Ultrastructural architecture and mechanical properties of attachment pads in *Tettigonia viridissima* (Orthoptera Tettigoniidae)

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Abstract Natural releasable attachment systems of insect legs, where attachment-detachment performances are often very fast, seem to be optimized to get a maximum of real contact to the substratum. Tarsi of *Tettigonia viridissima* bear flexible attachment pads with unusual ultrastructural architecture of the cuticle. The indentation of the attachment pads was measured under different loads using a force-tester. Since the mechanical properties are influenced by material structure, the freeze-substitution experiments were undertaken to investigate the influence of loads on material structure. Both profile changes of the surface and the orientation of cuticle microfibrils were visualized by means of scanning electron microscopy followed by fracturing of the frozen material. The results show that the flexible pad material deforms replicating the substrate profile down to the micrometer roughness. The pad material showed both elastic and viscous behavior under loads. Elastic deformation of the pad occurred under normal force applied for 4–6 s (elastic modulus 27.2 ± 11.6 kPa). Two viscous relaxation processes were found, of time constants $\tau_1 = 1.88 \pm 0.616$ s and $\tau_2 = 41.2 \pm 9.95$ s. Low stiffness of material studied here aids in surface replication and increase of area of real contact between the pad and the underlying substrate.

Key words Ultrastructure · Cuticle · Material properties · Elasticity · Viscosity

Introduction

Attachment forces mediated by friction or adhesion are usually proportional to the area of real contact between two surfaces (Persson 1998). Attachment systems, where attachment-detachment performances are often very fast, have to be optimized to achieve a contact to the substratum in the shortest time (Radhakrishnan 1998). There are two alternative designs of such natural releasable systems used in locomotion. Pads of flies, beetles, and earwigs are covered by relatively long deformable setae (Bauchhenss and Renner 1977; Stork 1980), which presumably can bend and thus repeat the surface profile. The second type of pads, so called arolia and euplantulae, occur in cockroaches, grasshoppers, bees, and bugs (Snodgrass 1956; Arnold 1974; Henning 1974; Ghasi-Bayat et al. 1980a). These organs are smooth and soft, but have an exceptional flexibility (Slifer 1950; Roth and Willis 1952).

Insects with smooth type of attachment pads can walk on a variety of plant surfaces and always deal with an unpredictable roughness of the substrate. In spite of this, the attachment system allows an insect to attach to the surface and to detach from it easily. It has been previously shown that attachment of a great green bushcricket *Tettigonia viridissima* (Orthoptera: Tettigoniidae) onto a surface is strongly dependent on the deformation of the pads (Jiao et al. 2000). However, the ability of leg attachment pads to adapt to a variety of surface profiles remained a hypothesis which needed to be proven experimentally.

Despite numerous literature sources devoted to the microsculpture and ultrastructural architecture of tanned cuticle of insect sclerites (Hepburn 1985), very little work has been done on the structure of the flexible cuticle (Vincent and Prentice 1973; Carruthers and Davie 1983; Hackman and Goldberg 1985). Previous authors have shown that material of a smooth pad differ from common structure of the cuticle. Pad cuticle is not layered and consists of rod-like fibers oriented at some
angle to the cuticle surface (Slifer 1950; Roth and Willis 1952; Kendall 1970). Since mechanical properties of a biological material are determined by its structure, the ultrastructural study of the surface and material fractures by means of scanning electron microscopy followed by freezing-substitution experiments was undertaken to visualize material behavior of the pad under loads. We have hypothesized that pads, due to their flexibility, are able to replicate the surface profile down to the microscale roughness.

Normal forces ranging from nano- to micronewtons have been used in studies of the mechanical deformation of cells (Bausch et al. 1998, 1999). To study the mechanical properties of attachment pads, a force tester, capable of generating and detecting greater forces, was applied (Scherge and Schaefer 1999; Gorb and Scherge 2000).

**Materials and methods**

**Microscopy and freeze-substitution experiments**

Living specimens of *T. viridissima* were captured in Ilmenau, Germany. The distal euliplata of a living insect was lightly pressed against smooth and rough surfaces and frozen in this condition with liquid N₂. As rough surfaces, silicon plates with cut-cone-shaped outgrowths were used. The outgrowths were 3.983 μm (SD = 0.178, n = 20) high and 7.122 μm (SD = 0.279, n = 20) in diameter at their bases. The distance between outgrowths from center to center was 11.955 μm (SD = 2.822, n = 20) (Fig. 5A, B). In a control experiment, we used euliplata not contacting the substrate.

The freeze-substitution technique is, meanwhile, a standard method in cell science (Schwarz and Humbel 1988; Meissner and Schwarz 1990). In this study, we tried to apply this method to understand behavior of biological material under loads. Usually, frozen biological material can recover to a certain extent after being thawed. The OsO₄ substitution was necessary to fix all material deformations in the frozen condition and keep such a deformation in the course of the whole microscopic procedure. The shock-frozen pads from different experiments were fractured using a razor blade and transferred to 0.5% OsO₄ 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 orientation of inner structures. Semi-thin sections (0.5–2.0 μm) of pads, embedded in Spurr-resin (Spurr 1969), were sectioned using a diamond knife. The sections were picked up on pioloform-covered glass cover-slips, treated with Maxwell’s solution (Maxwell 1978) for 2–5 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 scanning electron microscope at 20 kV.

**Force tester**

The force tester (Tetra, Ilmenau, Germany; Fig. 1A) included three main parts, a platform, a glass spring, and a fiber optical sensor. The lower sample was mounted on the platform, the upper sample was attached to the glass spring with a spring constant of 106.9 N m⁻¹. Driven by a motor, the platform was capable of moving the lower sample up and down. Spring deflection was detected by the fiber-optic sensor connected to a computer used for data acquisition. The resulting force between two samples was recorded.

**Sample preparation**

A living animal was immobilized on the platform using sticky tape (Fig. 1A); one leg was firmly fixed with wax, the distal pad was used as the lower sample. A smooth silicon chip was cleaned in an ultrasonic bath, and then in ethanol was attached on the spring as the upper sample. All experiments were carried out at room temperature (22–24 °C) at a relative humidity of 47–56%.