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SYMPOSIUM

Robust Performance of Spider Viscid Silk on Hairy and Smooth Insect Substrates

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Synopsis Spider viscid silk adheres to insects in orb webs and is a "smart-adhesive" that quickly changes droplet size, viscosity, and adhesiveness in response to atmospheric humidity. Different species of spiders "tune" water uptake to match the humidity of their foraging environments, achieving a similar "universal" viscosity that optimizes tradeoffs in spreading versus cohesive bulk energy needed to enhance adhesion. Too much water lowers viscosity so that the glue spreads well, but cohesive failure occurs easily, generating poor adhesion. However, the optimal viscosity model of adhesion is based on experiments using smooth glass. Here we test the hypothesis that a less viscous, "over-lubricated" glue, which shows poor adhesion on smooth glass, will be stickier on hairy insects because of its greater ability to spread across three-dimensional rough surfaces. We ran adhesion tests of the furrow spider (*Larinioides cornutus* [Clerck 1757]) viscid silk on honey bee (*Apis mellifera*) thorax, with and without hairs, in either high or medium humidity. Our results show that "over-lubricated" glue increases adhesion on hairy surfaces, performing equally as well as an optimally viscous glue.

Introduction

Viscid silk is used by araneoid orb-weaving spiders to build the capture spirals of their webs, which are supported by the dry, stiff, and strong, major ampullate silk radii and frame. Viscid silk consists of an extensible flagelliform silk axial thread coated by secretions from the aggregate gland arranged in evenly spaced glue droplets. After a flying insects impacts an orb web and its flight energy is dissipated by the major ampullate silk, viscid silk sticks to the prey and holds on to the prey until the spider reaches it. However, many insects escape from orb webs before being subdued by the spiders (Nentwig 1982; Eberhard 1990; Blackledge and Zevenbergen 2006). Therefore, the adhesive properties of viscid silk play an important role in determining orb spider foraging success.

The aggregate secretions in viscid silk contains glycoproteins (Tillinghast et al. 1981; Vollrath and Tillinghast 1991; Choresh et al. 2009), a cocktail of hygroscopic, low molecular weight organic and inorganic compounds (LMMC) (Anderson and Tillinghast 1980; Vollrath et al. 1990; Jain et al. 2015), and water. The LMMC in addition to absorption of atmospheric water directly interacts with and stabilize the glycoproteins (Sahni et al. 2014). The LMMC also plays an important role when the wet glue comes in contact with a surface by sequestering the water away from the glue–substrate interface to maintain adhesion in humid conditions (Jain et al. 2018; Singla et al. 2018).

The amount of water in the aggregate glue controls its viscosity and this helps the spreading of glue across surfaces while maintaining cohesion strength and firm attachment to the underlying flagelliform silk (Amarpuri et al. 2015, 2017). Glue viscosity can change up to a 1000-fold with atmospheric humidity and the glue from different spider species achieves

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optimal viscosity at different atmospheric humidities, potentially allowing them to maximize adhesion for specialized foraging microhabitats. Viscid silk adhesion can further be affected by the surface energy of smooth substrates (Opell et al. 2011). In addition to how a single viscid glue droplet attaches to a surface, the arrangement of aggregate glue into a beads-on-a-string morphology on the flagelliform silk also plays an important role in optimizing adhesion strength. This geometry allows multiple glue droplets to deform during pull off, forming a "suspension bridge" morphology (Opell and Hendricks 2007). Multiple crack fronts therefore have to be overcome for a viscid silk thread to release from the substrate and adhesive force is greatest when glue viscosity maximizes the number of droplets that deform without detaching from the substrate. However, most of this research on structure and function of viscid silk has been conducted using synthetic smooth substrates such as glass and metal to help in improving the repeatability across experiments.

In contrast, orb spiders capture a wide variety of insects whose cuticles vary greatly in roughness and morphology. Many insects have complex micro- and nanoscale topography on their otherwise smooth exoskeletons and most insects are covered with a variety of macroscale setae and/or scales that function in protection, communication, and sensing the environment. Setae could reduce viscid silk adhesion by preventing the kind of smooth, even contact seen with glass, but could also present greater surface area for glue contact and induce shearing during detachments. Few studies have investigated adhesion on such three-dimensional rough surfaces. Opell and Schwend (2007) found that viscid silk adhesion varied 3.5-fold across smooth and hairy insects, in part because setae sometimes prevented glue droplets from making contact. And Eisner et al. (1964) showed that moths use sacrificial scales that adhere to viscid silk but easily detach from the cuticle to escape spider webs. However, neither of these studies considered how glue viscosity might influence viscid silk adhesion. Glue viscosity changes prey retention times at different humidities, as the viscid silk of an orb weaving spider held house flies up to 11 s longer at the optimal humidity of its aggregate glue (Opell et al. 2017). Moth-specialist spiders use an "over-lubricated" glue, ~1000× less viscous than is optimal on glass for other spider species, to rapidly flow around moth scales and adhere to the underlying cuticle (Diaz et al. 2018, 2020). This importance of spreading suggests the hypothesis that more typical orb spider's viscid silk might also adhere better to rough, hairy insect substrates when "over-lubricated" at viscosities lower than the optimum seen on synthetic smooth substrates. We test this hypothesis by comparing the adhesion of spider viscid silk to honey bees at both "optimal" and "over-lubricated" humidities.

Materials and methods

Spider care and silk sample collection

Larinioides cornutus (Clerck, 1757) were collected because it was locally abundant and its adhesive silk is well-studied. *Larinioides cornutus* specimens were collected in, Akron, Ohio. Spiders were kept in the lab inside cages made of plexiglass and wire screen (37 cm width × 38 cm height × 11.5 cm depth) with a water dish placed at the bottom of each container, to help them thrive. The spiders were fed a diet of crickets at least once a week, or after every web collected. Silk samples were collected from the capture spiral portion of the web below the hub. Samples were collected with thin cardboard containing a 12.58 mm width square cut, secured using Elmer's® glue, and cut using a soldering iron to prevent any pre-straining that could alter the physical properties of the specimens. After collection, silk specimens were placed in a container that blocked UV radiation, dust, and any other factors that might lead to degradation of the sample over time, until being tested 2–3 weeks later.

Preparation of substrates

Honey bees, *Apis mellifera*, were used to test how the aggregate glue adheres to different physical morphologies by altering its cuticle physically, without altering the chemicals such as waxes found on the cuticle. Bee thorax was altered in three ways for this study: removal of all hairs on the thorax, removal of some long hairs on thorax, and all hairs left intact on thorax. These substrate alterations were labeled smooth, some, and hairy, respectively (Fig. 1). A stereo microscope Leica S6D (Leica Microsystems, Wetzlar, Germany) was used to view the hair removal process—curved forceps were used to carefully pluck hairs and/or rub hairs off from the thorax, for both the "smooth" and "some" substrates. *Apis mellifera* were collected from local bee farms and frozen overnight. *Apis mellifera* were allowed to thaw and dry for 48 h before preparation. Borosilicate glass was used for the control group and was prepared by cleaning with 3:1 ratio of isopropyl alcohol and de-ionized (DI) water, followed by rinsing with DI water, and finally air dried prior to testing.

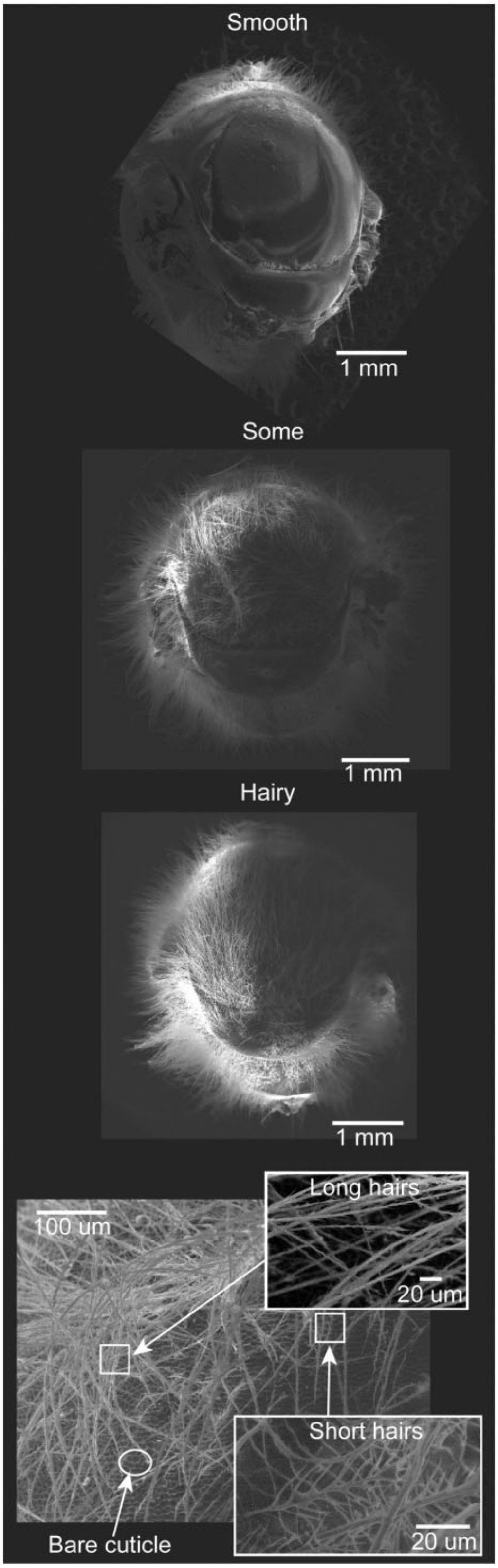

Fig. 1 SEM images of bee thorax substrates: hairy, some, and smooth. The image on the bottom is a closeup showing microstructures of different components present in the bee thorax: long hairs, short hairs, and honeycomb-like dimples in the cuticle of the bee.

A representative of each group of bee substrates was used to take images under an environmental scanning electron microscope (FEI Quanta 200 ESEM). Substrates were sputtercoated with gold for 30 s prior imaging.

Adhesion testing

A total of four threads each from 17 webs built by 15 different spiders (two spiders built two webs on different days) were tested across all four substrates so that any variation among webs in stickiness was evenly distributed across treatments (Supplementary Table S1). Due to experimental difficulty, we were not able to test five webs on all substrate (see Fig. 2 for sample sizes). Adhesion testing was performed using an MTS Nano Bionix test system (Agilent Technologies, TN, USA) inside a humidity chamber. By running nitrogen gas through a column filled with DI water we were able to increase or decrease the relative humidity (RH). Two humidities were used in this study, 50% RH (optimal) and 90% RH ("over-lubricated"). Samples were allowed to equilibrate for 2–3 min at its respective humidity to acclimate prior to testing. To test adhesion a 12.58 mm specimen of viscid silk was brought in contact with the substrate until a 15 μN preload, held for 6 s, and then brought back up until detachment. Work of adhesion, or energy it takes to unstick from the substrate, was calculated as the area under the load–extension curve. Maximum force during pull-off and total distance pulled was also measured.

Qualitative analysis

Adhesion experiments were recorded using a Photron SA4 (Photron, Tokyo, Japan) at 60 fps through a stereo microscope Leica S6D (Leica Microsystems). Videos were used to understand behavior of viscid silk during pull-off from substrates at each humidity. Additionally, number of droplets in contact with substrate was quantified using photoshop (CC 2019, Adobe, San Jose, CA). Because of video quality we were unable to quantify every test and only a subsample per substrate per humidity was used (n/N, where n is the total threads and N is the number of webs; glass 50% 10/3, 90% 10/3; smooth 50% 4/3, 90% 10/5; some 50% 7/2, 90% 9/3; and hair 50% 7/3, 90% 9/3).

Statistical analysis

The mean work of adhesion, maximum force of adhesion, and distance pulled observed for each web per substrate per humidity was obtained by taking the

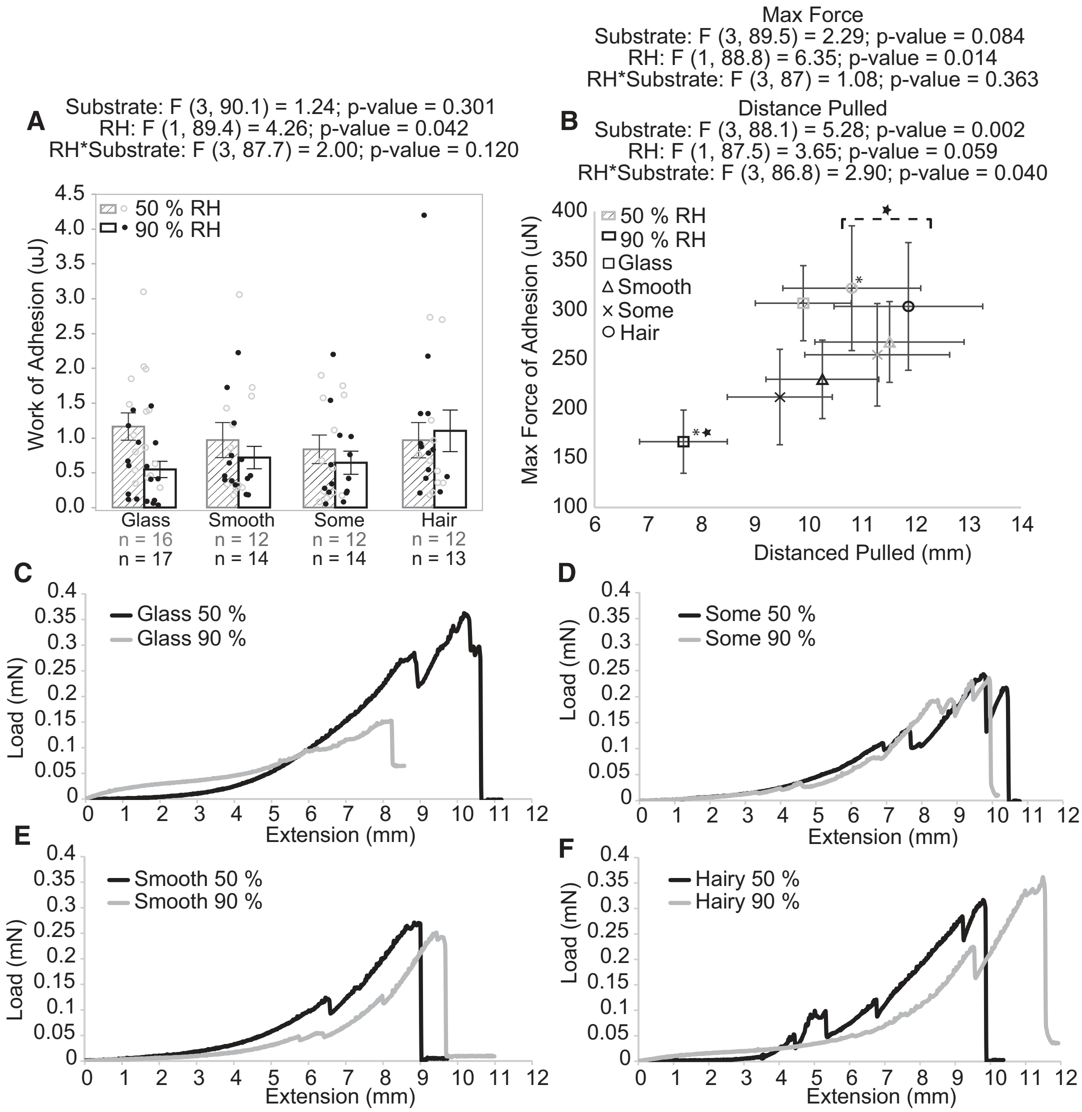

Fig. 2 Work of adhesion, maximum force of adhesion, and distance pulled of viscid silk against four substrates (hydrophilic glass, and hydrophobic bee substrates smooth, some, and hairy). **A**) Our results show that regardless of aggregate glue viscosity (50% "optimal" and 90% "over-lubricated") work of adhesion was similar for bee substrate, while over-lubricated aggregate glue did not perform as well in the hydrophilic glass. Bars represent mean + standard error from the mean. Each data point represents the mean of four viscid silk measurements per web for each substrate at each humidity. **B**) Maximum force of adhesion on the *y*-axis and displacement in the *x*-axis. Asterisk (*) shows statistical difference in force: glass at 90% was significantly lower than hairy at 50%. Star shows significant differences in displacement: glass at 90% was significantly lower than hairy, some, and smooth at 50%, and hairy at 90%. Values represent mean ± standard error from the mean. **C**–**F**) Representative load–extension curves for all four substrates at two viscosities.

mean of four viscid silk measurements per web for each substrate at each humidity. Webs built by the same spider on different days were treated as independent data points. To determine the adhesion performance of viscid silk on different substrates at two different humidities, we used a factorial mixed-model analysis of variance (ANOVA) to compare work of adhesion, maximum force of adhesion, and distance pulled as a function of substrate (glass, smooth, some, or hairy), the testing humidity (50% or 90% RH), and their interaction. Individual web was modeled as a random effect in all analyses. A factorial ANOVA tested if number of droplets in contact differ between substrates, the testing humidity, and their interaction. All statistical analyses were performed using JMP Pro 14 (SAS Institute, Inc., Cary, NC).

Results

Adhesion testing

We tested the adhesion performance of viscid silk from *L. cornutus* on four different substrates at optimal humidity (50% RH) and very humid

Table 1 Comparison of glue properties between humidities for each substrate

		Substrate	Percentage difference between humidities	Standard error	P-value
Glue properties	Work of adhesion (μJ)	Glass	67	0.20	0.069
		Smooth	15	0.23	0.938
		Some	11	0.23	0.984
		Hair	21	0.23	0.998
	Max force of adhesion (μN)	Glass	54	47.67	0.077
		Smooth	0	54.07	0.979
		Some	3	54.07	0.966
		Hair	2	54.84	1
	Distance pulled (mm)	Glass	20	0.79	0.165
		Smooth	4	0.90	0.945
		Some	2	0.90	0.638
		Hair	17	0.91	0.843
		Substrate	**Number**	**Standard error**	
	Droplets coming in contact	Glass	63.2	5.09	
		Smooth	22.71	2.16	
		Some	16.38	1.64	
		Hair	19.88	2.19	

Percentage difference was calculated as the difference of the values divided by the average of the two values. Standard error from the mean and *P*-values from Tukey's *post hoc* test. Number of droplets coming in contact quantified from videos.

conditions where aggregate glue is "over-lubricated" (90% RH). We found that overall humidity had an effect on the work adhesion of viscid silk (Fig. 2A; $F[1, 89.4] = 4.26$, $P = 0.042$). The control glass substrate showed a marginal (statistically significant) drop in performance when detaching at high humidity, while the work of adhesion was similar between humidities for all three bee substrates (Table 1 and Fig. 2A). Maximum force of adhesion showed a similar trend with humidity having an effect ($F[1, 88.8] = 6.35$, $P = 0.014$) and pairwise comparisons showing a drop in force when viscid silk detatches from the glass substrate during high humidity, but only marginally significant (Table 1 and Fig. 2B). Force of adhesion was significantly different between the non-optimal glass at 90% RH and hairy substrate at 50% RH.

The extensibility of the viscid silk represented as distance pulled before detachment from the substrate showed a different pattern as the two other properties. Substrate and the interaction between substrate and humidity have an effect in the total distance pulled (Fig. 2B; $F[3, 88.1] = 5.28$, $P = 0.002$ and $F[3, 86.8] = 2.90$, $P = 0.04$, respectively) as glass at 90% RH extended significantly lower than hairy bee at 90% ($P = 0.002$) and all three bee substrates at 50% RH (hairy $P = 0.049$, some $P = 0.01$ and smooth $P = 0.004$).

Qualitative analysis

Number of droplets in contact with the substrate was higher for glass than any of the bee substrates, due in part to the curvature of the natural bee thorax substrates ($F[3, 3]$: 41.39, $P < 0.01$). Neither humidity nor the interaction of humidity and substrate had a statistically significant effect on the number of droplets contacting the substrate ($F[1, 1]$: 1.23, $P = 0.27$; $F[3, 3]$: 0.81, $P = 0.49$; respectively). Because humidity had no effect in the number of droplets in contact within each substrate, values were pooled across humidity and reported for each substrate in Table 1.

Discussion

In this study we looked at viscid silk performance of the spider *L. cornutus* on a range of substrates with different roughness and under two environmental conditions that changed the viscous properties of the viscid silk. We tested the hypothesis that an "over-lubricated" glue will adhere better to hairy insect surfaces due to better spreading across the surface compensating for fewer droplets contacting the surface (Table 1). Overall, we found that viscid silk adhesion was reduced on glass at high humidity, previously identified as "sub-optimal" for *L. cornutus* (Amarpuri et al. 2015). However, adhesion was similar between "optimal"

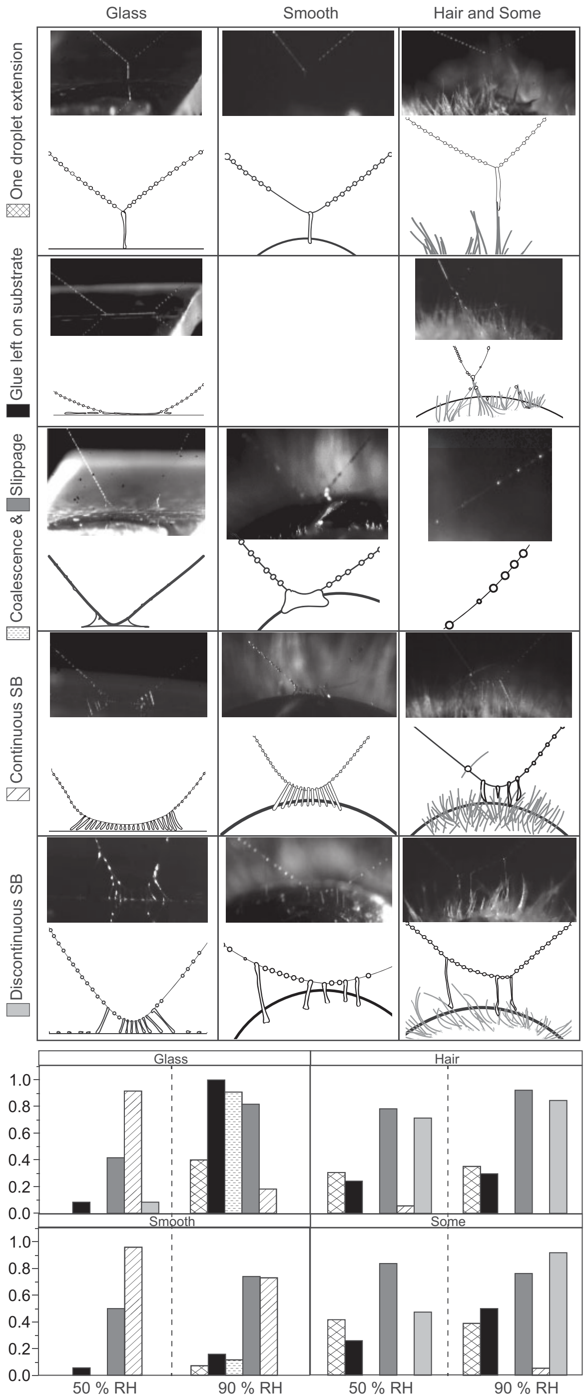

Fig. 3 We have quantified the frequency of the pull-off behavior of the viscid silk on the four substrates per humidity. We classified the behaviors as: (1) one droplet extension, (2) glue left behind on the substrate, (3) coalescence of droplets, (4) slippage of droplets, (5) continuous suspension bridge, and (6) discontinuous suspension bridge. We see that the behavior of viscid silk is similar for both "optimal" and "higher" humidities for the more complex substrate (some and hair), while pull-off behavior for the glass differs between the two viscous conditions of the aggregate glue.

and "higher" humidities for both smooth and hairy bees, suggesting that spider silk adhesion is robust to changes in viscosity when adhering to natural surfaces.

We propose two hypotheses for why changes in glue viscosity have a greater effect on adhesion to glass versus insect substrates. First, the glass is hydrophilic and this may increase the tendency of the water-based aggregate glue to overspread at high humidity. The overspreading has shown to result in lower adhesion (Amarpuri et al. 2015, 2017). Second, there is slightly more variation among tests on natural substrates compared with glass, especially on hair substrates, and this may obscure any modest effect of viscosity on adhesion.

The decreased adhesion of viscid silk on glass when over-lubricated was largely driven by changes in pull-off behavior (Fig. 3). "Over-lubricated" glue droplets coalesced into sheets that failed cohesively at much lower force than they did when forming a suspension bridge at the "optimal" viscosity (Fig. 3). In contrast, pull-off behavior did not vary greatly between viscosities on natural bee substrates. This robust performance is noteworthy particularly because we did find differences in pull-off behavior between bee surfaces. Continuous suspension bridges formed mostly on the smooth bee substrates, at both high and low humidities, and there was greater evidence of glue left behind on, and hence cohesive failure, the hairy substrates. We suspect this cohesive failure was driven by shearing along the setae.

We show that the viscid silk of one species of orb web maintains good adhesive contact with a natural bee surface even at the very high humidity. The robust adhesion of viscid silk to bee despite a 1000-fold change in viscosity suggests that typical orb spiders aggregate glue may be less-specialized for specific foraging microhabitats than sometimes suggested (Amarpuri et al. 2015). The production of LMMC in aggregate glue is likely metabolically expensive—as their composition changes as spiders become hungry (Townley et al. 2006) and because most of the spiders known to recycle silk in their webs' use aggregate glue to make their webs adhesive (personal observation). LMMC composition varies widely across spider species (Jain et al. 2018) and helps to determine how glue viscosity changes with humidity and also provides the necessary stability to solvate the glycoproteins (Sahni et al. 2014). For species that forage over a wide range of humidity, the LMMC plays an important role at low humidity in maintaining the water content necessary for the aggregate glue to be tackier and adhesive. Because of our observations that *L. cornutus* aggregate glue maintains good adhesion with the bee substrate at high humidity, the excess water uptake by LMMC is not detrimental to adhesion. Therefore, there may be interesting tradeoffs in how spiders in different environments invest in LMMC—based on metabolic production versus humidity—mediates effects on adhesion. Moreover, most studies on orb spider diets find them to be generalist predators, necessitating the ability of webs to adhere to a broad range of insect cuticle morphologies (Nentwig 1987). Thus, our finding that viscid silk maintains functionality across a broad array of ecological conditions may not be as surprising as it first seems.

Conclusion

Previous research proposed that the viscid silk of different species of spiders foraging in different microhabitats has specialized viscid silk that is optimized to stick strongly to surfaces at different relative humidities. In contrast, we found that viscid silk adhesion on natural insect substrates was robust to changes in glue viscosity. Moreover, strength and work of adhesion were not influenced by the presence of hairs on insects, even though glue failure occurred in different ways on smooth versus hairy insects. This robust performance should facilitate the generalist predatory strategies of most orb spider species. Adhesion in humid conditions is challenging for both natural and synthetic systems and viscid silk presents a good strategy to adhere to rough surfaces at high humidity.

The data underlying this article are available as supplementary material in Supplementary Tables S1 and S2.

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Supplementary data

Supplementary data are available at *ICB* online.

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