Biological microtribology: anisotropy in frictional forces of orthopteran attachment pads reflects the ultrastructure of a highly deformable material

Stanislav Gorb¹ and Matthias Scherge²

¹Biological Microtribology Group, Department of Biochemistry, Max-Planck-Institute of Developmental Biology, Spemannstrasse 35, D-72076 Tübingen, Germany (stas.gorb@tuebingen.mpg.de)

²Microtribology Group, Institute of Physics, Ilmenau Technical University, Weimarer Strasse 32, D-98684 Ilmenau, Germany (matthias.scherge@physik.tu-ilmenau.de)

Evolutionarily optimized frictional devices of insects are usually adapted to attach to a variety of natural surfaces. Orthopteran attachment pads are composed of hexagonal outgrowths with smooth flexible surfaces. The pads are designed to balance the weight of the insect in different positions and on different materials. In a scanning electron microscopy study followed by freezing–substitution experiments, the ultrastructural architecture of the pad material was visualized. In friction experiments, the interaction was measured between the attachment pad and a polished silicon surface. The inner structure of this material contains distally directed rods, branching close to the surface, and spaces filled with fluid. The specific design of the pad material provides a higher frictional force in the distal direction. Frictional anisotropy is more enhanced at higher normal forces and lower sliding velocities. It is concluded that optimal mechanical functionality of biosystems is the result of a combination of surface structuring and material design.

Keywords: microfriction; materials design; ultrastructure; SEM; TEM; cuticle

1. INTRODUCTION

Materials scientists continue to look for new types of materials with controlled friction and adhesion (Russell & Kim 1999; Crevoisier *et al.* 1999). A rich source of design principles can be found in nature. Biological motion systems are highly adapted micromechanical units. These systems exhibit an optimized combination of surface structure and material design. Therefore, biological systems are excellent candidates for models for micro-electro-mechanical systems. However, natural frictional systems have been poorly studied with respect to their mechanical as well as structural properties. Despite the vast literature devoted to the microsculpture and ultrastructural architecture of the tanned cuticle of insect sclerites (Hepburn 1985), very little work has been done on the structure of the flexible cuticles (Vincent & Prentice 1973; Carruthers & Davey 1983; Hackman & Goldberg 1985). Two types of membranous deformable cuticle have been previously reported in insects. The first type is a highly extensible membrane found in the locust abdomen that can extend up to ten times its length (Vincent & Wood 1972; Vincent 1975, 1981). This cuticle is highly specialized in its protein composition (Hackman & Goldberg 1987). The second type is a folding laminated membranous cuticle with a somewhat lower stretching capacity reported from the abdomen of the tsetse fly, *Glossina morsitans* (Hackman & Goldberg 1987), and the bug *Rhodnius* (Hackman 1975; Reynolds 1975). However, none of these studies has addressed the mechanical issues in depth, and thus exact values for friction or elasticity are lacking. At the present time, the majority of tribology research into biosystems is restricted to lubrication mechanisms of human and animal joints (Persson 1998). It is believed that this study is the first attempt to correlate the microtribological properties of a biological system with its material structure.

During a long period of evolution, insects have solved the problem of attachment to smooth and sculptured plant surfaces. The attachment system must allow the insect to adhere to the surface and to detach from it easily. In evolution, two principal types of insect attachment pads used in locomotion have been developed: hairy, such as fly pulvilli (Bauchhens 1979; Gorb 1998b) or beetle pads (Stork 1983), and smooth, such as the arolia and euplantulae of grasshoppers (Slifer 1950; Kendall 1970) and cockroaches (Arnold 1974; Roth & Willis 1952). Both systems are similar in terms of providing maximum contact independent of the substrate microsculpture. However, their designs are completely different. The first type is composed of numerous flexible hairs fitting well to the surface roughness of the substratum. The second type has a relatively smooth surface, but contains highly deformable material also designed to adapt quickly to various surface profiles.

For this study, we have chosen the great green bush-cricket (*Tettigonia viridissima*) euplantulae, belonging to the second type of attachment pads as mentioned above, owing to their specific material design. The insects have an average mass of 1 g leading to a normal force of 800 μ N per single pad. In order to gain detailed microtribological information, the friction force of the attachment pads was measured under different normal forces in living and dead insects using a microtester. Profile changes of the surface and the orientation of cuticle microfibrils were tested by means of scanning electron microscopy followed by freezing–substitution experiments.

2. MATERIAL AND METHODS

(a) Force tester

In friction experiments, the interaction was measured between the attachment pad and a polished silicon surface. The

Figure 1. Microtester set up for friction measurements. The oscillatory motion is provided by means of a piezoelectric stack (x -piezo). The lower sample holder (equipped with a living insect) is attached to the x -piezo. A silicon plate attached to a glass spring serves as the upper sample. A laser beam reflected by a mirror (attached to the spring) is used to detect deflection of the spring by interferometry. In the z -direction an additional piezo (z -piezo) is attached to adjust the normal force. S, sample; G, glass spring consisting of the glass body and two $100\mu\text{m}$ wide beams serving to detect the deflection; Si, silicon sample; R, reflector for the laser beam; L, laser beam.

pad was attached to a sample holder oscillated by a piezotransducer (x -piezo) at constant velocity. To achieve constant sliding velocity (except at both turning points), the x -piezo was powered by a saw-tooth signal. The sliding velocity can be calculated by $v = 2\Delta x/T = 2f\Delta x$, where Δx is the experimental travelling distance of the x -piezo, f is the frequency of the saw-tooth signal and T is the time-period of one oscillation.

The silicon sample ($5\text{ mm} \times 5\text{ mm}$) was attached to a double-leaf spring (figure 1). The spring is deflected due to friction, and this deflection is measured by a single-beam laser interferometer with a resolution of 1 nm . The measured length multiplied by the spring constant yields the tangential force. The maximum deflection of the spring that can be detected is $30\mu\text{m}$. Thus, with a spring constant of 50 N m^{-1} a force range from 50 nN to 1.5 mN can be covered. The normal force is set by a second piezotransducer (z -piezo) to range between 500 nN and 1 mN . Both samples are first approached by means of mechanical micropositioners. The final engagement is then achieved by expanding the z -piezo. When the first deflection of the spring is detected, contact is determined with an accuracy of 10 nN . The normal force is adjusted by controlling the voltage of the z -piezo. The friction tester is housed in an environmental chamber. For maximal vibration isolation, the tester and the chamber were positioned on a concrete block with a mass of 3 tonnes .

(b) Animals and sample preparation

Males and females of *T. viridissima* were captured in Ilmenau (Germany), immobilized using sticky tape and attached to the holder connected to the x -piezo. Each insect was positioned upside down, and orientated in a horizontal direction along the x -axis. The tarsus was firmly attached to the underlying surface using wax. The silicon sample was positioned parallel to the

distal euplantula. In this condition, the whole insect was moved together with its tarsus along the x -axis. The frictional force was measured on the euplantula of the pre-terminal tarsomere in two series of experiments: first, at different normal forces ranging from $50\mu\text{N}$ to $650\mu\text{N}$ at constant frequency (0.5 Hz); and second, at different frequencies ranging from 0.05 Hz to 2 Hz at constant normal force ($87\mu\text{N}$). All experiments were carried out at 25°C and 58% relative humidity.

The experimental work with small biological samples indicates that the tests have to be performed with living substrates. Owing to fast evaporation, dead samples are not suitable. Friction and indentation experiments made clear that the interpretation of the data must result from the combination of physical and biological approaches.

(c) Ultrastructural study of the pad material

The distal euplantula of a living insect was lightly pressed against a smooth surface and frozen in this condition with liquid nitrogen. As a reference, we used euplantulae not contacting the substratum. The freeze-substitution technique was used (Schwarz & Humbel 1988; Meissner & Schwarz 1990). The shock-frozen pads were then fractured using a razor blade and transferred to 0.5% osmium tetroxide solution in absolute acetone at -80°C for 48 h , washed in absolute acetone at -20°C , transferred to absolute ethanol and critical-point dried.

An additional technique was used to obtain information about the orientation of inner structures. Semi-thin sections ($0.5\text{--}2.0\mu\text{m}$) of pads embedded in Spurr resin were sectioned using a diamond knife. The sections were picked up on pioloform-covered cover-slips, treated with Maxwell's solution (Maxwell 1978) for $2\text{--}5\text{ min}$ in order to remove the resin, washed in absolute ethanol and critical-point dried.

All preparations were mounted on holders, sputter-coated with gold-palladium (10 nm) and examined in a Hitachi S-800 (Tokyo, Japan) scanning electron microscope at 20 kV .

3. RESULTS

(a) Characteristics of the pad surface and pad material

Our experiments and ultrastructural studies showed that the cuticles of orthopteran pads are natural friction-active materials with a specific inner and outer structure. Each tarsus of *T. viridissima* contains three or four euplantulae. The pre-terminal tarsomere has the biggest euplantula ($1.7\text{--}2.5\text{ mm}$ wide) (figure 2). In the light microscope, its surface appears smooth. In reality, it is composed of hexagonal structures (area $14.7\mu\text{m}^2$; s.d. = 1.96 ; $n = 22$) (figure 3) with underlying tiny rods (diameter $0.08\mu\text{m}$; s.d. = 0.01 ; $n = 25$) (figure 4). These rods are branches of thicker rods (diameter $1.12\mu\text{m}$; s.d. = 0.09 ; $n = 20$) located deeper in the cuticle. In sections and fractures, the uppermost layer resembles a thin film (180 nm thick; s.d. = 39 ; $n = 20$). The thickness and the non-fibrous structure correspond to the epicuticle, the outermost layer of insect integument.

(b) Frictional properties of the pad

By oscillating the sample over a distance of $10\mu\text{m}$ along the x -axis (distal-proximal) at a low normal force, we were able to measure the frictional properties of the pad surface in both directions (F_d and F_p , respectively) (figure 5a). The selection of this low normal force

Figure 2. Tarsus of the third leg of *T. viridissima* with four attachment pads (euplantulae). d, distal direction.

Figure 3. Scanning electron micrographic image of the pad surface.

(ca. 80–100 µN) was necessary to measure the initial processes during contact formation of pad and counter-surface without getting into visco-elastic deformation of the pad. The static friction force was calculated from the force plot (figure 5a). The static friction is the value that occurs just before sliding. In other words, the forces between zero and the onset of sliding were interpreted as static friction. These values are shown in figure 5a for both proximal and distal directions. In general, the friction force increased with increasing normal force (figure 5b). With increasing frequency, frictional force decreased first at frequencies of 0.1–0.5 Hz and then slightly increased at frequencies from 1 to 2 Hz (figure 5c). In figure 5b,c data obtained for several pads of living animals were pooled together.

Figure 4. Shock-frozen pad after substitution and fracture. Outermost branches of rods are sloped at a certain angle. d, distal direction; EPI, epicuticle; RD, rods.

The experiments showed that the static friction during proximal movement was larger and stable compared to that during distal movement. For example, the static friction force, shown in figure 5, in the distal direction was about 5 µN and in the proximal direction 23 µN. During distal movement, friction slowly increased. This effect is reflected in the rising part of the curve. The dependence of the frictional force on normal force is shown in figure 6a. The anisotropy slightly increases with increasing normal force (figure 6b). The frictional behaviour of the pad changes with frequency. Minimum friction force occurred at 0.5 Hz ($10 \mu\text{m s}^{-1}$) and increased at lower and higher frequencies (figure 7a). The anisotropy decreases with increasing frequency (figure 7b). The difference between distal and proximal movements was clearly present in every set of experiments with all animals we tested. However, due to differences in the absolute values that occurred in experiments with different animals, there was significant overlap between distal and proximal movements. Therefore, we decided not to pool all the obtained data together but just present case studies. However, the anisotropy effects presented in figures 6 and 7 were absolutely similar to those obtained in experiments with different animals.

4. DISCUSSION

(a) Relationship between directionality of frictional force and structure of the pad material

The anisotropy can be explained by interpreting the information provided by the shock-freezing experiments. Since the rods have an initial pre-determined slope of 45–70°, the normal force—due to the weight of the insect—will decrease the slope to 5–10° (figures 8 and 9). Leg movements directed proximally also decrease the slope. As the rods deform, the shape of the hexagonal outgrowth also changes. It is assumed that the rods, together with their branches and the flexible cuticle, assure optimal contact with the substrate by adjusting the pad to micro- and meso-scale roughness.

Figure 5. Frictional behaviour of the pad. (a) Friction measured in different directions. F_d , friction force measured during pad movement in a distal direction; F_p , frictional force measured during pad movement in a proximal direction. (b) Friction force versus normal force at a frequency of 0.5 Hz ($n = 9$, $N = 3$). (c) Friction force versus frequency at normal force of 100 μN ($n = 3$, $N = 2$), error bars are standard deviations.

If the pad moves distally, some critical force is needed to overcome the rod stiffness and slope the rods in the other direction (figure 10). Thus, friction force increases slowly since energy is dissipated continuously due to the bending of the rods. This hypothesis also explains why anisotropy increases with increasing normal force. However, beyond a certain normal force, the anisotropy

Figure 6. (a) Friction force versus normal force at a frequency of 0.5 Hz. Solid circles and line are values obtained when the pad moved distally. Open circles and broken line are values obtained when the pad moved proximally. (b) Difference in friction force during proximal and distal movements versus normal force.

no longer increases. It is assumed that at this normal force the reorientation of the rods in the opposite direction will be limited. Presumably, the anisotropy in frictional force is of importance during fast movements, such as jumping and landing, when normal forces act in different directions.

(b) Forces involved in a resulting frictional force

The friction force remains low as long as a critical normal force of about 600 μN is not exceeded. It has already been shown (§ 1) that due to the mass of the insect a single pad has to bear a normal force of about 800 μN . For normal forces higher than 600 μN at an amplitude of 10 μm the pad and substrate come into intimate and permanent contact so that sliding disappears. The permanent contact is observed on the force curve, in which the sliding region disappeared at higher normal forces. It is presumed that in this condition the insect may adhere safely to the substrate, even when upside-down, and gravity is balanced.

Studies investigating the chemical composition of insect footprints and cuticle lipids indicate that the liquid that covers the pads is mainly composed of hydrocarbons with hydrophobic surface properties (Kosaki & Yamaoka 1996). Therefore, capillary action due to water can be

Figure 7. (a) Friction force versus frequency at a normal force of $87 \mu\text{N}$. Solid triangles and line are values obtained during distal movement. Open triangles and broken line are values obtained during proximal movement. (b) Difference in friction force during proximal and distal movement versus frequency.

neglected. However, the hydrocarbons do wet in order to form a capillary neck. In addition to capillarity, an attractive force can also be caused by dispersion forces when the distance between the two samples is lower than about 10 nm (Israelachvili 1992). Dispersion forces represent the most effective type of Van der Waals interaction arising from quantum mechanical effects. In both cases the contact area plays a crucial role in the strength of adhesion and friction. The actual nanoscopic mechanisms are a combination of the discussed influences and are a topic for further investigation.

(c) Conclusions and outlook

Via the process of natural selection, nature has optimized biological frictional systems (Gorb 1998a). The experiments show that the inner and outer architecture of orthopteran pads provides stability and extreme flexibility. This allows the pad material to adapt to different substrate roughnesses, which are unpredictable for mobile insects, such as grasshoppers and cockroaches. The first interesting feature of the system studied is the specific orientation of stiff components in the composite material, which results in higher friction in one particular direction. Another feature of the system is that the differently sized areas of rods seem to be adapted to different scales of roughness (micro- and meso-scale roughness). At normal forces exceeding $600 \mu\text{N}$, which corresponds to the force generated by the weight of the animal, pads stop

Figure 8. Free pad that has not been in contact with the substratum. Shock-frozen samples after substitution and fracture. Rods are sloped in the distal direction at an angle of about 45° . d, distal direction.

Figure 9. Pad that has been pressed against the substratum. Shock-frozen samples after substitution and fracture. Rods are sloped in the distal direction at an angle of about 5° . d, distal direction.

sliding and come into contact with the substrate. Such an attachment force seems to be sufficient to balance gravity in all kinds of positions.

When the insect is standing upside-down, pads experience a set of forces that are different from those experienced on the vertical surface. In the upside-down situation, the attachment will be influenced by both the pulling force of the insect's mass and the adhesion of the

Figure 10. Hypothetical deformability of initially sloped rods (a) in a composite material, (b) in direction of predetermined deformation, and (c) in the opposite direction.

pad. The current experiments were targeted to evaluate the lateral force (friction) acting on the pad surface when an insect is on a slope or vertical surface. With this set-up we cannot explain adhesion force, which will presumably mainly contribute to attachment in the upside-down position. Friction in the initial process of contact formation is necessary to achieve an optimized contact with the surface. However, friction alone does not cause the permanent contact observed in our experiments. This is accomplished by the combination of friction-induced contact optimization and adhesion. Adhesion will be addressed in further experiments. Additionally, further studies on the cuticle ultrastructure and its contribution to mechanical behaviour will aid the understanding of the design of natural composite materials in relation to their functions. It is hoped that the approach used in this investigation will initiate further studies on natural frictional and releasable attachment systems.

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