Underwater locomotion in a terrestrial beetle: combination of surface de-wetting and capillary forces

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For the first time, we report the remarkable ability of the terrestrial leaf beetle *Gastrophysa viridula* to walk on solid substrates under water. These beetles have adhesive setae on their feet that produce a secretory fluid having a crucial role in adhesion on land. In air, adhesion is produced by capillary forces between the fluid-covered setae and the substrate. In general, capillary forces do not contribute to adhesion under water. However, our observations showed that these beetles may use air bubbles trapped between their adhesive setae to walk on flooded, inclined substrata or even under water. Beetle adhesion to hydrophilic surfaces under water was lower than that in air, whereas adhesion to hydrophobic surfaces under water was comparable to that in air. Oil-covered hairy pads had a pinning effect, retaining the air bubbles on their feet. Bubbles in contact with the hydrophobic substrate de-wetted the substrate and produced capillary adhesion. Additional capillary forces are generated by the pad’s liquid bridges between the foot and the substrate. Inspired by this idea, we designed an artificial silicone polymer structure with underwater adhesive properties.

**Keywords:** adhesion; beetle; biomimetics; bubble; de-wetting; under water

1. INTRODUCTION

Some beetle species can walk freely on flat vertical surfaces such as smooth plant leaves. This ability is attributed to the presence of specialized adhesive setae on their tarsi [1]. These structures were previously studied by scanning and transmission electron microscopy [2,3], and their adhesive forces were measured using force transducers [4]. Beetle adhesive setae are supplemented with a liquid secretion that is responsible for generating capillary forces on various surfaces [5,6]. Earlier studies have demonstrated that beetle pads adhere well to dry substrates [1,4]. However, the ability of terrestrial insects to walk on smooth surfaces under water has not been previously demonstrated. In nature, plants may be covered by water for quite a long period of time, especially after heavy rain. Tropical insects that inhabit rain forests may regularly encounter this problem.

Underwater adhesion is difficult owing to the problem of displacing water from the adhesive interface and the ability of water to decrease the strength of chemical bonds [7]. Nevertheless, nature provides examples of underwater attachment based on complex polymer glues [7–10]. Recently, geometry-related effects have been shown to contribute to underwater adhesion, such as a mushroom-shaped fibrillar microstructure that significantly enhanced a suction effect under water [11].

Animals capable of attachment to the water–solid interface may be capable of controlling the surface wettability. In many cases, such biological surfaces are covered by fine microstructures and waxy hydrophobic materials. Many plant and animal surfaces have a combination of surface geometry and chemistry to keep the surface water-repellent. Some super-hydrophobic plant surfaces have a self-cleaning property [12]. Water-repellent feet of water striders enable locomotion on the water’s surface [13]. Backswimmers from the genus *Notonecta* and midges *Clunio* are capable of keeping an air bubble attached to the surface for breathing under water [14,15]. This air bubble is affected by hydrostatic pressure depending on the depth of the animal under water. In the case of the midge *Clunio*, the bubble is stable up to a depth of 3 m [15].

In the present study, we discovered, for the first time, the remarkable phenomenon of the underwater adhesion ability, based on the trapped air bubble, in the terrestrial chrysomelid beetle *Gastrophysa viridula* (figure 1). Using an experimental approach on living beetles, we clarified the physical background of this ability based on the combination of (i) microstructured surfaces keeping an air bubble under water, (ii) the de-wetting property of such a bubble, and (iii) adhesion generated by the air bubble itself, and additionally by capillary forces, possibly mediated by oil bridges of beetle setae on the islands of de-wetted substrate.

We also designed an artificial microstructured polymer structure with a similar ability and tested it, using various surfaces. The ‘air bubble effect’ was estimated quantitatively using a force transducer. We aimed to verify the effectiveness of the structure for generating underwater adhesion on smooth substrates with various surface energies. This discovery and our biomimetic prototypes may provide an unconventional method for the development of underwater adhesives.
2. MATERIAL AND METHODS

(a) Observation of the contact area of setae under water

Beetles, G. viridula (Coleoptera: Chrysomelidae), were collected from their host plant Rumex obtusifolius. We used 29 beetles in the experiment. Beetles were placed in a small, square polystyrene case in order to observe the contact area of setae using the transparent bottom of a water bath. The case was filled with tap water and sealed with a cover. The air–solid and liquid–solid interfaces between the adhesive pad of the beetle and the glass substrate were observed using an optical microscope (Olympus-BH2-UMA, Nagano, Japan) in total reflection mode.

(b) Substrate preparation

Nine flat substrates were prepared for the experiment: soda-lime glass (g), smooth polymerized low-viscosity epoxy resin based on a modified Spurr embedding kit (Serva Electrophoresis) (w), smooth polycarbonate discs (a, c, A, B, C, D), and smooth polystyrene (p). Substrates a and A had a chemical vapour deposition (CVD) coating of Lipocer (Plasma Electronic GmbH, Neuenburg, Germany), whereas substrates B, c, C and D had a CVD coating of Aquacer (Plasma Electronic GmbH). The contact angles of water droplets on the substrates were measured using an automatic contact angle measuring device (OCA 30; Dataphysics Instruments GmbH, Filderstadt, Germany).

Before the experiment, the glass substrate was cleaned with ethanol and distilled water and dried using a nitrogen jet. Static electricity was eliminated from polymer plates using a gas ionizer (DC nozzle, Ionizing Air Nozzle; Tantec Inc., Schaumberg, IL, USA).

(c) Traction force of beetles on various substrates

A load cell force transducer with a 98 mN capacity (World Precision Instruments, Sarasota, FL, USA) was clamped to a holder perpendicular to the horizontal substrate surface. Beetles were anaesthetized with carbon dioxide and weighed. Each beetle was then attached by its dorsal surface to a human hair using a drop of molten beeswax. Each beetle was weighed again after 1 h of recovery from anaesthesia. The free end of the human hair was mechanically attached to the force transducer. When walking, the tethered beetle pulled the force transducer via the attached hair. Each beetle was tested once on different surfaces in a random order. The force data were recorded digitally, and the maximum pulling force during a 60 s long pulling period was estimated for each trial. The experiments with 29 individual female beetles were conducted on various substrates in air and under water (figure 2).

(d) Pull-off force between the structured polymer and various substrates

A silicone polymer with pillar-like outgrowths (figure 3a) was used to study the possible role of the microstructure in underwater adhesion. This experiment was conducted to verify whether the underwater adhesion mechanism of beetles was based on using the trapped air bubble. The pillar structure had a length of 2.4 mm, the curvature of the peripheral part was 200 μm, the diameter of the central part of the pillar head was 600 μm and the distance between pillars was 1.5 mm.

The contact angle of the water droplet on the flat silicone was 113.9° ± 1.8° (average ± s.d.). The side bearing the pillar structure was turned towards the bottom of the water bath and placed at a depth of 15 mm at room temperature (23–25°C). An air bubble with a volume of 0.1–0.3 ml was placed on the pillar structure, under water, using an injector. The microstructured surface was then pushed onto the substrate placed at the bottom. The silicone polymer was pulled in a direction (i) parallel (friction) and (ii) perpendicular to the bottom (pull off) at a speed of 50–250 μm s⁻¹ (single-axis motorized micromanipulator; Narishige Co., Tokyo, Japan). Friction and pull-off forces were measured using a load cell force transducer with a 245 mN capacity (World Precision Instruments). Both forces were estimated as the maximum of the detected force after deleting noise by calculation of the average value.

The experiment was repeated five or six times with each substrate (see above) (figure 3b). The pull-off force, shown in figure 8, was calculated by subtracting the weight of the sample from the value recorded.

3. RESULTS AND DISCUSSION

(a) Observation of the leaf beetle walking in water

The leaf beetle walked on a wooden stick until it was submerged under water where it then made a transition to the...
smooth bottom of the water bath. The beetle continued to walk freely on a smooth horizontal surface in water (figure 1). The leg attached to the smooth transparent surface under water was observed using an optical microscope in the total reflection imaging mode, which detected white areas indicating that air bubbles were trapped between the adhesive setae of the tarsomere (figure 4a). The dark points visible within the air bubble area in figure 4a are the setal tips contacting the de-wetted surface. The dark area around the air bubble corresponds to water surrounding the outside of the setae. On the smooth substrate in air, we could only observe the dark contact sites of setal tips (figure 4b). Under water, the air bubble moved with the leg movement when the beetle began to walk. This indicated that the bubble was trapped securely within the spaces between the setae. The volume of the air bubble on the feet varied depending on whether it was attached to one part of the sole only or to the whole sole. The foot surface, where the bubble was attached, was in a Cassie wetting state [16], and the bubble-free part, was in a Wenzel state [17]. The volume of the air bubble under water did not change, compared with that in air (104° for m and 108° for c, respectively). A strong traction force was detected on polymer surfaces a, c, m, when the performance of the beetle under water was compared. The relationship between the traction force under water and the contact angle of water droplets on corresponding substrates (figure 6) shows that the traction force is significantly lower on a hydrophilic surface with a contact angle of 43° than on all other surfaces. This phenomenon is proved below (§3d).

If a tiny drop of a non-ionic surfactant (Triton X) was added to the water, the beetle immediately lost its contact with the solid substrate and came to the surface. Thus, dissolving the surfactant in water made the beetle's locomotion under water impossible. This experimental result suggested that the air bubble, trapped by the beetle's soles, was the main mechanism responsible for beetle adhesion under water.

(b) Traction force generated by leaf beetles under water and in air

The data were analysed with one-way analysis of variance (ANOVA). Then pairwise multiple comparisons by Holm–Sidak method were carried out between the forces on various surfaces under water and in the air. Degrees of freedom (d.f.) was 7. The ANOVA test statistics ($F$) was 15.389. Probability value ($p$) was less than 0.0001. Detailed data comparison is shown in table 1. The results showed that the traction force on hydrophilic substrates under water was significantly lower (43° for g and 59° for c, respectively) than that on the same substrates in air (figures 5 and 6; the numbers in brackets are values of a water drop contact angle). The traction force on hydrophobic surfaces under water did not change, compared with that in air (104° for m and 108° for a, respectively). A strong traction force was detected on polymer surfaces a, c, m, when the performance of the beetle under water was compared. The relationship between the traction force under water and the contact angle of water droplets on corresponding substrates (figure 6) shows that the traction force is significantly lower on a hydrophilic surface with a contact angle of 43° than on all other surfaces. This phenomenon is proved below (§3d).
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Table 1. Traction force of female beetles G. viridula on various surfaces in air and under water. The results of all pair-wise multiple comparison procedures by the Holm–Sidak method performed after one-way ANOVA (analysis of variance) ($F = 15.389$, $p < 0.001$) $g$ ($n = 29$), $g$ ($n = 10$), glass plate; $a$ ($n = 9$), $a$ ($n = 10$), polycarbonate disc with the Lipocer coating; $c$ ($n = 9$), $c$ ($n = 10$), polycarbonate disc with the Aquacer coating; $m$ ($n = 10$), $m$ ($n = 10$), polymerized low-viscosity epoxy resin plate) in the air and under water. Underlined font indicates under-water conditions; $F_i$ the ANOVA test statistics; $p$, the probability value; $t$, the t-test value.

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Figure 5. Female beetle traction forces generated by walking on different substrates ($g$ ($n = 29$), $g$ ($n = 10$), glass plate; $a$ ($n = 9$), $a$ ($n = 10$), polycarbonate disc with the Lipocer coating; $c$ ($n = 9$), $c$ ($n = 10$), polycarbonate disc with the Aquacer coating; $m$ ($n = 10$), $m$ ($n = 10$), polymerized low-viscosity epoxy resin plate) in the air (not underlined letters, white columns) and under water (underlined letters, grey columns). According to pair-wise multiple comparison procedures (the Holm–Sidak method performed after one-way ANOVA), $F = 15.389$, $p < 0.001$. Different letters indicate statistically significant differences ($p < 0.005$). Ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. The detailed data comparison is shown in table 1.

Figure 6. Dependence of the female beetle traction force, under water, on the water contact angle on solid substrates used in the experiment. White bars, air; grey bars, water.

(c) Role of the surface microstructure in adhesion mediated by an air bubble in water
The role of the polymeric surface microstructure in air bubble adhesion was investigated in order to understand underwater adhesion of beetles. This experiment was conducted using a silicone plate covered with small pillar structures (figure 3a), with a smooth structure made of the same material, as a control. When the silicone plate and substrate were smooth, no adhesive force was detected by the force sensor. The relative position of the bubble on the smooth substrate and smooth silicone surface moved freely. In contrast to adhesion between smooth plates, the bubble was always trapped between the pillars in the same position, and it acted as an adhesive under water.

This result showed that the surface microstructure had an important role in holding the air bubble to the insect’s feet under water and is responsible for the generation of adhesive force under water.

(d) Underwater adhesion to hydrophobic and hydrophilic surfaces
The air–solid interface under water formed a complex shape that was held in place by pillar microstructure (figure 7). When the structured polymer with the air bubble was pulled perpendicularly from the substrate in water, the force was rather strong. The air–solid interface on the substrate was affected by the hairy structure. When the shape of the air–solid interface on the substrate changed, the pull force also changed.
Pull-off experiments performed with the structured polymer on various flat surfaces demonstrated that adhesion of the air bubble increased with an increasing contact angle of water on these surfaces (figure 8). These results were comparable with the experiments obtained on beetles.

Adhesion of the structured polymer with an air bubble on the surface with a low contact angle of water had a negative value caused by buoyancy. A surface with a low contact angle of water creates a thin water layer between an air bubble and the substrate (B). The existence of this thin water layer results in the bubble being separated from the substrate and the appearance of buoyancy corresponding to the volume. According to a previous study [18] on the adsorption of air bubbles, bubbles adhere strongly to hydrophobic surfaces and weakly to hydrophilic surfaces. The same tendency was observed in the present study. A stable aqueous film (wetting film) is formed between the air bubble and the solid surface if the solid surface is hydrophilic. The aqueous film is

Figure 7. Air bubble with a volume of 0.3 ml trapped by the pillar-like polymer structure observed from below through the transparent substrate. The dark grey line between pillars is the air–liquid interface attached to the transparent substrate. The area surrounded by a black line is the air–solid interface.

Figure 8. The relationship between the water contact angle of the solid substrate and the pull-off force generated by a structured polymer with trapped air bubbles, under water. The polymer was pulled in the direction perpendicular to the substrate. Each point shows an average value of five or six single pull-off force values with an air bubble of 0.3 ml obtained for each substrate (g, glass; m, polymerized low-viscosity epoxy resin; A–D, polycarbonate discs; ps, polystyrene). Substrate A had a chemical vapour deposited (CVD) layer of Lipocer, while substrates B–D had a CVD layer of Aquacer. The structured polymer was pulled in a direction perpendicular to the substrate. Error bars indicate s.d. of mean.

Figure 9. The relationship between the trapped air volume and pull-off force generated by the structured polymer with and without pre-load on a polystyrene surface under water. The polymer was pulled in a direction perpendicular to the substrate. Light grey bars, experiment without pre-load; dark grey bars, experiment with pre-load; white bars, experiment in the air. Each point shows an average value of five single force values. Error bars indicate s.d. deviation of mean.

Figure 10. The relationship between the trapped air volume under water and the friction force generated by the structured polymer with and without pre-loading. The polymer was pulled in a direction parallel to the substrate. Light grey bars, experiment without pre-load; dark grey bars, experiment with pre-load; white bars, experiment in the air. Each point shows an average value of five single force values. Error bars indicate s.d. of mean.
thick (40–200 nm) if it is affected by the double-layer force, whereas it is thin (5–15 nm) if it is affected only by van der Waals forces. That is why, in the case of a hydrophobic surface, the aqueous film is not formed between the bubble and the solid surface. The bubble adheres directly to the hydrophobic surface. In beetles, the bubble makes direct contact of setae with the substrate surface possible. This is the reason why the measured force on the hydrophobic surface did not change between air and underwater conditions.

On the hydrophilic surface, the beetle traction force was strongly decreased, when compared with the force in air; however, beetles still produced a small force. The force on the hydrophilic (with 60° of contact angle) surface under water might be additionally enhanced by the capillary bridges caused by an oily secretion on the setal tips of the de-wetted dry surface.

The plotted data in figures 9 and 10 show the maxima of the measured force. Forces measured in both directions (perpendicular and parallel to the substrate) increased with an increase in air volume (figure 9) and were stronger at higher pre-loading (figure 9). When the structured polymer was pulled parallel to the substrate, pre-loading had an especially big influence (figure 10). Interestingly, the contact area of the air bubble with the substrate and the length of the boundary layer between air–solid–liquid were not strongly influenced by pre-loading (figure 11). However, the contact area, the length of the fluid–air interface and the distance between the polymer plate and substrate must influence the measured forces if the capillary force is the main driving force. Because these three factors are not affected by pre-loading, it is possible that the increase in the measured traction force by pre-loading is caused by an increase in the solid–solid interaction between pillar tips and the substrate.

With an increasing volume of the bubble, the traction force in water was close to the force values measured in the air (figure 10). This result helps to understand the reason why traction force by the insect on the hydrophobic substrates did not change in water and in air. The setae of the beetles were able to make contact directly to the solid surface under water because of de-wetting of the solid surface by the air bubble.

Our study has clearly demonstrated that beetles have the ability to fix the bubble in place by using hairy structures, which bring the adhesive pads to Cassie wetting state under water. We have proved that such an air bubble, attached to the hairy surface, is the mechanism enabling beetle attachment and locomotion under water.

Figure 11. (a) The dependence of both the trapped air interface area and (b) the length of the boundary between air and water, attached to the substrate, on the trapped air volume. Light grey bars, without pre-load; dark grey bars, with pre-load. Each point shows an average value of the force data. Error bars indicate s.d. of mean.

Figure 12. A plastic toy bulldozer adhering to the substrate using the structured polymer with trapped air bubbles under water. (a) Substrate slope of 30°. (b) Air bubbles trapped between pillars of the structured polymer. (c) Substrate slope of 90°.
(c) Potential for bio-inspired underwater adhesion
Our discovery demonstrates the beetle's ability to use trapped air for underwater adhesion, and shows some potential for the design of artificial surfaces having a similar ability. Artificial hairy or pillar-like microstructures can also effectively fix a bubble to the surface. Biomimetic surfaces such as these were efficient in holding a toy plastic bulldozer (7.0 g) underwater (figure 12). The toy contained tires made of a silicone polymer with pillar-like microstructures (figure 3a) capable of trapping air bubbles.

4. CONCLUSIONS
We have discovered, for the first time, that the leaf beetle G. viridula can walk freely under water by using air bubbles trapped between adhesive hairs. Attachment to the hydrophobic surfaces (with a contact angle of about 100–110°) under water was as good as adhesion in the air, and was significantly stronger than that of the hydrophilic ones with a contact angle of 40°. The mechanism was successfully demonstrated in an experiment using a silicone polymer covered by pillar-like microstructures. Furthermore, we designed a new, underwater adhesive device based on the discovered adhesive effect.

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