Friction force reduction triggers feet grooming behaviour in beetles

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In insects, cleaning (grooming) of tarsal attachment devices is essential for maintaining their adhesive ability, necessary for walking on a complex terrain of plant surfaces. How insects obtain information on the degree of contamination of their feet has remained, until recently, unclear. We carried out friction force measurements on walking beetles \textit{Gastrophysa viridula} (Coleoptera, Chrysomelidae) and counted grooming occurrence on stiff polymer substrata with different degrees of nanoroughness (root mean square: 28–288 nm). Since nanoscopically, rough surfaces strongly reduced friction and adhesion without contaminating feet, we were able to demonstrate, for the first time to our knowledge, that friction force between tarsal attachment pads and the substrate provides an insect with information on the degree of contamination of its attachment structures. We have shown that foot grooming occurrence correlates not only with the degree of contamination but also with the decrease of friction force. This result indicates that insects obtain information about the degree of contamination, not statically but rather dynamically and, presumably, use mechanoreceptors monitoring either tensile/compressive forces in the cuticle or tensile forces between leg segments.

\textbf{Keywords:} adhesion; attachment; grooming behaviour; sensory control; Coleoptera; Chrysomelidae

1. INTRODUCTION

Insects bearing specialized hairy (setal) attachment devices are able to adhere to and walk along various vertical surfaces and even ceilings [1–3]. These systems rely on arrays of hairs (setae) located on the ventral surface of the tarsus (foot). It has been previously reported that setae produce droplets of a fluid secretion in the contact zone between each setal tip and the substrate [2,4–7]. The presence of the fluid in the contact zone leads to the enhancement of capillary forces, which together with intermolecular interactions are mainly responsible for the generation of strong adhesion [8]. Setal tips of most attachment devices in insects bear spatula-like (tape-like) terminal plates responsible for proper contact formation between setae and substrates of different geometry [9,10].

Since insects mainly live on plant surfaces and often must walk on vertical stems or the underside of leaves, a strong attachment force between feet and substrate is an important requirement for proper functioning of their locomotory systems. However, most plant surfaces are covered by small dust particles, pollen grains or even fluid droplets [11]. That is why adhesive setae, bearing fluid drops on their tips, can be easily contaminated by environmental particles. Plants possessing self-cleaning surfaces [12] are covered with wax crystals, which in many cases can break when insects walk on them and contaminate attachment devices [3,11,13–17] (figure 1). It has been previously demonstrated that attachment forces are strongly reduced on contaminated feet [11,17].

To reduce contamination of attachment pads, insects actively clean their tarsi by applying a complex sequence of rubbing movements by the ipsilateral or contralateral legs (figure 2a–c). Comb-like arrays of setae, located on the lateral surface of legs, are often used to brush and clean contaminated adhesive setae (figure 2d). As previously mentioned, in the contaminated foot both the friction and adhesion between attachment devices and the substrate are reduced. Therefore, it is plausible to assume that such a reduction of the contact forces between the foot and the substrate during locomotion and not just the presence of contamination itself is responsible for triggering foot grooming behaviour in insects.

This study was undertaken in order to test this hypothesis by applying traction experiments with living beetles \textit{Gastrophysa viridula} (Coleoptera, Chrysomelidae). In our previous studies, we have reported that fine surface asperities with nanoscale roughness have a strong effect on insect adhesion ability [3,18,19]. In the present study, we prepared nanostructured surfaces (root mean square (r.m.s.): 28–288 nm) by the use of aluminium vapour deposition on silicon wafers and a two-step moulding technique [20]. Foot grooming frequency was recorded on different substrates and compared with friction forces generated by tethered walking beetles.

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2. MATERIAL AND METHODS

(a) Beetles

Leaf beetles *G. viridula* (Coleoptera, Chrysomelidae) were collected on their host plant *Rumex obtusifolius* in the forest areas of Stuttgart (Germany) and kept in the laboratory in plastic cages containing fresh plant material of *R. obtusifolius*. Average weight of tested beetles was 13.5 mg (s.d. = 1.4, n = 10, five males, five females).

(b) Substrates

A metal vapour deposition method was applied for template preparation of surfaces varying in their surface roughness. Surface roughness was controlled using aluminium thin films deposited on silicon wafers. It ranged from entirely smooth (r.m.s. about 28 nm) to the r.m.s.-roughness of about 288 nm. The aluminium films were used as templates to prepare polymer substrata (figure 3) with the same surface roughness.

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Figure 1. Clean (a,b) and contaminated (c,d) attachment devices of *G. viridula*. The contamination is caused by crystalline waxes of the pitcher plant *Nepenthes alata* after beetle has walked on the waxy surface of the pitcher. SH, shaft; SP, Spatula; WX, wax crystals.

Figure 2. (a) Female *G. viridula* grooming the first and second feet of the right body side (arrows). (b) Grooming second and third feet of the right body side (arrows). (c) Female *G. viridula* grooming first foot and antenna of the right body side (arrows). (d) The grooming tool consisting of a comb-like array of cuticle outgrowths located on the edge of the tibia (arrow).
profile for their further use in traction experiments with tethered beetles. Negative moulds of original substrata were prepared using a two-component dental wax (President Light Body, Coltene, Switzerland) [20]. In a further step, positive moulds were obtained from negatives by the use of methacrylate low viscosity resin polymerized at 70°C for 8 h [21]. The surface roughness (r.m.s.) and grain width were estimated by an atomic force microscope Nanoscope-III (Digital Instruments, USA). Grain width was estimated as an averaged diameter of asperities.

(c) Force measurements

A 10 g load cell force transducer (World Precision Instruments, Sarasota, FL, USA) was clamped to a holder perpendicular to the horizontal plane of the substrate tested (for further details see Gorb et al. [17]). Prior to the experiment, each substrate was briefly cleaned with ethanol and distilled water and dried in a nitrogen jet. Static electricity was eliminated by a gas ionizator (DC-nozzle, Ionizing Air Nozzle, Tantec Inc.). Dust particles were removed from resin surfaces with a nitrogen jet.

Beetles were anaesthetized with carbon dioxide, and attached, on their dorsal surface, to a human hair using a droplet of molten beeswax. After recovery from anaesthesia (1 h), the beetles were again weighed. Then the free side of the human hair was mechanically attached to the force transducer. Tethered beetles walked on various surfaces that were offered to them in a random order, and pulled the force transducer through the attached hair. The friction force signal was digitally recorded in the computer, and a maximum friction force during the pulling time of 60 s was estimated from each run. Maximal friction force values, obtained for different substrata, were normalized to the force measured on the smooth glass substrate.

(d) Grooming behaviour

Individual tethered beetles were allowed to walk on different substrata for 60 s. During this period of time, presence or absence of grooming was recorded. The occurrence of grooming was estimated as the proportion of time (0–100%) that beetles spent grooming during each individual run (10 runs per substrate for each beetle, 19 substrates, 10 beetles).

(e) Forces and grooming behaviour on contaminated substrata

In order to obtain the relationship between the friction force and grooming behaviour on contaminated feet, the force measurements were performed on the clean glass substrate (control) and glass substrate contaminated by the glass beads of two different diameters (2 and 100 μm). Friction forces and grooming behaviour in three individual males and three females were measured as described above.

3. RESULTS AND DISCUSSION

The surfaces depicted in figure 3a had small protrusions (average width 0.9 μm, the r.m.s. varied from 28.3 to 99.6 nm). When a leaf beetle walked over such a surface, the traction force decreased with an increase of the surface roughness (figure 4a). The traction force generated by beetles was strongly reduced on the surface with the roughness of 100 nm. The surfaces shown in figure 3b had surface protrusions of the larger width (3.1–4.2 μm) and r.m.s. ranging from 177 to 288 nm. When a leaf beetle walked over such a surface, the traction force was rather high even on the surface with the roughness of 177 nm (figure 4), presumably because the contact area of a single spatula on the
substrate protrusion with the larger width is larger than on the protrusion with the small width. The traction force decreased with an increase of the surface roughness and on the substrata covered with small protrusions.

We have selectively studied feet of beetles in the scanning electron microscope after traction experiments on various tested substrata. As expected, attachment devices were not contaminated. However, we have observed a strong correlation between the normalized friction force, which was the friction force measured on the tested substrate, divided by the friction force measured on the smooth sample, and grooming occurrence. We have performed this analysis separately for female and male individuals, and obtained very similar results independently of the beetle’s sex (figure 5).

At normalized force values close to 1, almost no grooming behaviour was observed. Frequency of foot grooming events increased with the decreasing friction force on nanostructured surfaces. If the normalized force became smaller than 0.1, the insect had difficulty walking, and spent most of the time actively grooming its feet. Although insects did not have contamination on their feet, they actively groomed them depending on the friction force achieved on the particular substrate.

Beetles walking on the glass substrata, contaminated with glass beads of different diameter, demonstrated very low attachment force and high occurrence of grooming movements in comparison with insects walking on a clean glass surface (figure 6). The feet of the beetles, walking on contaminated glass substrate, were strongly contaminated by glass beads, especially in the case of small beads (figure 7).

The above results clearly indicate that insects obtain information about contamination of attachment structures not statically from contaminated surface mechanoreceptors, located on the ventral surface of legs, but rather dynamically from mechanoreceptors, responsible for sensing friction forces between the foot and the substrate. Potential candidates for this sensory function could be receptors sensing tensile or compressive forces within the cuticle (campaniform sensilla) [22–24] or stretch

**Figure 5.** Relationship between friction force and foot grooming occurrence in *G. viridula*. The diagram is based on 190 single observations of 10 individual beetles. Normalized force of 1 corresponds to the results obtained on the smooth surface, where the friction force was the strongest, and insects were able to walk freely without any traces of disability. Forces on various rough surfaces were lower than those on the smooth surface, and that is why the normalized force dropped. With the decrease of normalized force, the feet grooming occurrence increased. Linear regression analysis for pooled data on both males and females demonstrates strong correlation between grooming rate and friction force: grooming frequency (‰) = 69.3 – (59.1 ~ normalized force), $r^2 = 0.8$. Comparison of linear regression model with original data using one-way ANOVA: $F = 30.1$, $p < 0.001$. Thick striped lines, females; filled bars, males.

**Figure 6.** Relationship between friction force and foot grooming occurrence in *G. viridula*. Six individual animals (three males and three females) were measured first on clean glass and then on the glass surface contaminated with spherical glass beads of two different diameters (2 and 100 μm). Forces on contaminated surfaces were significantly lower than on the control clean surface. With the force decrease, the feet grooming occurrence increased. Tukey-test after ANOVA on the force: 2 μm beads versus glass, $p < 0.001$; 100 μm beads versus glass, $p < 0.001$; 2 μm beads versus 100 μm beads, $p = 1$.

**Figure 7.** Adhesive setae of *G. viridula* females after walking on the glass surface contaminated with spherical glass beads of two different diameters: (a) 100 μm and (b) 2 μm.
receptors (chordotonal organs) located inside the leg and sensing tension between different segments of the leg [25,26].

4. CONCLUSION
This study demonstrates, for the first time to our knowledge, that friction force between tarsal attachment pads and substrate provides an insect with information about the degree of attachment structure contamination. We have shown that foot grooming occurrence correlates, not with the degree of contamination, but rather with the degree of the friction force decrease. This result indicates that insects measure the degree of contamination not statically, but dynamically and presumably use mechanoreceptors measuring either tensile/compressive forces in the cuticle or tensile forces between leg segments.

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