Wetting of the tarsal adhesive fluid controls underwater adhesion in ladybug beetles: Supplementary material

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S1.1 Simulation method: Single capillary bridge

Capillary force due to a single adhesive fluid or bubble meniscus (termed "capillary bridge") is calculated by performing simulations in Surface Evolver[1], similar to the method described by De Souza et al.[2]. A simple cubic geometry, mimicking the capillary bridge, of constant volume, V, is defined as the initial condition with an interfacial tension, γ, with the surrounding medium. Interfacial tension of the capillary bridge with the substrate is given by $\gamma \cos\theta$, where θ is the corresponding contact angle inside the bridge. For the case of a bubble meniscus, θ is defined w.r.t. the surrounding water, since θ can also directly characterise the substrate wettability. The capillary bridge spans a gap distance d between the top face and the substrate. The boundary conditions are set corresponding to a pinned contact line of diameter D on the top face and constant interfacial tension with the substrate on the bottom. All lengths are normalised relative to length $s = (3V/4\pi)^{1/3}$. An appropriate geometry refinement routine is chosen to evolve the capillary bridge shape to its minimum energy state. The normalised total capillary force, $\hat{f} = f/\gamma s$, is the sum of the Laplace pressure and surface tension contributions , where:

$$f = f_{laplace} + f_{surface\,tension} = \Delta P_{laplace} A_{_{bottom}} + 2\pi R_{bottom} \gamma \sin\theta \tag{S1.1}$$

Here, $\Delta P_{laplace}$ is the Laplace pressure of the equilibrium capillary bridge, A_{bottom} is the contact area of the capillary bridge with the substrate at bottom and R_{bottom} is the corresponding radius of contact, all obtained from the simulation output for the equilibrium surface.

The gap distance d is varied stepwise and the capillary force is calculated each time to obtain force-distance curves for a particular choice of D and θ.

S1.2 Single capillary bridge: Effect of volume

Surface Evolver simulation results showing the effect of volume on the maximum capillary force of a single fluid bridge. Since the fluid is pinned at the top to the same diameter, D, a smaller volume would result in high interfacial curvatures, which increases the capillary force due to the negative Laplace pressure. In this case, small contact angles lead to a greater increase in adhesion.

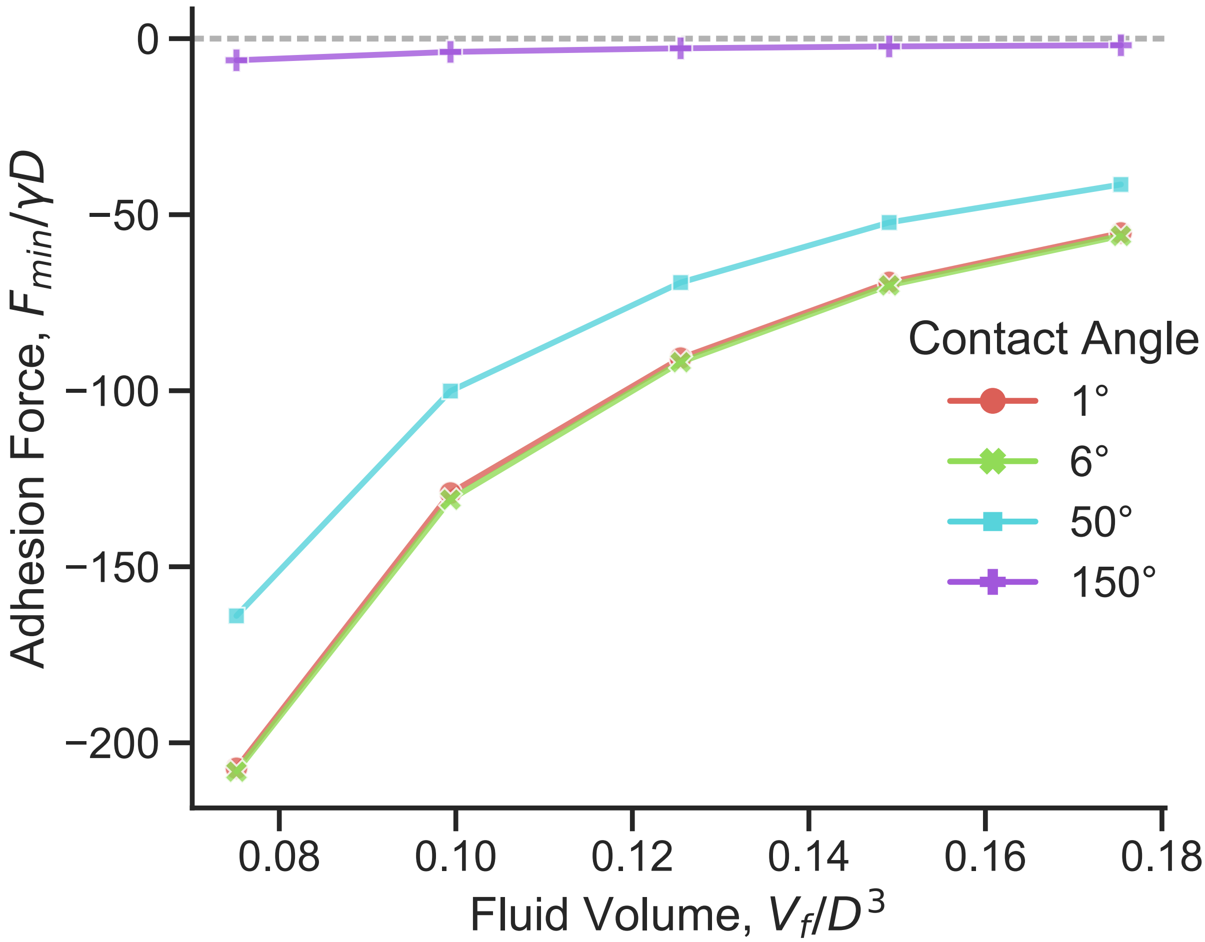

Figure S1.1: Normalised maximum capillary force for a single bridge as a function of fluid volume

S1.3 Capillary Bridge Model: Effect of hair diameter at constant fluid volume

Here, instead of scaling the fluid volume relative to the hair diameter, we now assume a fixed total fluid volume distributed equally among the N hairs. Total fluid volume, $V_{total} = NV_f = 2000$. Hair diameter is varied while keeping the total hair contact area constant. Length is in arbitrary units. Forces increase at a much smaller rate on decreasing diameter when compared to the case with self-similar scaling of fluid volume (Figure 8 in main text).

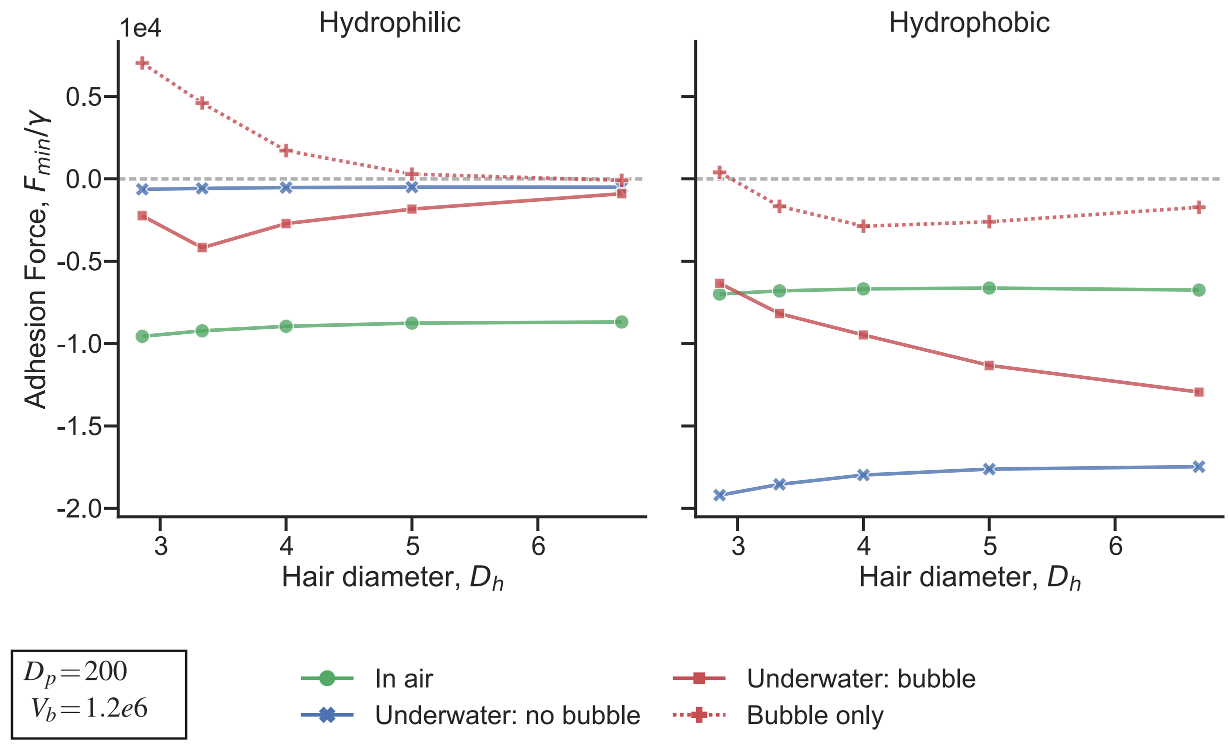

Figure S1.2: Normalised adhesion force of hairy pad system on a hydrophilic and hydrophobic substrate as a function of hair diameter (D_h), calculated from the capillary bridge model. The total adhesive fluid volume is fixed to 2000. Adhesion forces are calculated from minima of the respective force-distance curves. Negative force value represents attraction. The bubble's contribution to the net force for an *underwater: bubble* contact is denoted by plus symbols. Bubble volume and pad diameter are kept fixed. All lengths are scaled relative to D_p.

S1.4 Statistical comparison

Pairwise statistical comparison of single leg adhesion force measurements of the ladybug beetle (*Coccinella septempuctata*) for each contact type and substrate are shown (Table S1.1). The uncorrected p-values and Common Language Effect Size (CLES) are obtained from post-hoc pair-wise Student t-test between A and B while keeping the third parameter fixed. p-values showing statistically significant difference between A and B are in boldface. CLES represents the statistical proportion of samples under A with higher adhesion than under B. The condition for statistical significance is based on the Bonferroni-corrected critical p-value of 0.008.

Table S1.1

Fixed	A	B	p-value	CLES
In air	PFOTS	Glass	0.959	0.48
Underwater: bubble	PFOTS	Glass	0.011	0.96
Underwater: no bubble	PFOTS	Glass	$<$ **0.001**	1.0
PFOTS	In air	Underwater: bubble	0.897	0.48
PFOTS	In air	Underwater: no bubble	0.828	0.48
PFOTS	Underwater: bubble	Underwater: no bubble	0.721	0.44
Glass	In air	Underwater: bubble	**0.002**	1.0
Glass	In air	Underwater: no bubble	$<$ **0.001**	1.0
Glass	Underwater: bubble	Underwater: no bubble	0.07	0.84

S1.5 Field desorption mass spectroscopy

To test whether the ladybug beetles retain their adhesive fluid underwater unchanged, we performed a Field Desorption Mass Spectroscopy (FDMS) using a ZAB 2-SE-FPD spectrometer (VG Instruments, Malaysia). The measurements were done on the extracted secretions before and after submerging its legs under water. The middle leg of an Asian ladybird (*Harmonia axyridis*) was immersed in 50 µL Tetrahydrofuran (THF) for 20 min and then transferred to the measurement chamber of the FDMS. As a reference, pure THF was used. The second middle leg of the same ladybird was subsequently immersed in 100 µL Milli-Q water for 15 min (close to the time-scale of the underwater adhesion tests), then in THF for 20 min and then transferred to the measurement chamber of the spectrometer. Molecular weights were extracted from the peak positions of the FDMS spectra. Probable compostion was identified from the molecular weights by assuming the secretion fluid consists possibly of hydrocarbons, alcohols, aldehydes or carboxylic acids.

FDMS results confirm the presence of adhesive fluid on the Asian ladybird's leg when underwater (Table S1.2). Molecular weights of the epicuticular grease extracted from the beetle's leg, without and after immersion in water, show that, except for two molecular weights (406.8 g mol^{-1} and 331.6 g mol^{-1}), the chemical fingerprint remained unchanged. This let us assume that the tarsal adhesive fluid was not washed away underwater. Probable compounds in the fluid, corresponding to the resultant molecular weights, include mostly aliphatic hydrocarbons with traces of aldehydes. Our preliminary measurements on *Harmonia axyridis* let us conjecture that, even for the case of *Coccinella septempuctata*, the tarsal adhesive fluid was not washed away from the hairs during the underwater experiments. A detailed study is however essential to ensure that the difference in the ladybug species between our adhesion and FDMS tests doesn't qualitatively change the above outcome. Further, the detected compounds would also include cuticular secretions from the leg apart from the tarsal fluid. Thus, a more precise fluid extraction method is necessary to definitely confirm the above conjecture, and will be a subject of future study.

Table S1.2: Molecular weights of the tarsal adhesive fluid secretion of *Harmonia axyridis* without and after submerging the beetle's leg in water.

Without submerging (g mol^{-1})	After submerging (g mol^{-1})	Probable compounds
324.5	324.5	$C_{23}H_{48}$, $C_{22}H_{44}O$
	331.6	$C_{24}H_{44}$
350.5	350.5	$C_{25}H_{50}$
352.5	352.5	$C_{25}H_{52}$, $C_{24}H_{48}O$
378.5	378.5	$C_{27}H_{54}$
404.6	404.5	$C_{29}H_{56}$
406.8		$C_{29}H_{58}$
432.8	432.7	$C_{31}H_{60}$

S1.6 Capillary force due to a bubble

Capillary force of a single bubble against a PFOTS surface are compared for two different volumes. The volumes correspond to the expected range in the case of the trapped bubble in a ladybug. Here, the bubble is pinned to a micropatterned PDMS substrate on the top. The maximum adhesion force of any of the bubble never exceeds 50 µN, significantly lower than the beetle's underwater adhesion to the same substrate ($>$ 400 µN). Thus, the bubble's contribution to adhesion in the "*underwater: bubble*" contact of a ladybug's pad should be negligible. Measurement videos are included in the supplementary material (S6).

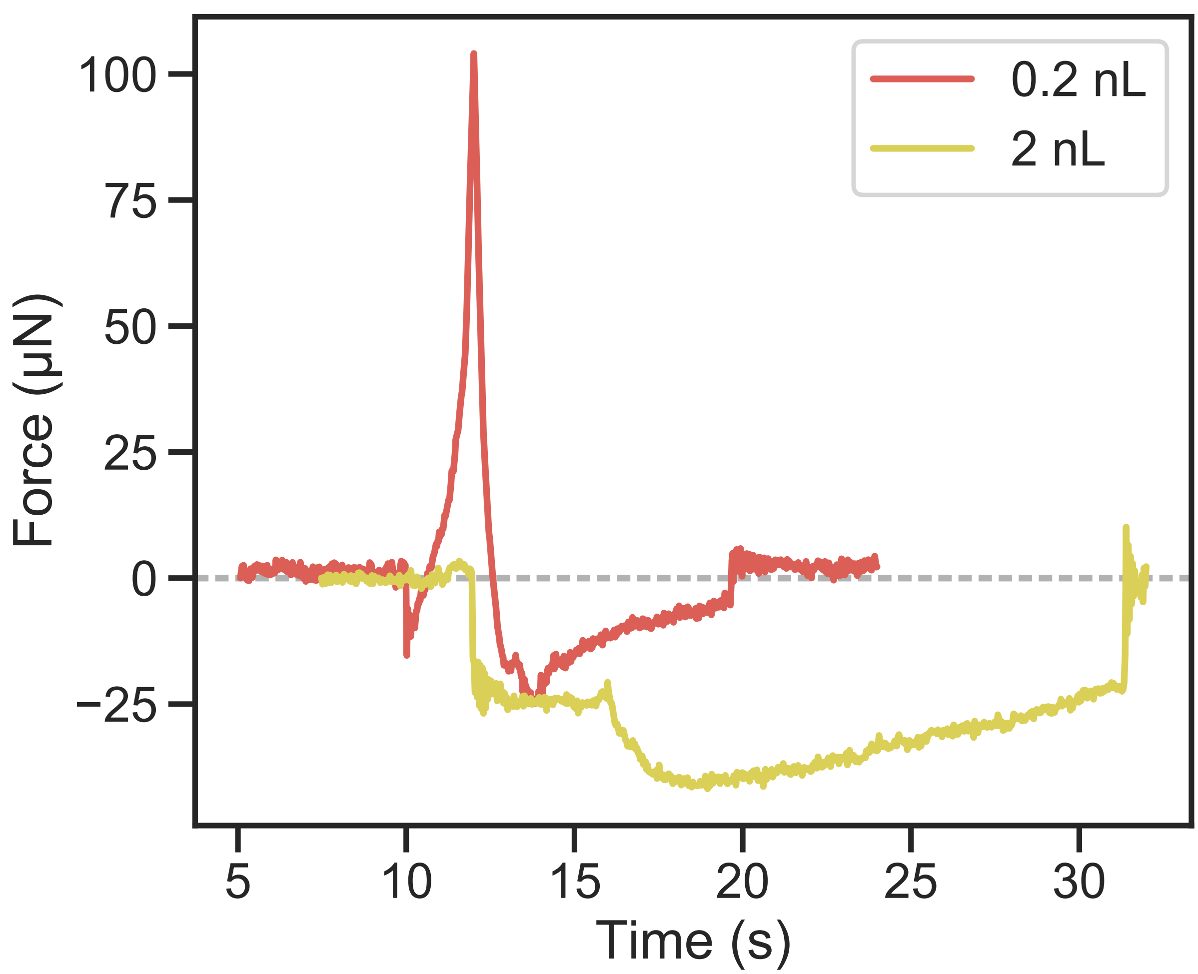

Figure S1.3: Capillary force of the bubble

References

(1) Brakke, K. A. The surface evolver. *Experiment. Math.* **1992**, *1*, 141–165.

(2) De Souza, E. J.; Brinkmann, M.; Mohrdieck, C.; Arzt, E. Enhancement of Capillary Forces by Multiple Liquid Bridges. *Langmuir* **2008**, *24*, 8813–8820.