Insect tricks: two-phasic foot pad secretion prevents slipping

Jan-Henning Dirks, Christofer J. Clemente and Walter Federle*

Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

Many insects cling to vertical and inverted surfaces with pads that adhere by nanometre-thin films of liquid secretion. This fluid is an emulsion, consisting of watery droplets in an oily continuous phase. The detailed function of its two-phasic nature has remained unclear. Here we show that the pad emulsion provides a mechanism that prevents insects from slipping on smooth substrates. We discovered that it is possible to manipulate the adhesive secretion in vivo using smooth polyimide substrates that selectively absorb its watery component. While thick layers of polyimide spin-coated onto glass removed all visible hydrophilic droplets, thin coatings left the emulsion in its typical form. Force measurements of stick insect pads sliding on these substrates demonstrated that the reduction of the watery phase resulted in a significant decrease in friction forces. Artificial control pads made of polydimethylsiloxane showed no difference when tested on the same substrates, confirming that the effect is caused by the insects’ fluid-based adhesive system. Our findings suggest that insect adhesive pads use emulsions with non-Newtonian properties, which may have been optimized by natural selection. Emulsions as adhesive secretions combine the benefits of ‘wet’ adhesion and resistance against shear forces.

Keywords: insect adhesion; friction; tribology; biomechanics; emulsion

1. INTRODUCTION

Many insects are able to climb on vertical and inverted surfaces with the help of tarsal attachment pads that adhere via small volumes of fluid secreted into the contact zone (Walker et al. 1985; Lees & Hardie 1988; Gorb 2001; Vötisch et al. 2002). Chemical analyses of this fluid in beetles showed that it contains saturated and unsaturated linear hydrocarbons of C_{20}–C_{28} chain length, fatty acids and alcohols (Ishii 1987; Attygalle et al. 2000; Betz 2003), as well as true waxes (Kosaki & Yamaoka 1996). A recent study on locusts detected not only shorter chain fatty acids (C_{16}–C_{20}) but also significant amounts of polar and amphiphilic components such as saccharides, amino acids and cholesterol (Vötisch et al. 2002). From the low contact angles of footprints on glass, it was concluded that the secretion consists of lipid nano-droplets in an aqueous fluid (Vötisch et al. 2002). However, in vivo observations using interference reflection microscopy (IRM) in ants and stick insects showed that the emulsion’s continuous phase is oily and contains volatile, hydrophilic droplets (Federle et al. 2002). It has been hypothesized that the emulsion might act as a coupling agent, helping pads to adhere to diverse (hydrophilic and hydrophobic) substrates (Vötisch et al. 2002; Gorb 2008). However, since hydrophobic fluids adhere well to both hydrophilic and hydrophobic substrates, it is unclear how exactly the addition of a hydrophilic phase could enhance adhesion. The detailed function of the fluid’s two-phasic composition is still unknown.

To climb safely on smooth substrates, insects not only have to produce adhesion but also considerable friction forces. Shear forces help animals to support their body on vertical surfaces and are also essential for walking upside-down, when the sprawled leg posture gives rise to large centripetal forces. Many climbing pads can only resist pull-offs when they are strongly sheared towards the body, and detach as soon as this shear force is released (Autumn et al. 2006; Yao & Gao 2006, 2007; Gravish et al. 2008).

One might suppose that large shear forces are in conflict with a fluid-based adhesion mechanism as found in insects. While fluid in the contact zone can enhance adhesion to rough substrates (Drechsler & Federle 2006), it should act as a lubricant on smooth surfaces, causing pads to slide. A previous analysis of the pattern of interference fringes at the edge of the pad contact zone in stick insects suggested that the fluid film between pad cuticle and surface has a thickness of 90–160 nm (Federle et al. 2002). A continuous fluid film of this thickness should cause excessive sliding even for relatively viscous fluids.

However, insect pads clearly defy this prediction. In all insects studied to date, maximum shear forces greatly exceed maximum adhesive forces (Walker et al. 1985; Gorb et al. 2002; Federle et al. 2004). Moreover, insect pads are able to generate considerable static friction (i.e. a static force parallel to the surface resisting sliding of the pad), even when pad secretion has accumulated on a surface (Drechsler & Federle 2006). This suggests that the fluid itself plays a role in maximizing friction, and

*Author for correspondence (wf222@cam.ac.uk).

possibly by means of its two-phase composition. It is known that many emulsions are more viscous than their pure components and often exhibit non-Newtonian properties (Barnes 1994). This could provide an explanation for the independent evolution of two-phase secretions in insect foot pads (Federle et al. 2002; Vötsch et al. 2002) and in the adhesive prey capture apparatus of rove beetles (Kölsch 2000).

In this study, we test the effect of the pad secretion’s two-phase nature on friction forces by selectively reducing the hydrophilic phase of a stick insect’s emulsion in vivo, using a polymeric, water-absorbing substrate.

2. MATERIAL AND METHODS

2.1. Study animals

Adult stick insects (*Carausius morosus*; 715 ± 196 mg, mean ± s.d., *n* = 12) were taken from a laboratory colony. Both for IRM and for single-pad force measurements, we enclosed them in a hollow glass tube and the protruding tarsus of one front leg was fixed to a stiff wire with dental cement (ESPE, Protemp).

2.2. Preparation of substrates and polydimethylsiloxane control ‘pads’

We used polyimide substrates spin-coated onto glass to selectively remove the dispersed, hydrophilic phase of insect adhesive emulsion in vivo. Polyimide resins are transparent and can absorb significant amounts of water (Sacher & Susko 1979; Paplham et al. 1995). The polyimide PI-2611 (HD Microsystems) used in our experiments has a typical moisture uptake of 0.5 per cent (weight gain). Polyimide substrates were prepared by spin-coating 18 mm × 9 mm × 0.1 mm glass coverslips and curing them for 6 h under atmospheric conditions at 350°C. ‘Thin’ substrates were spin-coated for 5 min at 100 rps and ‘thick’ substrates for 1 min at 30 rps. The thickness of the spin-coated substrates was measured using a Dimension AFM (D3100, Veeco) to be 3023 ± 808 nm for thin and 679 ± 235 nm for thin substrates (means ± s.d., *n* = 6); the AFM measurement also showed that both surfaces were perfectly smooth (roughness average *Rq* < 5 nm).

Mica slides were split immediately before use to obtain a clean and smooth surface. Polystyrene (Sigma Aldrich, solved in toluene), poly(methyl methacrylate) (PMMA; Sigma Aldrich, solved in toluene), and Teflon® AF 1600 (Dupont, solved in FC-75) substrates were prepared by spin-coating onto 10 mm × 5 mm glass coverslips; curing time and temperature were adapted to the requirements of the coating materials.

‘Dry’ polydimethylsiloxane (PDMS) rubber control pads were prepared by polymerizing droplets of Sylgard 184 (Dow Corning) on the end of an insect pin. The obtained hemispheres were smooth and yielded contact areas comparable in size to those of the stick insects used (26 849 versus 73 421 μm²).

2.3. Interference reflection microscopy

IRM was used to visualize the adhesive contact zone of *C. morosus* (Federle et al. 2002, 2006). IRM measures the reflectivity of a specimen under monochromatic illumination. If the specimen contains thin layers of different refractive indices, light reflected from closely adjacent interfaces has an optical path difference, leading to interference (Gingell & Todd 1979; Rädler & Sackmann 1993; Wiegand et al. 1998). We used a Leica DMR-HC upright microscope with a bandpass filter in the epi-illumination path to isolate the 546 nm line from the spectrum of a 100 W mercury arc lamp.

To study the adhesive emulsion of stick insects, pads were brought into contact with the PI-2611 polyimide substrates (refractive index 1.9) under the microscope using a micromanipulator. The pad contact zone was viewed with a 100 ×/1.25 oil objective and an illuminating numerical aperture of 0.79; images were recorded with a 12 bit monochrome digital camera (QIC-FM12, QImaging).

2.4. Cryo-scanning electron microscopy

We used a Gatan Alto 2100 cryo-transfer system in an FEI XL30 ESEM FEG operating in a high-vacuum mode. A glass coverslip with *C. morosus* footprints was frozen in liquid nitrogen in the slushing chamber, and transferred via the preparation chamber to the cold stage in the microscope. The sample was then warmed up to a temperature of −90°C for 3 min to remove condensed ice crystals by sublimation. Images were taken at −120°C with an accelerating voltage of 3 kV and a working distance of 7.9 mm.

2.5. Single-pad force measurements

A self-built two-dimensional bending beam force transducer equipped with 350 Ω foil strain gauges (Vishay SR-4) was used to measure friction forces of single adhesive pads. The spring constants of the adhesion and friction axes were 57.0 and 11.9 N m⁻¹, and the resolution 0.07 and 0.05 mN, respectively. Voltage output was amplified (GSV1T8, ME-Systeme) and recorded on a data acquisition board (PCI-6035E, National Instruments) with a sampling frequency of 1 kHz. The force transducer was moved by a three-dimensional motor positioning stage (M-126PD, C-843, Physik Instrumente). Motor movements, video trigger and force recording were controlled with a custom-made LanVIEW (National Instruments) programme that included a normal force feedback. Adhesive pads were brought into contact with one of the two (thick and thin) polyimide-covered glass coverslips (each 18 mm × 9 mm × 0.1 mm) mounted side by side at the end of the force transducer. Contact area was recorded under reflected light using a Redlake PCI 1000 B/W camera at 10 Hz. A schematic view of the setup is shown in figure 1.

Before each friction measurement, the pads were brought into contact with the substrate for 2 s with a normal force of 0.5 mN. Sliding movements were performed over a distance of 2 mm in the proximal direction (imitating a pull of the leg towards the body), with a motor velocity of 0.05 mm s⁻¹. The normal force was kept constant at 0.5 mN via force feedback. For each pad, paired measurements were performed on thick and thin polyimide in randomized order. Each
slide was performed on a fresh area of the substrate. Before each recorded slide, a ‘cleaning’ slide was performed on the surface of interest to minimize any possible effect of the previous surface contact on fluid production. The movement patterns of the cleaning slides were similar to the ones of the recorded slides. Friction and shear stress were measured under the same conditions for stick insect arolia and PDMS control pads.

To measure ‘remaining’ friction and the relationship between shear stress and sliding velocity, pads were kept in contact for 180 s after the end of the motor movement, during which the pad continued sliding with exponentially decreasing velocity (figure 5a). This exponential decay part of the force curves was used to measure the relationship between shear stress and sliding velocity (figure 5c). The deflection of the force transducer was calculated from the force and the beam’s spring constant. As the pad itself was mounted statically, the change of deflection represents the sliding velocity of the pad on the polyimide surface. The displacement curves were filtered using the predicted mean square error quintic spline algorithm (Walker 1998) to calculate the sliding velocity. We plotted shear stress against sliding velocity and performed a model II (standardized major axis) regression (Sokal & Rohlf 1995). To improve the independence of the regression data points, shear stress and velocity values were only included for successive intervals of 2 s. The slope and intercept of the regression line were measured for each individual and were statistically compared between thin and thick polyimide surfaces.

3. RESULTS AND DISCUSSION

In vivo IRM of the adhesive contact zone of stick insect pads confirmed our earlier finding that the adhesive secretion is a water-in-oil emulsion (Federle et al. 2002). The volatile, hydrophilic droplets of the emulsion were abundant on a variety of surfaces, both hydrophilic and hydrophobic (glass, mica, polystyrene, PMMA and Teflon AF). However, when adhesive pads were brought into contact with polyimide PI-2611 substrates of 2–4 μm thickness, only the oily bulk phase was visible in the contact zone (figure 2a). We discovered that this effect disappeared for very thin PI-2611 substrates of 0.6–0.9 μm thickness. Here, the hydrophilic droplets were visible as on most other substrates (figure 2b). This indicates that the watery droplets are absorbed by the polyimide and that on very thin polyimide substrates, the absorption is smaller owing to the insufficient volume of the polymer layer.

To confirm that the polyimide-coated surfaces absorb water from the pad contact zone we deposited small water droplets (diameter 5–30 μm) on thick polyimide-coated substrates using an ultrasonic humidifier (Honeywell, BH-860 E). When C. morosus pads were placed onto the substrate, some water droplets became trapped in the adhesive contact zone and were visible as bright spots with a high-contrast interference fringe pattern, similar to fluid A droplets. The size of these droplets decreased over time and smaller droplets completely disappeared. This effect was absent on glass, mica, polystyrene, PMMA and Teflon AF, thus excluding an uptake of water into the cuticle or into the adhesive fluid itself.

Further observations revealed that the visible hydrophilic droplets originate by coagulation from droplets that are smaller than the resolution limit of the light microscope. Cryo-scanning electron microscopy (cryo-SEM) images of C. morosus footprints showed that droplets contained high densities of nanostructures, even after sufficient evaporation time (diameter 100–240 nm), which probably represent small hydrophilic
droplets (figure 3). Similar nanostructures have been observed in footprint droplets of flies and locusts (Gorb 2001; Vötsch et al. 2002). When we rapidly shifted pads in contact with thin substrates by 50 µm in the proximal direction (using a micromanipulator), the number and size of visible hydrophilic droplets immediately increased (figure 4). Some coagulated droplets even appeared on thick substrates, where no droplets had been visible before the pad displacement. The visible droplets thus represent only a fraction of the actual volume of the hydrophilic phase in the contact zone because some volume is present in the form of submicroscopic droplets within the continuous hydrophobic phase. These observations also show that even on the thick polyimide substrate, the absorption was not complete. Under the IRM, the hydrophilic droplets usually appeared bright without any interference fringes, showing that they were flat and compressed (figure 2b; maximum height less than ca 0.15 µm, average diameter 0.46 ± 0.20 µm). IRM observations of sliding pads showed that the hydrophilic droplets remained either attached to the surface, or moved together with the pad. Droplets attached to the surface were visibly deformed when pad areas in close contact were sliding across them (video S2 in the electronic supplementary material).

We used these thick and thin PI-2611 substrates as a tool to reduce the hydrophilic phase of the adhesive emulsion in vivo and to investigate the effect of the secretion’s two-phasic nature on friction forces. Single-pad friction forces of stick insects were consistently lower on the thick polyimide substrate (figure 5a,b). The differences in friction and shear stress between both substrates were highly significant (paired t-tests, friction $t_{11} = 4.42$, $p = 0.001$; shear stress $t_{11} = 3.59$, $p = 0.004$). The effect on adhesion was weaker but still significant ($t_{11} = 2.80$, $p = 0.017$; figure S1 in the electronic supplementary material).

On both polyimide substrates, the stick insect pads exhibited an approximately linear relationship between shear stress and sliding velocity, with a positive intercept (figure 5c), consistent with previous results obtained for ant pads sliding on PMMA (Federle et al. 2004). This intercept corresponds to the quasi-static friction force that remained after the end of the

Figure 2. Adhesive emulsion of stick insects (C. morosus), visualized by IRM. (a) Contact zone of C. morosus on thick (2–4 µm) polyimide-coated surface and (b) on thin polyimide (0.6–0.9 µm). The hydrophilic component of the adhesive emulsion (droplets, arrows) was only visible for pads in contact with the thin polyimide; fringes at the edge of the contact zone (right) show a wedge of the bulk hydrophobic phase. Scale bar, 5 µm.

Figure 3. Cryo-SEM image of a footprint of C. morosus on glass. Before freezing, the footprint was allowed to evaporate at room temperature and 30 per cent humidity for 6 h. Note that the fluid droplets contain nanostructures (arrows). Scale bar, 2 µm.

Figure 5. Friction and shear stress for stick insect pads on thick polyimide (2–4 µm) and thin polyimide (0.6–0.9 µm) substrates. (a) Normalized friction (relative decrease) and (b) shear stress as a function of sliding velocity. (c) Linear relationship between shear stress and sliding velocity with a positive intercept (quasi-static friction force) for both substrates. (d) Friction (a) and shear stress (b) as a function of time for single-pad measurements on thick polyimide (2–4 µm) and thin polyimide (0.6–0.9 µm) substrates.
motor movement (figure 5a). Model II regression intercepts were significantly smaller on the thick polyimide substrate (paired t-test, \( t_{11} = 2.95, p = 0.015 \)). Thus, both dynamic and static friction were reduced by the removal of the hydrophilic droplets.

To confirm that the observed effects are a consequence of the insect’s fluid-based adhesive system, we performed an identical set of control experiments on the same surface pairs using dry ‘model pads’ made of polymerized PDMS droplets. Here, friction and shear stress showed no difference between the thick and thin polyimide substrates (paired t-test, \( t_{12} = 0.07, p > 0.5 \)).

Our results demonstrate that the two-phasic nature of adhesive secretion increases the insect’s ability to withstand shear forces. Friction forces in the presence of the hydrophilic droplets were higher despite large volumes of fluid being present in the contact zone. This result could be explained by direct effects of the larger, coagulated droplets within the contact zone. It is possible that the larger droplets adhere to both the cuticle and the surface, giving rise to some additional shear stress. Droplets only adhering to the substrate could also increase friction by mechanically deforming the pad cuticle during sliding. An alternative explanation is that the secretion film may become more viscous when the volume fraction of the hydrophilic phase is larger. Such an effect is well established for emulsions and theoretical models for their rheology have been developed by Einstein and others (Einstein 1906; Pal 2001). The emulsion’s viscosity is greater than that of the continuous phase owing to the close packing of droplets and, for larger volume fractions, owing to the deformation of droplets. When confined to thin films, emulsions can show even more complex non-Newtonian behaviour, which may no longer be dominated by bulk-continuum properties but by
processes at the interface (Yan & Kuroda 1997; Clasen & McKinley 2004). Many emulsions are shear-thinning and exhibit Bingham flow, where shear stress is linearly dependent on shear rate, with a positive intercept (Tadros 1994). Thus, Bingham fluids require an initial minimum ‘yield stress’ before they start to flow. The stick insects’ relationship between shear stress and sliding velocity (figure 5c) is consistent with Bingham flow. A yield point of the two-phase pad secretion provides an explanation for the significant static friction observed in insects. In our experiments, the remaining, quasi-static friction was smaller on the thick polyimide substrate, confirming that the emulsion’s yield stress had been reduced by the removal of the hydrophilic droplets. However, the remaining friction did not completely disappear. This may be explained by the finding that the watery droplets were not fully absorbed even by the thick polyimide (droplets with diameters smaller than approximately 150 nm are invisible with IRM and might be stabilized by amphiphilic substances in the secretion). Even if the volume fraction of the dispersed phase is low, non-Newtonian behaviour might be inherent in the oily bulk phase itself, in particular when long-chained molecules are present and when films are confined to molecular dimensions at the points of closest contact (Israelachvili et al. 1988; Thompson et al. 1992). The minimum separation distance between the pad cuticle and the substrate is still unclear, and it is possible that such zones of close contact exist.

A Bingham-type fluid between the pad and the substrate may provide a particularly wear-free way of producing static friction. It allows the pad to yield and slide when large shear forces are acting and thus prevents stresses from reaching levels that would damage the cuticle. High-wear stick-slip behaviour occurs when the static friction exceeds the kinetic friction; it is prevented in this case by the ascending force–velocity dependence. Indeed, undamaged insect adhesive pads slide smoothly and do not exhibit stick-slip. Recent work on geckos shows that fibrillar dry adhesives exhibit similar smooth and wear-free sliding, which in this case may arise from randomly occurring nanoscopic stick-slip events (Gravish et al. in press).

Emulsions are widely used in cosmetics and food technology as well as for paints and coatings. The fine-tuning of rheological properties is critical for these applications (Tadros 1994). The insects’ use of an emulsion as an adhesive fluid conveys the benefits of ‘wet’ adhesion (better adhesion to rough surfaces, wear resistance) without sacrificing the essential ability to withstand shear forces. Our results suggest that insect adhesive organs take advantage of non-Newtonian properties of emulsions and these properties may have been optimized by natural selection.

Thus far, engineers have concentrated on biomimetic adhesives inspired by the dry, fibrillar system of geckos (del Campo & Arzt 2007). However, the insects’ secretion-aided attachment systems also provide an as yet unexplored source of inspiration for novel biomimetic adhesives. Moreover, understanding the detailed function of the insect adhesive system may lead to the development of a new type of non-toxic, wear-resistant insect-repellent coating.

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