

Slippery pores: anti-adhesive effect of nanoporous substrates on the beetle attachment system

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Traction experiments with adult seven-spotted ladybird beetles *Coccinella septempunctata* (L.) were carried out to study the influence of surface structure on insect attachment. Force measurements were performed with tethered walking insects, both males and females, on five different substrates: (i) smooth glass plate, (ii) smooth solid Al₂O₃ (sapphire) disc, and (iii–v) porous Al₂O₃ discs (anodisc membranes) with the same pore diameter but different porosity. The traction force of beetles ranged from 0.16 to 16.59 mN in males and from 0.32 to 8.99 mN in females. In both sexes, the highest force values were obtained on smooth solid surfaces, where males showed higher forces than females. On all three porous substrates, forces were significantly reduced in both males and females, and the only difference within these surfaces was obtained between membranes with the highest and lowest porosity. Males produced essentially lower forces than females on porous samples. The reduction in insect attachment on anodisc membranes may be explained by (i) possible absorption of the secretion fluid from insect adhesive pads by porous media and/or (ii) the effect of surface roughness. Differences in attachment between males and females were probably caused by the sexual dimorphism in the terminal structure of adhesive setae.

Keywords: absorption; adhesion; attachment; insect; roughness; porosity

1. INTRODUCTION

Most recent data on insect hairy attachment systems have demonstrated their excellent attachment properties and high reliability of contact (Gorb 2001, 2005; Federle 2006). However, many microstructured surfaces of plants may strongly reduce insect attachment (Stork 1980*a*, 1986; Edwards 1982; Edwards & Wanjura 1990; Eigenbrode 1996; Eigenbrode *et al.* 1998, 1999; Eigenbrode & Kabalo 1999; Brennan & Weinbaum 2001; Eigenbrode & Jetter 2002; Gaume *et al.* 2002, 2004; Gorb & Gorb 2002; Rutledge & Eigenbrode 2003; Gorb *et al.* 2005, 2008). To explain anti-adhesive properties of these plant substrates, which are covered with microscopic wax crystals, four hypotheses were previously proposed (Gorb & Gorb 2002). (i) Wax crystals cause microroughness, which considerably decreases the real contact area between the substrate and setal tips of insect adhesive pads (roughness hypothesis). (ii) Wax crystals are easily detachable structures that contaminate pads (contamination hypothesis). (iii) Structured wax coverage may absorb the fluid from the setal surface (fluid absorption hypothesis). (iv) Insect pad secretion may dissolve

wax crystals (wax dissolving hypothesis). This would result in the appearance of a thick layer of fluid, making the substrate slippery.

Recently, only the first two hypotheses were tested. The influence of surface roughness on insect attachment has been proven experimentally in several studies (Gorb 2001; Peressadko & Gorb 2004; Voigt *et al.* 2008; Bullock & Federle 2009; Gorb & Gorb 2009). It has been shown that insects generate much higher forces on either smooth or rough surfaces with an asperity size exceeding 3.0 µm than on those with the roughnesses ranging from 0.3 to 3.0 µm. This effect has been explained by the specific geometry of spatula-like terminal elements of insect tenent setae that are able to generate sufficient contact with large surface irregularities. The worst attachment has been observed on substrates with a roughness of 0.3 and 1.0 µm. In this case, because of the small size of asperities, the area of real contact between these substrates and the tips of insect setae was very small. Since adhesion force depends on the area of real contact, insects were not able to attach successfully to surfaces with such a microroughness.

The contamination of insect pads by plant wax crystals has been demonstrated for several insects and in a

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series of plant species (Gaume *et al.* 2004, 2005; Gorb & Gorb 2006). It has been found that plants differ essentially in their contaminating effects on insect pads (Gorb & Gorb 2006). The degree of contamination depends on the micromorphology of waxes. The analysis of the relationship between the contaminating ability and geometrical parameters of wax crystals has shown that the contamination is related to both the largest dimension and the aspect ratio of crystals.

To date, the wax dissolving hypothesis and the fluid absorption hypothesis have not been experimentally tested. In the present paper, we used nanoporous substrates with the same pore diameter, but different porosity (area of voids in a material surface, normalized over the total area), in order to test the fluid absorption hypothesis. According to this hypothesis, absorption of the insect pad fluid by the porous substrate results in a reduction in the fluid thickness between the terminal plates of the insect pads and the substrate, and this leads to a reduction in capillary interaction in the contact area. We measured traction forces of tethered adult seven-spotted ladybird beetles *Coccinella septempunctata* (L.) (Coleoptera, Coccinellidae) walking on five different substrates and performed inversion tests with the insects on these substrates. Three types of nanoporous membranes and two smooth solid surface samples were used. The following questions were addressed. (i) Do insects perform differently on smooth and porous surfaces? (ii) How does the porosity influence the traction force? (iii) Do females differ from males in their attachment on smooth and porous surfaces?

2. MATERIAL AND METHODS

2.1. Insects

The seven-spotted ladybird beetle *C. septempunctata* was used as a model insect species because of its comparatively large size (body length: 6–8 mm) and commercial availability. It originated from Europe, and was repeatedly introduced and then established in the USA for biological control of aphids (Rech & Garde 2003). Nowadays, it has Palaearctic distribution (Klausnitzer & Klausnitzer 1997).

Adult beetles were obtained from a commercial supplier (Katz Biotech AG, Baruth, Germany) and kept in small ventilated cages ($15 \times 8 \times 10 \text{ cm}^3$) at a temperature of 22–24°C, 60–75% relative humidity and a 16L:8D photoregime for 2 days. Beetles were fed with grain aphids *Sitobion avenae* (Fab.) (Homoptera, Aphididae) obtained from the commercial supplier. Water in the cages was changed daily and the cages were sprayed with water twice a day.

2.2. Surfaces used

Anodiscs (Whatman, Schleicher and Schuell, Whatman International Ltd, Maidstone, UK) are porous, disc-shaped Al_2O_3 membranes. We selected three different types of membranes with pores of about 200 nm in diameter, but with different porosity. As a control, two different smooth surfaces were used: sapphire ($\alpha\text{-Al}_2\text{O}_3(0001)$) and hydrophilic soda-lime glass.

2.3. Microscopy

The topography of the anodisc membranes was visualized using scanning electron microscopy (SEM). Small parts of samples were mounted on holders by means of conductive carbon double-sided adhesive tape, sputter-coated with gold–palladium (6 nm) and studied in a SEM Hitachi S-4800 (Hitachi High-Technologies Corporation, Tokyo, Japan) at 3 and 20 kV accelerating voltage. Pore diameter, thickness of walls between pores and sample porosity were quantified from digital images by using SigmaScan Pro 5 software (SPSS Inc., Chicago, IL, USA).

To study the attachment organs of the beetle, insects were air-dried, mounted dorsally on holders, sputter-coated with gold–palladium (6 nm) and examined in the SEM at 3 kV.

2.4. Estimation of the free surface energy

For smooth surface samples (glass and sapphire), contact angles of a series of fluids (water, diiodomethane and ethylene glycol) were measured, and the free surface energy as well as its polar and dispersion components were estimated using a high-speed optical contact angle measuring device OCAH 200 (DataPhysics Instruments GmbH, Filderstadt, Germany). We used 1 μl drops and ellipse fitting for evaluation of static contact angles (for a detailed description of the method, see Gorb *et al.* (2004)). For each sample, 5–10 measurements of the contact angle of each liquid were performed. The free surface energy and its components were calculated according to the universal Owens, Wendt and Kaelble method (Owens & Wendt 1969).

2.5. Estimation of absorption properties of nanoporous membranes

Absorption rates of two fluids—polar water and non-polar mineral oil (Mobil DTE Medium, viscosity $43.4 \text{ mm}^2 \text{ s}^{-1}$ at 40°C (Varenberg & Gorb 2009))—by three nanoporous anodisc membranes were estimated by comparing the evolution of contact angles of the fluids. The oil was employed as a rough approximation of the modelled oily pad secretion found previously in beetles (Ishii 1987; Kosaki & Yamaoka 1996; Eisner & Aneshansley 2000; Attygalle *et al.* 2002). Smooth solid sapphire and glass samples served as reference surface samples. We used 1 μl sessile drops, video-recorded (10 frames s^{-1}) the behaviour of the drops on test surfaces during the first 60 s, and then performed dynamic contact angle measurements using the contact angle measuring device mentioned above. For each surface sample (altogether five), five experiments were carried out with each fluid.

2.6. Traction force measurements and inversion tests

To measure the attachment forces of beetles on different surface samples, traction experiments with tethered walking insects were carried out with a load cell force transducer (10 g capacity, Biopac Systems Ltd, Santa Barbara, CA, USA). Both males and females were used in experiments.

Beetles were narcotized with CO₂ for approximately 1 min in order to glue together their elytra and to attach a human hair to the insect with a small droplet of molten beeswax. After recovery for approximately 30 min, the insects were then used for force tests (for a detailed description of the method, see Gorb *et al.* (2008)). With each insect individual, experiments on five substrates were performed: (i) the smooth solid glass plate, (ii) the smooth solid sapphire disc, and (iii–v) anodisc membranes (ano-28, ano-42 and ano-51) with pores of the same diameter but with different porosity. The force produced by the insect moving on the horizontal test substrate was recorded. Since the beetles were constrained to pulling parallel (not at an angle) to the measurement axis of the transducer, the registered force corresponded to the total pulling force. Force–time curves of the beetles stretching the hair for approximately 5–25 s were used to estimate the maximal traction force generated by the beetles. Tests were carried out at a temperature of 24–25°C and 50–56% relative humidity. Ten females and 10 males were tested on each surface. In all, 100 force measurements were conducted. The behaviour of the insects during traction force experiments was observed and registered.

Additionally, inversion tests were performed with each individual beetle after each traction force measurement. A tested substrate with an insect attached to it was inverted to angles of 90° and 180°. Adhesion was considered as *good* when beetles remained attached to the inverted surface for more than 10 s. When beetles were not able to remain attached for 10 s and fell from the surface, adhesion was estimated as *poor*.

3. RESULTS

3.1. Surface topography of porous substrates

Anodisc samples bear pores of round shape (figure 1). Although pore diameters varied from approximately 220 to 250 nm in each membrane type, they showed no significant difference between samples (one-way analysis of variance (ANOVA) on ranks: $F_{2,99} = 1.900$, $p = 0.155$). However, the difference in the thickness of the walls separating the pores into different anodisc types (ano-28: 181.78 ± 112.10 nm, $n = 76$; ano-42: 127.62 ± 83.84 nm, $n = 76$; ano-51: 74.87 ± 52.15 nm, $n = 94$) was highly significant (one-way ANOVA on ranks: $F_{2,245} = 151.859$, $p < 0.001$), and, therefore, the surface porosity of the samples also differed, being 28, 42 and 51 per cent, respectively.

3.2. Physico-chemical properties of smooth control samples

Glass and sapphire samples showed considerable differences in contact angles of all liquids measured (figure 2*a*). While glass was readily wetted with water, the sapphire had a much higher contact angle with this liquid (Mann–Whitney rank sum test: $T = 15.000$, $p = 0.003$). In the case of another polar liquid, ethylene glycol, a similar tendency was found (Mann–Whitney rank sum test: $T = 15$, $p = 0.003$); however, it was not so pronounced. As for non-

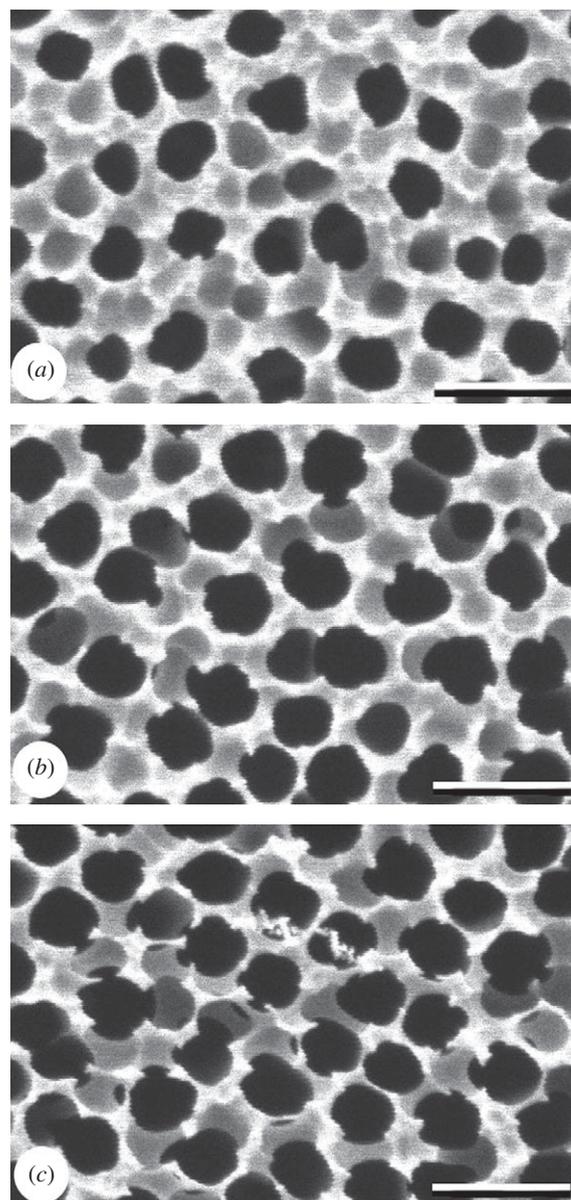


Figure 1. SEM micrographs of porous membranes with porosity of (a) 28%, (b) 42% and (c) 51%. Scale bars: 500 nm.

polar diiodomethane, the contact angle was higher on glass than on sapphire (t -test: $t = 15.149$, $p < 0.001$), although both surfaces were rather unreadyly wetted with this liquid (contact angles greater than 50°).

The surfaces differed essentially in their free surface energy values (figure 2*b*). In the glass sample, it was rather high, with a highly predominating polar component. The free surface energy of the sapphire sample was more than threefold lower, with a high contribution of the dispersion component.

3.3. Fluid absorption on nanoporous anodisc membranes

The behaviour of fluid drops differed essentially on solid samples compared with nanoporous ones. On both glass and sapphire substrates, after a drop had been stabilized, the contact angles changed gradually with time in the case of water (figure 3*a*) or remained almost

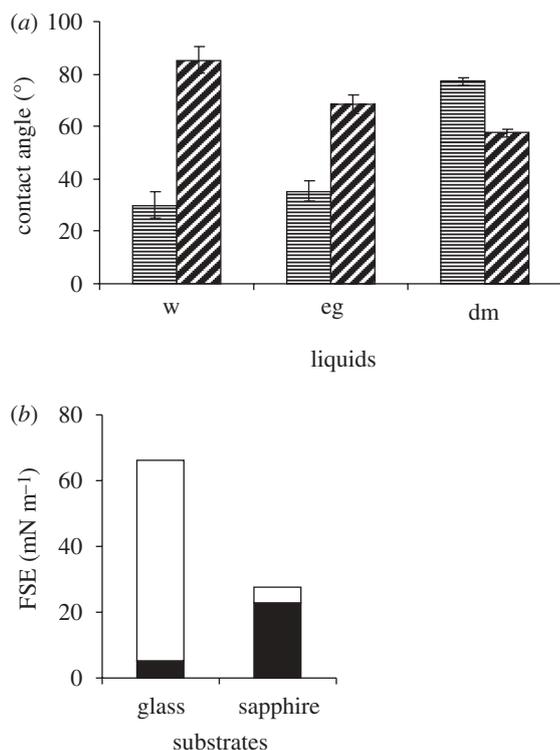


Figure 2. Physico-chemical properties of smooth solid glass and sapphire samples. (a) Contact angles of water (w), ethylene glycol (eg) and diiodomethane (dm). (b) Free surface energy (FSE) and its dispersion and polar components. (a) Bars with horizontal lines, glass; bars with cross shadings, sapphire. (b) Filled bars, dispersion; open bars, polar.

the same in the case of oil (figure 3c). The decrease in contact angle of water had a linear character that apparently indicates the evaporation of the fluid in air.

On all nanoporous membranes, angle–time curves showed two distinctly different parts (figure 3b,d). First, an immediate rapid decrease in the contact angle during the first 5–15 s for water and approximately 20 s for oil occurred, which was especially pronounced on samples with higher porosity. When small angles were achieved, fluids showed linear dependencies of the angle on time similar to those observed with solid samples. Such behaviour of fluid drops may be explained by rapid absorption of fluids by porous media at the beginning of the experiments, which was followed by fluid evaporation in the case of water.

3.4. Structure of the beetle attachment system

The tarsus of *C. septempunctata* is composed of three tarsomeres and is equipped with two claws curved ventrally (figure 4a,d,k,l). Attachment pads belong to the hairy type of locomotory devices in insects. The ventral side of the two first proximal tarsomeres (1–2) bears numerous tiny tenent setae (figure 4b,c,e,f). Among the setae, several types were distinguished: (i) with a pointed, usually sharp tip (figure 4g), (ii) with a flattened and widened end plate (rounded or ellipsoid), called the spatula (figure 4i), (iii) a transitional type, often with a pointed tip and a rather narrow elongated end plate (figure 4h), and (iv) with a flat discoid end plate (figure 4j). There is a distinct sexual dimorphism

in the morphology of adhesive pads: males have all four setae types, whereas females have only the first three types.

While no strong differences were observed in the distribution of different types of setae between tarsomeres in females, males showed noticeable differences. In females, the first tarsomere of each leg is covered with pointed-tipped/transitional setae, which are rather similar in appearance (figure 4e). The second tarsomere has pointed-tipped setae on its proximal and lateral margins, spatula-bearing setae on the distal margin and various transitional setae in the middle (figure 4f).

In males, setae with discoid terminal elements occur either on both tarsomeres (forelegs and midlegs, figure 4a,k) or only on the second one (hindlegs, figure 4l). These setae are situated in the central part of the tarsomere: exactly in the middle (first tarsomere, figure 4b) or somewhat laterally (second tarsomere, figure 4c). The area occupied by this setae type (about 50% of the total tarsomere area) was almost constant between forelegs and midlegs in the case of the first tarsomere (figure 4a,k), but changed considerably in the second tarsomere from 10 to 30 per cent of the total tarsomere area in the series: hindlegs—midlegs—forelegs (figure 4a,k,l). The distribution of other setal types in males' pads was similar to that in females.

3.5. Beetle attachment ability

The traction force—produced by insects on different substrates—ranged from 0.16 to 16.59 mN in males and from 0.32 to 8.99 mN in females (figure 5).

Beetles generated much stronger traction forces on smooth solid surfaces (glass and sapphire samples) than on porous ones (anodisc membranes) with the difference being highly significant (Mann–Whitney rank sum test: $T = 3215$, $p < 0.001$). The comparison (i) between both smooth substrates pooled together with each porous one (Kruskal–Wallis one-way ANOVA on ranks: $H_{3,99} = 72.667$, $p < 0.001$) and (ii) between each smooth substrate with each porous one (Kruskal–Wallis one-way ANOVA on ranks: $H_{4,99} = 73.556$, $p < 0.001$) also showed significantly different values of the traction force.

For smooth surfaces, both the sex of the insect individuals and the surface type affected the force, whereas there was no statistically significant interaction between these two factors (table 1). We measured significantly higher forces on the glass sample than on the sapphire sample (Tukey test after two-way ANOVA: $q = 4.249$, $p = 0.005$; figure 5a). On both smooth surfaces pooled together (Tukey test after two-way ANOVA: $q = 7.294$, $p < 0.001$) and on each smooth surface (Tukey test after two-way ANOVA, glass: $q = 4.269$, $p = 0.005$; sapphire: $q = 6.047$, $p < 0.001$), males performed much better than females. Females showed different forces on glass than on sapphire (Tukey test after two-way ANOVA: $q = 3.894$, $p = 0.009$), whereas males produced similar forces on both samples.

The comparison between different anodisc membranes also showed the influence of the sex of insect individuals and the surface on the traction force and no interaction between these factors (table 2). As for

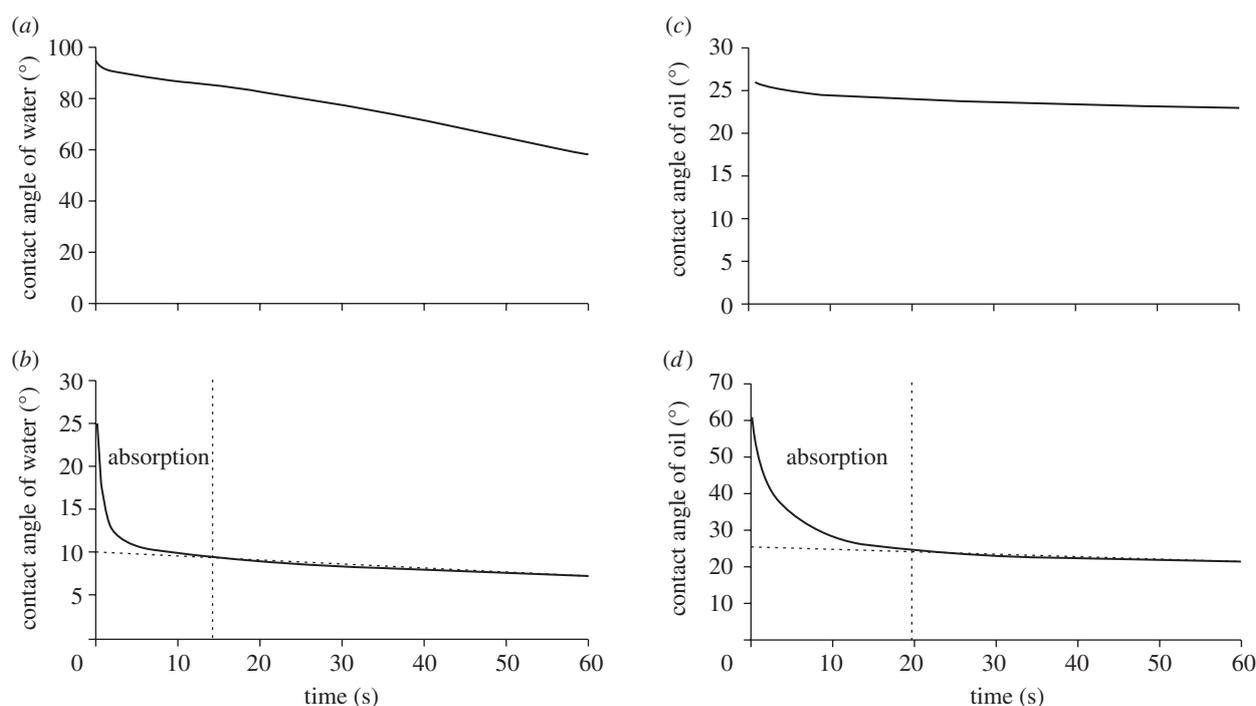


Figure 3. The dynamic of contact angles of water (*a,b*) and oil (*c,d*) on the sapphire sample (*a,c*) and nanoporous membrane with the porosity of 42% (*b,d*). Dotted lines in (*b*) and (*d*) confine parts of curves showing the absorption of fluids.

these surfaces, the only difference found was between the anodisc samples with 28 per cent (higher forces) and 51 per cent porosity (lower forces) (Tukey test after two-way ANOVA: $q = 3.751$, $p = 0.028$; figure 5*b*), whereas the forces were similar in other pairs of surfaces. Females attached much more strongly than males on all porous surfaces pooled together (Tukey test after two-way ANOVA: $q = 4.932$, $p = 0.001$) and on the anodisc membrane with a porosity of 42 per cent (Tukey test after two-way ANOVA: $q = 3.606$, $p = 0.014$). There was no significant difference in traction forces between any pair of surfaces within either males or females.

Experimental insects performed well and showed normal locomotion on both smooth solid glass and sapphire samples. On all porous membranes, beetles usually were not able to get a grip and slid over the surfaces, refused to walk and came to a standstill or even turned over on their backs. When substrates were inverted to both 90° and 180°, insects remained attached to both the glass and sapphire surfaces (*good* adhesion), but failed in cases of all porous samples (*poor* adhesion).

4. DISCUSSION

4.1. Comparison of insect attachment on smooth versus porous substrates

Our data on the traction force of *C. septempunctata* beetles showed that insects, both males and females, performed much better on smooth solid substrates than on porous ones. This effect may be primarily explained by the absorption of insect adhesive fluid. It is known that insects produce and deliver a fluid pad secretion into a contact zone, in order to wet the

contact between each tenent seta and substrate, and to increase adhesion owing to capillary forces (Ishii 1987; Kosaki & Yamaoka 1996; Eisner & Aneshansley 2000; Attygalle *et al.* 2002; Federle *et al.* 2002; Voetsch *et al.* 2002). Since the beetle secretion (or at least a part of it) consists of oily substances, the anodisc membrane surface should be readily wetted with the secretion. We show clearly here that nanoporous substrates can absorb both polar (water) and non-polar (oil) fluids. Therefore, we conclude that, owing to their high porosity, such porous samples absorb the fluid from beetle setal tips, and this may lead to a reduction in insect adhesion force (as suggested previously by the absorption hypothesis according to Gorb & Gorb (2002)).

An alternative explanation of the force reduction on the porous samples is the difference in roughness of the substrates tested (roughness hypothesis according to Gorb & Gorb (2002)). The influence of the surface roughness on insect attachment has been previously revealed in experiments with the fly *Musca domestica* (Diptera, Muscidae) and the beetles *Gastrophysa viridula* and *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae), where insects showed higher forces on smooth artificial substrates than on nano- and micro-rough ones (Gorb 2001; Peressadko & Gorb 2004; Voigt *et al.* 2008; Bullock & Federle 2009; Gorb & Gorb 2009). The critical roughness, reducing the attachment, was 0.3 and 1 μm , whereas surfaces with larger asperity sizes (3, 9, 12 μm) ensured only slightly lower forces than on smooth substrates. Anti-adhesive properties of such micro-rough substrates have been explained by a decrease in the real contact area between surface irregularities and terminal elements of insect setae (Peressadko & Gorb 2004).

In our experiments, porous substrates can be considered as non-smooth (rough) ones. Our data confirm

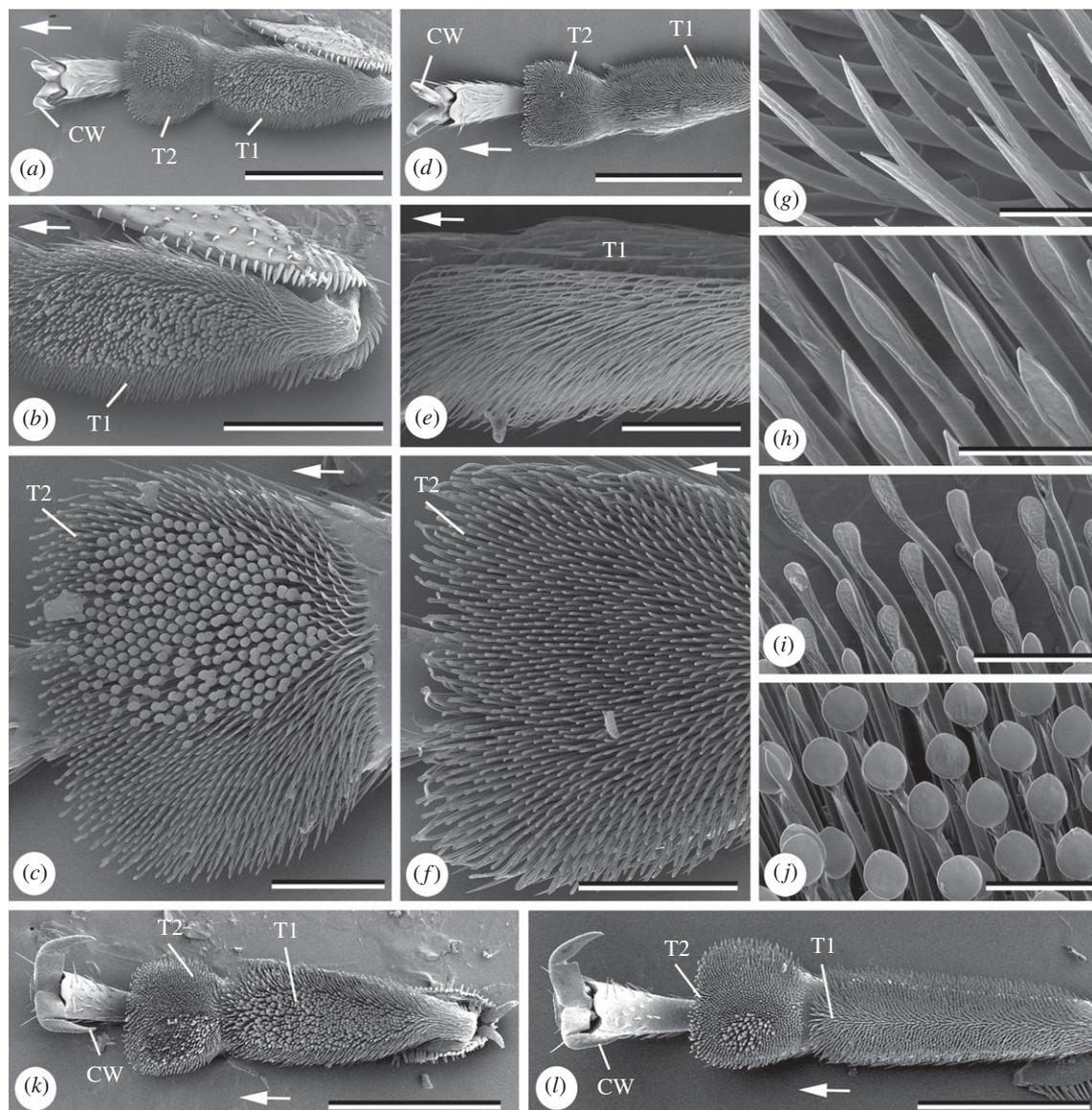


Figure 4. SEM micrographs of attachment devices in the beetle *Coccinella septempunctata*. (a,k,l) Tarsi of forelegs (a), midlegs (k) and hindlegs (l) in males. (d) Tarsus of the foreleg in females. (b,e) The second proximal tarsomere of forelegs in males (b) and females (e). (c,f) The first proximal tarsomere of forelegs in males (c) and females (f). (g–j) Different types of tenent setae. (a–c,g–j): male; (d–f): female. Arrows in (a–f,k,l) show the distal direction. CW, claw; T1, first proximal tarsomere; T2, second proximal tarsomere. Scale bars: 500 μm in (a,d,k,l); 300 μm in (b); 200 μm in (e); 100 μm in (c,f); 20 μm in (i,j); 10 μm in (g,h).

previous results that micro- and nano-roughness of the substrate strongly reduce contact forces of insect attachment devices. All porous (rough) surfaces reduced force by up to 10 times when compared with the smooth solid surface made of the same material. Assuming that the thickness of walls between pores in anodisc samples corresponds to the size of surface asperities in rough samples studied previously, we obtained sizes of 0.18, 0.13 and 0.07 μm for anodisc membranes with 28, 42 and 51 per cent porosity, respectively. Since the dimensions of these structures are close to the critical roughness found previously, we may interpret the reduction in the attachment force by the effect of the substrate roughness.

However, all our substrates have smaller surface structures than those used before (smaller than 0.3 μm). In fact, the rough substrates used in the

present work have filled the gap in information about the performance of insect attachment devices between smooth substrates and those with structure dimensions above 0.3 μm . The results of the present study revealed a drop in the attachment force with a decrease in the size of surface irregularities, found previously for other micro-rough substrates.

4.2. Difference in traction forces between two smooth solid substrates

Although both glass and sapphire substrates were samples with smooth surfaces, beetles generated significantly higher traction forces on the glass sample than on the sapphire sample. We can explain this result by the influence of physico-chemical properties of substrates on adhesion. It is known that the free surface

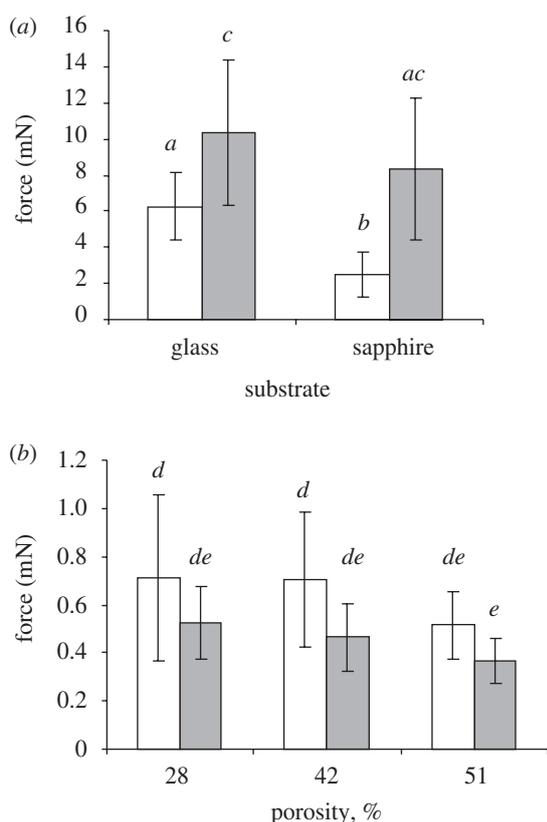


Figure 5. Traction force of male and female beetles *Coccinella septempunctata* on smooth solid (a) and porous surfaces (b). According to the Holm–Sidak method and Tukey test of multiple comparisons of means, performed after one-way ANOVA ($F_{3,39} = 12.141$, $p < 0.001$) and Kruskal–Wallis one-way ANOVA on ranks ($H_{5,59} = 14.690$, $p = 0.0012$), respectively, means with different letters differ significantly from one another. (a,b) Open bars, female; shaded bars, male.

Table 1. Effects of sex and surface on traction force measured on different smooth surfaces (results of the two-way ANOVA: d.f., degrees of freedom; F , the ANOVA test statistics; m.s., mean square; p , probability value; s.s., sum of squares).

source	d.f.	s.s.	m.s.	F	p
sex	1	246.369	246.369	26.605	<0.001
surface	1	83.588	82.588	9.027	0.005
sex * surface	1	7.320	7.320	0.790	0.380

energy of the substrate is an important parameter, affecting adhesion between two contacting bodies (Johnson *et al.* 1971; Kendall 1971). In solids, an increase in surface energy leads to an increase in the real contact area, which results in a high attracting force (Israelachvili 1992). In our study, the decrease in contact angles of polar fluids (especially water) and higher surface energy of the glass substrate correlated with a stronger traction force in the beetles. For the sapphire surface, a lower value of the surface energy resulted in an essential reduction in attachment force. A similar effect has been previously reported for *G. viridula* on hydrophilic and silanized hydrophobic glass (Gorb & Gorb 2009). Centrifugal experiments carried out with

Table 2. Effects of sex and surface on traction force measured on different porous surfaces (results of the two-way ANOVA: d.f., degrees of freedom; F , the ANOVA test statistics; m.s., mean square; p , probability value; s.s., sum of squares).

source	d.f.	s.s.	m.s.	F	p
sex	1	0.541	0.541	12.164	<0.001
surface	2	0.354	0.177	3.972	0.025
surface * sex	2	0.023	0.011	0.256	0.775

C. septempunctata on hydrophilic and hydrophobic glass surfaces (D. Voigt 2008, unpublished data) confirmed our results obtained in traction tests with this insect species.

4.3. Why do males and females perform differently?

For both smooth solid and porous surfaces, the sex of insect individuals was the most important factor influencing the traction force. However, its effect differed between surface types. Males generated considerably higher forces on smooth substrates than females, whereas females performed better than males on rough anodisc membranes. Similar results were obtained previously for the beetles *L. decemlineata* and *G. viridula* on smooth and micro-rough surfaces (Voigt *et al.* 2008; Bullock & Federle 2009).

The difference in the attachment ability to different substrates presumably results from the sexual dimorphism in the microstructure of adhesive pads. Besides setae with spatula-like and pointed tips, males also bear rather large fields of numerous setae with disc-like terminal plates, adapted for holding on to females strongly and for a long time during pairing (Stork 1980b, 1983). The experiments performed on smooth surfaces with artificial polymeric bio-inspired materials have demonstrated that discoid terminal elements provide fast and simple generation of reliable adhesive contact, and can stay ‘passively’ adhered without any external support (Gorb & Varenberg 2007a,b; Varenberg & Gorb 2008). They are more efficient for long attachment, presumably, because of their ability to resist cracks, which appear in the centre of the disc rather than at its edge. It has been shown experimentally that it is necessary to apply high pull-off force to rupture the contact between discoid tips and the smooth surface. Since females of *C. septempunctata* bear only setae with spatula-shaped or pointed tips, they were not able to provide such strong adhesion to a smooth surface as did males.

Spatula-shaped terminal elements are able to generate adhesion only if shear force is applied (Autumn *et al.* 2000; Gorb & Varenberg 2007a,b; Bullock & Federle 2009). Such contacts can be easily ruptured when the shear force stops acting. Therefore, systems with spatula-like tips are well adapted for short-term temporary adhesion during locomotion requiring fast detachment. Moreover, the adaptability of spatula-like plates to rough substrates is presumably higher than in disc-like terminal elements because of the difference

in the terminal plate thickness. Low bending stiffness of the spatula-like terminal plate was theoretically predicted owing to its very flat geometry (according to the model of Persson & Gorb (2003)). Thus, on rough substrates, adhesion in both males and females relied principally on these setae establishing sufficient contact with tiny surface irregularities in islands of solid material between pores. Such geometry and mechanical properties of setal tips provides high adaptability to the profile of rough substrates (Persson & Gorb 2003; Peressadko & Gorb 2004) and, consequently, higher adhesion force than setae with disc-like terminal plates. Therefore, the predominance of spatula-shaped setae in females probably resulted in their higher attachment to rough samples.

For smooth solid substrates, the comparison of surfaces within the sexes showed a significant difference in forces for females. We explain this result by the effect of the differences in the surface energy of smooth solid substrates tested on the adhesion of the spatula-like terminal elements (§4.2). It has been previously shown for artificial mushroom-like micro-contacts that such terminal plate geometry seems to act like a passive suction device (Varenberg & Gorb 2008). Therefore, their adhesion is less dependent on physico-chemical properties of the substrate. Similar effects can be expected in male beetles with similar geometry of the terminal contacts. However, this remains to be experimentally proven in future studies. In females, contact breakage between spatulae and substrate is achieved by peeling (S. N. Gorb 2007, personal observation), the force of which relies on the surface energy of the substrate (Kendall 1971). Surprisingly, geometry is sufficient to explain why we observed no difference in male adhesion between smooth solid substrates with different surface energy. Of course, potential differences in the material chemistry and/or chemical composition of the pad fluid between males and females may also influence attachment abilities of different sexes, but we have no evidence about a chemical difference of their pads. Since all porous samples used in this study were made of the same material (aluminium oxide) and, therefore, must have the same surface energy, we found here no differences in traction forces for both sexes.

The results of our experiments with artificial nanoporous surfaces of anodisc membranes are in agreement with the data obtained previously for other insects on nano- and microstructured artificial and natural substrates, such as plant surfaces covered with prominent epicuticular wax crystals. From this, we can conclude that an essential reduction in insect attachment may be achieved by the submicron substrate structuring, regardless of whether the substrate consists of outgrowths or holes.

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