

RESEARCH ARTICLE

Leaf beetle attachment on wrinkles: isotropic friction on anisotropic surfaces

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SUMMARY

The influence of surface roughness on the attachment ability of insects has been repeatedly reported. In previous experiments, complex surface topographies were used as test substrates, whereas periodical structures have so far been neglected. In the present study, traction experiments with adult beetles *Gastrophysa viridula* and *Leptinotarsa decemlineata* were carried out to study the influence of surfaces, structured with periodical wrinkles, on insect attachment. Force measurements were carried out on male and female insects, both intact and after removal of claws, performing tethered walking on five polydimethylsiloxane substrates: (i) smooth, non-structured (control), (ii–v) structured with wrinkles of different wavelengths (366, 502, 911 and 25,076 nm). In two test series, beetles walked either perpendicular or parallel to the wrinkle alignment. Adults of *G. viridula* produced generally higher forces than those of *L. decemlineata*. The results show that the alignment of wrinkles had no significant influence on the force generation by beetles, probably because of the skewed position of their tarsomeres relative to the substrates. In both sexes, the highest force values were obtained on surfaces with wrinkles of 25 µm wavelength. On other wrinkled substrates, forces were significantly reduced in both males and females compared with the smooth, flat control, with the minimum force achieved on wrinkles with a wavelength of 911 nm.

Key words: cuticle foldings, insect, plant surface, traction force, wrinkles.

INTRODUCTION

Plant surfaces offer terrain for most insect species of the world. The co-evolutionary progress indicates insects' successful attachment and locomotion on a diversity of epidermal plant structures, including for example cell concavities, trichomes, wax crystals and cuticle folds (Southwood, 1986).

The direct influence of different plant surface characteristics on insect attachment has been experimentally shown previously for representatives of Coleoptera: Chrysomelidae (Stork, 1980b; Stork, 1980c; Gorb and Gorb, 2002; Gorb and Gorb, 2006; Gorb and Gorb, 2009), Coccinellidae (Gorb et al., 2005; Gorb and Gorb, 2011), Staphylinidae (Betz, 2002); Diptera: Calliphoridae (Gaume et al., 2004; Gorb et al., 2004), Drosophilidae (Gaume et al., 2002); Hemiptera: Aphididae (Lees and Hardie, 1988), Pyrrhocoridae (Gorb et al., 2004), Miridae (Voigt et al., 2007; Voigt and Gorb, 2010); and Hymenoptera: Formicidae (Federle et al., 1997; Federle et al., 2000; Gaume et al., 2002). Nano-rough plant surfaces covered with wax crystals were reported to prevent attachment in the majority of investigated insect species. Recent studies with artificial, well-defined rough substrates clarified the effect of different surface asperities on insect attachment (Gorb, 2001; Peressadko and Gorb, 2004; Voigt et al., 2008; Al Bitar et al., 2010; Gorb et al., 2010; Bullock and Federle, 2011). The strongest attachment was measured on smooth and rough surfaces having a particle asperity size of >1 µm. The attachment, i.e. friction forces, significantly decreased on substrates with an asperity size of 0.3 and 1.0 µm (root mean square roughness 90.0 and 238.4 nm), which corresponds to the dimensions of plant epicuticular wax crystals. The impact of this roughness was previously explained

by the reduction of the contact area between small surface irregularities and the characteristic size and shape of the setal tips of adhesive pads (Peressadko and Gorb, 2004).

However, periodic anisotropic rough surfaces have not been considered so far in experimental investigations of insect attachment. Such regular structures are present in the natural terrain of insects as cuticle folds on the surfaces of plant flowers, leaves, stems, fruits and seeds (Fig. 1) (Kurer, 1917; Barthlott and Ehler, 1977; Knapp, 1993; Koch et al., 2009; Prüm et al., 2012). There is a large diversity of folding patterns in plants (Bringmann and Kühn, 1955). The dimensions and shape of cuticle folds may vary depending on plant habitat, species, organ and developmental stage. On flower petals, folds occur very frequently, and smooth epidermis can almost never be found (Kurer, 1917). The density of folds has been reported to range between 2 and 35 folds per 20 µm (Barthlott and Ehler, 1977). Recently, the width (*w*), the distance (*d*) and in some cases the height (*h*) of folds have been measured in flower petals of *Viola tricolor* L., Violaceae (260 nm *w*, 450 nm *d*), *Dahlia pinnata* Cav., Asteraceae (390 nm *w*, 210 nm *d*), *Rosa chinensis* Jacq., Rosaceae (410 nm *w*, 210 nm *d*), *Cosmos atrosanguineus* (Hook.) Voss, Asteraceae (600 nm *w*, 460 nm *d*) (Schulte et al., 2011), *Cyclamen persicum* Mill., Primulaceae (500 nm *w*, 500–1500 nm *d*, 500 nm *h*) and on leaf surfaces of *Litchi chinensis* Sonn., Sapindaceae (adaxial: 100–400 µm *w*, irregular distance; abaxial: 500 nm *w*, 500 nm *d*, 500–1000 nm *h*), *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg., Euphorbiaceae (adaxial: 500–1500 nm *w*, 500 nm *d*; abaxial: 2000–5000 nm *w*, 8000–15,000 nm *d*, 8000–14,000 nm *h*) (Prüm et al., 2012). Folds may run (1) without order (randomly oriented), (2) anisotropically,

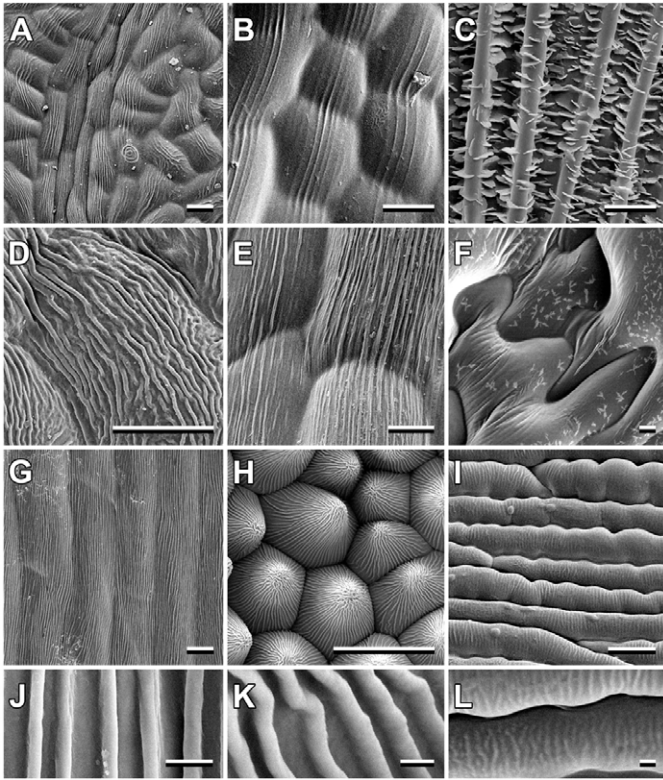


Fig. 1. Cryo-scanning electron microscopy (cryo-SEM) micrographs showing examples of anisotropically oriented cuticle folds on plant surfaces. (A) The upper (adaxial) leaf surface of *Malus domestica* Borkh. 'Boskoop' (Rosaceae). (B) *Rumex obtusifolius* L. (Polygonaceae), main vein on the lower (abaxial) leaf side. (C) The lower leaf surface of *Polygonatum multiflorum* (L.) All. (Asparagaceae). (D,E) *Rheum rhabarbarum* L. (Polygonaceae), upper leaf side (D) and stem (E). (F) *Solanum tarnii* Hawkes and Hjert. (Solanaceae), lower leaf side. (G–L) The upper flower surface (petals) of *Paeonia* Hybr. (Paeoniaceae) (G,J), *Cardamine pratensis* L. (Brassicaceae) (H,K), and *Taraxacum officinale* L. (Asteraceae) (I,L). Scale bars: A,B,D,E,G,H,I 20 μ m; C,F,J,L 2 μ m; K 200 nm.

predominantly in one direction or (3) regularly parallel (Barthlott and Ehler, 1977). Several functions are reported for plant cuticle folds: (1) to stabilise thin cell walls, (2) to decrease wettability with water and contamination by both cuticle hydrophobicity and its microscopic sculpture (contact area reduction) and (3) to set-up surface reflection properties (Barthlott and Ehler, 1977; Koch et al., 2009). In addition, (4) in hierarchically, anisotropically structured surfaces of carnivorous plant traps and kettle trap flowers, cuticular folds in combination with downward pointing papillae or ideoblasts are assumed to interfere with insect attachment, probably by preventing claw hooking, and creating a micro-roughness, thus reducing the contact area of insect adhesive pads (Pohl, 1927; Ehler, 1974; Poppinga et al., 2010). In traction experiments with different plant species, traction forces of male Colorado potato beetles were reduced by an average of 88% on hydrophobic surfaces with isotropic folds of about 0.5–14 μ m in height, 0.5–2 μ m in width and a distance of 0.5–15 μ m, compared with smooth plant surfaces (Prüm et al., 2012).

Entomophilous flower surfaces have generally been observed to provide structures promoting attachment for pollinators (Kugler, 1970; Kevan and Lane, 1985; Whitney et al., 2009; Whitney et al., 2011; Koch et al., 2010). Because anisotropic cuticle folds occur

frequently on flower surfaces, one could suppose them to be involved in the attachment support for pollinators. We wonder whether and how anisotropic cuticle folds of different dimensions and orientation influence foot attachment during insect locomotion in different directions relative to the orientation of the folds.

In order to clarify the impact of periodical surface topography, we used artificially wrinkled surfaces having different wavelengths but identical surface chemistry. The surfaces were created by controlled wrinkling of elastomeric substrates: wrinkles with well-defined wavelength can be produced by coating an elastomeric material with a thin hard layer and exposing the system to strain. This effect provides a versatile approach for structuring surfaces on the micrometre or submicrometre scale (Schweikart and Fery, 2009; Schweikart et al., 2010a). As periodicities of these artificial surface wrinkles correspond well to cuticle folds of flowers (Schweikart et al., 2010b), they provide suitable, comparable substrates for attachment experiments with insects on well-defined periodic surface structures without the influence of surface chemistry, which strongly varies in different plant species.

Previously, in comparison to a smooth, flat surface, a decrease in sliding friction force was observed for a rigid spherical lens, sliding over a surface-wrinkled, elastomeric substrate (Rand and Crosby, 2009). The decrease was greater for sliding perpendicular rather than parallel to the wrinkles. Moreover, the average sliding force was directly proportional to the applied load for both wrinkled and non-wrinkled surfaces.

In the present study, we measured traction forces of two leaf beetle species (Chrysomelidae) on structured polymer surfaces, in order to ascertain the influence of wrinkle dimensions and orientation relative to the direction of beetle movement on the force generation by intact and clawless adult beetles. Two leaf beetle species of different body weight and adhesive pad dimensions were compared.

MATERIALS AND METHODS

Insects

Leaf beetles *Gastrophysa viridula* De Geer and *Leptinotarsa decemlineata* Say (Coleoptera, Chrysomelidae) were used in the experiment. Imagos of both species were obtained from laboratory rearings 5 days after hatching from pupae [*G. viridula* (see Voigt et al., 2011); *L. decemlineata*: Bayer CropScience AG, BCS-R-I-BI-SE-E, Entomology, Bayer CropScience AG, Monheim, Germany]. During experiments, beetles were kept in ventilated plastic containers with ceaprene stoppers (16 ml; Greiner Bio-One GmbH, Frickenhausen, Germany) at 22 \pm 0.8°C and 32 \pm 3.1% relative humidity (RH). The structure and performance of tarsal attachment devices in both beetle species have previously been well studied (e.g. Peressadko and Gorb, 2004; Schuppert et al., 2005; Voigt et al., 2008; Voigt et al., 2010; Gorb and Gorb, 2009; Bullock and Federle, 2009; Bullock and Federle, 2011; Clemente et al., 2010; Hosoda and Gorb, 2011; Prüm et al., 2012). Both species bear spatula-shaped lanceolate and filament-shaped tarsal adhesive setae in males and females, and additionally setae with discoid terminal tips only in males (Stork, 1980a; Pelletier and Smilowitz, 1987).

Substrates

Wrinkled substrates were created by uniaxial stretching of a silicon elastomer, polydimethylsiloxane (PDMS), exposing it to an oxygen plasma in order to convert the surface to silica, and subsequent relaxation. Stable wrinkles with one dominant wavelength were obtained. Wrinkles were aligned perpendicular to the applied strain (Bowden et al., 1999). Both the wavelength and the amplitude could be controlled *via* the plasma dose applied (Pretzl et al., 2008). In

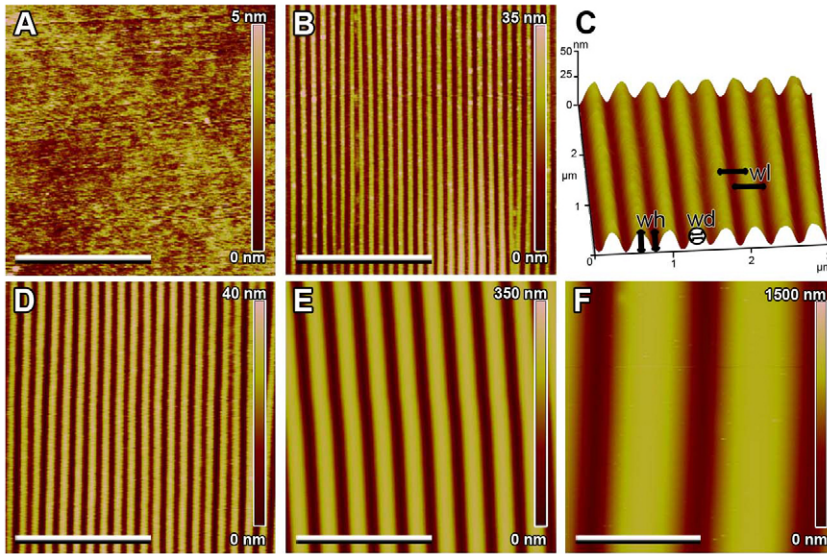


Fig. 2. Atomic force microscopy (AFM) images (tapping mode) of polydimethylsiloxane (PDMS) surfaces. (A) Non-structured control surface, average surface roughness $R_a < 1$ nm. (B–F) Wrinkled surfaces show a sinusoidal surface topography with further surface roughness on the nanoscale, which can be neglected when compared with the height of the wrinkles (Volinskii et al., 2000; Chung et al., 2011). λ : B, C 366 nm; D 502 nm; E 911 nm; F 25,076 nm. wh, wave height; wd, wave tip diameter; wl, wavelength. Scale bars: A, B, D, E 5 μ m; F 30 μ m.

detail, the PDMS elastomer was prepared by mixing Sylgard 184 (Dow Corning, Midland, MI, USA) with a 10:1 ratio by mass of pre-polymer to curing agent. The mixture was stirred and poured into a carefully cleaned, plain glass dish. After 24 h at room temperature and baking at 60°C for 2 h, the cross-linked PDMS was cut into 30×60×2 mm (width×length×thickness) strips. These were stretched along the long side of the PDMS sample in a custom-made apparatus to a linear strain of 25%. The stretched substrates were exposed for variable times to an oxygen-plasma at 0.2 mbar (20 kPa) using a plasma etcher operating at 0.1 kW (flecto10, Plasma Technology GmbH, Herrenberg-Gültstein, Germany) to convert the topmost layer to silica. For the largest periodicities, oxidation was carried out by a UV-ozone treatment using a commercial UV-ozone system (PSD Pro Series, Novascan, Arnes, IA, USA) and an exposure time of 30 min. In all cases, after cooling, the strain was slowly released from the specimen and stable uniform wrinkles were obtained and evaluated with an atomic force microscope (Veeco Dimension 3000 AFM, Veeco Instruments GmbH, Mannheim, Germany) operating in tapping mode. The two treatments result in comparable, silica-like surface structures. Surfaces are hydrophilic after surface oxidation and subsequently turn moderately hydrophobic because of contamination in air. Typical contact angles on surfaces exposed to identical treatment are around 60 deg for laboratory conditions (A.S., personal observation). The elasticity modulus of PDMS is of the order of 1 MPa and it is not significantly altered by the plasma/UV/ozone treatment as the surface layers introduced by these procedures are of nanoscale dimensions (Erath et al., 2010). A smooth, non-structured control surface was fabricated by cutting a 30×60×2 mm strip of cross-linked PDMS. To ensure comparable surface chemistry

of the wrinkled samples, the specimen was exposed to oxygen plasma for 10 s. AFM measurements revealed an average surface roughness (R_a) less than 1 nm.

Five different substrates were prepared for traction force experiments: a smooth, non-structured control surface (i), and wrinkled samples having wavelengths of (ii) 366 nm, (iii) 502 nm, (iv) 911 nm and (v) 25,076 nm (Fig. 2, Table 1).

Traction force experiment

Traction force measurements were carried out as previously described (Gorb et al., 2004). Insects were carefully clamped with tweezers, and attached to a 10 cm long, thin polyamide monofilament sewing thread (Transfil 70; Amann & Söhne GmbH and Co. KG, Bönningheim, Germany) with a small molten wax droplet. Their elytra were glued together. Then, beetles were weighed using an analytical balance (AG 204 Delta Range; Mettler Toledo GmbH, Greifensee, Switzerland). The free end of the thread was connected to the force sensor. Using a FORT-10 force transducer (10 g capacity, Biopac Systems Ltd, Santa Barbara, CA, USA), the traction force was measured in males and females of both beetle species (*G. viridula* and *L. decemlineata*). Individuals of each species were first measured intact and again after their claws were amputated. Beetles were immobilised by 1 min CO₂ anaesthesia to enable the amputation of claws using razor blades, then allowed 2 h recovery time before the experiment was resumed.

Each specimen was tested (1) intact, walking on the non-structured surface (i), (2) intact, walking on structured surfaces (ii–v) perpendicular to wrinkles, (3) intact, walking on structured surfaces (ii–v) parallel to wrinkles, (4) clawless, walking on the non-structured surface (i), (5) clawless, walking on structured surfaces

Table 1. Surface profile characteristics (nm)

PDMS sample no.	Mean wavelength (error)	Mean wave height (error)	Wave tip diameter (mean \pm s.d.)
i (non-structured)	–	–	–
ii	365.8 (18.3)	20.5 (3.6)	135 \pm 19
iii	501.6 (31.7)	29.7 (4.5)	238 \pm 21
iv	911.3 (402.6)	255.6 (3.0)	496 \pm 34
v	25,076 (1392.4)	847.7 (76.9)	10,474 \pm 398

See Fig. 2C for definitions of wavelength, wave height and wave tip diameter ($N=10$). PDMS, polydimethylsiloxane.

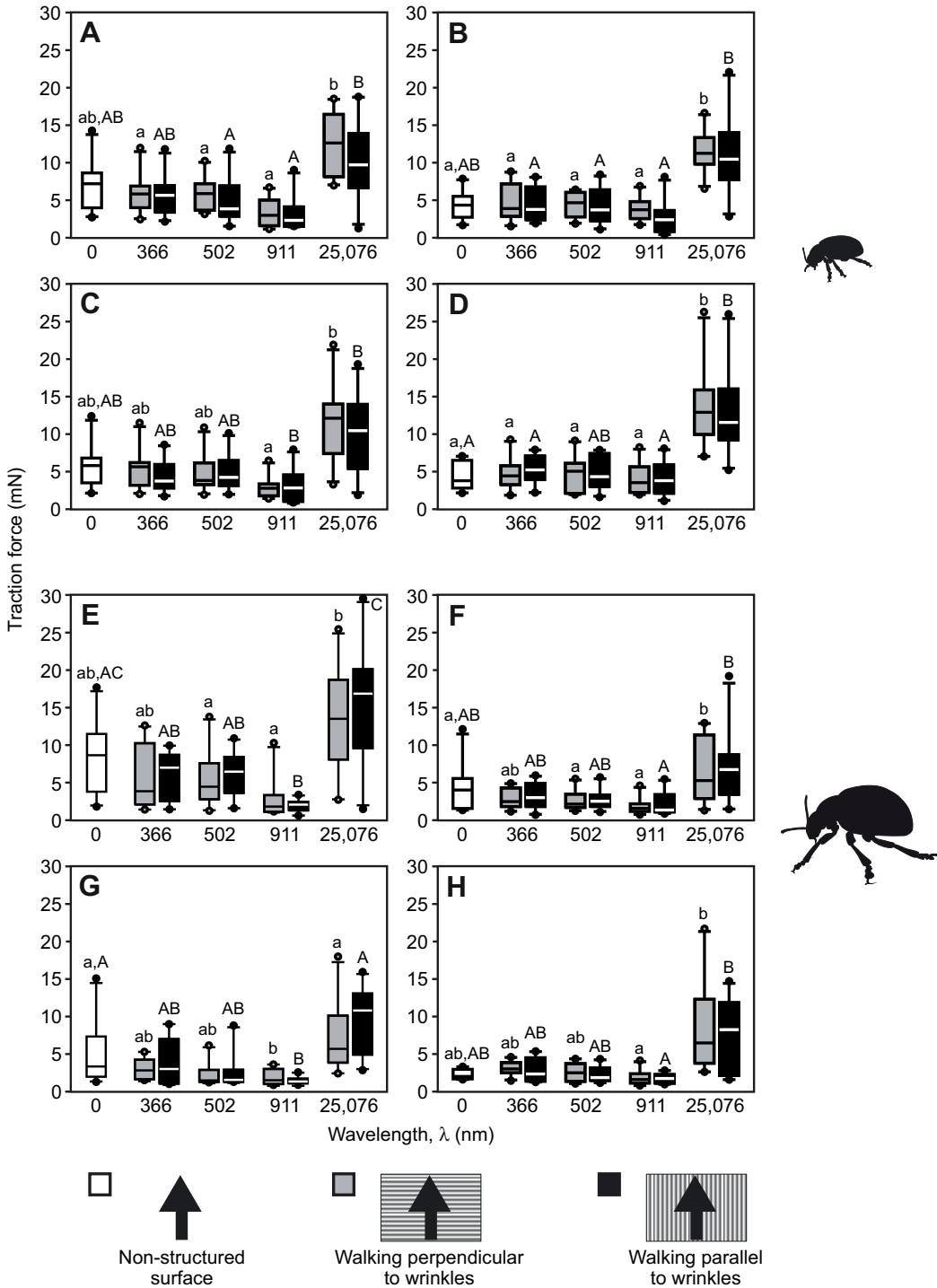


Fig. 3. Forces generated by male (A,C,E,G) and female (B,D,F,H) *Gastrophysa viridula* (A–D) and *Leptinotarsa decemlineata* (E–H), intact (A,B,E,F) and clawless (C,D,G,H) beetles in traction force experiments on smooth, flat and structured PDMS surfaces. Different letters indicate statistical differences between force values generated by beetles walking perpendicular (lowercase) and parallel (uppercase) to the wrinkle alignment within a single subfigure. See Table 2 for statistics considering differences in traction forces on wrinkled surfaces between different wavelengths, and Results for differences in traction forces between intact and clawless, male and female beetles, traction parallel and perpendicular to wrinkles, and between species.



(ii–v) perpendicular to wrinkles, and (6) clawless, walking on structured surfaces (ii–v) parallel to wrinkles.

The different substrates (i–v) were presented randomly during the experiment. Using AcqKnowledge 3.7.0 software (Biopac Systems Ltd), force–time curves were recorded to estimate the maximum traction force produced by a single beetle during three consecutive runs at 5 s per test substrate. On each surface, 10 males and 10 females per species were tested individually. In total, 720 individual tests with 40 different individuals were carried out: $N=9$ surfaces (I, ii–v perpendicular to wrinkles, ii–v parallel to wrinkles), $n=20$ individual tests per species, (1) intact and (2) without claws. Kruskal–Wallis one-way ANOVA on ranks followed by all pair-wise multiple

comparison procedures (Tukey test) was applied to determine differences in forces between test surfaces (SigmaStat 3.1.1, Systat Software, Inc., Richmond, CA, USA). Mann–Whitney rank sum test (MWT) indicated statistical differences between (a) walking directions perpendicular and parallel to wrinkles, (b) males and females, (c) intact and clawless individuals, and (d) the two species.

RESULTS

Body weight varied significantly between species (for both males and females: MWT, $t=55$, $P\leq 0.001$) and between sexes in *L. decemlineata* (MWT, $t=59$, $P\leq 0.001$). Adults of *L. decemlineata* (males 80.4 ± 13.55 mg, females 120.0 ± 17.20 mg) were 8–10 times

Table 2. Statistical differences in traction forces on wrinkled surfaces between different wavelengths (Kruskal–Wallis ANOVA on ranks)

Species	Sex	Walking direction to wrinkle alignment	Beetle condition	H-statistics, P-value
<i>Gastrophysa viridula</i>	Male	Perpendicular	Intact	$H_{4,45}=25.4, P\leq 0.001$
			Clawless	$H_{4,45}=21.9, P=0.003$
		Parallel	Intact	$H_{4,45}=16.2, P\leq 0.001$
	Female	Perpendicular	Clawless	$H_{4,45}=15.0, P=0.005$
			Intact	$H_{4,45}=22.6, P\leq 0.001$
		Parallel	Intact	$H_{4,45}=21.8, P\leq 0.001$
<i>Leptinotarsa decemlineata</i>	Male	Perpendicular	Clawless	$H_{4,45}=19.1, P\leq 0.001$
			Intact	$H_{4,45}=20.4, P\leq 0.001$
		Parallel	Intact	$H_{4,45}=18.6, P\leq 0.001$
	Female	Perpendicular	Clawless	$H_{4,45}=20.2, P\leq 0.001$
			Intact	$H_{4,45}=21.9, P\leq 0.001$
		Parallel	Clawless	$H_{4,45}=23.7, P\leq 0.001$
		Perpendicular	Intact	$H_{4,45}=12.8, P=0.012$
			Clawless	$H_{4,45}=22.1, P\leq 0.001$
		Parallel	Intact	$H_{4,45}=13.0, P=0.011$
		Clawless	$H_{4,45}=12.9, P=0.012$	

See also Fig. 3.

heavier than those of *G. viridula* (males 10.2 ± 1.88 mg, females 12.5 ± 3.46 mg).

In traction experiments on structured surfaces having the same wavelength, no statistical differences in force between walking directions (perpendicular and parallel to the wrinkle alignment) were found. However, the dimensions of the wrinkles significantly influenced the traction of beetles (Fig. 3, Table 2). Individuals of both species performed statistically best on the substrate structured with wrinkles of 25,076 nm wavelength, independently of the alignment of the wrinkles. The lowest forces were measured on wrinkles of 911 nm wavelength. Intact *L. decemlineata* males performed statistically better than clawless individuals on the same substrate in walking perpendicular (MWT, $t=74, P=0.021$) and parallel (MWT, $t=140, P=0.009$) to wrinkle alignment at 502 nm wavelength. Forces of males were significantly higher than those of females. Forces differed in intact (MWT, $t=78, P=0.045$) and clawless (MWT, $t=77, P=0.038$) *L. decemlineata* on the non-structured, flat substrate.

Gastrophysa viridula generated significantly higher traction forces than *L. decemlineata* on the smooth substrate (clawless females, MWT, $t=71, P=0.011$), and on structured substrates when walking perpendicular to wrinkle alignment at wavelengths of 366 nm (clawless males, MWT, $t=-137, P=0.017$), 502 nm (clawless males, MWT, $t=69, P=0.007$; intact females, MWT, $t=136, P=0.021$), 911 nm (intact females, MWT, $t=24, P=0.002$) and 25,076 nm (intact females, MWT, $t=136, P=0.021$), and when walking parallel to wrinkle alignment on wavelengths of 366 nm (clawless females, MWT, $t=137, P=0.017$), 502 nm (intact females, MWT, $t=133, P=0.038$; clawless females, MWT, $t=73, P=0.017$) and 911 nm (intact females, MWT, $t=71, P=0.011$; clawless females, MWT, $t=133, P=0.038$).

DISCUSSION

Wrinkle alignment and beetle attachment

Traction force corresponds to the friction between the insect tarsi and the substrate. In our experiments, beetles generated force during walking by pulling horizontal to the surface. That may be the reason why no significant difference in the traction force of both tested beetle species between the parallel and perpendicular anisotropic wrinkle alignment was found. Another explanation for the similar performance of beetles walking perpendicular and parallel to wrinkle alignment is the skewed position of the beetle feet during walking and traction (Fig. 4). An influence of anisotropic surface structures on friction forces is actually to be expected (Rand and Crosby, 2009) because of the previously reported distal and distolateral orientation of adhesive setae resulting in the anisotropy of frictional forces in hairy attachment pads (Niederregger et al., 2002; Bullock and Federle, 2009). Although the body axis of leaf beetles in our experiments is obviously aligned perpendicular or parallel to the wrinkles, feet and single tarsomeres are not oriented in the same way. Independently of the surface structure, each tarsus adheres to the substrate in a discrete position and orientation that differ from those of the other tarsi. As shown in Fig. 4, tarsi of forelegs and hindlegs are frequently twisted at an angle of about 45 deg, and those of midlegs at an angle of about 90 deg relative to the body axis (which is aligned along or across the wrinkle axis). Additionally, the positions of different, flexibly joined tarsomeres on a single tarsus may differ from each other. Tarsomeres were observed permanently tilting and shearing, even in obviously resting beetles (D.V., unpublished data), and even when attached to the ceiling (Fig. 4C). Thus, the alignment of adhesive setae of the walking beetle never corresponds to the alignment of surface wrinkles. That is why we did not find any anisotropic effects of

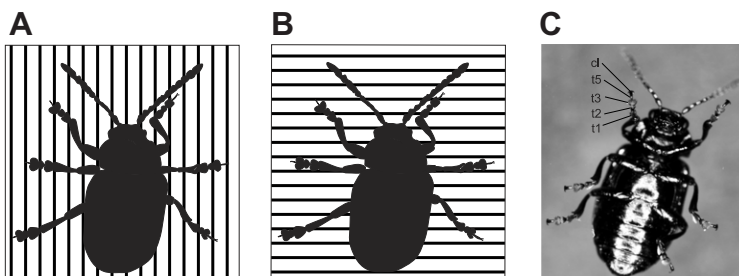


Fig. 4. (A,B) Schematic top view showing the position of the horizontally walking beetle and its feet on structured substrates in the traction experiment, parallel (A) and perpendicular (B) to wrinkles. Legs on the left side of the body are drawn without claws. (C) A digital image of the beetle *G. viridula* adhering upside down to a horizontal Plexiglas® surface. Feet are held at narrower angles to the body. Note the irregular alignment of the different feet (tarsi) and feet segments (tarsomeres) in A–C. cl, claw; t1–5, tarsomeres 1–5 (t4 is not visible in the picture).

surface structures on the beetle traction force. Leaf beetles performed similarly on horizontal, anisotropically wrinkled surfaces (present study) and horizontal, isotropically rough ones (Peressadko and Gorb, 2004; Voigt et al., 2008; Bullock and Federle, 2009; Gorb and Gorb, 2011).

On plant surfaces, which prevent or promote insect attachment, wrinkles (i.e. cuticle folds) are frequently involved in surface structure complexes, consisting of several hierarchical levels. For example, the combination of convex epidermal cells or papillae and a coverage of cuticle folds may result in superhydrophobic surfaces, occurring in flower petals (Koch et al., 2008; Koch et al., 2010; Schulte et al., 2009; Schulte et al., 2011), and anti-adhesive properties in traps of carnivorous plants (Bohn and Federle, 2004; Poppinga et al., 2010). We assume there would be a different impact of wrinkle alignment on insect attachment in combination with other prominent surface structures in contrast to the smooth, flat, wrinkled surfaces used in the present study. This assumption is supported by previous reports on flower petal surfaces, where conical cells act as tactile cues for pollinating bees and bumblebees (Kevan and Lane, 1985; Whitney et al., 2009; Whitney et al., 2011), because those conical cells are frequently covered with cuticle folds. Probably, the feet of flower-visiting insects, which are known to walk and attach properly on prominently wrinkled petal surfaces, are more strongly adapted to cuticle folds than those of the beetle species used in the present study. To test this proposal and the flower surface selectivity for the attachment of specific insect species, further studies should explicitly include insect pollinators.

Wrinkle dimensions and beetle attachment

Wrinkle wavelength and amplitude have previously been reported to influence the adhesive properties of a wrinkled surface in experiments measuring the maximum separation force of a flat cylindrical probe from wrinkled surfaces (Davis and Crosby, 2011).

Leaf beetles of both species generated the highest forces on the substrate with the most prominent wrinkles (wavelength 25,076 nm, wave height 848 nm, wave tip diameter 10,474 nm; significant result) (Fig. 3). The wavelength and wave tip diameter apparently correspond to roughness values of surfaces where claw interlocking has previously been discussed in studies using centrifugal force measurements (Peressadko and Gorb, 2004; Voigt et al., 2008; Al Bitar et al., 2010; Bullock and Federle, 2011). However, our results showed no significant importance of claws on the tested wrinkled surfaces, notwithstanding the fact that beetles tended to generate lower forces after claw removal. For the successful mechanical claw interlocking with the substrate texture, the average asperity diameter has to be much bigger than the insect claw tip diameter (Dai et al., 2002). Accordingly, beetles in our study were able to grasp the irregularities on the most prominently wrinkled surface, when comparing the wave tip diameter of about 10,500 nm and the claw tip diameter of about 210 nm in *L. decemlineata* and 190 nm in *G. viridula* (D.V., unpublished data).

Other effects may also contribute to the obtained results. The wave height roughly corresponds to the R_z value (the average roughness of n individual roughness depths over a specified length) of previously studied substrates, which have been shown to decrease insect attachment, particularly at a so-called critical roughness between 0.3 and 1.0 μm of nominal asperity size (Peressadko and Gorb, 2004; Voigt et al., 2008; Al Bitar et al., 2010). Possibly, the depth of spaces between asperities and not necessarily their size is the crucial factor for claw interlocking.

However, data obtained in this study allowed us to deduce that tarsal adhesive setae dominate force generation in beetles on the

tested surfaces. Attachment to rough substrates is ensured by the presence of spatula-shaped thin tips of setae, providing an optimum adaptability to substrates as a result of the low bending stiffness and viscoelastic behaviour of the terminal plate (Persson and Gorb, 2003; Gorb and Varenberg, 2007; Eimüller et al., 2008; Varenberg et al., 2011). We assume that such an optimum setal tip adaptation to the substrate with the largest wrinkles exists. On smaller wrinkles, traction forces significantly decreased, with the minimum force at 911 nm wavelength, 256 nm wave height and 496 nm wave tip diameter. A similar decrease of friction force has previously been reported from centrifugal force experiments on surfaces with nominal asperity sizes from 0.05 to 1 μm for *G. viridula* (Peressadko and Gorb, 2004; Bullock and Federle, 2011) as well as of 0.3 and 1 μm for *L. decemlineata* (Voigt et al., 2008). The effect has been explained by the reduction of setal contact area between small surface irregularities and the characteristic size and shape of setal tips (Peressadko and Gorb, 2004). Similarly, males of Colorado potato beetles generated about 88% lower traction forces on hydrophobic plant surfaces with cuticle folds in comparison to smooth surfaces (Prüm et al., 2012). Folds were about 0.5–14 μm in height, 0.5–2 μm in width, had a distance of 0.5–15 μm , and roughly corresponded to the wrinkled PDMS surfaces that resulted in a decrease of traction forces of the beetles in our study. However, the data cannot be directly compared because the plant surfaces also varied in their material properties and wettability.

Beetle performance

Although one would expect that the larger, 8–10 times heavier *L. decemlineata* should have more muscle mass and generate higher forces than *G. viridula*, the latter performed significantly better in traction force experiments on 50% of the tested substrates (Fig. 3). These results contradict previous ones on the same two beetle species tested in centrifugal force experiments, where hydrophobic, rigid, fractal rough resin surfaces were used (Peressadko and Gorb, 2004; Voigt et al., 2008; Bullock and Federle, 2011). In the present study we used hydrophobic, elastomeric PDMS substrates structured with wrinkles. According to Rand and Crosby, the average sliding force of a rigid lens on flat and wrinkled, elastomeric PDMS surfaces is directly proportional to the applied load (Rand and Crosby, 2009), which would also lead us to expect *L. decemlineata* to be stronger. But in contrast to the rigid lens, tarsal adhesive setae are rather viscoelastic and have specific geometry and dimensions depending on the insect species. The better performance of *G. viridula* can probably be attributed to its smaller and thinner setae (tip width 4 μm , tip thickness 0.19 μm) compared with those of *L. decemlineata* (tip width 9 μm , tip thickness 0.21 μm) [data obtained from figures in our previous publications (Voigt et al., 2008; Gorb and Gorb, 2004)]. Narrower, thinner setae are much more adaptable to substrate irregularities, forming more intimate contact with the surface.

Generated traction forces ranged from 2.7 mN in intact females on the 911 nm wavelength surface to 13.2 mN in clawless females on the 25,076 nm wavelength surface in *G. viridula*, and from 1.4 mN in clawless males on the 911 nm wavelength surface to 15.6 mN in intact males on the 25,076 nm wavelength surface in *L. decemlineata*. These force values correspond to 22–106 times (*G. viridula*) and 2–19 times (*L. decemlineata*) body weight. These values are commonly named safety factors. The values obtained for *G. viridula* are comparable to those measured in previous centrifugal force studies on rough epoxy resin substrates (Peressadko and Gorb, 2004); however, they are higher than those recently found in another study (Bullock and Federle, 2011). Adults of *L. decemlineata* performed distinctly worse on anisotropically wrinkled PDMS

surfaces than on fractal rough, resinous ones in previous studies (Voigt et al., 2008). This knowledge helps towards the development of materials preventing beetle attachment in the context of Colorado potato beetle control.

Males and females of *G. viridula* generated similar traction forces on all test surfaces (Fig. 3). Males of *L. decemlineata* were statistically stronger on the non-structured, flat substrate. These data confirm our previous results (Voigt et al., 2008). But in contrast to previous findings on Spurr epoxy resin, adults of both leaf beetle species showed generally less attachment ability with the PDMS substrate. Reasons for this may be found in the elastic nature of PDMS (Mata et al., 2005) and complex interface molecular mechanisms influencing the friction behaviour (Bistac and Galliano, 2005).

Moreover, the surface free energy (SFE) differs distinctly between Spurr (SFE 27.3 mN m⁻¹, disperse components 17.6 mN m⁻¹, polar components 9.7 mN m⁻¹; D.V., unpublished data) and PDMS [SFE 19.9 mN m⁻¹, disperse components 17.9 mN m⁻¹, polar components 2.0 mN m⁻¹ (Owen, 1998)]. The low SFE value and very small polar portion of PDMS suggest limited free bonding forces and low wettability. Thus, beetle tarsal adhesive setae and the tarsal adhesion-mediated fluid cannot form an intimate contact with the PDMS surface, resulting in relatively low traction forces.

Conclusion and outlook

The present study shows the ability of insects to attach to a variety of surface structures of different geometries, dimensions and material properties. Male and female *G. viridula* and *L. decemlineata* leaf beetles attached and pulled on anisotropically wrinkled surfaces in both a perpendicular and parallel direction. We explain the obtained isotropic force data on anisotropic wrinkled surfaces as being due to the skewed positions of their tarsi and tarsomeres relative to the orientation of wrinkles during walking.

Gastrophysa viridula, bearing smaller and thinner adhesive setae, seem to adapt better to the surfaces covered with wrinkles than *L. decemlineata*. Generally, the wrinkle dimensions significantly influenced the traction force of beetles. The minimum force on the surface with a wavelength of 911 nm may potentially contribute to the development of artificial surfaces with anti-adhesive properties, for example for designing insect traps or protective foils.

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