Effect of shear forces and ageing on the compliance of adhesive pads in adult cockroaches

Yanmin Zhou, Adam Robinson, Christine Viney and Walter Federle

Department of Zoology, University of Cambridge,

Downing Street, Cambridge CB2 3EJ, UK

ABSTRACT

The flexibility of insect adhesive pads is crucial for their ability to attach on rough surfaces. Here we use transparent substrates with micropillars to test in adult cockroaches (*Nauphoeta cinerea*) whether and how the stiffness of smooth adhesive pads changes when shear forces are applied, and whether the insect's age has any influence. We found that during pulls towards the body, the pad's ability to conform to the surface microstructures was improved in comparison to a contact without shear, suggesting that shear forces make the pad more compliant. The mechanism underlying this shear-dependent increase in compliance is still unclear. The effect was not explained by viscoelastic creep, changes in normal pressure, or shear-induced pad rolling, which brings new areas of cuticle into surface contact. Adhesive pads were significantly stiffer in older cockroaches. Stiffness increased most rapidly in cockroaches aged between 2.5 and 4 months. The increase in stiffness is likely based on wear and repair of the delicate adhesive cuticle. Recent wear (visualised by methylene blue staining) was not age-dependent, whereas permanent damage (visible as brown scars) accumulated with age, reducing the pads' flexibility.

Keywords

biomechanics, adhesion, insect cuticle, material properties, ageing, wear

Summary statement

Using transparent micropillar substrate, we show that the compliance of smooth adhesive pads in insects increases with shear forces but decreases with the insect's age.

List of symbols/abbreviations

AFM: Atomic Force Microscopy

λ:effective pad compliance (as defined by equ. 1)

PDMS : polydimethylsiloxane

SEM : Scanning Electron Microscopy

s.e.m.: standard error of mean

W: work of adhesion

E_{eff}: effective elastic modulus

s: pillar spacing

d: pillar diameter

h: pillar height

INTRODUCTION

The locomotion of insects is often constrained by their ability to attach to surfaces in their environment. In order to climb successfully on a wide range of rough surfaces found in nature, insects have evolved attachment organs which include claws and soft attachment pads (Beutel and Gorb, 2001). Claws can interlock with most surface asperities if larger than the diameter of the claw tips, while soft pads are most useful when surface protrusions are too small for the claws to grip (Dai et al., 2002; Bullock and Federle, 2011).

The mechanisms by which insect adhesive pads cope with surface roughness are still not well understood. The pads need to be compliant to be able to deform and achieve sufficient contact (Gorb et al., 2000; Clemente et al., 2009). In smooth adhesive pads of insects, compliance at different length scales is achieved by a hierarchically organized cuticular structure. The specialised cuticle of smooth pads consists of large inner rods oriented at an angle almost perpendicular to the surface. They branch out into finer fibrils near the surface, conveying smaller-scale compliance. These fibres are covered by a thin epicuticle, which in stick insects is folded longitudinally (Scholz et al., 2008; Bennemann et al., 2014).

A further key feature of animal attachment organs is their direction-dependence. Forces of adhesive pads are usually maximised when insect legs are pulled toward the body and they detach easily when this force is released or when they are pushed (e.g. Federle et al., 2001; Federle and Endlein, 2004; Autumn et al., 2006; Clemente and Federle, 2008). Although this direction-dependence is wide-spread among animals with adhesive footpads, the mechanical systems underlying it can be diverse. In hairy adhesive systems, direction dependence is caused by the default position of seta tips, which need to be bent by a pull in order to make full contact to the substrate. In smooth adhesive pads, the direction-dependence can be based on the unfolding of the adhesive pad, or on a hydraulically mediated contact area increase.

It is still unclear, however, whether the ability of the pad to deform around surface asperities is also dependent on the shear force acting on the pad. The internal fibrous cuticle structure of smooth pads would allow a shear-dependent change in compliance. It has been shown that the internal fibres assume a more oblique orientation when the pad is pulled. This may not only result in a more compact packing of the cuticular rods (thereby potentially increasing stiffness) but also reduce the thickness of the cuticle (thereby reducing its bending stiffness). It is unclear which of the two effects dominates, or whether they will cancel each other out. If there is a change in pad stiffness mediated by a pull, it could have different effects, depending on the substrate. On a smooth substrate, peeling or fracture mechanics models would predict a stiffer pad to achieve higher adhesion (Maugis and Barquins, 1978; Bartlett et al., 2012). On a rough substrate, compliant adhesives will adhere better as they will achieve a larger contact area. Here, we study how shear forces influence the ability of smooth adhesive pads to make contact to a transparent micro-structured substrate.

The stiffness of tarsal pads has been found to be affected by the insects' age. The pads of aged cockroaches were found to be less flexible than those of younger ones, concurrent with a reduced climbing performance (Ridgel et al., 2003; Ridgel and Ritzmann, 2005), but it is still unclear to what extent these changes are the result of wear and damage to the cuticle. Insect pads are often found with brown 'scars' which are much stiffer than the rest of pad. Lai-Fook (1966) demonstrated that, due to the action of phenolases, a permanent localised darkening and hardening of the cuticle (sclerotisation) occurred in insects after superficial abrasions. Insect pads are affected by this 'scaring' process with age, which affects their flexibility.

Age may also provide a possible explanation for the observed, high intraspecific variation of adhesion in insects. Even on the same substrates, insects from the same colony can show very different shear and adhesive stresses (e.g. Clemente et al., 2009; Zhou et al., 2014).

To quantify the stiffness of smooth insect pads, previous studies measured load-displacement curves of whole pads (Gorb et al., 2000; Jiao et al., 2000), or indented pads with spherical tips or used Atomic Force Microscopy (AFM) to characterise the micromechanics with high spatial resolution (Scholz et al., 2008). In these studies the material properties were measured at a single point under approximately static conditions.

To assess the effective stiffness of the whole adhesive pad contact zone, we used a transparent micropillar substrate with a gradient of pillar spacings. Microstructured substrates are powerful tools to study insect adhesion (Clemente et al., 2009; Zhou et al., 2014). Smooth adhesive pads of insects make full contact (touching the substrate between the pillars) where the pillars are spaced widely but only partial contact (touching the pillar tops only) where the pillar spacing is narrow (Zhou et al., 2014). The effective elastic modulus of pads can be estimated from the transition point from full to partial contact (Zhou et al., 2014).

When the surface of the cuticle has been damaged, the water-proofing wax layer of the epicuticle may be destroyed, making the cuticle permeable to water-soluble dyes such as methylene blue (Slifer, 1950). Following damage, the lipid layer can be repaired and become again impermeable (Wigglesworth, 1945; Slifer, 1950). For soft insect cuticle, more than superficial damage can result in sclerotisation through the action of phenolases in the cuticle, leading to permanent brown scars (Lai-Fook, 1966).

Here we studied the cuticular damage to the arolium by examining blue (stain) and brown (permanent scar) colouration in both young and aged adults.

We address the following questions:

(1) how do pulling forces affect the stiffness of adhesive pads?

(2) does the compliance of pads change with age?

(3) how does damage accumulate over time in cockroaches?

RESULTS

Effect of shear forces on the compliance of the arolium

When cockroach adhesive pads were brought into contact with the gradient micropillar substrate (Fig. 1), they made full contact on the side with large pillar spacings, and partial contact on the the side with dense pillar arrays. The transition from full to partial contact mostly coincided with a change in pillar spacing on the substrate (Fig. 2). When pads were pulled across the substrate, the transition changed significantly from larger to smaller pillar spacings (supplementary material Movie 1). The values of λ (indicating compliance) for cockroach pads increased significantly with sliding distance (Page's L test, $L_{21,60} = 1.73\times10^5$, $P < 0.001$; Fig. 3). The position of the contact transition approached a steady state during the pulling movement. The change was fastest when the pulling movement started. In the course of sliding, the pads' total projected contact area increased, compared to the initial contact (Wilcoxon signed rank test, $V = 272$, $n = 46$, $P = 0.003$).

The changes in pad compliance (λ) cannot be explained by simple creep, as λ did not change significantly from five seconds before to the start of sliding (Wilcoxon signed rank test: $V = 57$, $n = 34$, $P = 0.445$). However, pad compliance (λ) then increased significantly with sliding (Wilcoxon signed rank test, start of sliding vs. 5 seconds thereafter: V = 52, n = 34, P = 0.008; P values corrected for multiple testing using the Holm-Bonferroni method; Holm, 1979).

In order to investigate whether the observed change in compliance is based on a change in material properties or caused by different areas of the pad cuticle coming into contact, we made an attempt to track 'landmark' points in the adhesive contact zone. Unfortunately only one adhesive pad had a visible air bubble which could be tracked as a landmark (see Fig. 2). In this cockroach, λ increased within the first second from 0.087 to 0.096 µm. The distance of the air bubble from the proximal edge of the contact zone increased by 19.6 µm within 3 seconds after the pulling movement had started. This suggests that the pad underwent a small amount of rolling, bringing new areas of adhesive pad cuticle into contact on the proximal side. However, as the movement of the air bubble was considerably shorter than the length of the adhesive pad (97.1 µm, see Fig. 2), the bulk of the adhesive contact area during initial contact was still in contact during the steady-state sliding phase. To determine whether the 'new' areas on the proximal side of the pad could be responsible for the measured change in pad stiffness, we tested whether pads are more compliant on the proximal side than on the distal one. We measured the transition from partial to full contact separately for the distal and the proximal half of the pad contact zone for pads in initial contact and after sliding. We did not find any evidence for a higher compliance on the proximal side, neither at initial contact nor after sliding; there was even a slight trend towards higher compliance on the distal side (Fig. 4; Wilcoxon signed rank tests for initial contact: $V = 572.5$, $n = 60$, $P < 0.001$; after sliding: $V = 32$, $n = 60$, $P = 0.114$). This indicates that the observed decrease in stiffness after shear is not caused by simple rolling of the pads.

Effect of age on the flexibility and adhesion of the cockroach arolium

The transition from full to partial contact changed and hence the value of λ increased gradually to a steady state during the pulling movement (Fig. 3). To compare the pad compliance for cockroaches of different age, we used the steady-state value of λ (measured at t = 50 second). Pad compliance significantly decreased with age (Spearman's Rank correlation, $r_s = -0.588$, $n = 60$, $P < 0.001$, Fig. 5). This decrease appeared to be relatively abrupt. Specifically, the difference in compliance was significant between consecutive age groups only for the cockroaches 2.5 and 4 months old (Mann-Whitney *U* test: $W = 100$, $n = 20$; $P < 0.001$, all other comparisons $P > 0.05$; P values corrected for multiple testing) (Holm, 1979).

In order to verify that the observed 'age' effect represented a real change and not a difference between separate groups of cockroaches kept in different boxes, we collected a smaller series of data using untested cockroaches from the '2.5-months' group, tested three months later, i.e. after 5.5 months ($n = 10$). Again, pad compliance was significantly higher for the 5.5-months old cockroaches (Mann-Whitney *U* test: $W = 84$, $n = 20$, $P = 0.017$; Fig. 6). The two groups tested at 5.5 months age were not significantly different from one another (Mann-Whitney *U* test: $W = 61$, n

= 20, P = 0.418; P values corrected for multiple testing) (Holm, 1979).
We compared the adhesion forces of cockroaches aged 0.5 and 4.5 months on a microrough test substrate. The adhesion forces of the younger cockroaches were significantly higher than those of the aged ones (Mann-Whitney U test: W = 82, n = 20, P = 0.014; fig 7). This suggests that the softer pads in younger cockroaches help them to deform and achieve sufficient contact on rough surfaces.

Age-dependent wear of cockroach arolium

We assessed both methylene blue staining (indicating recent wear) and brown cuticle colouration (indicating cuticle repair and sclerotisation) of the arolium in newly hatched, 3-month old and 7-month old cockroaches. There was no correlation between the amount of blue arolium staining and the age of the cockroaches (Spearman's Rank correlation, r_s = 0.081, n = 26, P =0.694). However, brown arolium colouration increased significantly with age (Spearman's Rank correlation, r_s = 0.672, n = 26, P < 0.001; fig 8). A repeated analysis of the same pads of the newly hatched cockroaches showed that there was a strong correlation between the amount of blue staining on day 3 and the brown colouration on day 10 (Spearman's Rank correlation, r_s = 0.628, n = 18, P = 0.005). The brown scars appeared to reduce the pads' flexibility. We visualised this effect in a 7-day old adult cockroach with a single brown scar on one of its arolia. This arolium was brought into contact with a pillar substrate with 1.4 µm pillar height and diameter and 4 µm spacing (fig 9). In the scarred area, the arolium cuticle was unable to deform around the pillars to make full contact on this substrate, whereas all other areas were soft enough to achieve full contact.

DISCUSSION

Effect of shear forces on adhesive pad compliance

Our results show that the ability of cockroach adhesive pads to compensate surface roughness increases when the pads are pulled toward the body. When sheared, the pads made full contact on pillar arrays with a smaller spacing than before the shear movement. The median effective compliance parameter (estimated from the topography) increased by 22% within the first nine seconds of the start of the pull, and stayed approximately constant thereafter (Fig. 3). What is the mechanism underlying this increase in compliance?

(1) The change in stiffness could be explained by time-dependent viscoelastic properties of the pad cuticle. Viscoelasticity has been demonstrated for smooth adhesive pads of other insects at the level of whole pads (Gorb et al., 2000), but its importance for the pad cuticle itself is still unclear. After bringing the pads into contact with the gradient pillar substrate, the pad cuticle did not show any significant creep, i.e. the compliance parameter λ remained approximately constant over the first five seconds of contact. Only with the start of sliding, the pad's compliance increased. Thus, the

observed changes in stiffness cannot be explained by viscoelasticity. Strong viscoelasticity may generally be undesirable for adhesive pads of climbing animals, as they have to attach and detach rapidly during locomotion.

(2) Shearing results in some rolling of the pad, bringing new areas of cuticle into contact at the proximal side. Thus, it is possible that different areas of pad cuticle are in contact during initial contact and after shearing. However, our findings indicate that not only was the amount of rolling relatively small (so that only about 20% of the pad's contact area were new), but there was also no evidence that regions on the proximal side of the pad were any softer than those on the distal side. Thus, although some rolling may have occurred, it cannot explain the observed shear-induced increase in pad compliance.

(3) The transition from partial to full contact of the pad could be influenced by the normal pressure in the pad contact zone. Although a constant normal force was maintained during our experiments, it would be possible that a shear-induced reduction in contact area (as observed for rubber hemispheres pressed against glass; Savkoor and Briggs, 1977) increases the pressure within the contact zone, leading to a shift of the compliance parameter λ. However, our results showed that the overall projected contact area *increased* when the pads were sheared, so that there is no evidence for a pressure-induced transition from partial to full contact.

(4) In the absence of evidence in favour of the previous three explanations, the observed increase in pad compliance may indicate a change in cuticle material properties in response to a pull. A change in cuticle material properties could be based on the pad's internal fibrillar ultrastructure. In stick insects, the default angle of the larger cuticular rods was measured to be 57° or 71° to the pad surface in the proximal-to-distal orientation (Dirks et al., 2012; Bennemann et al., 2014). A pull reduces this angle (Dirks et al., 2012). The consequences of this angle change for effective cuticle stiffness are non-trivial. Because of the smaller angle, both the main rods and the finer cuticular fibrils of the outer 'branching' zone may bend more in the tangential direction, thereby increasing compliance. On the other hand, the distance between adjacent rods will decrease, making the pad cuticle more compact, potentially increasing the coupling between rods and reducing pad compliance.

It is likely that the observed stiffness change is an adaptation to enhance adhesion on rough surfaces when the foot is pulled toward the body. So far, however, it is unclear whether the change in compliance is reversible and whether it occurs only when the pad is sheared in the pulling direction.

Effect of age on adhesive pad compliance

The compliance of cockroach arolia (values of λ) decreased significantly with age. This decrease appeared to be relatively abrupt, with the greatest change occurring in cockroaches between 2.5 and

4 months of age. As the pads' compliance parameter λ decreased from around 0.14 to 0.10 µm, their elastic modulus may have increased by ca. 40% (assuming no change in the work of adhesion with age). This stiffening occurred in pads without any visible damage or brown colouration. Thus, if pads with visibly damaged pads (and sclerotized scars) had been included, an even greater effect would have been recorded.

In a previous study we quantified pad stiffness in *N. cinerea* cockroaches without considering their age, and found a mean compliance parameter λ of 0.15 µm (Zhou et al., 2014). This value corresponds well to the pad compliance of young cockroaches found here, suggesting that most insects used in our previous study were less than four months old. This age bias may be based on our selection of insects and pads without any visible damage (Zhou et al., 2014).

Wear and repair of arolium cuticle

Adhesive pads are subject to damage and wear throughout an insect's lifetime. We monitored wear and damage of the cockroach arolia by observing cuticle colouration and its stainability with methylene blue.

Pad staining by methylene blue indicates recent damage by abrasion of the impermeable wax layer on the surface of the epicuticle (Wigglesworth, 1945; Slifer, 1950). The wax layer can be repaired, thereby restoring its water-proofing function (Slifer, 1950; Lai-Fook, 1966). Consistently, the level of methylene blue staining in our study did not increase with age but remained approximately constant. It was found for *Calpodes* fly larvae that wounded cuticle regions started to darken (indicating sclerotization by phenolic tanning) before they lost permeability to methylene blue (Lai-Fook, 1966). When larvae were prevented from sclerotization by dipping them in a suspension of phenylthiourea, their permeability to methylene blue persisted longer (Lai-Fook, 1966). These findings suggest that repair of the wax layer and cuticle sclerotization are correlated. Therefore, it is likely that the increased pad stiffness observed in our study was also based on a weak sclerotization of the cuticle by phenolic tanning (Vincent and Wegst, 2004; Andersen, 2010), even though no brown scars were visible. The detailed mechanism underlying the invisible stiffening is still unclear; it could be simply based on smaller amounts of melanin produced during the repair of small and superficial cuticle wounds (Slifer, 1950; Lai-Fook, 1966), or by a different mechanism of cuticle sclerotization (Andersen, 2010).

Brown scars were more frequent and occupied larger areas on the pads of older insects. The stiffening of adhesive pads reduces the insects' adhesive performance on rough surfaces, and it may contribute to the age-dependent decline in locomotor activity of cockroaches (Ridgel et al., 2003). Stiffening may represent an unwanted by-product of cuticle repair mechanisms, and selection should favour physiological mechanisms that allow the repair of the wax layer in adhesive pads with a minimal stiffness increase.

In many insect species, individuals of different age vary in their activity, leading to a simple decline in spontaneous locomotion with age as reported in flies (Le Bourg, 1987), or a complex division of labour between age groups in some social insects (Seeley, 1995). Age-dependent activity could result in a different frequency of adhesive pad surface contacts which contribute to wear (Ridgel and Ritzmann, 2005). The fast decrease in pad compliance between 2.5 and 4 months of age may be interpreted by a higher locomotory activity for cockroaches of this age, although no higher methylene blue stainability was observed.

The microstructured, transparent substrates with standardised topographies are a powerful tool to study the performance of natural adhesives on rough surfaces. The gradient pillar substrates used in this study allow an instantaneous assessment of pad stiffness over the whole contact zone and under different experimental conditions.

MATERIALS AND METHODS

Study animals

Adult cockroaches (*Nauphoeta cinerea*; body mass 549.3 ± 14 mg; mean ± s.e.m., $n = 60$) were taken from laboratory colonies. In order to group the cockroaches by their age, newly hatched adult cockroaches were collected and kept in separate plastic boxes together with those hatched in the same month. Newly hatched adult cockroaches were easily recognized by their completely white cuticle; most parts of the exoskeleton turn brown in the course of cuticle sclerotization within less than one day. We are therefore confident that the collected cockroaches were less than one day old. We separated 6 groups of cockroaches, within each group all insects had hatched within the same month. The cockroaches were kept at 24°C on a 12:12 hour light-dark cycle and fed on dog food. There was a significant increase of body mass with age (Spearman's Rank correlation, $r_s = 0.490$, $n = 60$, $P < 0.001$; body mass, newly hatched: 419 ± 10.2 mg, $n = 10$; 7-months-old: 634 ± 28.2 mg, $n = 10$; mean ± s.e.m.). For the experiments with microstructured substrates, cockroaches were anaesthetised with CO_2 and immobilised by tying them on their back using Parafilm to a microscope slide glued on a glass tube. One of the hind legs was fixed with Vinyl Polysiloxane impression material (Elite HD+ light body, Zhermack, Badia Polesine, Italy) to a piece of soldering wire attached to the microscope slide, so that the whole tarsus was immobilised and the adhesive pad stood out as the highest point. To ensure that only the adhesive pad comes into contact with the test substrate, we trimmed the tips of the claws. We only tested arolia without any visible damage.

Fabrication of microstructured substrates

Microstructured transparent substrates were fabricated using photolithography and nanoimprinting.

The fabrication method followed a previous study (Zhou et al., 2014). In brief, a lithography shadow mask was designed and produced (Compugraphics Intl. Ltd, Glenrothes, Fife, Scotland). SU-8 2002 photoresist (viscosity 7.5 cSt; MicroChem, Newton, Massachusetts, USA) was spin-coated onto a silicon wafer for 30 seconds at 2000 rpm, resulting in a feature height of 1.4 μm. The features were produced on the silicon wafer by exposing the photoresist with UV light through the mask, followed by developer treatment. A soft polydimethylsiloxane (PDMS; Sylgard 184; Dow Corning, Midland, Michigan, USA) mould was then used to transfer the features from the silicon wafer to transparent epoxy (PX672H/NC; Robnor Resins, Swindon, Wiltshire, UK) on glass coverslips (18 mm × 18 mm × 0.1 mm).

Using this method, transparent substrates were produced with a 525 μm × 2 mm 'gradient' pattern consisting of cylindrical pillars arranged in a series of 12 square arrays increasing in pillar spacing (Fig. 1). The pillars were 2 μm in diameter and 1.4 μm in height. The centre-to-centre pillar spacing increased from 3 to 8 μm in steps of 0.5 μm (Fig. 1).

Scanning electron microscopy (SEM)

For scanning electron microscopy (SEM) of microstructured substrates, the surfaces were mounted on SEM stubs and sputter-coated with gold to prevent charging. Samples were viewed using a Philips XL 30 FEG microscope (Philips, Amsterdam, Netherlands) with a beam voltage of 5kV.

Visualization of the adhesive pad contact zone

Following previous studies (Drechsler and Federle, 2006; Bullock et al., 2008), a custom-made setup was used to perform pulling movements of single adhesive pads of live cockroaches on the gradient micropillar substrate. The spring constant of the 2-D force transducer used was 31.8 N/m in the normal direction and 41.9 N/m in the lateral direction. The force transducer was mounted on a three-dimensional motor positioning stage ((M-126PD, C-843, Physik Instrumente, Karisruhe, Germany), controlled by a custom-made LABVIEW (National Instruments, Austin, Texas, USA) program. The contact of insect pad through the transparent substrate was recorded at 10 Hz using an externally triggered Redlake PCI 1000 B/W video camera (Cheshire, Connecticut, USA).

A coverslip with the gradient micropillar substrate was mounted on the force transducer so that the axis of the gradient was aligned to one of the axes of the motor. The hind leg adhesive pad of a cockroach was brought into contact with the centre of the micropillar gradient with a normal force of 1 mN. The pad was brought close to the surface manually, followed by 10 seconds approach using the force feedback system. The axis of the leg was perpendicular to the gradient, and the pad touched each of the zones with different spacings. The substrate was then moved for 20 seconds across the insect pad, away from the insect (corresponding to a horizontal pull of the leg toward the body), while maintaining a constant normal force of 1 mN (feedback control frequency 20 Hz) and a sliding velocity of 0.05 mm/s. Due to the alignment of the substrate, the pad moved perpendicular to the gradient, and therefore stayed in the centre of the pattern throughout the movement. At the end of the sliding movement, the pad was left in contact with the same normal force for another 30 seconds before the substrate was pulled off.

The image of the contact area was used to estimate the compliance of the arolium. As the pattern was designed to be slightly narrower than the width of the cockroach pads, a transition from partial contact (pad only in contact with the top of the pillars) to full contact (pad also in contact with the area in between the pillars) was always observed. We measured the pad's total contact area during initial contact and at the end of sliding as twice the (fully visible) pad half-area on the side with larger pillar spacing; the pad mid-line was defined relative to the visible left and right pad edges.

Pad stiffness estimation

We measured from the contact images the pillar spacing where the pad changed from full to partial contact (Fig. 2). The position of this transition was measured relative to the visible left and right margins of the gradient to find the smallest pillar spacing on which the pad could still make full contact. This spacing was used to estimate the pad's flexibility. One image per second was analysed for the first 30 seconds from when the pulling movement started, while one image per five seconds was analysed for the last 20 seconds since the contact hardly changed during this period.

The contact model used in our previous study (Zhou et al., 2014) predicts that a smooth pad should make full contact to a substrate patterned with cylindrical pillars if

$$\frac{W}{E_{eff}} > \frac{h^2}{\pi\left(\sqrt{2}s - d\right)} \equiv \lambda, \qquad (1)$$

where W is the work of adhesion, E_{eff} is the effective elastic modulus, s is the pillar spacing where the transition from full to partial contact happened, d is the pillar diameter, h is the pillar height and λ is a summarizing topography parameter with dimensions of length, which can be used as a proxy for the pad's compliance and ability to conform to rough surfaces. A high compliance λ indicates that the contact transition occurred for a small pillar spacing, which requires a low effective elastic modulus of the pad.

To evaluate whether 'new' areas on the proximal side of the pad could mediate a shear-induced change in pad stiffness, we measured λ separately for the distal and the proximal half of the pad contact zone (see Fig. 2).

Adhesion force measurement

In order to evaluate how the variation in pad compliance affects adhesion, we measured pull-off forces of single cockroach pads on a micro-rough substrate. A piece of aluminium oxide polishing paper (asperity size 50 nm; Ultra Tec, Santa Ana, California, USA) glued to a glass coverslip (18 mm × 18 mm × 0.1 mm) was mounted at the end of a metal bending beam with a spring constant of 14.3 N/m. The bending beam was mounted on a three-dimensional motor positioning stage (see above). A fibre optic sensor (D12, PHILTEC, Annapolis, Maryland, USA) measured the beam's deflection as the distance to a smooth reflective metal foil target glued onto the beam just before the substrate. The fibre optic sensor was used in its near field (ca. 30-130 µm distance to reflective target), and was calibrated to obtain force. The substrate was moved into contact with the insect pad with a normal force of 1 mN, kept in contact for 30 seconds (feedback frequency 20 Hz), and then pulled off perpendicularly at a velocity of 0.1 mm/s. The peak forces were used for further analysis. The cockroaches used in this experiment were 0.5 and 4.5 months old (body mass: 480 ± 31.1 mg and 645 ± 20.3 mg; mean ± s.e.m., n = 10 each).

Visualization of wear in the arolium of cockroaches

We visualized damage and wear of the adhesive pad cuticle with a method similar to that used by Slifer (1950). A 0.1% solution of methylene blue was obtained by dissolving methylene blue powder (general purpose grade, Fisher Scientific, Hampton, New Hampshire, USA) in deionised water. A piece of tissue paper was folded, fully soaked with methylene blue solution, and laid out in a petri dish. A cockroach was then placed on the tissue paper with a petri dish lid over it. This ensured that the cockroach could not escape and its tarsi were in contact with the dye. Tissue paper was used rather than liquid methylene blue solution to prevent the cockroach from drinking the dye. The insect was kept in the petri dish for one hour and was then transferred to a clean petri dish, which was rinsed four times (with the cockroach inside) to remove any surplus dye. The cockroach was then anaesthetised by cooling at - 10°C for approximately 2 minutes until it stopped moving so that it could be observed under a stereomicroscope.

In order to study the age dependence of wear, cockroaches were randomly selected from newly hatched (n = 6), 3-months-old (n = 10) and 7-months-old (n = 10) groups. In order to score the arolia for blue (methylene blue stain) and brown (sclerotized cuticle) colouration, each pad contact area was evenly divided into six segments of equal size (three distal and three proximal). Each pad segment was given a score from 0 to 3 for the intensity of blue and brown colouration. We calculated the average score of blue and brown colouration for each cockroach (pooling across all measurements and arolia).

Captions

Fig. 1. **SEM micrographs of the transparent 'gradient' microstructured epoxy substrate with square arrays of cylindrical pillars 1.4 µm in height and 2 µm in diameter.** (*a*) from left to right, the centre-to-centre spacing increases from 3.5 to 6.5 µm in steps of 0.5 µm. (*b*) transition from 3.5 to 4.0 µm spacing. (*c*) transition from 6.0 to 6.5 µm spacing. Scale bars: 20 µm (*a*), and 5 µm (*b*, *c*).

Fig. 2. **Contact area image of cockroach arolium (*Nauphoeta cinerea*) pulled across the 'gradient' pillar substrate.** The pillar spacings increase from 3 µm (left) to 8 µm (right) in steps of 0.5 µm. Scale bar: 100 µm.

Fig. 3. **Shear-dependent change in compliance of cockroach arolia pulled across the 'gradient' pillar substrate.** (*a*) Typical shear-induced change in compliance λ (see equ.1) for the pad of a 5.5-months old adult cockroach. (*b*) Relative pad compliance (percentage of each pad's steady-state compliance λ at t = 50 seconds) increased and gradually approached a steady state. *Centre lines* and *boxes* represent the median within the inner quartiles, *whiskers* show the 10th and 90th percentiles and *circles* indicate outliers.

Fig. 4. **Shear-dependent change in compliance (measured as λ, see equ.1) of cockroach arolia pulled across the 'gradient' pillar substrate, measured separately for the proximal and distal half of the pad.** *Centre lines* and *boxes* represent the median within the inner quartiles, *whiskers* show the 10th and 90th percentiles and *circles* indicate outliers.

Fig. 5. **Arolium compliance (measured as λ, see equ.1) in different age groups of *N. cinerea* cockroaches.** *Centre lines* and *boxes* represent the median within the inner quartiles, *whiskers* show the 10th and 90th percentiles and *circles* indicate outliers.

Fig. 6. **Change in arolium compliance (measured as λ, see equ.1) in *N. cinerea* cockroaches from the same experimental group, measured 2.5 and 5.5 months after hatching.** *Centre lines* and *boxes* represent the median within the inner quartiles, *whiskers* show the 10th and 90th percentiles and *circles* indicate outliers.

Fig. 7. **Effect of age on the adhesion of cockroach arolia on a microrough surface (asperity size 50 nm).** Adhesion was weaker for the older cockroaches. *Centre lines* and *boxes* represent the median within the inner quartiles, *whiskers* show the 10th and 90th percentiles and *circles* indicate

outliers.

Fig. 8. **Age-dependent increase of brown cuticle colouration in arolia of *N. cinerea* cockroaches.** The level of colouration was scored from 0 to 3. *Circles* represent individual insects.

Fig. 9. **Effects of cuticle damage on arolium flexibility in *N. cinerea*.** (*a*) arolium with a brown scar (arrow). (*b*) the same pad contacting a microstructured square array of pillars with 1.4 µm diameter, 1.4 µm height and 4 µm spacing. *Arrow* in (*b*) shows the loss of contact caused by scar shown in (*a*). Scale bar: 100 µm.

Competing interests

The authors declare no competing or financial interests.

Author contributions

W. F. and Y. Z. designed this study. A. R. designed and fabricated the substrates. Y. Z. and C. V. conducted the experiments and collected data. Y. Z. analysed the data. W. F. and Y. Z. interpreted the results and wrote the article.

Funding

This study was supported by a studentship from the Cambridge Overseas Trust (to Y.Z.) and research grants from the UK Biotechnology and Biological Sciences Research Council [BB/I008667/1], the Human Frontier Science Programme [RGP0034/2012] and AkzoNobel.

References

Andersen, S. O. (2010). Insect cuticular sclerotization: A review. *Insect Biochem. Mol. Biol.* **40**, 166-178.

Autumn, K., Dittmore, A., Santos, D., Spenko, M. and Cutkosky, M. (2006). Frictional adhesion: a new angle on gecko attachment. *J. Exp. Biol.* **209**, 3569-3579.

Bartlett, M. D., Croll, A. B., King, D. R., Paret, B. M., Irschick, D. J. and Crosby, A. J. (2012). Looking beyond fibrillar features to scale gecko-like adhesion. *Adv. Mater.* **24**, 1078-1083.

Bennemann, M., Backhaus, S., Scholz, I., Park, D., Mayer, J. and Baumgartner, W. (2014). Determination of the Young's modulus of the epicuticle of the smooth adhesive organs of *Carausius morosus* using tensile testing. *J. Exp. Biol.* **217**, 3677-3687.

Beutel, R. G. and Gorb, S. N. (2001). Ultrastructure of attachment specializations of hexapods (Arthropoda): evolutionary patterns inferred from a revised ordinal phylogeny. *J. Zool. Syst. Evol. Res.* **39**, 177-207.

Bullock, J., Drechsler, P. and Federle, W. (2008). Comparison of smooth and hairy attachment pads in insects: friction, adhesion and mechanisms for direction-dependence. *J. Exp. Biol.* **211**, 3333-3343.

Bullock, J. M. R. and Federle, W. (2011). The effect of surface roughness on claw and adhesive hair performance in the dock beetle *Gastrophysa viridula*. *Insect Sci.* **18**, 298-304.

Clemente, C. J., Dirks, J.-H., Barbero, D. R., Steiner, U. and Federle, W. (2009). Friction ridges in cockroach climbing pads: anisotropy of shear stress measured on transparent, microstructured substrates. *J. Comp. Physiol. A* **195**, 805-814.

Clemente, C. J. and Federle, W. (2008). Pushing versus pulling: division of labour between tarsal attachment pads in cockroaches. *Proc. R. Soc. B* **275**, 1329-1336.

Dai, Z., Gorb, S. N. and Schwarz, U. (2002). Roughness-dependent friction force of the tarsal claw system in the beetle *Pachnoda marginata* (Coleoptera, Scarabaeidae). *J. Exp. Biol.* **205**, 2479–2488.

Dirks, J.-H., Li, M., Kabla, A. and Federle, W. (2012). In vivo dynamics of the internal fibrous structure in smooth adhesive pads of insects. *Acta Biomater.* **8**, 2730-2736.

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.

Federle, W., Brainerd, E. L., McMahon, T. A. and Hölldobler, B. (2001). Biomechanics of the movable pretarsal adhesive organ in ants and bees. *Proc. Natl. Acad. Sci. USA* **98**, 6215-6220.

Federle, W. and Endlein, T. (2004). Locomotion and adhesion: dynamic control of adhesive surface contact in ants. *Arthropod Struct. Dev.* **33**, 67-75.

Gorb, S., Jiao, Y. and Scherge, M. (2000). Ultrastructural architecture and mechanical properties of attachment pads in *Tettigonia viridissima* (Orthoptera Tettigoniidae). *J. Comp. Physiol. A* **186**, 821-831.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65-70.

Jiao, Y., Gorb, S. and Scherge, M. (2000). Adhesion measured on the attachment pads of *Tettigonia viridissima* (Orthoptera, Insecta). *J. Exp. Biol.* **203**, 1887-1895.

Lai-Fook, J. (1966). The repair of wounds in the integument of insects. *J. Insect Physiol.* **12**, 195-226.

Le Bourg, E. (1987). The rate of living theory. Spontaneous locomotor activity, aging and longevity in *Drosophila melanogaster*. *Exp. Gerontol.* **22**, 359-369.

Maugis, D. and Barquins, M. (1978). Fracture mechanics and the adherence of viscoelastic bodies. *J. Phys. D Appl. Phys.* **11**, 1989-2023.

Ridgel, A. L. and Ritzmann, R. E. (2005). Insights into age-related locomotor declines from studies of insects. *Ageing Res. Rev.* **4**, 23-39.

Ridgel, A. L., Ritzmann, R. E. and Schaefer, P. L. (2003). Effects of aging on behavior and leg kinematics during locomotion in two species of cockroach. *J. Exp. Biol.* **206**, 4453-4465.

Savkoor, A. R. and Briggs, G. A. D. (1977). The effect of tangential force on the contact of elastic solids in adhesion. *Proc. R. Soc. Lond. A* **356**, 103-114.

Scholz, I., Baumgartner, W. and Federle, W. (2008). Micromechanics of smooth adhesive organs in stick insects: pads are mechanically anisotropic and softer towards the adhesive surface. *J. Comp. Physiol. A* **194**, 373-384.

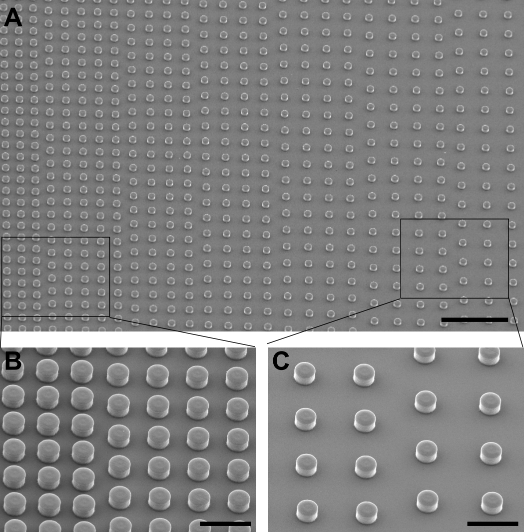
Seeley, T. D. (1995). The wisdom of the hive. Cambridge Mass, London: Harvard University Press.

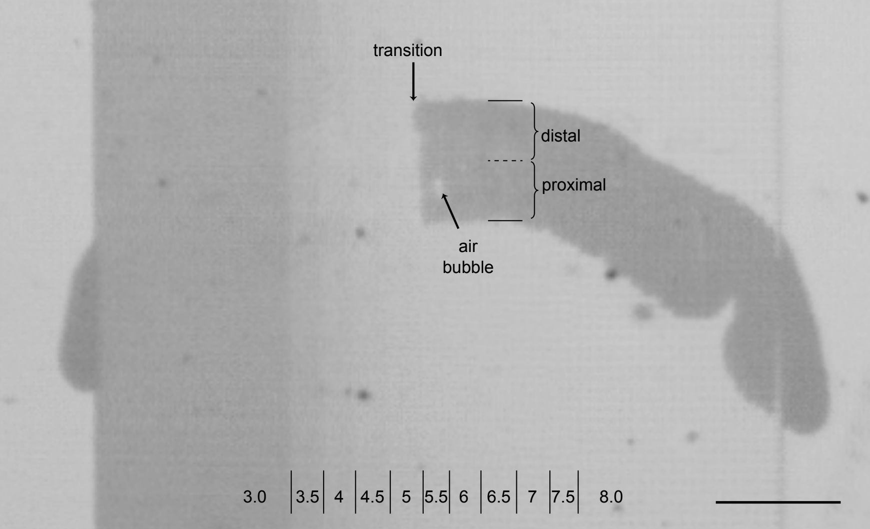
Slifer, E. H. (1950). Vulnerable areas on the surface of the tarsus and pretarsus of the grasshopper (Acrididae, Orthoptera) with special reference to the arolium. *Ann. Entomol. Soc. Am.* **43**, 173-188.

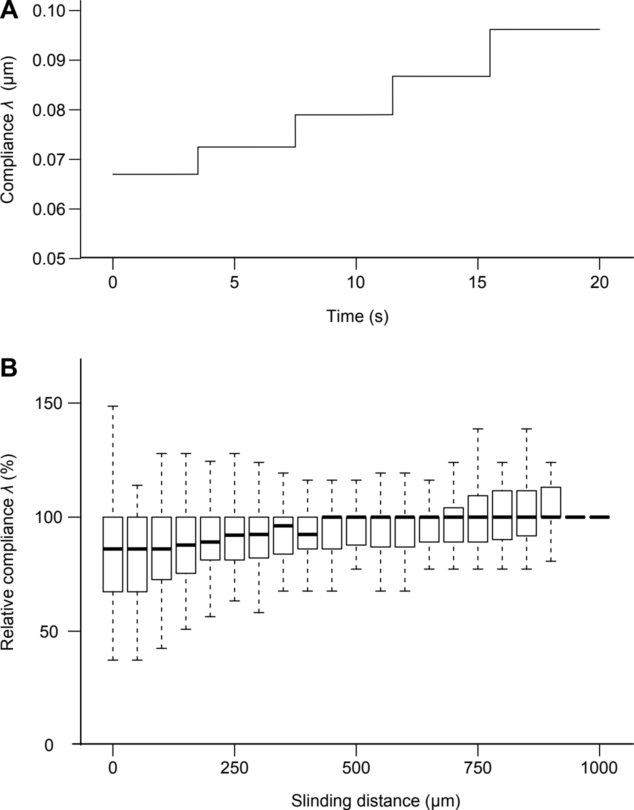
Vincent, J. F. V. and Wegst, U. G. K. (2004). Design and mechanical properties of insect cuticle. *Arthropod Struct. Dev.* **33**, 187-199.

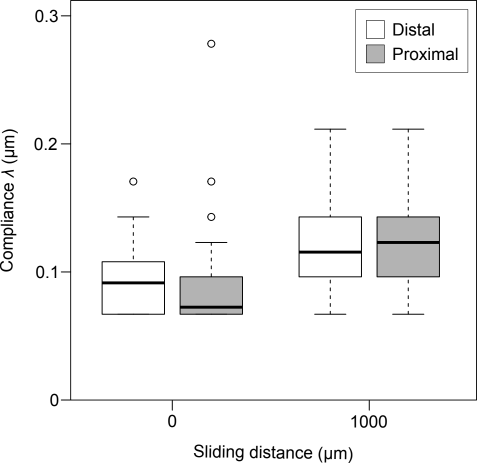
Wigglesworth, V. B. (1945). Transpiration through the cuticle of insects. *J. Exp. Biol.* **21**, 97-114.

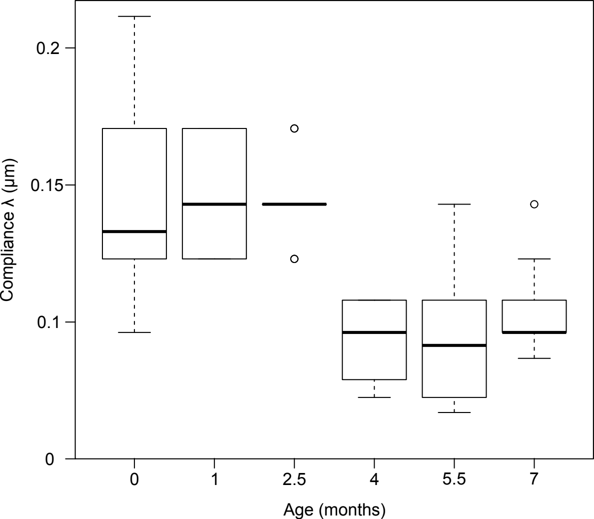
Zhou, Y., Robinson, A., Steiner, U. and Federle, W. (2014). Insect adhesion on rough surfaces: analysis of adhesive contact of smooth and hairy pads on transparent micro-structured substrates. *J. R. Soc. Interface* **11**, 20140499.


A
B
C

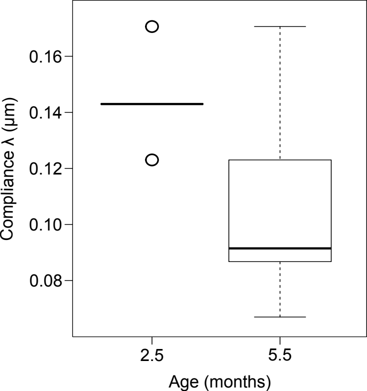

transition
distal
proximal
air
bubble
3.0
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8.0


A
0.10
0.09
0.08
0.07
0.06
0.05
Compliance λ (μm)
0
5
10
15
20
Time (s)
B
150
100
50
0
Relative compliance λ (%)
0
250
500
750
1000
Slinding distance (μm)

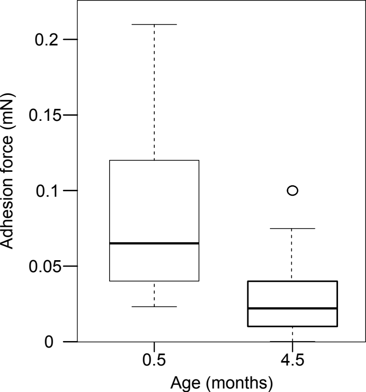

Distal
Proximal
0.3
0.2
0.1
0
Compliance λ (μm)
0
1000
Sliding distance (μm)



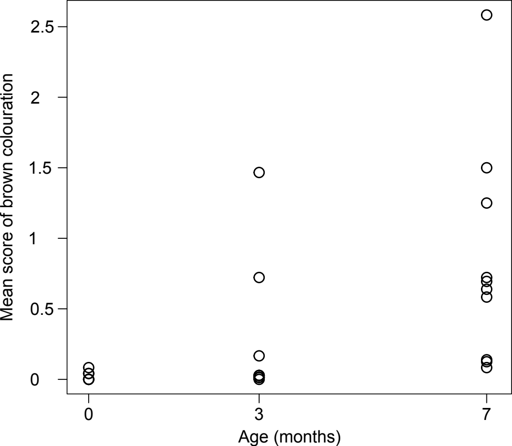
Compliance λ (μm)
0.2
0.15
0.1
0
0
1
2.5
4
5.5
7
Age (months)

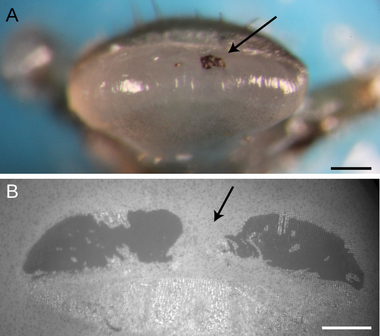


0.16
0.14
0.12
0.10
0.08
Compliance λ (µm)
2.5
5.5
Age (months)



0.2
0.15
0.1
0.05
0
Adhesion force (mN)
0.5
4.5
Age (months)


Mean score of brown colouration
2.5
2
1.5
1
0.5
0
0
3
7
Age (months)


A
B