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THE ORGANS OF ADHESION IN THE APHID *MEGOURA VICIAE*

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Summary

1. The anatomy of the adhesive organs is described in *Megoura viciae* Buckton. The claws serve as grappling hooks which are locked in position by the retractor unguis tendon. The pulvilli, pliable cuticular sacs, are everted from the tibiotarsal articulation. They bear a surface adhesive, a non-volatile oil, which is secreted through the cuticle. The pulvilli are everted by blood pressure and are withdrawn inside the tibia by contraction of the tibial muscles.

2. The adhesion of these organs to various types of surface was tested by covering over the pulvilli or by claw amputation. Pulling forces were applied by progressively loading the aphid with weights. The pulvilli perform best on relatively smooth surfaces. Aphids are dislodged from the underside of polished glass by a mass of approx. 9–14 mg (a pulling force of $8{\cdot}8\text{–}13{\cdot}7\times10^{-5}$ N). The claws cannot engage a completely smooth surface but are highly effective on rough ones, withstanding a load of approx. 57 mg on dry filter paper. Adhesion of the pulvilli to waxy surfaces is only slightly less than on glass but is greatly reduced on surfaces with still lower free energies, such as Teflon.

3. On a glass surface the pulvilli sometimes leave 'footprints' consisting of oily droplets of variable size. Nevertheless, in many instances such deposits are small or absent, indicating that the bond sometimes breaks between the adhesive and the substratum. In a selected series of footprints with large deposits, their volume was found to be sufficient to form a layer at least $17{\cdot}7\times10^{-9}$ m in thickness over the area of pulvillar contact. This is consistent with the view that surface tension forces would adequately account for adhesion, an additional limiting factor being the physical properties (wettability) of the substratum. Viscous forces would impede the very rapid (less than 0·02 s) removal of the pulvilli, were it not for the retraction mechanism which peels off the pulvillus from the distal edge, like a piece of adhesive tape.

4. The ultrastructure of the pulvillar gland is described. The epidermal cells discharge into a cavity which is confluent with a further reservoir within the spongy endocuticle, itself a meshwork of dissociated cuticular microfibrils. The points of exit of the secretion appear to be the epicuticular filaments. The product of the gland, which can be collected by pricking the pulvilli, is water soluble and

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proteinaceous, differing entirely from the hydrophobic lipid-soluble end-product on the pulvillar surface. We presume that the lipoprotein precursor is dissociated enzymatically at the level of the inner epicuticle.

Introduction

The climbing organs of insects have long been a favourite object of study. Apart from the usually well-developed tarsal claws, a wide variety of organs specialized for adhesion are found on tarsus or tibia. These are of two kinds: discrete cushions of integument bearing several thousands of adhesive tenent hairs with spatulate tips; and pad-like structures devoid of hairs. Recent experimental work has focused particularly on the housefly and other higher Diptera (Hasenfuss, 1977; Walker, Yule & Ratcliffe, 1985) and on chrysomelid beetles (Stork, 1980*a*). These are all species which utilize tenent hairs for clinging to smooth surfaces. Hairless adhesive pads occur on the arolium of cockroaches, together with the euplantulae or pulvilli associated with each of the tarsomeres (Roth & Willis, 1952; Arnold, 1974). Adhesive pads are also found in the coreid Heteroptera (Ghazi-Bayat, 1979) and in Pentatomidae (Ghazi-Bayat & Hasenfuss, 1980). In the leafhopper *Empoasca fabi*, the pulvilli are located on the pretarsus and, together with the claw-bearing unguifers, form a pouch-like structure which adheres well to glabrous but not to pubescent plant leaves (Lee, Kogan & Larsen, 1986). Eversible pulvilli or *Solenbläschen* are common in the Aphididae (Weber, 1930) and were briefly described in *Megoura viciae* by Buckton in 1876. In this species the pulvilli are well-developed sacs that are everted from the tibiotarsal articulation when the tarsus is resting on the substratum but which are retracted inside the tibia when the leg is withdrawn. All instars can readily walk on glass in an inverted posture.

Interest in the physiology of these organs has usually been focused on the mode of adhesion to smooth surfaces, such as glass, on which the claws can gain no purchase. Several mechanisms have been proposed (see Stork, 1980*a*, for review). The view that adhesion in the housefly is due to the production of a sticky secretion is of some antiquity (e.g. Dewitz, 1884). Recent confirmation of this theory has come from the researches of Hasenfuss (1977, 1978) and Walker *et al.* (1985), who showed that in *Musca* and *Calliphora*, respectively, the adhesive agent is a non-volatile oil whose presence can be detected as footprints on a glass surface. According to Hasenfuss (1977, 1978), the lipid secretion in *Musca* is conducted to the tenent hairs through a series of grooves (*Rillen*) running from the upper to the lower seta-bearing surface of the pulvillus. In *Calliphora*, Bauchhenss (1979) considers that the secretion is stored in the spongy cuticle of the pulvillus and is transported through the pore canal system. In *Phormia terraenovae*, Lewis (1954) also refers to the 'gelatinous endocuticle' of the pulvillus.

Measurements by Walker *et al.* (1985) of the pulling force required to detach *Calliphora vomitoria* from a glass surface, together with estimates from footprints of the thickness of the lipid layer, were consistent with the hypothesis that adhesion arises from the surface tension of the lipid layer separating the tips of the

setae from the substratum. An alternative suggestion, first proposed by Gillett & Wigglesworth (1932) in their 'seizure' model in *Rhodnius prolixus*, placed more emphasis on the molecular adhesion of solid surfaces. In the absence of any clear evidence of lipid secretions, a modified form of this hypothesis is also favoured by Stork (1980*a*) for the chrysomelid beetle, *Chrysolina polita*.

With pad-like pulvilli, as in *Megoura*, the separate areas of adhesion are comparatively large, and the adhesive, an oil, is undoubtedly secreted by the highly modified epidermal cells of the pulvillus itself. We have examined the ultrastructure of this organ and have measured the forces the pulvillus can sustain on various surfaces.

Materials and methods

Adult apterae were collected 0–3 days after the final ecdysis, from bean plants (*Vicia faba*) growing in long-day conditions. Various anatomical features connected with the mechanism of pulvillar eversion and retraction can be seen most easily in recently moulted and still untanned aphids which are virtually transparent when viewed with the light microscope. Examination of the external details of the pulvilli is greatly facilitated by ether anaesthesia which causes these organs to evert.

The respective contributions made by claws and pulvilli to adhesion on various surfaces were tested either by amputating the claws with a razor blade fragment while the aphid was at rest on a nylon block or by covering the pulvilli with a droplet of cellulose paint. For the latter purpose the aphids were first etherized and laid on their backs. Several hours were allowed for recovery from anaesthesia and from any possible after-effects of the amyl acetate solvent.

The pulling force that the aphids can resist when resting in an inverted position on a horizontal surface was estimated by loading them with weights. A small copper wire hook (mass approx. 1·5 mg) was first cemented onto the dorsum with superglue. Additional tared wire hooks were then added until the aphid became dislodged. Larger weights were added first and then progressively smaller ones up to the point when dislodgement was expected to occur. The total sustainable mass includes those of both the aphid and the hook. This simple method could not, of course, be used with some experimental surfaces on which adhesion proved to be insufficient to support even the mass of the aphid. In such instances aphid and hook were suspended from the arm of a VDF torsion balance with a 2·5-mg range which was brought into exact balance by the addition of counterweights. The experimental surface was then advanced upwards with a micromanipulator until it was just grasped by the tarsi. After operating the torsion lever the point at which detachment occurred was recorded. The calculation of the pulling force did not then include the mass of the aphid and hook. *Megoura* is a species that readily releases its hold and drops from the host plant in response to mechanical and probably pheromonal disturbance. After handling procedures (attaching hooks etc.) a 1- to 2-h recovery period was always allowed. Nevertheless, it was still

essential to reject measurements if the aphid became agitated and was clearly reluctant to hold on under any circumstances.

Electron microscopy

Scanning electron microscopy

Adult aphids taken from their host plants were immersed directly in acetone. Under these conditions the pulvilli were retracted. However, aphids with everted pulvilli could be obtained by etherization prior to submersion. After a week or more the specimens were critical-point dried, coated with gold–palladium and examined with a Philips SEM 500 scanning electron microscope.

Transmission electron microscopy

Tibiotarsal joints from newly moulted adult apterae were isolated under cold primary fixative (formaldehyde/glutaraldehyde; Karnovsky, 1965). After 1–1·5 h at 4 °C the tissue was briefly rinsed in cacodylate buffer before secondary fixation in osmium tetroxide for 1 h. Dehydration in acetone and embedding in Epon 812 were followed by double staining of thin sections in uranyl acetate and lead citrate (see Hardie, 1980, for details). Sections were viewed with a Philips EM 300 electron microscope.

Results

Anatomy of the claws and structures associated with the eversion and retraction of the pulvilli

Fig. 1 illustrates diagramatically a number of features in the tibia and tarsus that are important in the grasping and adhesive functions of the claws and pulvilli. The hooked claws of the pretarsal segment are fused structures which are operated by the long retractor unguis tendon which passes through both tarsus and tibia to muscle insertions in the femur (Fig. 1C). The pear-shaped tendon 'end-piece' is articulated to a stout unguiretractor plate at the base of the claws. The pretarsus is also attached to the tarsus by a cuticular bridge which forms a dorsal hinge (Fig. 1A, *h*). Contraction of the tendon muscle buckles the hinge and draws the pretarsus into the tarsal 'socket'. This clamps the claws in their normal position with their tips directed ventrally. If the tendon muscle is relaxed (or the tendon severed by cutting through the tarsus), the claws tilt upwards, becoming disengaged from any irregularities in the substratum as the leg is drawn towards the body (Fig. 1E).

The pulvillus is a specialization of the ventral intersegmental membrane between tibia and tarsus (Figs 2, 3). The cuticular wall is tough yet highly pliable. The area of adhesion of the everted organ can be examined with the light microscope when the aphid is hanging from the underside of a glass coverslip (Fig. 4). The surface bears faint ridges and there are often larger folds whose pattern changes if the foot is withdrawn and replaced on the substratum. The area of contact depends on the posture of the aphid but when the leg is in its normal

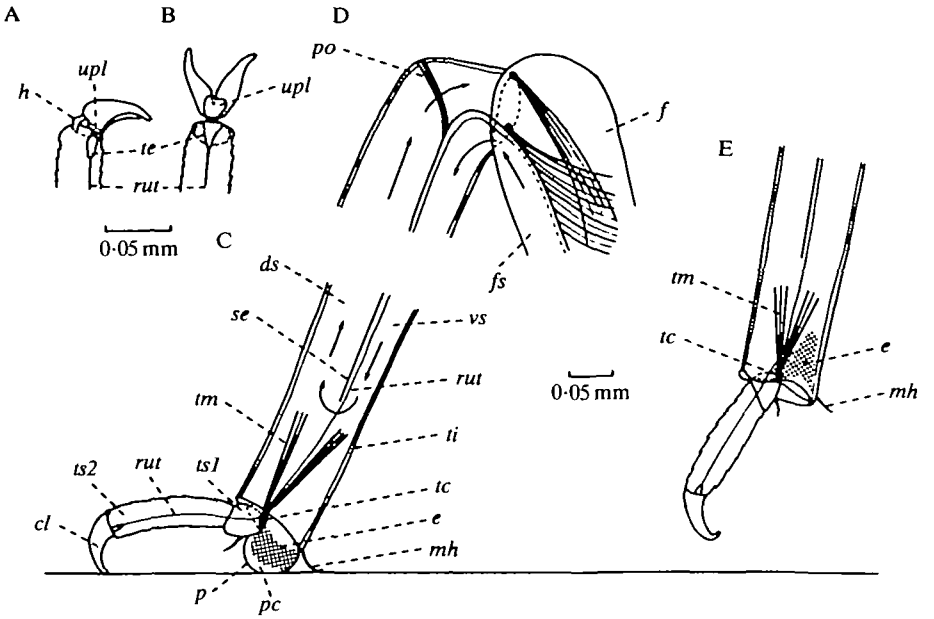
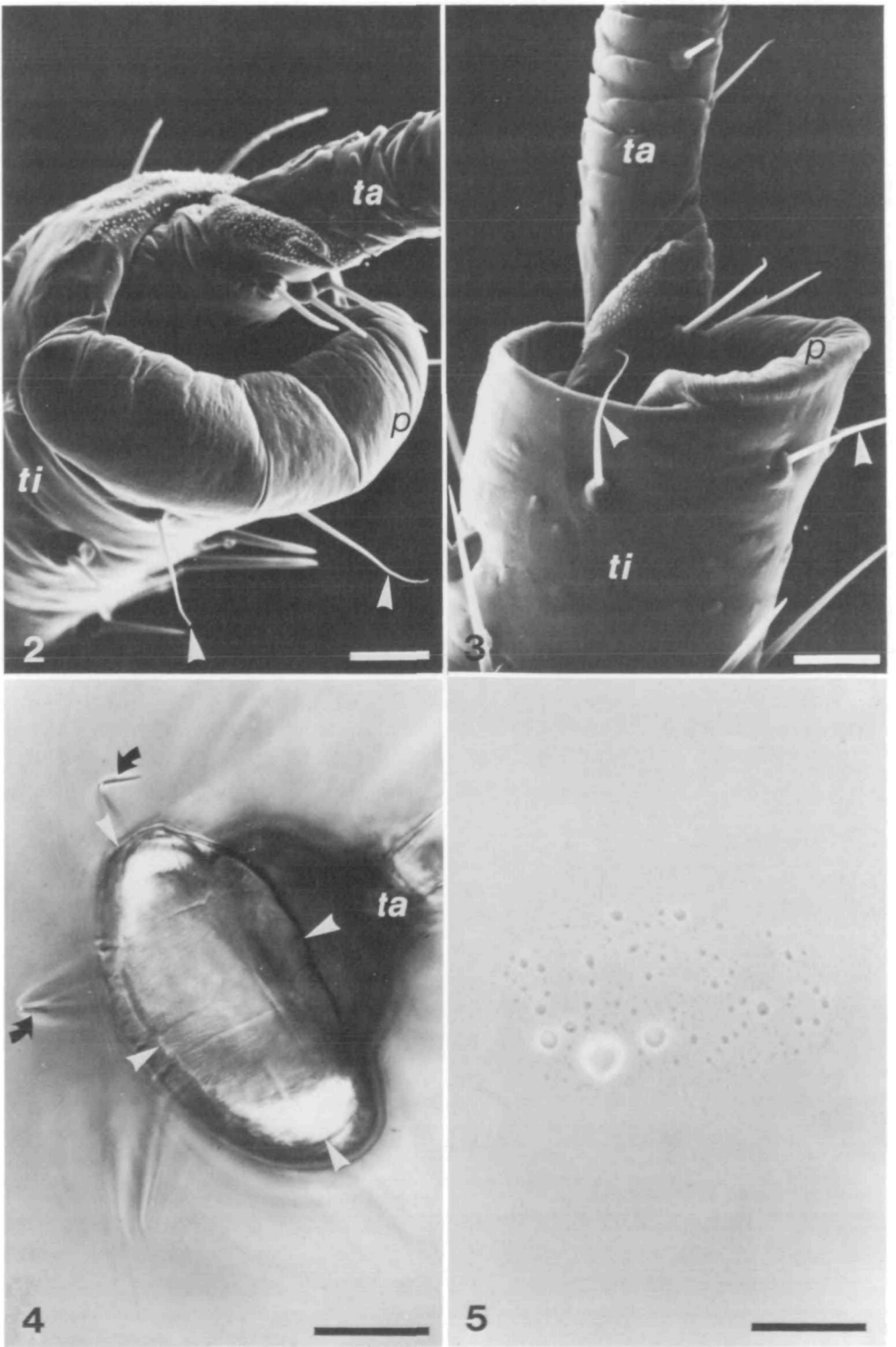


Fig. 1. Anatomy of the leg showing diagramatically the features associated with adhesion and grasping. (A) Pretarsus disarticulated after severing the unguiretractor tendon. (B) The same in ventral view with pretarsus bent dorsally (hinge concealed). (C) Disposition of tibia and tarsus when the leg is applied to a smooth surface, showing the everted pulvillus. (D) The tibiofemoral joint showing the position of the pulsatile organ. Arrows indicate the direction of the blood circulation. (E) The tibiotarsus with pulvillus retracted after the leg is detached from the surface. *cl*, claw; *ds*, dorsal sinus; *e*, pulvillar epidermis; *f*, femur; *fs*, femoral sinus; *h*, hinge; *mh*, mechanosensory hair; *p*, pulvillus; *pc*, pulvillar cavity; *po*, pulsatile organ; *rut*, retractor unguis tendon; *se*, septum; *tc*, tarsal condyle; *te*, tendon endpiece; *ts1*, tarsal segment 1; *ts2*, tarsal segment 2; *ti*, tibia; *tm*, tibial muscles; *upl*, unguiretractor plate; *vs*, ventral sinus.

attitude each pulvillus occupies an oval measuring approximately $3{\cdot}0\times10^{-9}\,m^2$, or $1{\cdot}8\times10^{-8}\,m^2$ for the total surface of the six pulvilli. Smaller areas of contact are observed if the leg is tilted sideways so that only the side of the pulvillus touches the glass. The mean radius of each area of contact, obtained by averaging the larger and smaller diameters, was estimated as $3{\cdot}1\times10^{-5}\,m$.

Associated with each pulvillus are six delicate setae borne at the distal extremity of the tibia (not three as previously stated by Lees, 1984) (Figs 2, 3). The tips of the two dorsal setae are stouter and only just reach the substratum; the two pairs of ventral and lateral setae have fine flagelliform tips which can be seen to be bent against the glass and may be dragged across the surface in a whip-like manner if the pulvillus slips laterally on the glass surface, as frequently occurs when the leg is flexed (Fig. 4). These hitherto-undescribed sensilla are evidently mechanoreceptors that register the proximity of the substratum.

The tarsus is two-jointed. The small basal joint, which bears a row of three setae of distinctive shape (Figs 2, 3), terminates proximally in a single sclerotized


ta
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condyle (Fig. 1C, *tc*). This is the site of attachment of two substantial muscles with insertions in the tibia about one-fifth of their length from the distal end. These tibial muscles (*tm*) presumably represent the levators and depressors of the tarsus which are attached to separate condyles in many insects (Snodgrass, 1935). In the aphid they evidently contract synchronously, serving both as tarsal depressors and as pulvillar retractors. This action arises from the ventral position of the condyle. Tibial muscle contraction causes the condyle, together with the attached folds of pulvillar epithelium, to be further withdrawn into the lumen of the tibia while the whole tarsus hinges downwards around a flexible line of cuticle on the dorsal side of the tibiotarsal joint (Fig. 1E). The pulvilli remain retracted as long as the legs are out of contact with the substratum. We believe that tibial muscle relaxation may be initiated by the flagelliform setae of the tibia as they touch the surface. The pulvilli then evert and the tarsus is raised, thus forming an angle with the tibia as seen in the natural stance (Fig. 1C).

The pulvillus is everted by blood pressure. A significant element in the control of the peripheral blood circulation is the pulsatile organ located at the proximal end of the tibia (Fig. 1D, *po*). The tibia is itself traversed by a horizontal septum which divides it into dorsal and ventral sinuses. The muscle band that forms the 'accessory heart' is attached to the dorsal wall of the tibia and to the septum. Blood circulation can readily be observed in newly moulted aphids by following the motion of haemocytes and aggregations of fatty material in the haemolymph. The rapid beating of the pulsatile organ aspirates blood from the femur into the narrower ventral compartment of the tibia. The flow of blood continues peripherally to a point nearer the attachment of the tibial muscles where the tibial septum ends. Particles can be seen rounding this point and returning, sometimes in jerky motion, up the wider dorsal sinus to the level of the pulsatile organ where they pass directly into the femur without mixing with the centripetal bloodstream. The septum is continued into the femur and may be associated with a valve (see Brocher, 1909; Kaufman & Davey, 1971).

Although the pulsatile organ may make a small contribution towards the maintenance of the haemolymph hydrostatic pressure, it seems probable that

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Fig. 2. Scanning electron micrograph of the tibiotarsal articulation showing the everted pulvillus (*p*) and the whip-like mechanosensory hairs (arrowheads). *ta*, tarsus; *ti*, tibia. Scale bar, 20 μm.

Fig. 3. Scanning electron micrograph of the tibiotarsal articulation with the pulvillus (*p*) retracted. Arrows indicate the mechanosensory hairs. *ta*, tarsus; *ti*, tibia. Scale bar, 20 μm.

Fig. 4. Phase-contrast light micrograph from a newly moulted adult clinging to the underside of a glass coverslip. The outline of the ovoid region of pulvillar contact is shown with arrowheads. A number of cuticular folds can be seen in the region of contact; two of the long mechanosensory hairs make contact with the coverslip (curved arrows). Scale bar, 20 μm.

Fig. 5. Phase-contrast light micrograph showing a footprint of oil droplets left on a coverslip after removal of the pulvillus. Scale bar, 20 μm.

there is a more general mechanism associated with the thorax since the everted pulvilli of anaesthetized aphids do not immediately collapse if the abdomen is transected. The tibial muscles are sufficiently powerful to overcome both the hydrostatic pressure and the forces of adhesion. Indeed, video recordings show that the retraction of the pulvillus is remarkably rapid, occupying less than 0·02 s (one frame). Although direct observation was not feasible, it seems probable that the pulvillus is peeled off from the distal edge as the tarsus is withdrawn.

Mode of adhesion of the pulvillus

Only three of the possible mechanisms of adhesion discussed by Stork (1980*a*) have been considered in *Megoura*. No evidence was found for the existence of residual electrostatic charges since the ability of aphids to walk upside down on glass was not impaired by high humidities or by discharging an electrostatic gun in their vicinity. Some attention was given to the possibility that the pulvillus might act as a sucker since the apical lumen of the tibia, which is virtually occluded by the voluminous secretory epithelium, has some superficial resemblance to a piston. However, as the tibial muscles are attached to the tarsal condyle and do not penetrate the pulvillus (see Fig. 1C), there is no mechanism by which the centre of the pulvillus could be drawn away from the substratum, thus generating the necessary pressure reduction. Aphids cannot be held for more than a few seconds in a vacuum without becoming anoxic. However, isolated legs detached with forceps from etherized aphids remained hanging from glass *in vacuo* for several minutes if the severed tibia was first sealed with wax to prevent immediate pulvillar withdrawal.

The adhesive secretion

If a 4-day-old aptera is allowed to rest on the under surface of a clean coverslip, careful examination of the glass near the pulvilli sometimes reveals the presence of footprints which take the form of an array of oily droplets often varying considerably in size (Fig. 5). The elementary properties of this oil appear to be similar to those described by Bauchhenss (1979) and by Walker *et al.* (1985) for species of *Calliphora*. The water-insoluble droplets readily dissolve in lipid solvents (hexane, benzene, etc.) but are only very sparingly stained by Black Sudan B. The material shows little birefringence and is relatively non-volatile, the droplets remaining unchanged in diameter after exposure to the air for 24 h. The adhesive properties of the lipid are most easily appreciated if etherized aphids are laid on their backs and a fine needle is brought into contact with the everted pulvillus. The needle immediately adheres and cannot be removed without dragging the leg sideways. The pulvilli are also everted during ecdysis and, together with the claws, serve to anchor the exuvium to the substratum as the legs are withdrawn from the old cuticle. The everted pulvilli of cast skins are also tacky and remain so for many days.

We attempted to estimate the volume of adhesive oil liberated during single pulvillar attachments. When apterae are observed clinging to the underside of a

coverslip, their normal behaviour is gradually to draw the legs towards the body with the pulvillus still attached, as if seeking some surface irregularity for the claws to engage. At the end of this manoeuvre the pulvillus is held stationary for some moments before being suddenly released and the leg again extended. The path of the sliding pulvillus and its final resting place were therefore examined and photographed. The volumes of any residual secretion were estimated by measuring the diameters of the droplets and assuming that each was hemispherical. The results were as follows.

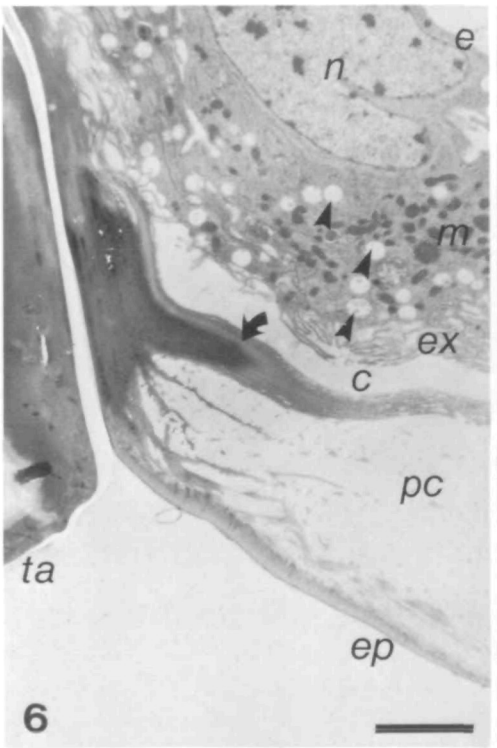
The path of the sliding pulvillus is usually entirely devoid of adhesive. Stationary footprints were characterized by deposits that varied greatly in volume. Among 32 'footprints' left by 15 aphids, no traces at all were visible in 15 instances; a small deposit, consisting of a few small droplets, was found in 10 examples; and a copious deposit, sometimes containing large droplets of irregular shape, was found in seven (Fig. 5). In the latter examples the average volume was estimated to be $53{\cdot}1\times10^{-18}\,m^3$ (range $6{\cdot}9–144{\cdot}3\times10^{-18}\,m^3$). Assuming the surface area of the pulvillus to be $3{\cdot}0\times10^{-9}\,m^2$, this volume of secretion would form a layer of average thickness $17{\cdot}7\times10^{-9}\,m$.

Since the oil deposits left on glass vary so substantially it is clear that their volume does not afford a reliable guide to the thickness of the adhesive layer between the pulvillus and the substratum. Much of the secretion evidently remains on the pulvillus. In this context it is noticeable that if aphids that have been walking steadily for some time are tested by touching the everted pulvilli with a fine needle, the surface remains tacky, showing no indication that the adhesive is depleted.

Ultrastructure of the pulvillus

Although scanning electron microscopy shows the surface details of the retracted and everted pulvillus (Figs 2, 3) it failed to reveal the mode of liberation of the adhesive secretion. No secretory pores were visible and any evidence of lipid secretion on the surface was, of course, removed during the preparative procedures. The surface of the expanded organ is drawn into a series of puckered folds, similar to those seen in aphids clinging to glass (cf. Figs 2, 4), but these are not thought to be a significant part of the mechanism of adhesion.

Transmission electron microscopy is more informative. If the junction of the normal tibial cuticle with the pulvillar cuticle is examined, the compact laminae of the endo- and exocuticle are seen to become separated and 'frayed' (see Fig. 6), eventually giving way to typical pulvillar cuticle where the short microfibrils are randomly distributed (Figs 6–8). The irregular alignment of the microfibrils produces a pulvillar cuticle which is unusually thick (see Fig. 6), forming a resilient, flexible and spongy layer beneath the thin epicuticle. The latter is 300 nm in depth and consists of a thin electron-dense cuticulin layer and a thicker, more electron-lucent inner epicuticle (Figs 7, 8). It is traversed by fine pores or filaments (10 nm in diameter), which sometimes appear to extend into the procuticle (Figs 7, 8).



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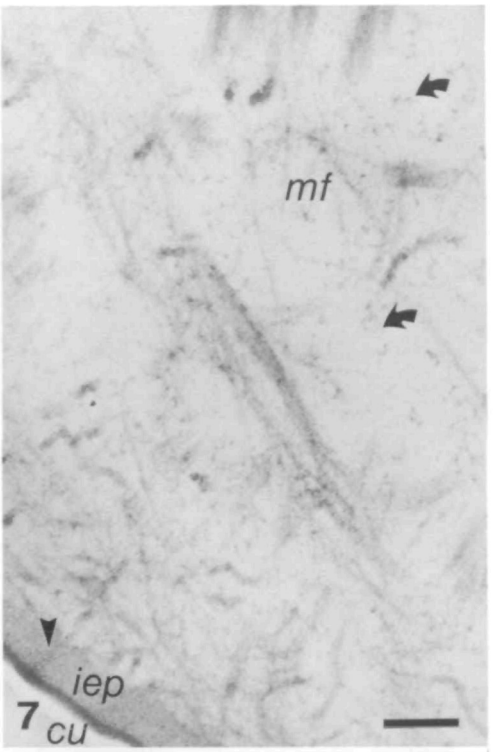

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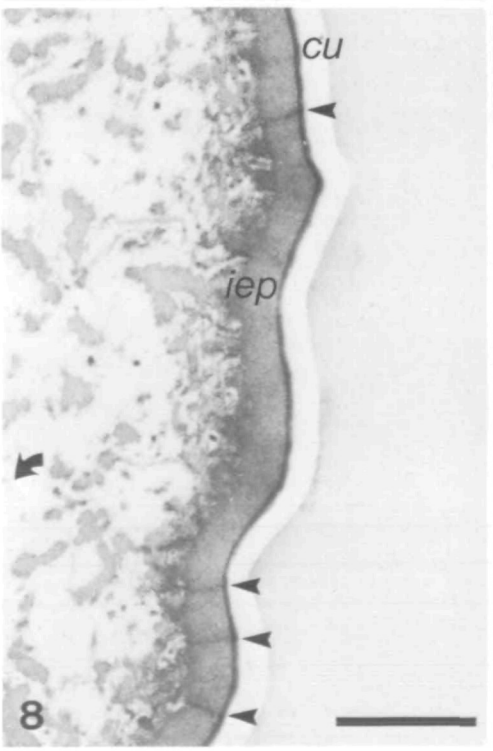

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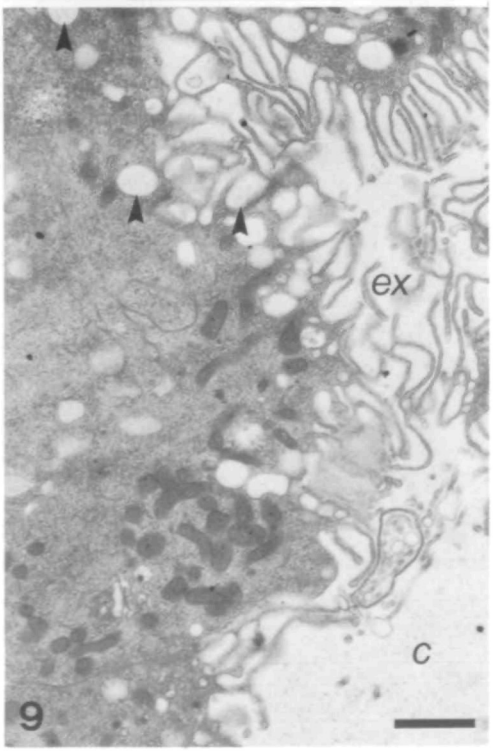

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The pulvillar cuticle is separated from the underlying epidermis by a cavity which contains flocculent material (Figs 6, 9). Similar material can also be detected within the pulvillar procuticle (Fig. 7) and in the abundant membrane-bound vacuoles found in the glandular epidermis (Fig. 9). This finding supports the view that the porous pulvillar procuticle provides an additional reservoir for storing the secretion or its precursor.

The epidermal cells of the pulvillus are larger than their counterparts in other regions of the leg, forming a layer approximately 10 μm in thickness compared with 3 μm elsewhere. The dense cytoplasm contains many ribosomes, sometimes on rough endoplasmic reticulum (not shown), numerous mitochondria and a variety of vesicular components (Figs 6, 9). The distal cell surfaces are extended to form a network of interconnecting processes which are presumably associated with the release of the vesicle contents into the pulvillar cavity (Fig. 9).

Some insight into the nature of this fluid can be obtained by puncturing the pulvillus with a fine tungsten needle. If the aphid is standing on a glass slide, the needle enters the organ from the side so that the possibility of contamination with haemolymph is slight. The released fluid rapidly dries to a glassy solid which has a waxy feel when scratched with the needle. It is insoluble in lipid solvents but is immediately soluble in water. If warmed gently, the material softens slightly but does not evaporate or melt; after further heating it loses water-solubility and eventually chars. Several colour tests for protein were applied to the small quantities of material available. The ninhydrin and Millon reactions proved positive, the Sakaguchi test for arginine negative. From these observations we conclude that the material stored in the pulvillus has entirely different properties

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Fig. 6. Low-power transmission electron micrograph showing one edge of the pulvillar region. Part of the tarsus (*ta*) is seen on the left. The pulvillar epidermis (*e*) contains nuclei (*n*), mitochondria (*m*) and secretory vesicles (arrowheads); the distal cell borders show interconnected ribbon-like extensions (*ex*). The epidermal cells are separated from the pulvillar cuticle by a cavity (*c*). The lamellae of the conventional tibial cuticle can be seen (curved arrow) but these fray out to form a thickened, spongy layer of flexible procuticle (*pc*). The epicuticle (*ep*) is also visible. Scale bar, 2 μm.

Fig. 7. High-power transmission electron micrograph of part of the pulvillar cuticle showing the outer electron-dense cuticulin layer (*cu*) and the inner epicuticle (*iep*) with associated filaments (arrowhead). The filaments appear to continue into the procuticle which also contains randomly distributed cuticular microfibrils (*mf*) and flocculent material giving a speckled appearance (curved arrows). Scale bar, 0·2 μm.

Fig. 8. High-power transmission electron micrograph of epicuticle showing the cuticulin layer (*cu*) and inner epicuticle (*iep*) with filaments traversing it (arrowheads) and continuing into the procuticle. Some flocculent material is also visible (curved arrow). The clear region adjacent to the cuticulin layer is an artefact resulting from the embedding medium pulling away from the cuticle. Scale bar, 0·5 μm.

Fig. 9. Part of the distal border of the pulvillar epidermis showing clear vesicles, some of which contain flocculent material (arrowheads). Folded and finger-like extensions of the cell surface (*ex*) occupy part of the pulvillar cavity (*c*) which also contains flocculent material. Scale bar, 1 μm.

from the adhesive oil liberated on the external surface of the organ and is presumably a lipoprotein precursor.

Effect of surface type and climbing attitude on adhesion

Adult apterae, with all the pulvilli covered with cellulose paint, or with their claws amputated, were tested for adhesion while resting on the underside of three surfaces of differing roughness (Table 1). On polished glass an average mass of 8·9 mg was sustained (equivalent to a pulling force of $8{\cdot}7\times10^{-5}$ N) when the pulvilli were intact, but there was no adhesion by the claws alone (or by cuticle just proximal to the claws). The ground glass surface, although appearing relatively smooth, provided sufficient asperities for the claws to gain a significant foothold, and a mass of 18·1 mg was required to dislodge the aphid. With pulvilli alone, adhesion was reduced to about half the value recorded for polished glass. However, the loose-textured fibres of dry filter paper provided an ideal surface for the claws to grasp, a mass of over 50 mg being sustainable. Near the point of release, the pulvilli and the base of the tarsi were usually pulled away from their normal alignment with the substratum. Aphids depending only on their pulvilli sustained a pulling force about equal to that on ground glass.

In a further series of experiments the performance of the pulvilli was assessed by comparing the adhesion to various surfaces before and after claw amputation. In experiments 1–7 in Table 2 we record the mass required to dislodge aphids at rest on the underside of horizontal surfaces. In experiment 8 the aphids were climbing up a vertical sheet of polished glass with hook and weights arranged so as to pull vertically downwards.

The mass sustainable on polished glass both before and after claw amputation was somewhat higher, at 12·1 and 14·4 mg, respectively, than in the previous series (see Table 1) – perhaps because larger and more robust aphids were selected for testing. Since a more natural substratum would be plant surfaces with waxy cuticles, particularly the stems and leaves of the normal vetch host *Lathyrus pratensis* or the bean *Vicia faba*, these types of surface have a special interest. Intact aphids showed a high degree of adhesion to the underside of broad bean

Table 1. *Pulling forces required to detach apterae from the underside of three horizontal surfaces after eliminating either the claws or the pulvilli*

Treatment	Surface	Mass (mg)	Pulling force ($N\times10^{-5}$)
Pulvilli covered	Polished glass	0·0	0·0
	Ground glass	18·1 ± 3·9	17·8 ± 3·8
	Filter paper	56·6 ± 5·5	55·5 ± 5·4
Claws amputated	Polished glass	8·9 ± 0·6	8·7 ± 0·6
	Ground glass	4·4 ± 0·6	4·3 ± 0·6
	Filter paper	4·2 ± 0·6	4·1 ± 0·6

Values are mean ± S.E.M. ($N = 10$–11).

Table 2. *Mass (in mg) required to detach apterae from several kinds of horizontal (expts 1–7) or vertical (expt 8) surfaces, before and after claw amputation*

Climbing surface and attitude	*N*	Intact aphids	Claws amputated
1. Polished glass, horizontal	20	12·1 ± 0·8	14·4 ± 1·0
2. Bean leaf	11	65·9 ± 5·6	13·2 ± 1·2
3. Brussels sprout leaf	9	48·6 ± 9·3	11·0 ± 0·8
4. Paraffin wax	17	18·2 ± 1·5	10·5 ± 0·8
5. Polyethylene	10	24·0 ± 2·1	11·7 ± 1·0
6. PTFE	14	16·2 ± 2·2	3·3 ± 0·5
7. Chalk dust	17	0·0	0·0
8. Polished glass, vertical	11	60·6 ± 3·5	58·1 ± 3·7

Values are mean ± S.E.M.

leaves and also to leaves of a non-host, Brussels sprout, which evidently provided ample purchase for their claws (Table 2, expts 2, 3). In aphids depending only on the pulvilli, the level of adhesion was only slightly less than on polished glass, there being little difference between bean leaves and the brassica leaves with a smooth but much heavier layer of wax. Adhesion of the intact insect to a sheet of low melting point paraffin wax was relatively poor, probably because of the softness of the material and the lack of firm surface irregularities. The adhesion to wax in aphids deprived of their claws was much the same as to the leaf surfaces (Table 2, expt 4). Several tests were also made with Brussels sprout leaves bearing a heavy powdering of wax particles left by previous colonies of the aphid *Brevicoryne brassicae*. No adhesion at all was observed with clawless *Megoura*. The significance of this observation is discussed later.

The level of adhesion of clawless aphids to polyethylene was very similar to their adhesion to waxy surfaces (Table 2, expt 5). With the sample of Teflon used there were sufficient surface asperities to provide intact insects with some assistance in maintaining a hold with the claws (Table 2, expt 6), but pulvillar adhesion was very slight (mean 3·3 mg), often less than the mass of the insect. Fine chalk dust or bentonite, either applied loosely on glass or smeared over the glass surface with the fingertip, presented the aphid with an unclimbable surface. There was no measurable adhesion by the pulvilli.

A very different outcome was seen if the aphid was climbing a vertical glass surface with the pulling force acting downards (Table 2, expt 8). Both intact and clawless aphids were able to support a mass of about 60 mg, nearly five times the sustainable mass of an aphid resting on the lower side of a horizontal surface. As the sustainable maximum was reached the aphids often slowly slid downwards

without releasing the pulvilli from the substratum. It was clear that in this attitude the capacity to resist a pulling force was also improved on surfaces other than glass. For example, on Teflon sheet the mean sustainable mass by clawless aphids was 13 mg, a fourfold increase (five specimens).

Discussion

The claws and pulvilli have differing but complementary functions in *Megoura*. The claws act as miniature grappling hooks which, when locked in position by the contraction of the retractor unguis muscle, engage the irregularities in rough-textured surfaces, while the adhesive pulvilli hold the tarsi level with the substratum. In this attitude the claws operate to maximum advantage as the leg is drawn towards the body, but are immediately released when the pull on the long retractor tendon ceases. The pulvilli, however, perform best on smooth surfaces. Although these would seldom be encountered by the insect in its natural environment, the pulvilli also adhere well to rougher surfaces such as filter paper or leaves: nevertheless, their contribution to the insect's ability to resist pulling forces due to wind or gravity is less than that of the claws alone.

The mode of adhesion of the pulvilli to different types of surface requires more detailed consideration. In the chrysomelid beetle *Chrysolina polita* the absence of any detectable secretion between the spatulate tips of the tenent hairs and the substratum, as well as their close contact (<100 nm) with a glass surface, led Stork (1980*a*) to conclude that molecular adhesion, possibly mediated by van der Waals forces, played a prominent role. However, recent work on fly (*Musca*, *Calliphora*) pulvilli has shown that the tenent hairs are moistened by an adhesive secretion, a non-volatile oil (Hasenfuss, 1978; Bauchhenss & Renner, 1977). Walker *et al.* (1985) have photographed the footprints which, in *C. vomitoria*, correspond to the distribution of the setae. Estimates of the volume of the secretion were consistent with the view that the surface tension of the lipid film between the hairs and the substratum could account for the observed adhesion of tethered flies to glass.

A non-volatile oil with properties similar to those described in flies is also liberated on the surface of the pulvillar pads in *Megoura*. Surface tension forces generated round the oil interface between a glass surface and the cuticle of the pulvillus are probably of major importance in this species also; but it is worth considering whether adhesion or detachment is also influenced by the viscous or viscoelastic properties of the adhesive. The relevant evidence concerns the pulling forces measured on glass, the estimates of the thickness of the oil deposits left as footprints and the adhesion to surfaces with lower free energies than glass.

The efficiency of *Megoura* pulvilli, expressed as the pulling force sustainable by a unit area of adhesive surface, is compared with that of two species with tenent hairs (Table 3). In *Megoura*, as in *Calliphora*, the values are based on the maximum pulling forces recorded in separating the insect from the substratum (see below). Table 3 shows that although the number of 'adhesive units' per insect is some thousands of times greater in the beetle *Chrysolina* or the fly *Calliphora* than

Table 3. *Comparison of the areas of adhesive surface in three species of insects and the pulling forces required to detach them from a polished glass substratum*

Species	Approx. body mass (mg)	No. of adhesive setae or pads/insect	Area of each seta or pad (m^2)	Total area of adhesive surface (m^2)	Sustainable mass (mg)	Maximum pulling force (N)	Pulling force ($N\,m^{-2}$) of adhesive surface	Author
Chrysolina polita, ♀	59	12 000	40×10^{-12}	48×10^{-8}	2450	$2{\cdot}4\times10^{-2}$	$5{\cdot}0\times10^{4}$	Stork, 1980*a*
Calliphora vomitoria	60	21 000	2×10^{-12}	$3{\cdot}5\times10^{-8}$*	2040*	2×10^{-2}*	$57{\cdot}0\times10^{4}$*	Walker, Yule & Ratcliffe, 1985
Megoura viciae	3·3	6	$0{\cdot}3\times10^{-8}$	$1{\cdot}8\times10^{-8}$	61	$5{\cdot}9\times10^{-4}$	$3{\cdot}3\times10^{4}$	This paper

* Values for five legs only attached to substratum.

in *Megoura*, the total areas of adhesive surface are not dissimilar, particularly in the latter two species. Nevertheless, the sustainable pulling force in *Megoura* is some 17 times less. While this is no doubt commensurate with the size of the aphid, whose mass is nearly 20 times less, the reduced adhesion requires some comment.

The relationship between the reduction in pressure (−P) within an adhesive secretion of surface tension γ and thickness 2r when a disc of radius R is pulled from a smooth surface of the same wettable material is given by Fender (1962) and Walker *et al.* (1985):

$$-P = \gamma\left(\frac{1}{R} - \frac{1}{r}\right). \quad (1)$$

Since $F = PA$, where F is the pulling force and A is the area of the disc, then:

$$F = -\gamma A\left(\frac{1}{R} - \frac{1}{r}\right). \quad (2)$$

In *Megoura*, $R = 3{\cdot}1\times10^{-5}$ m, $A = 1{\cdot}8\times10^{-8}$ m^2 and γ is assumed to have a value typical of a light mineral oil, namely 30 mN m^{-1} (see Walker *et al.* 1985). Reliable estimates of the thickness of the adhesive layer are hard to obtain since many 'footprints' on glass leave no oil deposits at all; and we have no estimates for the volume of secretion left on the pulvillus after the foot is detached. However, we have noted that, in seven instances where large deposits were left on glass, the oil, if evenly distributed, would be sufficient to form a layer of mean thickness $17{\cdot}7\times10^{-9}$ m, over the whole area of pulvillar contact. This is similar to the figure of 130×10^{-9} m given by Walker *et al.* (1985) for the thickness of the oil layer in *Calliphora*. If $17{\cdot}7\times10^{-9}$ m is accepted as a minimal value in *Megoura*, substitution in equation 2 suggests that a film of these dimensions would withstand a pulling force of $5{\cdot}9\times10^{-2}$ N, a value two orders of magnitude higher than the actual experimental value (Table 3). The pulling force would, of course, be reduced if the adhesion layer, 2r, was much more substantial than is suggested by the volume estimate.

In comparing *Megoura* with *Calliphora* the size disparity of the individual adhesive units is particularly striking. Thus the radius of the pulvillar pads, R, is $3{\cdot}1\times10^{-5}$ m in *Megoura* and approximately $7{\cdot}5\times10^{-7}$ m in the tenent hair discs of *Calliphora* (from micrographs by Walker *et al.* 1985). However, equation 1 shows that if the adhesion layer is of similar thickness in the two species the term 1/R becomes negligible in *Megoura*, whereas the pressure reduction within the adhesive layer of *Calliphora* would be diminished by some 10 %. If the total area of adhesive surface is approximately the same, a slightly greater adhesive efficiency would be expected in the aphid pulvilli than in the smaller tenent hairs. This cannot, therefore, account for any part of the lesser pulling forces sustainable by *Megoura* (Table 3).

A more probable explanation is that surface tension is not a limiting factor in adhesion and that in such species as *Calliphora* and *Megoura* (Table 3) the tenacity of adhesion depends to a marked extent on their behaviour. Thus Walker *et al.*

(1985) have shown that *Calliphora* clings to glass more effectively if the pulling force is tangential rather than normal to the surface. They attribute this difference to the ability of the fly to release its hold voluntarily in response to mechanoreceptors monitoring the leg attitude. The same relationship has been observed in *Megoura* in which the maximum sustainable pulling forces were recorded during vertical climbing (Table 2). In this circumstance surface tension forces are not operating to best advantage. The oil film is then subjected to shear and even acts as a lubricant at maximum load, sometimes allowing the aphid to slide down a glass surface. The aphid is much less inclined to cling to horizontal surfaces in an upside-down posture.

While the forces generated by surface tension are more than adequate to account for adhesion when the aphid is at rest, the viscous properties of the oil, which have a time dependency, might well impede the rapid separation of the pulvillus from the substratum. Bowden & Tabor (1950) show that when two rigid circular discs are pulled apart, large pulling forces normal to the surface are required before the viscous oil flows into the spaces left between the discs as they separate. If these have a radius R, the initial distance apart is h (= 2r) and the oil has a viscosity η, the force F necessary to pull the discs apart in time t is given by the expression:

$$F = \frac{3\pi\eta R^4}{4th^2}. \tag{3}$$

For the aphid pulvillus $R = 3{\cdot}1\times10^{-5}$ m, $t < 0{\cdot}02$ s (see above), $h = 17{\cdot}7\times10^{-9}$ m. If the oil is assumed to have the viscosity of a light mineral oil, namely 0·15 Pa·s, the theoretical force needed to pull the fully expanded pulvillus from a wettable surface in less than 0·02 s might be as large as 52 mN, equivalent to a mass of over 5 g. It is clear that this difficulty is circumvented by the pulvillar retraction mechanism which must peel off the pulvillus from the distal edge like a piece of adhesive tape. In tenent hairs that have no intrinsic retraction mechanism their small size might serve to diminish viscous drag and facilitate rapid removal of the legs from the substratum.

A further physical factor influencing adhesion is the nature of the bonding between pulvillus and substratum. From the variable quantities of secretion deposited on glass it is clear that three points of breakage should be considered: between the pulvillar cuticle and the adhesive oil; within the adhesive itself; and between the adhesive and the adherend surface. The first appears to be strongly bonded in *Megoura* as the pulvillus remains tacky, even after long walking bouts. It should nevertheless be borne in mind that the secretion will doubtless be constantly replenished from the pulvillar gland. Breakage may also occur within the adhesive itself when deposits are left on glass, the largest accretions possibly resulting from the build-up of oil on the pulvillar surface. Whether deposits are left on the adherend surface will presumably depend on the relative strength of the bonding within the adhesive and between adhesive and substratum. Although with glass these forces seem to be evenly matched, this will not necessarily apply to

other surfaces with a lower free energy. On paraffin wax or the waxy surface of leaves, pulvillar adhesion is only slightly less than on glass, but it may well be that the loss of valuable adhesive to the substratum would be correspondingly diminished. On 'non-stick' Teflon surfaces with their low wettability by lipids the very low pulling forces required to dislodge aphids are particularly striking (Table 2).

The total lack of adhesion of aphids to dusted surfaces is probably due as much to the absence of bonding between the dry powder and the substratum as to the adsorption of lipid by the dust. Stork (1980*b*) has also drawn attention to the role of powdery wax blooms in lowering the adhesion of the mustard beetle *Phaedon cochleariae* to glaucous-leaved brassicas. *Megoura* is not naturally found on host plants with heavy wax blooms. However, it is of some interest that this species, when deprived of the claws, cannot gain a foothold on leaf surfaces contaminated with *Brevicoryne* wax particles, although normal adhesion is seen on the smooth surface wax of a glossy brassica leaf. It is not surprising therefore that the aphid *Brevicoryne* has not evolved tarsal pulvilli (H. L. G. Stroyan, personal communication).

Ultrastructure of the pulvillus

No large surface pores are visible in scanning electron micrographs. Such channels have been detected in cockroach pulvilli (Arnold, 1974) but not in houseflies (Hasenfuss, 1977) or leafhoppers (Lee *et al.* 1986). However, sectioned material shows very fine filaments or pores comparable in diameter with the wax canals of Locke (1960), more recently termed epicuticular filaments by Filshie (1970). These structures terminate at the cuticulin layer or within it (Filshie, 1970), no external openings being visible in scanning electron micrographs. Their inward extensions are also characteristic of epicuticular filaments in general and, as such, would be expected to connect with the pore canal system when this is present in the underlying procuticle (Filshie, 1982). However, the procuticle of the aphid pulvillus lacks pore canals as do certain types of cuticle in the blowfly pulvillus (Bauchhenss, 1979), the wax-secreting cuticle of *Calpodes* larvae (Locke, 1960) and the flexible cuticle studied by Filshie (1982). We assume that the epicuticular filaments represent the sites of elaboration of the final secretory product (see below) and are also the loci for the extrusion of the adhesive oil. Whether pulvillar contact promotes the passive expulsion of the secretion or whether this is assisted by increased intraglandular pressure is uncertain (see Bauchhenss, 1979).

One of the most striking cytological features is the granular appearance of the ground substance which fills the epidermal cell vacuoles and which is also seen in the pulvillar cavity and among the disordered cuticular microfibrils of the procuticle. This may represent the fixed residue of the proteinaceous material released on puncturing the pulvillus. The evidence suggests that this secretion is a water-soluble lipoprotein which is presumed to be the precursor of the adhesive oil. It seems probable that the protein moiety is detached by the appropriate enzymes located at the level of the inner epicuticle which Locke (1959) has already

found to be rich in oxidases and esterases. It is noteworthy that a very similar relationship is found in the egg-waxing organ of ticks (Lees & Beament, 1948). Here, also, the water-soluble material present in the storage horns of the organ is proteinaceous in character whereas the wax which collects on the surface has the usual lipophilic properties.

In its internal structure, the aphid climbing organ resembles the blowfly pulvillus (Bauchhenss & Renner, 1977; Bauchhenss, 1979), except that in the latter insect the epidermis remains adherent to the cuticle whereas in the aphid it separates to form a cavity which, with the spongy procuticle, serves as a capacious reservoir for storing the epidermal cell secretion. Since the blowfly pulvilli are clothed with tenent hairs, the oily secretion must be transported to their spatulate tips. This requirement is unnecessary in the simpler aphid climbing organ.

We thank Jane Price for drawing Fig. 1.

References

ARNOLD, J. W. (1974). Adaptive features on the tarsi of cockroaches (Insecta: Dictyoptera). *Int. J. Insect Morph. Embryol.* **3**, 317–334.

BAUCHHENSS, E. (1979). Die Pulvillen von *Calliphora erythrocephala* Meig. (Diptera: Calliphoridae) als Adhäsionsorgane. *Zoomorphologie* **93**, 99–123.

BAUCHHENSS, E. & RENNER, M. (1977). Pulvillus of *Calliphora erythrocephala* Meig. (Diptera: Calliphoridae). *Int. J. Insect Morph. Embryol.* **6**, 225–227.

BOWDEN, F. P. & TABOR, D. (1950). *The Friction and Lubrication of Solids.* Oxford: Oxford University Press.

BROCHER, F. (1909). Sur l'organe pulsatile observé dans les pattes des Hémiptères aquatique. *Ann. Biol. Lacustre* **4**, 33–41.

BUCKTON, G. B. (1876). *A Monograph of the British Aphides*, vol. 1. London: Ray Society Monograph.

DEWITZ, H. (1884). Ueber die Fortbewegung der Thiere an senkrechten glatten Flächen vertmittelst eines Secretes. *Pflügers Arch. ges. Physiol.* **33**, 440–481.

FENDER, D. H. (1962). *General Physics and Sound.* London: English Universities Press.

FILSHIE, B. K. (1970). The resistance of epicuticular components of an insect to extraction with lipid solvents. *Tissue & Cell* **2**, 181–190.

FILSHIE, B. K. (1982). Fine structure of the cuticle of insects and other arthropods. In *Insect Ultrastructure*, vol. 1 (ed. R. C. King & H. Akai), pp. 281–312. New York: Plenum Press.

GHAZI-BAYAT, A. (1979). Zur Oberflächenstruktur der tarsalen Haftlappen von *Coreus marginatus* (L.) (Coreidae, Heteroptera). *Zool. Anz. Jena* **203**, 345–347.

GHAZI-BAYAT, A. & HASENFUSS, I. (1980). Zur Herkunft der Adhäsionsflüssigkeit der tarsalen Haftlappen bei den Pentatomidae (Heteroptera). *Zool. Anz.* **204**, 13–18.

GILLETT, J. D. & WIGGLESWORTH, V. B. (1932). The climbing organ of an insect, *Rhodnius prolixus* (Hemiptera; Reduviidae). *Proc. R. Soc. Ser.* B **111**, 364–375.

HARDIE, J. (1980). Differential sensitivity of neurosecretory and non-neurosecretory neurones of an aphid to sucrose during fixation for electron microscopy. *J. Microsc.* **118**, 483–487.

HASENFUSS, I. (1977). Die Herkunft der Adhäsionsflüssigkeit bei Insekten. *Zoomorphologie* **87**, 51–64.

HASENFUSS, I. (1978). Uber das Haften von Insekten an glatten Flächen – Herkunft der Adhäsionsflüssigkeit. *Zool. Jb. (Anat.)* **99**, 115–116.

KARNOVSKY, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* **27**, 137a–138a.

KAUFMAN, W. R. & DAVEY, K. G. (1971). The pulsatile organ in the tibia of *Triatoma phyllosoma pallidipennis. Can. Ent.* **103**, 487–496.

LEE, Y. I., KOGAN, M. & LARSEN, J. R. (1986). Attachment of the potato leafhopper to soybean plant surfaces as affected by morphology of the pretarsus. *Entomol. exp. appl.* **42**, 101–107.
LEES, A. D. (1984). Parturition and alate morph determination in the aphid *Megoura viciae*. *Entomol. exp. appl.* **35**, 93–100.
LEES, A. D. & BEAMENT, J. W. L. (1948). An egg-waxing organ in ticks. *Q. J. microsc. Sci.* **89**, 291–332.
LEWIS, C. T. (1954). Studies concerning the uptake of contact insecticides. I. The anatomy of the tarsi of certain Diptera of medical importance. *Bull. ent. Res.* **45**, 711–722.
LOCKE, M. (1959). Secretion of wax through the cuticles of insects. *Nature, Lond.* **184**, 1967.
LOCKE, M. (1960). The cuticle and wax secretion in *Calpodes ethlius* (Lepidoptera, Hesperidae). *Q. J. microsc. Sci.* **101**, 333–338.
ROTH, L. M. & WILLIS, E. R. (1952). Tarsal structure and climbing ability of cockroaches. *J. exp. Zool.* **119**, 483–517.
SNODGRASS, R. E. (1935). *The Principles of Insect Morphology*. New York, London: McGraw-Hill Book Co.
STORK, N. E. (1980*a*). Experimental analysis of adhesion of *Chrysolina polita* (Chrysomelidae: Coleoptera) on a variety of surfaces. *J. exp. Biol.* **88**, 91–107.
STORK, N. E. (1980*b*). Role of waxblooms in preventing attachment to brassicas by the mustard beetle, *Phaedon cochleariae*. *Entomol. exp. appl.* **28**, 100–107.
WALKER, G., YULE, A. B. & RATCLIFE, J. (1985). The adhesive organ of the blowfly, *Calliphora vomitoria*: a functional approach (Diptera: Calliphoridae). *J. Zool., Lond.* A **205**, 297–307.
WEBER, H. (1930). *Biologie der Hemipteren*. Berlin: Julius Springer.

Note added in proof

The attachment organs of the oak aphid *Myzocallis schreiberi*, recently described by Kennedy (1986), provide an interesting contrast with the pulvilli of *Megoura*. In *Myzocallis* attachment to the dense trichomes of the host leaf depends on the claws and a pair of finger-like empodia arising from their base. It seems, nevertheless, that these structures may also have some intrinsic adhesive properties as the aphids are able to sustain their own body weight on the underside of a glass surface.

Reference

KENNEDY, C. E. J. (1986). Attachment may be a basis for specialization in oak aphids. *Ecol. Ent.* **11**, 291–300.