8 individual steps, before being tested on the platform again. This cycle of experimentation continued until the frog displayed full recovery, with the slip and fall angles returning to the control measurements (or as near to this as possible). Once a frog had been used for an experiment, it was cleaned again, and not used for 24 h. Conducting the experiments in two separate ways – one with steps, one without – enabled us to test whether pad usage is important in the removal of beads.

Single toe pad force measurements

Adhesion and friction forces were measured using a custom-built force transducer, which consisted of a glass coverslip (as a surface for attachment of the frog's toe pad) connected to a bending beam. Forces were measured in two dimensions (lateral and normal directions, the x - and z -axes), and involved force feedback to maintain a constant load in the normal direction. The experimental set-up (Fig. 2) was developed from that used by Drechsler and

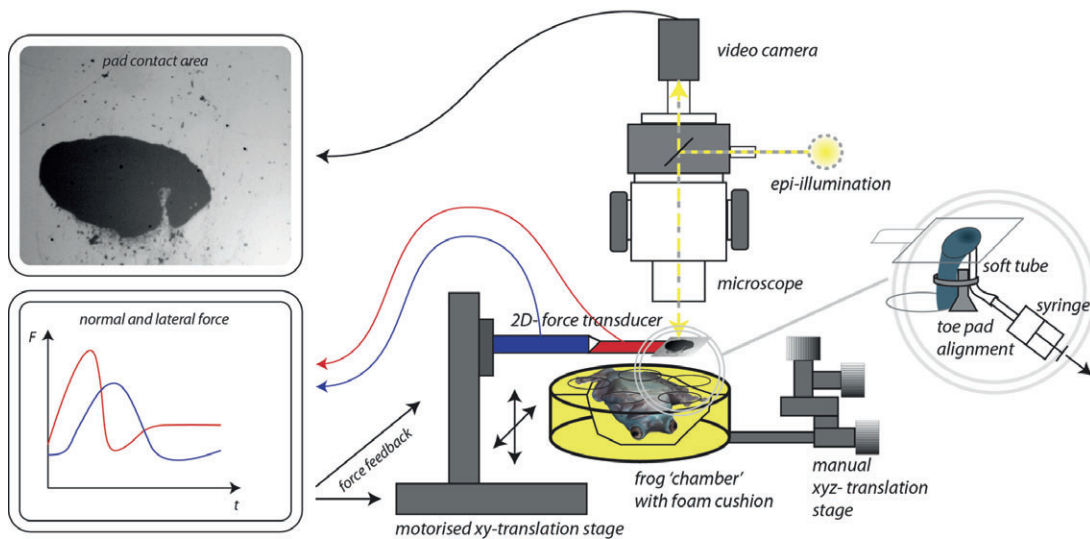


Fig. 2. Set-up used, showing the frog within the Petri dish, attached to a micromanipulator. Toe pad adhesion and friction forces were measured by a custom-built 2D-force transducer, used in a feedback loop to maintain a constant load during shear movements. The force transducer was manipulated using a motorised xz -translation stage, controlled by a computer. A digital video camera viewed the ventral pad area in contact with the transducer through a binocular microscope with epi-illumination that produced high contrast images of the area of the pad in contact with the force plate on a second computer. The inset shows how an individual toe pad (either the second or third toe of a forelimb) was held in the correct alignment with respect to the force plate.

Federle (Drechsler and Federle, 2006) and was similar to that used by Clemente and colleagues in their experiments on self-cleaning in insects (Clemente et al., 2010).

In order to measure the forces from a single toe pad, the frog was restrained in a plastic Petri dish using a foam cushion around the body of the frog as padding. The frog was then positioned upside-down so that a front leg was protruding out of a hole located in the bottom of the Petri dish. With the leg held in position by a piece of plastic tubing, a single toe pad was held in an extended position by gentle suction on the dorsal side of the pad using a hand-held syringe (see Fig. 2 inset). The force transducer was attached to a computer-controlled manipulating stage (model PD-126M, Physik Instrumente, Karlsruhe, Germany), which moves in 2 dimensions (x and z). A custom-built LabVIEW interface (LabVIEW Inc., National Instruments, Austin, TX, USA) was used to record forces and to manipulate the motor stage in a controlled fashion. Two programs were run – one involving a lateral drag across the coverslip on the x -axis (Fig. 3), referred to as the ‘drag’ program; and one that did not have a drag, and simply pushed the pad onto the coverslip using the z -axis (Fig. 4), called the ‘dab’ program. The movements controlled by the computer began with the toe pad adhering to the coverslip with 2 mN load applied (Fig. 3A), and, in the case of the drag program, was followed by a horizontal,

proximal 4 mm drag at 0.375 mm s^{-1} (load maintained at 2 mN by force feedback) to align the pad and measure maximum friction forces (Fig. 3B). Finally, the pad was pulled off from the coverslip at a 30 deg angle (Fig. 3C), an angle at which high adhesive forces occur (Barnes et al., 2006a).

Three control experiments were run to give an average pull-off force, before the pad was contaminated. The program was then run for eight measurements, unless recovery had occurred sooner. Contamination was achieved by pushing the pad downwards onto a monolayer of contaminant beads for $\sim 1\text{ s} - 2\text{ mN}$ of force for a full pad contamination and 0.5 mN of force to produce partial pad contamination. Fig. 5 shows the differing contamination levels produced by different loads of 2 and 0.5 mN. Peak values for friction and adhesive forces were extracted from the data using a custom-built MATLAB script (MathWorks, Natick, MA, USA).

A comparison of the amount of bead deposition on the glass, toe pad contact area and adhesive forces between dabs and drags was also conducted using the same setup as above. A video camera (100 frames s^{-1} , A602F, Basler, Ahrensburg, Germany), attached to the binocular microscope (Fig. 2), provided images of the pad in contact with the force plate throughout each experiment. Customised MATLAB scripts were used to calculate contact area and to count beads from pad images once the pad had been removed.

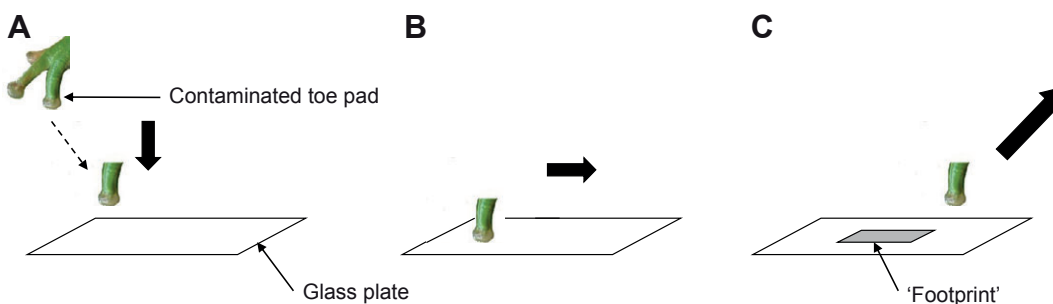


Fig. 3. Program involving a lateral ‘drag’. (A) The toe pad is brought into contact with the force plate. This is followed by (B) a proximally directed shear drag (horizontal movement towards the body), and (C) a 30 deg pull-off.

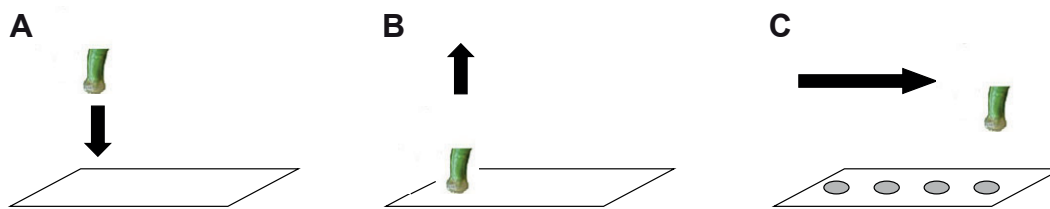


Fig. 4. Program involving no shear drag, simply a 'dab'. (A) The pad is brought into contact with the force plate, before (B) being removed vertically. (C) Such dabs were repeated along the plate.

Statistics

Page's non-parametric *L*-test was used for both experiments to test for progressive increases or decreases (i.e. trends) between measurements (Page, 1963), with results written as $L_{m,n}$, where m is the number of conditions and n is the sample size. All data were tested for normality, and then with Student's *t*-test (for normal data) or Wilcoxon's signed rank test (for non-normal data) as appropriate. Wilcoxon's signed rank test was also used for comparison of recovery of adhesion/friction between the two experimental situations in the unrestrained frog experiment. For the single toe pad experiments, two sample Student's *t*-test and Wilcoxon's signed rank test were used for comparisons, and Spearman's correlation test was used to test for a correlation between bead deposition and contact area. Statistical analyses for both experiments were done using MINITAB 15 (State College, PA, USA), and the statistical toolbox in MATLAB 7.6.0 (version R2008a). Data are given as means \pm s.d.

RESULTS

Evidence for self-cleaning in unrestrained frogs

Uncontaminated adult *L. caerulea* could not permanently hang upside-down from a smooth glass surface, but began slipping at an angle of 106.25 ± 26.13 deg, and detached at an angle of 141.75 ± 19.59 deg (4 measurements from each of 5 frogs). Maximum forces for the uncontaminated pads in this experiment (which for friction forces may not be the maximum forces that the frog can produce) depended on both mass and angle of slip/fall – 285.4 ± 94.5 and 255.3 ± 73.7 mN for friction and adhesion, respectively. In the box plots in Figs 6 and 7, the mean values for adhesion and friction for the three control trials carried out before a frog was contaminated represent 100%, and post-contamination results are plotted as a percentage of these values. Normalising the data in this way allowed us to combine the results from all five frogs on the same

plot. Contamination caused a significant reduction in both friction and adhesion forces, which recovered to pre-contamination values over the subsequent three trials.

Friction

For F_{friction} , contamination led to a decrease to $64.81 \pm 9.37\%$ of original forces (see Fig. 6, first trial), which was highly significant (*t*-test, $N=20$, $P<0.001$). As Fig. 6 also shows, both 'with steps' and 'without steps' trials showed a significant positive trend in recovery over the first three trials, after which forces had usually reached their pre-contamination values (Page's trend test, $L_{3,20}=448$, $P<0.001$ for 'with steps' measurements and $L_{3,20}=565$, $P<0.001$ for 'without steps' measurements). Wilcoxon's paired rank test conducted on each pair of trials (with and without steps) did not show a significant difference for either the first or the second trial ($N=20$, $P=0.586$ and $P=0.135$, respectively), while the third ($P=0.0245$) and fourth ($P=0.002$) trials showed significant differences between stationary frogs and frogs allowed to take steps, with the latter showing the faster recovery.

Adhesion

Contamination had a much greater effect on adhesive forces than on friction forces, with F_{adhesion} dropping to 0% after contamination (Fig. 7, first trial), but full recovery still occurred by the fourth post-contamination trial (Page's trend test, $L_{3,20}=448.5$, $P<0.001$ for 'with steps' data and $L_{3,20}=565$, $P<0.001$ for 'without steps' data). However, as our methodology did not usually measure maximum friction forces (as average slip angles exceeded 90 deg), this difference between the effects of contamination on adhesion and friction forces is exaggerated in our analysis. Recovery of adhesion forces was significantly faster when frogs walked in comparison to resting frogs. Paired tests showed that there were significant differences between each of the pairs found after the post-contamination measurement (first trial), with levels of significance

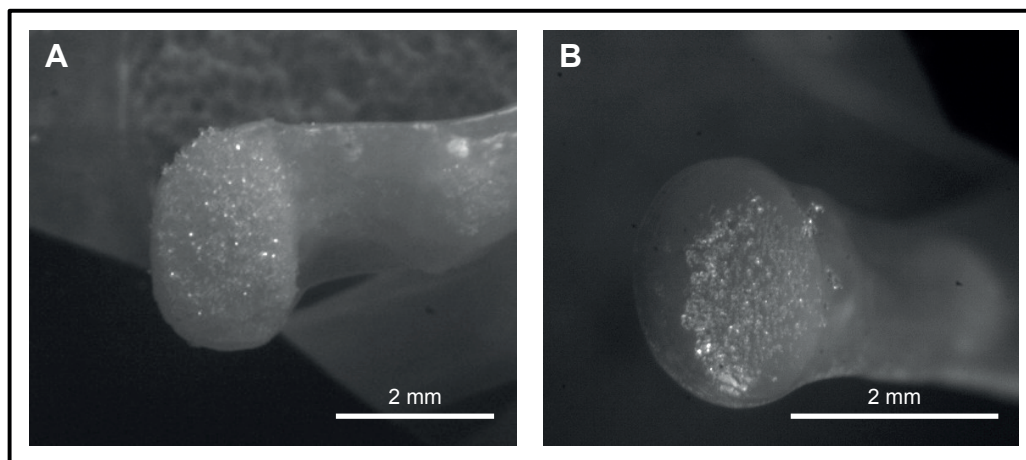


Fig. 5. Images showing frog pads contaminated with glass beads at two different levels of contamination. (A) A pad that has been pushed onto the beads with 2 mN force, and has subsequently become fully contaminated. (B) A pad that has been pushed onto the contaminant with 0.5 mN of force, which picks up fewer beads than with a stronger push, and therefore becomes partially contaminated.

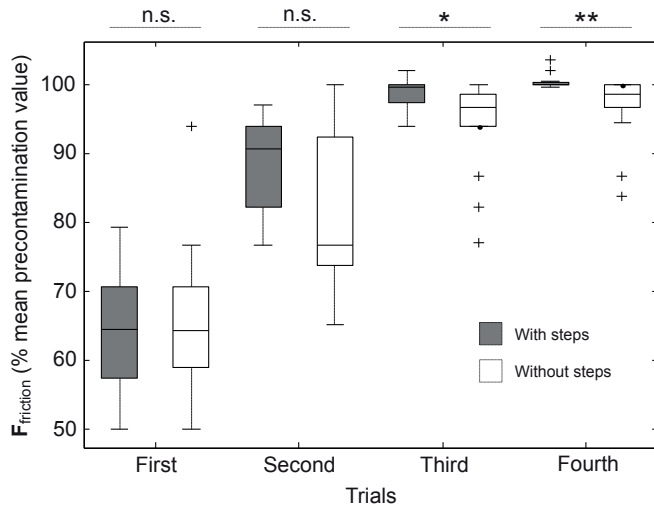


Fig. 6. Boxplot showing the recovery of frictional force (F_{friction}) for both 'with steps' and 'without steps' forms of the experiment as a percentage of the mean pre-contamination value. On each box, the central line is the median, the boundaries of the box are the 25th and 75th percentiles, the whiskers extend to include 99.3% of the data for a normal distribution, and outliers are plotted individually. The first measurement occurred immediately after contamination, and each subsequent measurement was separated by either a 1 min rest or a minute when the frog was allowed to take some controlled steps. Wilcoxon's paired rank tests were conducted on each pair, with the results for each shown above the plots. n.s., not significant, * $P < 0.05$, ** $P < 0.01$.

being $P = 0.022$, $P = 0.002$ and $P = 0.0029$ (t -test, $N = 20$) for the second, third and fourth trials, respectively.

Self-cleaning in single toe pads

To investigate the mechanisms involved in the self-cleaning of toe pads, single toe pad experiments were conducted on five frogs. The first experiment compared the adhesive force recovery of a fully contaminated toe pad for a drag and for a dab (Fig. 8). Contamination led to a large drop in F_{adhesion} for both trial methods, averaging 5.93% of original forces for the drag trials and 6.35% for the dab trials. Over a sequence of seven measurements following the first post-contamination measurement, a significant recovery trend was seen in the drag trials (Page's trend test, $L_{8,15} = 2856$, $P < 0.001$), while the dab trials showed no recovery within these eight measurements (Page's trend test, $L_{8,18} = 2714.5$, $P = 0.99$). Paired comparisons at each measurement for the two experiments showed that, for the first three measurements, there were no significant differences (t -test, $N = 18$, first $P = 0.5277$, second $P = 0.2145$, third $P = 0.0707$). However, by the fourth measurement a significant difference was seen (t -test, $N = 18$, $P = 0.004$), and the trend continued in all subsequent step comparisons (t -tests, $N = 18$, fifth $P = 0.0005$, sixth $P = 0.0004$, seventh $P < 0.0001$, eighth $P < 0.0001$). By the eighth step, 75.58% of pre-contamination adhesive force had been recovered by the pads in the drag experiment.

Although no recovery of adhesive force was seen in Fig. 8 for dab trials involving full pad contamination, experiments involving partial pad contamination (pad pushed onto contaminant with a force of 0.5 mN as opposed to 2 mN) showed significant recovery over the course of eight measurements (Page's trend test, $L_{8,13} = 2428.5$, $P < 0.001$) (Fig. 9). Interestingly, the drag measurements showed no loss of F_{adhesion} when partially contaminated (Fig. 9). Together, the data in Figs 8 and 9 indicate that there are differing degrees of force

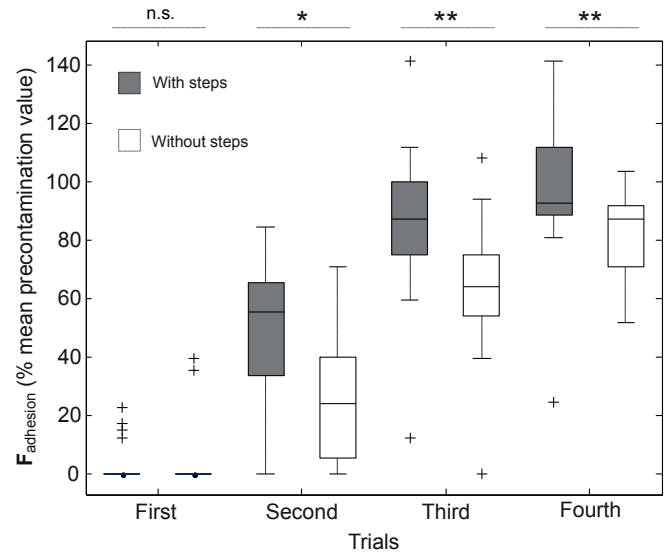


Fig. 7. Boxplot showing the recovery of adhesive force for both 'with steps' and 'without steps' forms of the experiment as a percentage of the mean pre-contamination value. The first measurement occurred immediately after contamination, and each subsequent measurement was separated by either a 1 min rest or a minute when the frog was allowed to take some controlled steps. Student's t -tests were conducted on each pair, with the results for each shown above the plots. n.s., not significant, * $P < 0.05$, ** $P < 0.01$. Other details as in Fig. 6.

recovery between these two types of trial, with the drag trials allowing a greater degree of self-cleaning than the dab trials.

Contact area and bead deposition

In order to gain an insight into the mechanism of self-cleaning, the area of pad in contact for each step was measured together with the number of beads deposited on the glass plate (a direct measure of self-cleaning), as well as the forces produced during each trial. This was done for partially contaminated pads as the rate of recovery was much faster. As seen in the previous experiment, the F_{adhesion} varied significantly between dab and drag trials (Fig. 10C), as did the percentage of the total pad area in contact with the surface (Fig. 10B) and the number of beads deposited by each step (Fig. 10A). The cumulative number of beads deposited in successive trials was then correlated with the area of pad in contact with the surface (Fig. 11), and a significant relationship was found for the drag trials (Spearman correlation test; $\text{RHO} = 0.5711$, $P = 0.0185$), while in the dab trials there was no significant correlation (Spearman correlation test; $\text{RHO} = -0.1496$, $P = 0.5175$). This indicates that pad contact area is improved by bead removal, and that an increased area of contact leads to increased forces. The drag trials resulted in significantly more beads being removed, as is apparent from Fig. 10A and Fig. 11 and can be seen directly in Fig. 12 and in videos of typical drag and dab trials (see supplementary material Movies 1, 2), resulting in a larger area of pad in contact with the plate and significantly larger forces over the first three steps. Drag movements of the pad over the surface are thus inherently more effective than dabs at producing self-cleaning.

DISCUSSION

Occurrence of self-cleaning

The whole-animal experiments provide evidence that the toe pads of tree frogs will self-clean over time. Adhesive and friction forces

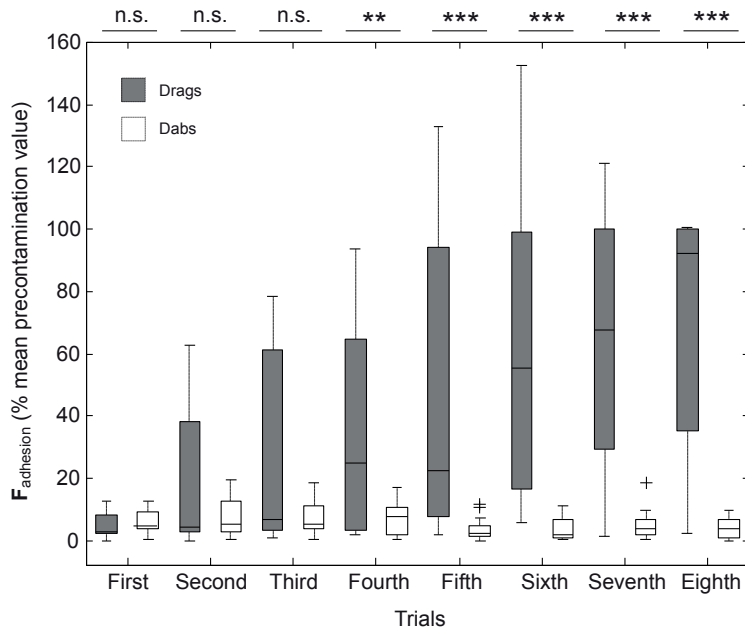


Fig. 8. Recovery of forces following full contamination. Boxplot comparing adhesive force (normal component of pull-off force) as a percentage of the mean pre-contamination value for both the drag and dab forms of the experiment (see Figs 3, 4) during recovery from full contamination (2 mN, see Fig. 5) in single toe pads. The boxes represent consecutive simulated 'steps' taken by the toe pad on the force plate following pad contamination. *t*-tests were conducted on each pair, with the results for each shown above the plots. n.s., not significant, ** $P < 0.01$, *** $P < 0.001$. Other details as in Fig. 6.

were significantly reduced after an initial contamination, but recovered with subsequent measurements. By the fourth step, 91.9% of all original adhesive forces and 98.5% of all original friction forces were being utilised by the frog. As self-cleaning has previously been shown to exist in geckos (Hansen and Autumn, 2005; Hu et al., 2012) and more recently in insects (Clemente et al., 2010; Orchard et al., 2012), it seems likely that it is ubiquitous in animals that use adhesion to climb. However, self-cleaning is not restricted to animals, as some plants (e.g. lotus) self-clean during rainfall. The leaf surfaces of lotus plants are ultrahydrophobic so that dirt particles are removed by water droplets rolling over the surface of the leaves. This is the so-called 'lotus effect' (Barthlott and Neinhuis, 1997), and has led to the development of self-cleaning paint (Lotusan, Sto Corporation, Atlanta, GA, USA). Nor can we exclude foot grooming in some species. There is no evidence for its use in geckos or tree frogs, but it is used in some insects. For example, an elegant study by Hosoda and Gorb showed that foot grooming is triggered by friction force reduction in the leaf beetle, *Gastrophysa viridula* (Hosoda and Gorb, 2011). Our whole-animal experiments also indicate that pad usage significantly increases the recovery of contaminated feet for both adhesion and friction forces – frogs

that utilised their pads showed a faster recovery rate than frogs that remained stationary.

Mechanisms of self-cleaning in tree frogs

Force measurements from single toe pads also showed evidence of self-cleaning, but at a slower rate than in unrestrained animals. The reasons for this are unclear, but an unrestrained frog would have a greater range of movements available to it during experimentation, any of which (including a longer shear slide) could enhance the cleaning process. However, the single toe pad results provide a greater insight into the mechanism of self-cleaning in tree frogs. Trials with simulated steps that included a drag movement recovered their pre-contaminated adhesive force after about eight trials, whereas trials in which the pad was simply pressed against the surface (dabs) showed little if any recovery (Fig. 8). However, some recovery did occur when the pads were only partially contaminated (Fig. 9). Shear movements of the pad over the surface are thus an important feature of self-cleaning. Indeed, some slipping of pads on smooth surfaces is a common feature of walking frogs, particularly on vertical surfaces (D. Samuel, T.E., W.J.P.B. and M. Riehle, manuscript in preparation), and slipping will be enhanced when contaminating particles

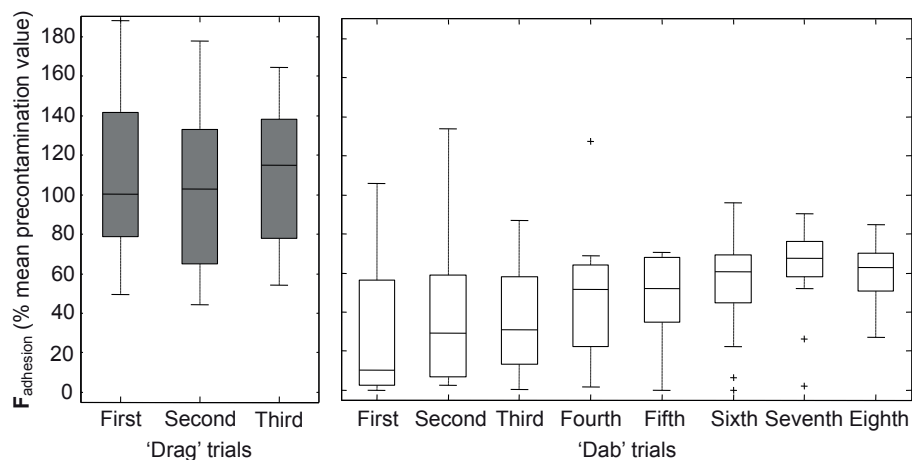


Fig. 9. Recovery of forces following partial contamination. Boxplot comparing adhesive force (normal component of pull-off force) as a percentage of the mean pre-contamination value for both the drag and dab forms of the experiment (see Figs 3, 4) during recovery following partial contamination (0.5 mN, see Fig. 5) in single toe pads. The boxes represent consecutive simulated 'steps' taken by the toe pad on the force plate following pad contamination. Other details as in Fig. 6.

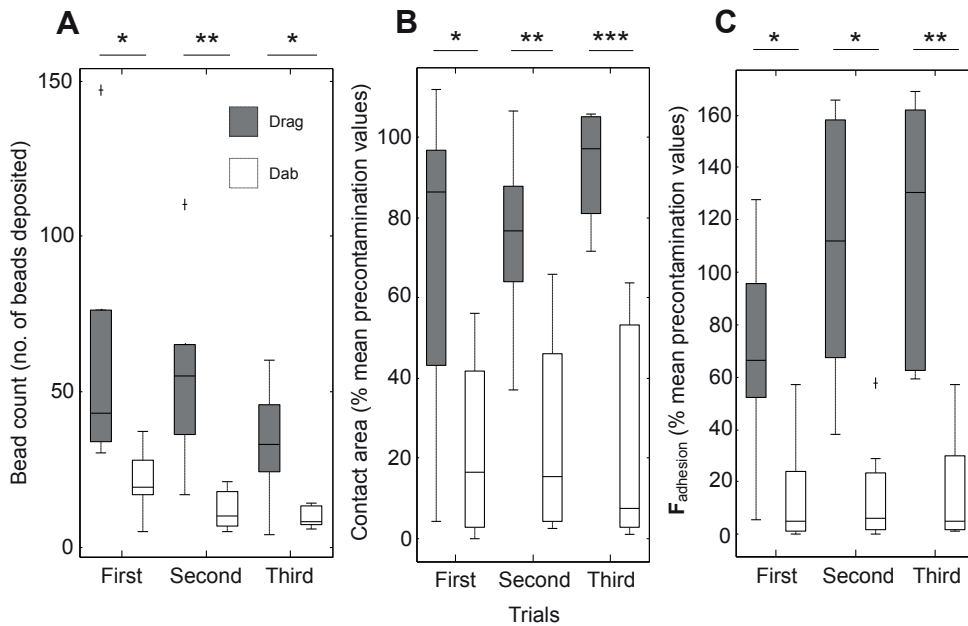


Fig. 10. Boxplot comparing various results for both the drag and dab forms of the experiment (see Figs 3, 4) during recovery from partial contamination in single toe pads. The boxes represent consecutive 'steps' taken by the toe pad on the force plate following pad contamination. (A) The bead deposition rate of each trial. (B) The contact area as a percentage of the pre-contaminated pads. (C) The adhesive forces as a percentage of the pre-contaminated pads. Student's *t*-tests were conducted on each pair, with the results for each shown above the plots. n.s., not significant, **P*<0.05, ***P*<0.01, ****P*<0.001. Other details as in Fig. 6.

reduce the area of close contact. Frogs will continually reposition their slipping pads on vertical or overhanging surfaces in a similar manner to flies (Wigglesworth, 1987), and so the drag movement in our measurements is a behaviour used by the frog, which is here shown to incorporate self-cleaning as well as maintaining adhesion to a surface.

In addition to measuring forces, these experiments also recorded pad contact area and the number of beads deposited on the glass plate during each trial. They clearly show a correlation between adhesive force (Fig. 10C) and contact area (Fig. 10B), so much so that normal stress (adhesive force per unit area) remained more or less constant [force per unit area values for drag controls ($1.08 \pm 0.24 \text{ mN mm}^{-1}$) and contaminated pads ($0.94 \pm 0.36 \text{ mN mm}^{-1}$) were tested for variance; Kruskal–Wallis test: *P*=0.0929]. Contaminants thus reduce adhesion by reducing the area of close contact between pad and surface. The number of beads deposited on the glass plate, an obvious measure of self-cleaning, was positively correlated with both force recovery and contact area (Fig. 10A). Videos of these experiments (see supplementary material Movies 1, 2) clearly show the role of pad sliding, and suggest that toe pad mucus could play a part in this process. Sliding between the pad and the glass plate moves the beads towards the distal end of the pad, and many get left behind in the mucus footprint left after each step. It thus appears that the mucus could have a 'flushing' action, aiding the movement of dirt particles to the tip of the pad and their subsequent removal from it – though further work is needed to verify this.

Comparisons with other animals

As our single pad experiments used a similar protocol to the experiments of Clemente and colleagues (Clemente et al., 2010), it is possible to compare our results with those of similar experiments carried out on beetles (hairy adhesive pads) and stick insects (smooth adhesive pads), both of which, like tree frogs, adhere by wet adhesion. As in our experiments, adhesion in stick insect pads depended upon the area of contact with the force plate, which, in different experiments, was reduced by contamination with beads of three different sizes. Recovery occurred as the beads were removed. In these insect experiments, some adhesive fluid was left behind as

a footprint following each step, with contaminant present in large quantities. It seems that large continuous quantities of fluid will simply clean away the particles, aided by friction between pad and surface (Clemente et al., 2010). In tree frogs, mucus production can be sporadic (Ernst, 1973), and so it is possible that recovery of forces will occur faster or slower depending on mucus volumes. Certainly, the mechanism of self-cleaning in tree frogs bears many similarities to that in insects, including those such as beetles that possess hairy adhesive pads. For instance, in insects the drag measurements lead to greater self-cleaning than the dabs (Clemente et al., 2010), as is

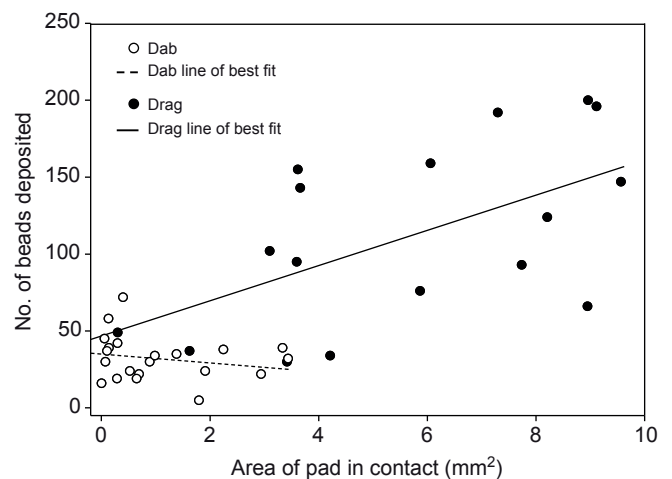


Fig. 11. Scatter plot showing the correlation between the number of beads deposited on the glass force plate (a cumulative total of beads from successive trials, i.e. no. for first trial=*x* beads, no. for second trial=*x* + *y* beads, no. for third trial=*x* + *y* + *z* beads) and the area of pad in contact with the glass surface (mm^2). Lines of best fit were calculated for both trial variations – with the drag trials showing a correlation significantly differing from zero (Spearman correlation test, *P*<0.05), while the dab trials showed no significant correlation (Spearman correlation test, *P*>0.05). Note that dab trials often resulted in the removal of fewer beads and were associated with smaller contact areas, while drag trials showed more bead deposition and greater contact areas.

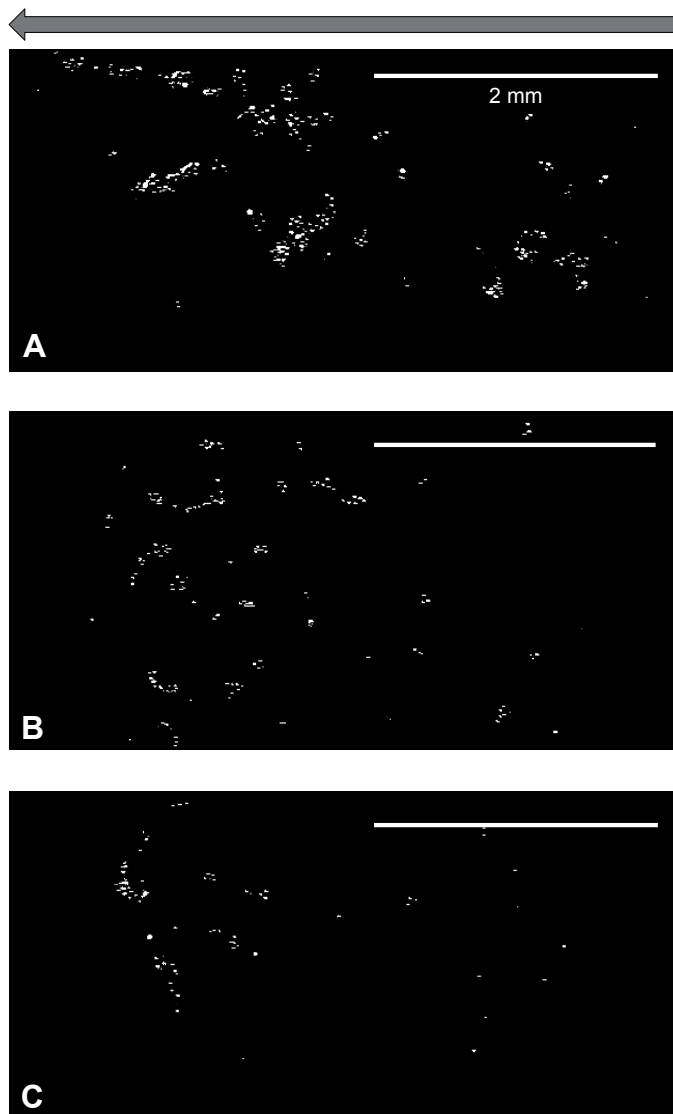


Fig. 12. Illuminated beads that have been deposited in a drag movement, as seen through the microscope for (A) the first trial, (B) the second trial and (C) the third trial. Beads were deposited in characteristic 'footprints', and frequently towards the end of the drag in clusters caused by the mucus. The arrow indicates the direction of the drag. Scale bars, 2 mm.

seen in frogs. The mechanism does, however, clearly differ from that proposed for geckos, for tree frog toe pads are, of necessity, hydrophilic, which means that dirt particles will not be lost as a result of their greater affinity for the substrate than the pad surface, nor are the pads peeled off *via* digital hyperextension (Hu et al., 2012). The adhesive fluid is essential for adhesion in both insects and tree frogs, and could be seen to play a role in self-cleaning too. Although exact comparisons cannot be made because of the different sizes and material properties of the contaminants used in different self-cleaning studies (Hansen and Autumn, 2005; Hui et al., 2006; Clemente et al., 2010; Orchard et al., 2012; Hu et al., 2012), it does appear that tree frogs self-clean as efficiently as other organisms studied to date.

Biomimetic implications

Several examples of bio-inspired designs have already arisen from adhesives in the natural world – Vogel and Steen's prototype

reversible adhesive device being a recent example that utilises wet adhesion by capillarity (Vogel and Steen, 2010). Man-made surfaces that could potentially self-clean would be overcoming arguably one of the biggest problems in the development of reversible adhesives – contamination of the adhesive surface. Until self-cleaning reversible adhesives are created, the adhesive pads of the natural world will remain vastly superior. However, the gap is closing, as Lee and Fearing have demonstrated a degree of self-cleaning in a synthetic gecko-inspired adhesive (Lee and Fearing, 2008).

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REFERENCES

- Autumn, K., Sitti, M., Liang, Y. A., Peattie, A. M., Hansen, W. R., Sponberg, S., Kenny, T. W., Fearing, R., Israelachvili, J. N. and Full, R. J. (2002). Evidence for van der Waals adhesion in gecko setae. *Proc. Natl. Acad. Sci. USA* **99**, 12252-12256.
- Barnes, W. J. P., Federle, W. and Drechsler, P. (2006a). Adhesion and friction forces generated by single toe pads of the frog *Litoria caerulea*. *Comp. Biochem. Physiol.* **143**, S94.
- Barnes, W. J. P., Oines, C. and Smith, J. M. (2006b). Whole animal measurements of shear and adhesive forces in adult tree frogs: insights into underlying mechanisms of adhesion obtained from studying the effects of size and shape. *J. Comp. Physiol. A* **192**, 1179-1191.
- Barthlott, W. and Neinhuis, C. (1997). Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta* **202**, 1-8.
- Clemente, C. J., Bullock, J. M. R., Beale, A. and Federle, W. (2010). Evidence for self-cleaning in fluid-based smooth and hairy adhesive systems of insects. *J. Exp. Biol.* **213**, 635-642.
- Drechsler, P. and Federle, W. (2006). Biomechanics of smooth adhesive pads in insects: influence of tarsal secretion on attachment performance. *J. Comp. Physiol. A* **192**, 1213-1222.
- Edwards, P. B. (1982). Do waxes of juvenile eucalyptus leaves provide protection from grazing insects? *Aust. J. Ecol.* **7**, 347-352.
- Eigenbrode, S. D. (2004). The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. *Arthropod Struct. Dev.* **33**, 91-102.
- Emerson, S. and Diehl, D. (1980). Toe pad morphology and mechanisms of sticking in frogs. *Biol. J. Linn. Soc. Lond.* **13**, 199-216.
- Ernst, V. V. (1973). The digital pads of the tree frog, *Hyla cinerea*. II. The mucous glands. *Tissue Cell* **5**, 97-104.
- Federle, W., Barnes, W. J. P., Baumgartner, W., Drechsler, P. and Smith, J. M. (2006). Wet but not slippery: boundary friction in tree frog adhesive toe pads. *J. R. Soc. Interface* **3**, 689-697.
- Green, D. M. (1979). Tree frog toe pads: comparative surface morphology using scanning electron microscopy. *Can. J. Zool.* **57**, 2033-2046.
- Hanna, G. and Barnes, W. J. P. (1991). Adhesion and detachment of the toe pads of tree frogs. *J. Exp. Biol.* **155**, 103-125.
- Hansen, W. R. and Autumn, K. (2005). Evidence for self-cleaning in gecko setae. *Proc. Natl. Acad. Sci. USA* **102**, 385-389.
- Hosoda, N. and Gorb, S. N. (2011). Friction force reduction triggers feet grooming behaviour in beetles. *Proc. R. Soc. Lond. B* **278**, 1748-1752.
- Hu, S., Lopez, S., Niewiarowski, P. H. and Xia, Z. (2012). Dynamic self-cleaning in gecko setae via digital hyperextension. *J. R. Soc. Interface* **9**, 2781-2790.
- Hui, C. Y., Shen, L., Jagota, A. and Autumn, K. (2006). Mechanics of anti-fouling or self-cleaning in gecko setae. In *29th Annual Meeting of the Adhesion Society*, pp. 29-31. Jacksonville, FL: Adhesion Society.
- Lee, J. and Fearing, R. S. (2008). Contact self-cleaning of synthetic gecko adhesive from polymer microfibers. *Langmuir* **24**, 10587-10591.
- Orchard, M. J., Kohonen, M. and Humphries, S. (2012). The influence of surface energy on the self-cleaning of insect adhesive devices. *J. Exp. Biol.* **215**, 279-286.
- Page, E. P. (1963). Ordered hypotheses for multiple treatments: a significance test for linear ranks. *J. Am. Stat. Assoc.* **58**, 216-230.
- Peattie, A. M. (2009). Functional demands of dynamic biological adhesion: an integrative approach. *J. Comp. Physiol. B* **179**, 231-239.
- Smith, J. M., Barnes, W. J. P., Downie, J. R. and Ruxton, G. D. (2006). Structural correlates of increased adhesive efficiency with adult size in the toe pads of hylid tree frogs. *J. Comp. Physiol. A* **192**, 1193-1204.
- Vogel, M. J. and Steen, P. H. (2010). Capillarity-based switchable adhesion. *Proc. Natl. Acad. Sci. USA* **107**, 3377-3381.
- Wigglesworth, V. B. (1987). How does a fly cling to the under surface of a glass sheet? *J. Exp. Biol.* **129**, 373-376.