ADHESION MEASURED ON THE ATTACHMENT PADS OF *TETTIGONIA VIRIDISSIMA* (ORTHOPTERA, INSECTA)

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Summary

The tarsi of the cricket *Tettigonia viridissima* bear flexible attachment pads that are able to deform, replicating the profile of a surface to which they are apposed. This attachment system is supplemented by a secretion produced by epidermal cells and transported onto the surface of the pad through the pore canals of the pad cuticle. This study shows that the secretion alone is necessary, but not sufficient, for adhesion. To account for the full adhesive force, the deformation of the pad and the resulting changes in contact area were considered. In two series of experiments, the adhesive properties of the secretion and the adhesion of the whole pad were measured using a force tester, the sensitivity of which ranged from micronewtons to centinewtons.

The adhesive forces of the secretion measured between a smooth sapphire ball with a diameter of 1.47 mm and a flat silicon surface ranged from 0.1 to 0.6 mN. In a control experiment on the silicon surface without secretion, no adhesive force was measured. There was no dependence of the adhesive force on the applied compressive force.

When an intact pad was pulled off a flat silicon surface, the adhesive force increased with increasing applied compressive force, but it did not increase further once the applied force exceeded a certain value. The saturated adhesive force, ranging from 0.7 to 1.2 mN, was obtained at applied forces of 0.7–1.5 mN. The hemispherical surface of the pad had a larger contact area and demonstrated greater adhesion under a larger applied force. Adhesion became saturated when a pad was deformed such that contact area was maximal. The tenacity (the adhesive force per unit area) was $1.7–2.2 \, \mathrm{mN \, mm^{-2}}$.

Key words: adhesion, epidermal secretion, biomaterial, cuticle, attachment device, cricket, *Tettigonia viridissima*.

Introduction

Insects have optimised their attachment to different surfaces over a long period of biological evolution. Leg attachment pads, used in locomotion, have an excellent ability to adapt to a variety of surface profiles. There are two alternative designs of such systems. The pads of flies, beetles and earwigs are covered by relatively long deformable setae, which can bend and, thus, replicate the profile of the surface. The second type of pad, so-called arolia and euplantulae, which occur in cockroaches, grasshoppers and bugs, are soft deformable structures with a relatively smooth surface. The first reports describing leg structures responsible for insect adherence and providing ideas about possible mechanisms of attachment were made during the nineteenth century (West, 1862; Dewitz, 1883; Dahl, 1884; Rombouts, 1884; Simmermacher, 1884). Different hypotheses for attachment, ranging from micro-suckers to the action of electrostatic forces, have been proposed (Gillett and Wigglesworth, 1932; Maderson, 1964).

The majority of investigators have clearly demonstrated the presence of an adhesive secretion in the contact area between pad surface and substratum (Bauchhenss, 1979a; Walker et al., 1985). Comprehensive electron microscopic studies have revealed that this non-volatile secretion is produced in flies by large cells located at the base of each pulvillus, that it is stored within a 'spongy' layer of cuticle and that it is delivered to the surface through a system of porous channels (Bauchhenss, 1979b) or through larger pores located under a plate-like structure at the top of each hair (Gorb, 1998).

Previous experimental studies on insect attachment abilities have supported the idea that this secretion is essential for successful attachment performance both in the 'hairy system' (Stork, 1980) and in the 'smooth system' (Lees and Hardie, 1988; Dixon et al., 1990). In fact, the attachment forces measured in previous studies consisted of two components, adhesive force and shear force, because the experiments had to be performed on the whole organism. The adhesive components of the attachment force and the adhesive properties of the secretion remain unknown. It has been reported that, among all the factors affecting friction, adhesion mainly contributes to the overall friction force in the micronewton range (>90 %) (Rabinowicz, 1995). It is believed,

therefore, that measurement of the adhesive component of the attachment force is a crucial goal in studies of biological attachment devices.

Which components of the living material contribute to overall adhesion? This paper will attempt to show that the secretion alone is necessary, but not sufficient, for adhesion. To account for the full adhesive force, the deformation of the pad and the resulting changes in contact area must be considered.

For this study, we have chosen *Tettigonia viridissima* (the great green bush cricket), whose limb pads (euplantulae) belong to the 'smooth' type of pad system. These insects can stand and walk on a smooth vertical surface, even upside-down under the surface. We used a recently developed micro-force tester, capable of sensing forces in the micro- to centinewton range, to study the adhesive properties of the secretion, adhesion of the pad and the relationship between adhesion and deformation of the pad. The tester allows measurements of forces to be made on single pads or even on localised parts of pads. A similar technique has recently been applied to the microtribological characterisation of technical surfaces (Scherge et al., 1999).

Materials and methods

Living males and females of *Tettigonia viridissima* L. were caught in Ilmenau, Thüringer Wald, Germany.

Light microscopy

The tarsi were carefully cut off, and the pads were pressed with fine tweezers against a clean glass coverslip to obtain a footprint. The fresh print was studied under the light microscope using phase contrast (Zeiss Axioscope). Another part of the print was mounted in distilled water, covered with a glass coverslip and again studied under the light microscope. Measurements of the droplets constituting the print were carried out from digital pictures using AnalySIS Software (Münster, Germany).

Force tester

The force tester (Tetra GmbH, Ilmenau, Germany) used for adhesion measurements (Fig. 1A) includes three main parts, a platform, a glass spring and a fibre-optic sensor. The lower sample is mounted on the platform, and the upper sample is attached to the glass spring with a spring constant of $106.9\,\mathrm{N\,m^{-1}}$. Driven by a motor, the platform is capable of being moved up and down. Spring deflection is detected by the fibre-optic sensor, and the signal is sent to a computer. The interacting force between two samples is recorded as a force *versus* time curve (Fig. 1B). To measure adhesion (adhesive force) between the two samples, the lower sample was moved towards the upper sample, then lowered after the two samples had remained in contact (remaining time) under a certain load force (applied force or normal force). The effects of the applied force on the resulting adhesive force were studied on the footprints and directly on the pad. The accuracy is determined
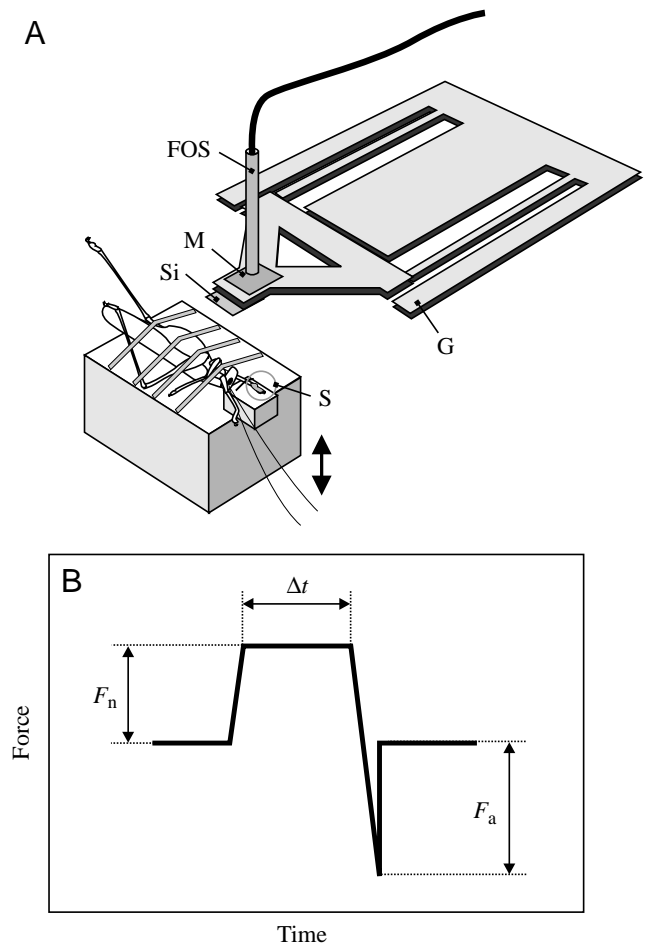

Fig. 1. Adhesion measurements. (A) The micro-force tester used for adhesion measurements. The lower sample (S), the pad of a living insect, is connected to the platform, and the upper sample (Si), a silicon chip, is attached to the spring. Driven by a motor, the platform moves the lower sample towards or away from the upper sample. The deflection of the glass spring (G) is monitored by the fibre-optic sensor (FOS) through the mirror (M). (B) Diagram of a typical curve of force recorded *versus* time. F_n, applied force; F_a, adhesive force; Δt, remaining time (see Materials and methods).

by the accuracy of the measurement of the spring deflection and the spring constant. With a length resolution of 10 nm and a spring constant of $106.9\,\mathrm{N\,m^{-1}}$, an accuracy of $1.069\,\mu\mathrm{N}$ was achieved. The spring constant was determined with an accuracy of approximately $\pm 2.5\,\mathrm{N\,m^{-1}}$. This leads to an error of 50 nN. All experiments were carried out at room temperature (22–24 °C) and at a relative humidity of 47–56 %.

Experimental arrangement and sample preparation
Experiment 1

The adhesive properties of the secretion were studied on footprints deposited on clean silicon surfaces. The pad of a freshly excised leg was pressed on a silicon surface and then taken away. This procedure was repeated several times with the same pad to obtain a sufficient amount of secretion on the silicon chip. The silicon chip was glued to the tester platform

as the lower sample. A sapphire ball 1.47 mm in diameter was attached to the spring as the upper sample. Both the silicon chip and the sapphire ball were smooth (R_a<1 nm, where R_a is the roughness of both the ball and silicon chip). Prior to the experiment, they had been cleaned in an ultrasonic bath filled first with acetone and then with ethanol. No adhesive force between the sapphire ball and a clean silicon surface in the control experiment could be measured (*n*=8, *N*=3 insects).

Experiment 2

Measurements of adhesion of the pad were made using the legs of living insects. A smooth silicon chip, cleaned as described above, was attached to the spring as the upper sample. A living insect was immobilised and attached with sticky tape to the platform (Fig. 1A). The tarsus of one foreleg was firmly fixed with wax as the lower sample. The measurements were made on the most distal pads of the tarsus (*n*=30, *N*=4 insects).

Experiment 3

An additional series of experiments was carried out with excised tarsi. To prevent evaporation, the cut end was sealed with a droplet of wax. The tarsus was firmly fixed to the platform with wax. All measurements were made with the most distal pads, as for experiment 2 (*N*=60, *N*=12 insects).

As a control for experiments 2 and 3, the deformation of wax under force applied by the sapphire ball was measured. This experiment showed that the hardness of the wax, measured in the same range of applied force as for pads, is comparable with that of the silicon surface. Hence, the effect of the wax supporting the pad on measurements could be ignored.

Experiment 4

To measure evaporation from excised tarsi, tarsi with the cut end sealed with wax were weighed on an analytical balance every 10 min for approximately 5 h. The results were compared with those obtained for pieces of similar size cut from the tibia (*N*=3).

Calculation of pad deformation

When a pad was pressed against a silicon surface attached to the glass spring, its surface was deformed. The indentation of the pad was obtained by comparing the spring deflections caused by the pad with those caused by a hard upper sample (e.g. the sapphire ball) (Fig. 2). The deflection of the glass spring pushed by the hard sample is equal to the displacement of the sample. However, when pushed by a soft pad, the spring deflects less because of the indentation on the pad. To give the same deflection of the spring, the pad has to be moved further towards the silicon surface. By subtracting the displacement of the hard sample from the displacement of the pad at the same spring deflection, the indentation of the pad under the force corresponding to that deflection was obtained.

The Hertz theory, which considers the deformation of two elastic bodies in contact under an external applied force, was used to calculate the deformation of the pad pressed onto the
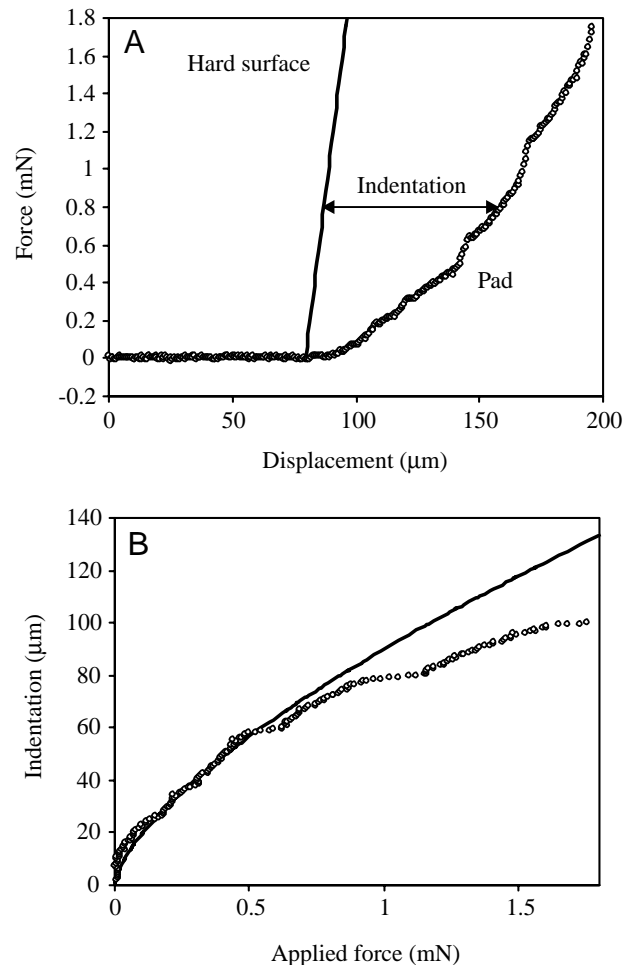

Fig. 2. Pad deformation. (A) Comparison of force–displacement curves obtained for the pad surface (individual points) and for a hard sample (solid line). The deflection of the spring pushed by a hard sample was equal to the displacement of the sample. When pushed by the soft pad, the spring deflected less because of the indentation of the pad. The indentation of the pad was calculated by subtracting the displacement of the hard surface from the displacement of the pad at the same spring deflection to give the indentation of the pad under the force corresponding to that deflection. (B) The indentation of the pad *versus* applied force (individual points). The solid line is the indentation data fitted according to the Hertz theory. The fluctuations in the data for the pad in A and B were presumably caused by the beating heart.

silicon surface (Hertz, 1881). The pad was treated as part of a sphere of radius *R* (Fig. 3), and the Hertz theory predicts the indentation on the pad δ under an external force F_n to be:

$$\delta = \frac{1}{K^{2/3}R^{1/3}} \, F_n^{2/3} \, . \tag{1}$$

where

$$\frac{1}{K} = \frac{3}{4} \, \frac{1 - \nu^2}{E} \, . \tag{2}$$

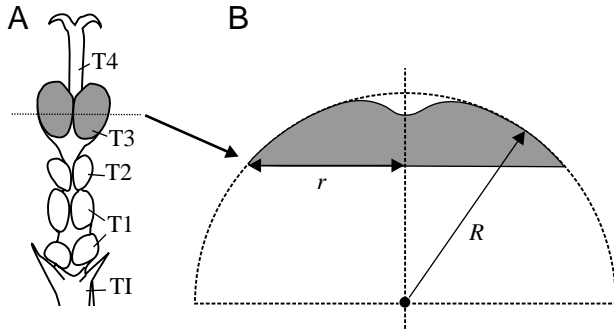*K* is the effective elastic modulus of the pad, ν is Poisson's

Fig. 3. The attachment pad of the *Tettigonia viridissima*. (A) Ventral view of the tarsus of the third pair of legs. TI, tibia; T1–T4, leg tarsomeres. The pad of the most distal tarsomere (grey-coloured area) was used in experiments. (B) Cross section of the pad (grey area) outlined by a semicircle. *R*, radius of curvature of the pad; *r*, pad width. Thickness of the middle part of the grey-coloured area is the pad thickness *d*.

ratio and *E* is the Young's modulus of the pad. Contact area *A* is related to the indentation δ and the applied force F_n as:

$$A = \pi a^2 = \pi R \delta = \pi (R/K)^{2/3} F_n^{2/3} \,, \qquad (3)$$

where *a* is the contact radius of the pad on the silicon surface.

Results

Pad morphology and observations on pad secretion

Euplantulae are hemispherically shaped and the material from which they are made is highly deformable. The surface of the pad has a hexagonal pattern in the scanning electron microscope. The surface often appears to be covered with a secretory material. In the light microscope, the footprints consisted of round droplets with a diameter of approximately 3–10 μm (Fig. 4). Inter-droplet distance on a dry glass surface was 7.582±1.793 μm (mean ± S.D., *N*=200). When the footprint was mounted in water, spherical droplets of the water-insoluble material occurred (Fig. 4B).

Adhesive properties of the pad secretion

Measurements of the adhesion of the pad secretion on a smooth silicon surface were carried out at eight locations on the surface. The adhesive forces varied from 0.1 to 0.6 mN (Fig. 5). Depending on the location selected, the adhesive force could increase (Fig. 5B) or decrease (Fig. 5A) monotonically or remain constant with increasing applied force ranging from 0.1 to 2.5 mN. The increase or decrease in adhesive force was normally approximately 0.1–0.2 mN for a 2.5 mN increase in applied force. In a few cases, the adhesive force oscillated randomly. The adhesive force depended strongly on the location of the sapphire ball on the silicon surface.

Pad adhesion to the smooth silicon surface

Individuals of *Tettigonia viridissima* have a mean live mass of approximately 1 g. For an insect standing on a surface, each of its six legs is pressed with a force of 1.6 mN. The adhesive
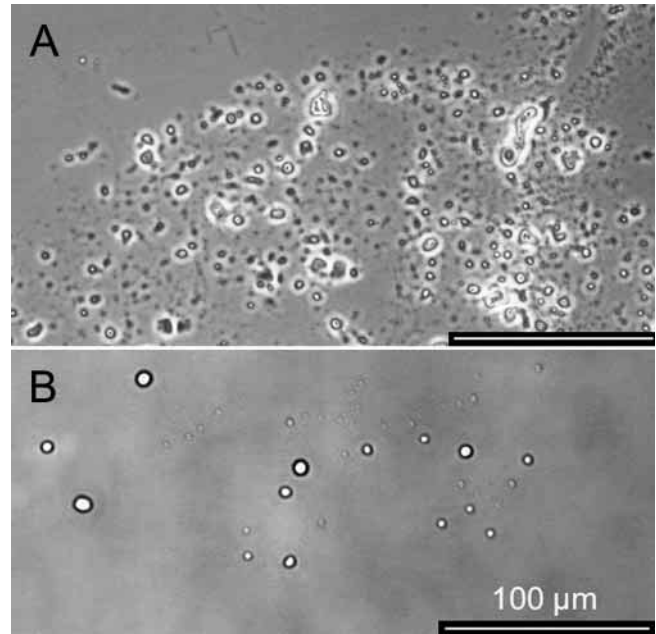

Fig. 4. Footprints of *Tettigonia viridissima*. (A) The unequal distribution of secretion on a glass surface (a coverslip). (B) Droplets occurring in footprint preparations mounted in distilled water.

force needed to release the front pad of the living insect was measured under an applied force ranging from 0.1 to 2.5 mN. In this experiment, the pad was deformed by an external force, which simulated the natural situation. It was found that the adhesive force increased at smaller applied forces (Fig. 6A) and remained constant when the applied force exceeded a certain value (Fig. 6B). The fluctuations observed in the force curve were caused by the heart beating (Fig. 6A, see also Fig. 2). In the case shown in Fig. 6B, the saturated adhesive force of 1.1 mN was reached at an applied force of 0.8 mN. These data were obtained from six trials performed on the same individual. The saturated adhesive forces of all six trials were very similar (1.11±0.03 mN, mean ± S.D.), and the adhesive force remained at around that value over the 3 h measurement period. In the different individuals studied, the saturated adhesive force ranged from 0.7 to 1.2 mN at applied forces of 0.7–1.5 mN (*N*=4).

Deformation

Typical data for indentation *versus* applied force are shown in Fig. 2B. The saturated adhesive force of 1.1 mN, given in Fig. 6B, was reached at an applied force of 0.8 mN, which generated an indentation of 75 μm (Fig. 2A). For all the pads used in the experiment (*N*=4), the indentation corresponding to the saturated adhesive force ranged from 40 to 80 μm.

Although the indentation of the pad was obtained by comparing the spring deflection caused by the pad with that caused by a hard lower sample, the contact area could not be measured or obtained in any other direct way. However, the indentation of the pad (Fig. 2B) was well modelled by equation 1 (from the Hertz theory) at applied forces of less than
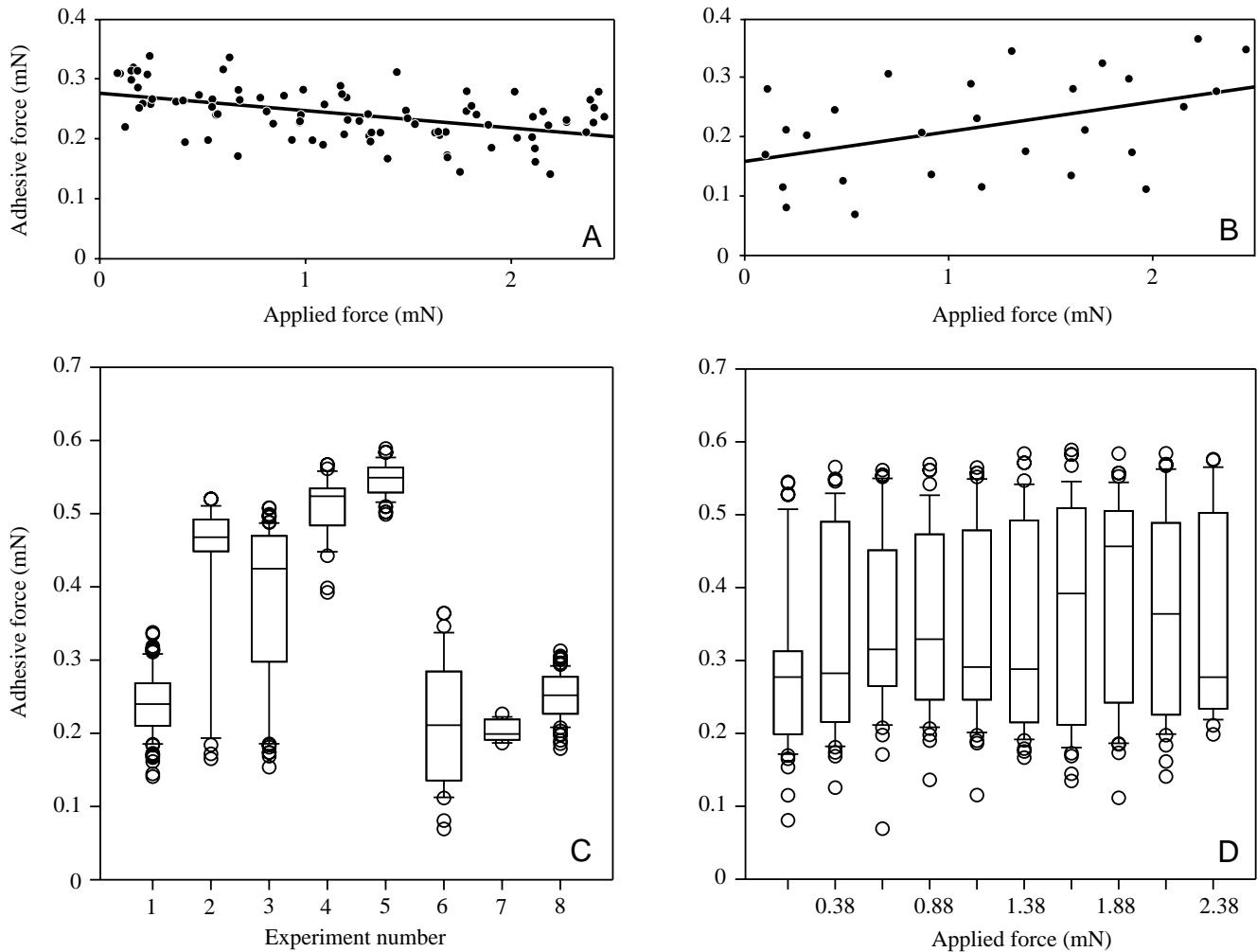
Fig. 5. Adhesive properties of the pad secretion distributed on the silicon chip and measured by a sapphire ball of diameter 1.47 mm. Because of the unequal distribution of secretion on the silicon surface, the adhesive force was location-dependent. Depending on the location selected, the adhesive force decreased ($y=0.28x-0.03$, $r^2=0.23$, $P<0.0001$) (A) or increased ($y=0.16x+0.05$, $r^2=0.19$, $P=0.02$) (B) with increasing applied force. (C) Mean adhesion at eight locations. (D) Adhesive force data obtained at eight locations pooled and plotted *versus* the applied force. The ends of the boxes define the 25th and 75th percentiles, with the median and error bars defining the 10th and 90th percentiles ($N=320$).

0.65 mN. Hence, the contact area caused by an applied force of less than 0.65 mN could be obtained from equation 3, which was used to derive the relationship between adhesive force and the deformation of the pad. With stronger applied forces, the deformations deviated from those predicted by the Hertz theory because the pad was only a part of a sphere. Taking 2.86 mm for the average radius of curvature of the pad R (1.5 mm for the pad radius r and 100 µm for the pad thickness d) (Fig. 3), the effective elastic modulus K of 21.9–64.1 kPa for pads was found by fitting the data for indentation *versus* applied force with equation 1.

Adhesion measured on the pad of an excised tarsus

To study the effects of a change in pad material on pad adhesion, measurements were carried out on a freshly excised leg. Surprisingly, the pads of freshly excised tarsi had the same adhesive force as a living pad. However, the adhesive force decreased to zero after 70–75 min (Fig. 7A). This effect can be

explained by the results of another experiment showing that the pad cuticle loses water more rapidly by evaporation than normal leg cuticle. After approximately 3 h, the pad had lost approximately 50 % of its initial mass as a result of evaporation (Fig. 7B).

Discussion

This study is the first account separately to quantify the contributions of the two main parts of an attachment system to overall adhesion. The system consists of the adhesive secretion on the pad surface and the highly deformable pad material. Both parts of the system work together during attachment to surfaces.

Composition and properties of the pad secretion

Fluid secretions have previously been reported from hairy adhesive pads of reduviid bugs (Edwards and Tarkanian,

1970), flies (Bauchhenss and Renner, 1977; Bauchhenss, 1979a,b; Walker et al., 1985) and coccinellid beetles (Ishii, 1987) and from the smooth pads of cockroaches (Roth and Willis, 1952), aphids (Lees and Hardie, 1988; Dixon et al., 1990) and bugs (Hasenfuss, 1977a,b, 1978; Ghasi-Bayat and Hasenfuss, 1980a–c). Pad secretion is an essential factor for attachment to smooth surfaces. For example, when treated with lipid solvents, the pads of the bug *Rhodnius prolixus* had a poorer attachment performance than non-treated pads (Edwards and Tarkanian, 1970). After a period of walking on silica gel, aphids (*Aphis fabae*) lose their ability to attach to smooth surfaces (Dixon et al., 1990).

The pad secretion of diverse insects contains a non-volatile lipid-like substance that can be observed in footprints under water (Hasenfuss, 1977a; Bauchhenss, 1979a) or in footprints stained by Sudan Black (Lees and Hardie, 1988). This fluid adheres well to both hydrophilic and hydrophobic surfaces, such as wax (Roth and Willis, 1952) and silanized glass (Dixon et al., 1990). In beetles, the chloroform-soluble part of the pad secretion is composed mainly of hydrocarbons, fatty acids and alcohols (Ishii, 1987; Kosaki and Yamaoka, 1996). However, the nature of the water-soluble fraction of the fluid remains unknown. It has been suggested that the fluid contains a surfactant that would make adhesion less sensitive to the nature of the surface (Dixon et al., 1990). Information about the adhesive properties of the fluid itself are lacking from the literature.

The surfaces of newly emerged orthopterans, such as *Melanoplus differentialis*, are impervious to water because of the thin layer of epicuticle (Slifer, 1950). The epicuticle later
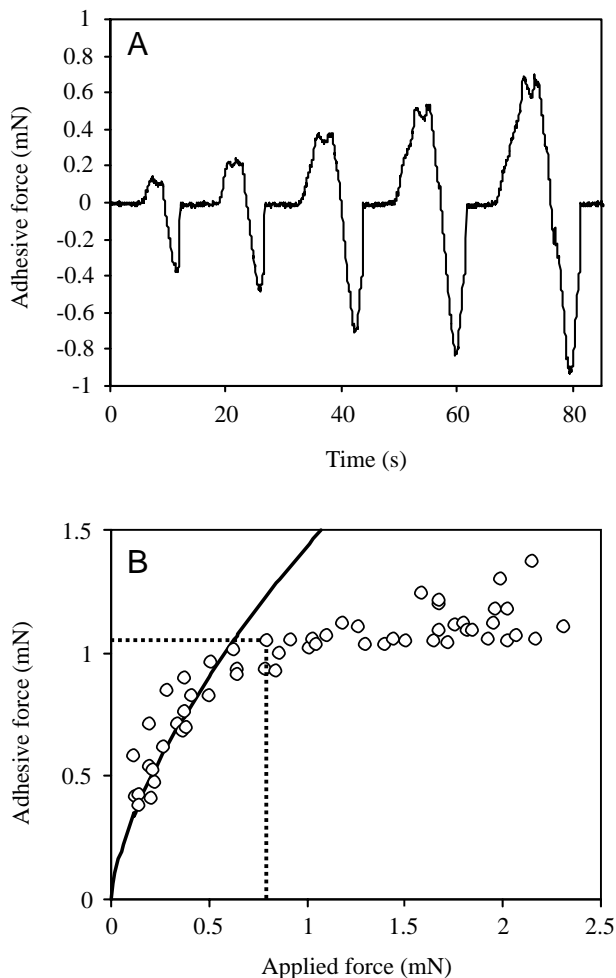

Fig. 6. Adhesive force measured on the pad of a living cricket. (A) An example of the force curves obtained experimentally. (B) Dependence of adhesion on applied force. At smaller applied forces, the adhesive force increased with increasing applied force; it became saturated at an applied force of more than 0.8 mN (broken line). The saturated adhesive force was approximately 1.1 mN. Assuming that the adhesive force (F_a) is proportional to the contact area caused by the applied force (F_n), the data were fitted in accordance with the Hertz theory $F_a = K_a A = \pi (R/K)^{2/3} K_a F_n^{2/3}$ (solid line), where K_a is a constant, A is the contact area, K is the effective elastic modulus and R is the radius of curvature of the pad.
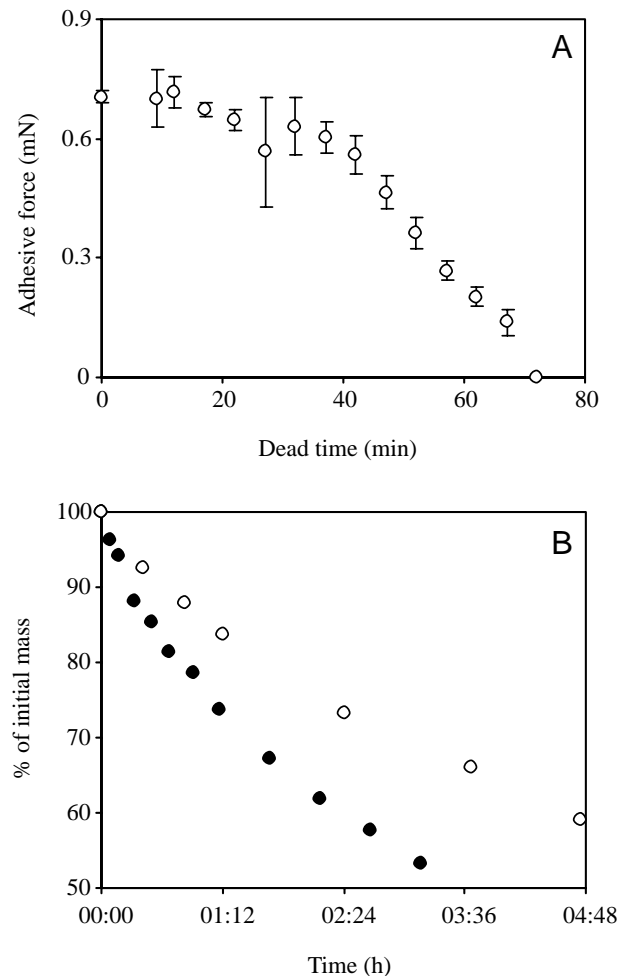

Fig. 7. Results of experiments with the tarsi excised from the body. (A) The decrease in adhesion measured on the pad of an excised tarsus. Values are means ± S.D. (N=3). The tarsus was excised immediately after adhesion measurements and tested repeatedly over 70 min. (B) Water loss (as percentage loss of mass) from pieces of leg excised from the body. Filled circles are data for tarsi (N=10); open circles are data for pieces of leg segment cut from the tibia (N=10).

becomes abraded because of locomotion on rough plant surfaces. However, the pad surface remains waterproof. This waterproofing mechanism presumably consists of a very thin layer of wax or wax-like material, which is soluble in xylol. This substance seems to be similar to that reported for cockroaches (Roth and Willis, 1952). These waxy substances may also be involved in the attachment mechanism.

The pad of *Tettigonia viridissima* is covered with an epidermal secretion released onto the cuticle surface. It has been suggested that the pad is moistened by a secretion originating in the region of the mouthparts (Hennig, 1973). The adhesive force of the living pad does not change over time, suggesting that the secretion originates from the pad itself. Pad-licking behaviour has, presumably, a cleaning function.

The purpose of the measurements with a sapphire ball on the silicon surface was to understand how the pad secretion contributes to the overall adhesive force. Adhesive force was detected only for secretion droplets distributed on a silicon surface, but not for a clean silicon surface. A droplet located at the first contact point of the sapphire ball would be spread and become thinner under a load, resulting in an increasing adhesive force with increased applied force (Fig. 5A). Droplets located away from the first contact point would be pushed aside, resulting in decreased adhesive force under higher applied force (Fig. 5B). In the cases in which droplets were located in an intermediate position relative to the sapphire ball, the adhesive force could either remain constant or oscillate slightly.

The secretion is probably highly viscous, and the measured adhesive force is mainly a result of the viscosity of the secretion. It is well known that the thinner the fluid film between two solids, the greater the pulling force required to separate the two surfaces (Bowen and Tabor, 1986). Unfortunately, it was impossible to study the viscosity of the secretion using our techniques. This will be performed by using atomic force microscopy in future studies. The indirect evidence for viscous force in our measurements was that, in some cases, a stronger adhesive force was measured on single droplets pressed by the sapphire ball. However, in the case of the pad, secretion droplets would presumably spread well on the pad surface even under low forces because of the soft material of the pad. This leads us to conclude that adhesion, measured directly on the pad, is mainly related to the contact area between the pad and the surface.

Relationships between adhesion and pad deformation

Material deformation is one of several factors responsible for the area of real contact between solids (Bowden and Tabor, 1986). Furthermore, the area of real contact may influence both adhesion and friction. Data on the material deformation of the pad aided in understanding the factors influencing the tribological properties of the pad.

Fig. 2 shows that the size of the indentation increased rapidly at smaller applied forces, and that this increase slowed down with greater applied force. This can be explained by the structure of the pad surface. Because of the hemispherical shape of the pad, both indentation and contact area at first increased rapidly in response to applied force. With increased contact area, however, it became harder to make an indentation in the surface of the pad. As a result, the indentation and the contact area increased more slowly with increasing applied force. The pad was only part of a sphere (Fig. 3). When mostly indented, it behaved almost like a hard sample. In this condition, the indentation increased very little with increasing applied force. Maximum contact area had been reached. At applied forces that caused the maximum contact area between the pad and the silicon surface, a saturated adhesive force was reached. The saturated adhesive force of 1.1 mN was obtained at an applied force of 0.8 mN (Fig. 6B). The corresponding indentation at this load was approximately 75 μm (Fig. 2), which was comparable with the thickness of the pad (approximately 100 μm). Before maximum contact was reached, both the contact area and the adhesive force increased with increasing applied force, indicating that the adhesive force was related to the contact area caused by the applied force.

Analysing the force curves in Fig. 6A, it was found that separation of the pad from the silicon surface was recorded as a 'jump off' and that the retraction part of the force curve was basically linear. This shows that the contact area did not change during the retraction process and that separation was a one-step event. Let us assume that the adhesive force F_a can be expressed as a function of the contact area A as $F_a = K_a A$, where K_a is a constant representing the adhesive force of the pad per unit area (tenacity). To check this relationship, we considered the following. (i) The adhesive force became saturated when the pad achieved maximum contact with the silicon surface. (ii) From equation 3, the adhesive force could be expressed as a function of applied force as:

$$F_a = K_a A = \pi (R/K)^{2/3} K_a F_n^{2/3} \qquad (4)$$

at applied forces of less than 0.65 mN. The data for adhesive force *versus* applied force were found to be in agreement with this relationship for applied forces of less than 0.65 mN (Fig. 6B) and K_a was 1.7–2.2 mN mm^{-2}. It was concluded that the attachment of *Tettigonia viridissima* to a smooth surface was strongly dependent on the contact area, i.e. the mechanical deformation of the pad.

In an individual of *Tettigonia viridissima* attached upside-down under a smooth surface, the adhesion of each leg needs to exceed the pulling force of 1.6 mN. In our experiments, the saturated adhesive force of the pad of one tarsomere ranged from 0.7 to 1.2 mN. This force is not sufficient to hold an insect upside-down. However, using additional tarsomeres could make this possible. In addition, other mechanisms may be involved in the attachment onto a surface. These are (i) the release of a larger amount of secretion; (ii) active control of the contact area by changing the haemolymph pressure or the air pressure in the air sacs; (iii) the contribution of lateral (frictional) forces to an overall attachment force.

The adhesive forces measured on pads of freshly excised legs were the same as those of intact insects because there was

some residual secretion on the pad surfaces and the pad material was almost unchanged. However, the pad of the excised tarsus loses its adhesive properties after approximately 1 h, probably because of the absence of any new secretion and the change in the material structure of the pad caused by dehydration.

Comparison with other insect attachment systems

Data on the hairy pad system of the adult reduviid bug *Rhodnius prolixus* led previous authors to suggest that mechanical interlocking between adhesive setae and irregularities in the substratum is responsible for attachment to the substratum (Gillett and Wigglesworth, 1932). In the beetle *Chrysolina polita*, the attachment force increases with the total number of adhesive setae (Stork, 1980). In the beetle hairy pad system, two forces make major contributes to the overall attachment force: (i) molecular adhesion between the setae and the substratum, and (ii) the surface tension of a thin layer of epidermal secretion. A similar attachment mechanism has been hypothesised for spatulate hairs of the adhesive pads of the *Gecko* (Ruibal and Ernst, 1965; Hiller, 1968; Russell, 1975; Stork, 1983b).

Molecular adhesion requires very close proximity between the surfaces in contact. This means that the setae or setal ends have to be composed of extremely flexible material. The flexibility of beetle setae has been confirmed by staining with Mallory's single stain (Stork, 1983a). For hairy pad systems, there are no data on the dependence of the attachment of pad material to the substratum on increasing loading force. For smooth pad systems, such as the arolium of the ant, the viscoelastic properties of the pad material have been suggested previously (Brainerd, 1994), but not proved experimentally.

The present set of experiments demonstrates the important role of the mechanical properties of euplantulae material in adhesion. The deformable cuticle of the pad has a different ultrastructure from that of the surrounding cuticle. It consists mainly of endocuticle (Roth and Willis, 1952; Henning, 1974), which is organised into characteristic rods orientated at an angle to the cuticle surface (Dewitz, 1884; Slifer, 1950). The rods, which are 1.0–2.5 μm wide at their bases, branch into finer rods close to the surface of the pad (Kendall, 1970). Examination of sections of pad material has provided evidence that, when the surface is compressed, the cuticle decreases in thickness, the long rods flattening close to one another against the inner layer. When the pressure is released, the rods return to their previous position (Slifer, 1950). In addition, the euplantulae contain air sacs branching from the tracheal trunk (Henning, 1974). Spaces within the tarsus are filled with haemolymph. The air sacs embedded in the fluid may provide an additional mechanism responsible for the flexibility of the pad material.

In summary, we conclude (i) that the adhesive secretion is essential for the attachment of the pad, and (ii) that the attachment is achieved by deformation of the pad. Further data on the composition of the secretion and its physical properties will contribute to our understanding of the function of this insect attachment system in locomotion. In a separate series of experiments, the relationship between pad deformation, its material properties and ultrastructure will be investigated.

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