INTERFACE

royalsocietypublishing.org/journal/rsif

Research

Cite this article: Diaz C, Maksuta D, Amarpuri G, Tanikawa A, Miyashita T, Dhinojwala A, Blackledge TA. 2020 The moth specialist spider *Cyrtarachne akirai* uses prey scales to increase adhesion. *J. R. Soc. Interface* **17**: 20190792. http://dx.doi.org/10.1098/rsif.2019.0792

Received: 24 November 2019
Accepted: 6 January 2020

Subject Category:
Life Sciences–Physics interface

Subject Areas:
biomechanics, biomaterials

Keywords:
spider silk, aggregate glue, adhesion, *Cyrtarachne*, moth, topology

Author for correspondence:
Candido Diaz
e-mail: candido.diaz.jr@gmail.com

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.4824060.

THE ROYAL SOCIETY PUBLISHING

The moth specialist spider *Cyrtarachne akirai* uses prey scales to increase adhesion

Candido Diaz[1], Daniel Maksuta[1], Gaurav Amarpuri[2], Akio Tanikawa[3], Tadashi Miyashita[3], Ali Dhinojwala[2] and Todd A. Blackledge[1]

[1]Department of Biology and Integrated Bioscience Program and [2]Department of Polymer Science, The University of Akron, OH 44325, USA
[3]Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

CD, 0000-0003-3705-2018; GA, 0000-0002-2655-5974; TAB, 0000-0002-8166-5981

Contaminants decrease adhesive strength by interfering with substrate contact. Spider webs adhering to moths present an ideal model to investigate how natural adhesives overcome contamination because moths' sacrificial layer of scales rubs off on sticky silk, facilitating escape. However, Cyrtarachninae spiders have evolved gluey capture threads that adhere well to moths. *Cyrtarachne* capture threads contain large glue droplets oversaturated with water, readily flowing but also prone to drying out. Here, we compare the spreading and adhesion of *Cyrtarachne akirai* glue on intact mothwings, denuded cuticle and glass to the glue of a common orb-weaving spider, *Larinioides cornutus*, to understand how *C. akirai* glue overcomes dirty surfaces. Videos show that *C. akirai*'s glue spreading accelerates along the underlying moth cuticle after the glue seeps beneath the moth scales—not seen on denuded cuticle or hydrophilic glass. *Larinioides cornutus* glue droplets failed to penetrate the moth scales, their force of adhesion thus limited by the strength of attachment of scales to the cuticle. The large size and low viscosity of *C. akirai* glue droplets function together to use the three-dimensional topography of the moth's scales against itself via capillary forces. Infrared spectroscopy shows *C. akirai* glue droplets readily lose free-flowing water. We hypothesize that this loss of water leads to increased viscosity during spreading, increasing cohesive forces during pull-off. This glue's two-phase behaviour shows how natural selection can leverage a defensive specialization of prey against themselves and highlights a new design principle for synthetic adhesives for adhering to troublesome surfaces.

1. Introduction

The ability of an adhesive to wet a substrate, create strong interfacial connections and maintain bulk cohesion determine the overall performance [1,2]. During wetting, the interfacial energy between the substrate and the liquid adhesive, the surface tension of the liquid adhesive and the energy of the medium (most commonly air) cause the liquid adhesive to form a droplet upon the surface [1,2]. As the surface energy of the substrate increases, droplets spread further, creating a lower contact angle and 'flatter' droplet [1,2]. Contaminants decrease adhesive strength by limiting substrate wetting as the contaminants block flow and lower the total surface area of connections between the substrate and glue [1,3]. During detachment, force is transferred to the contaminant rather than the substrate, leading to suboptimal energy dissipation during pull-off [1,3,4]. Overcoming contamination, therefore, is a major challenge for synthetic adhesives, ranging from household tapes to dental implants [4]. Adhesion to contaminated surfaces is also a problem in the natural world. For instance, geckos rely on the self-cleaning mechanism of their setae to retain adhesion strength over prolonged climbing, and mussels spread copious amounts of adhesive glue to create a stable attachment to

bio-fouled rock substrates [5,6]. Moths adhering to spider webs present an ideal model to investigate how natural adhesives overcome contaminated substrates because moths are covered in a sacrificial layer of scales that rub off on gluey spider silk such that moth scales are classically considered to be an effective anti-predator adaptation against spider webs [3,7–12].

Insect cuticle is composed of solid layers of chitin topped with a waxy layer of epicuticle that makes the cuticle hydrophobic [13,14]. Moths are unusual in that the top epicuticle is additionally covered in a layer of sacrificial scales that are loosely attached to the underlying cuticle at small junction points. These scales contain micro-ridging that makes them super-hydrophobic, even more readily shedding water from their surface than the base waxy layer [1,13,14]. This sacrificial layer of scales on moths lowers the adhesion of gluey spider silks at least 10-fold because the adhesive strength of the system is limited by the strength of attachment of the individual scales to the moth, rather than by the attachment of the glue to the upper surface of the scales [3,7,15]. One subfamily of spiders, the Cyrtarachninae (Araneae: Araneidae), have evolved webs that overcome this limitation and now specialize in the capture of moths, using only small numbers of viscid capture threads [7,12,16–18].

Cyrtarachne akirai in particular constructs a horizontal orb-web with long dangling capture threads covered in glue droplets roughly 8× larger than those used in typical orb webs [7]. These capture threads are then spaced exceptionally far from one another with prey often only coming into contact with a single thread but still being retained. This contrasts other orb webs that rely on prey contact with multiple capture threads and impact with thick radial threads, to absorb prey energy, to retain [7,12,18]. In addition to their unique web structure, *C. akirai* appears to limit its web building to a sub-ecosystem, only weaving webs in environments of greater than 80% RH [17]. Anecdotally, they can be seen quickly recycling their webs once the humidity begins to drop. Given their unique biological niche, studying the adhesive properties of *C. akirai* glue droplets could reveal how a natural system has evolved to overcome contaminants during adhesion.

Orb webs use viscid capture threads covered in aqueous glue to ensnare prey long enough to be subdued by the spiders [11,16,18–21]. These threads are usually great adhesives that maintain strength over extended periods of time and can repeatedly attach and detach to substrates [22,23]. Viscid capture threads are coated with aggregate glue, which is composed primarily of water, glycoproteins, which provide the adhesive strength, and a cocktail of low molecular mass compounds (LMMCs) and salts, which lubricate the glycoproteins and absorb humidity from the environment [24,25]. As humidity is increased, the LMMC passively absorbs water, thus increasing glue droplet size and decreasing droplet viscosity by over five orders of magnitude [24–26]. This has a significant effect on capture silk adhesion because of the interplay between droplet spreading and cohesive forces of the glue bulk, ultimately determining the failure mechanism of the adhesive during peeling [27].

Aggregate glue droplets absorb water under high humidity. This leads to larger droplets that spread further, increasing the surface area in contact with the substrate, while also diluting the glue and decreasing the cohesive forces; adhesion is maximal when these two competing properties are optimized [1,22]. At high humidity, overspreading of droplets results in cohesive failure between the capture thread and the bulk of the glue, with a substantial portion of the droplets being left on the substrate. Inversely, as humidity decreases, aggregate glue becomes less fluid and unable to flow into cracks or grooves present on the substrate, decreasing the effective contact area [1]. At an optimal water content, glue droplets on a thread will begin to stretch from the edges and form a suspension bridge as they are pulled from the substrate [21]. This suspension bridge distributes stress across the strand; this allows for higher work of adhesion where roughly half of the work is attributed to the stretching of glue droplets and the other half attributed to stretching of flagelliform threads [21]. The LMMC composition of different spider species' glue droplets is tuned to provide maximum adhesion at their common environmental humidity through a proper balance of cohesive strength and spreading [25–28].

Cyrtarachne akirai aggregate glue is noteworthy because of its exceptionally high water content that results in extremely large, widely spaced glue droplets that function at a viscosity approximately 1000 times lower than typical spider species [3,7,11,15,28]. In order to understand how these features influence *C. akirai* glue adhesion on moths, we compared adhesion strength and spreading behaviour to a well-studied orb-weaver, *Larinioides cornutus*. We chose *L. cornutus* because it is a model species for understanding aggregate glue adhesion and is closely related to *C. akirai* evolutionarily. *Cyrtarachne akirai* normally only forages under extremely high humidity and its glue rapidly dehydrates and loses adhesiveness below approximately 80% RH, thus we tested it at 90% RH. Tests involving *L. cornutus* were conducted at both 90% RH, where its viscosity matches *C. akirai*, and at 60%, where it normally shows maximum adhesion. This was done to independently look at both the adhesion strength lost on prey cuticle due to sacrificial scales and the hydrophobic nature of the cuticle. To further delve into the effect of hydrophobicity on adhesion strength, we created hydrophilic scaled moth substrates by plasma coating mothwings [29]. We hypothesized an increased strength of *C. akirai* glue on prey would be due to exceptional wetting, because of its low viscosity, of the hydrophobic prey cuticle relative to other species and that this spreading effect would be increased on hydrophilic substrates.

Freshly spun *C. akirai* glue is supersaturated with water and in an unstable state where adhesiveness is permanently lost as the glycoproteins collapse due to passive water loss in as little as 20 min even at extremely high humidity [12,28]. We hypothesize that differential spreading of glue components, with water-soluble components spreading faster than the glycoproteins, results in a similar collapse of the glycoproteins at small time scales during spreading on insects, thus explaining how a glue can simultaneously be 'overlubricated' and also show high cohesive forces [28]. We use infrared (IR) spectroscopy to measure the ratio of loosely bound to bound water over time. We synthesize the results of these experiments to create a conceptual model to explain the strong adhesion of *C. akirai* glue to 'dirty' moth prey.

2. Material and methods

2.1. Housing of spiders

Nine mature female *C. akirai* [30] were collected from tall grass surrounding several rice paddy fields in Chiba prefecture,

Japan, between June and September 2015–2017 (35.65635° N, 140.2425° E). Spiders were immediately shipped to the University of Akron (Akron, OH, USA) where they were housed in the laboratory for 2–12 weeks during June through December.

Spiders were housed in one of three sizes of plastic cages—height × length × width—27 cm × 17 cm × 16 cm, 20 cm × 20 cm × 20 cm or 37 cm × 24 cm × 21 cm. *Cyrtarachne* requires high humidity environments to induce web-spinning [17]. Cages were designed similar to those used to house *Pasilobus*, a sister clade that also builds similar webs only at high humidity [31]. The floor of each tank was filled with approximately 8 cm of water. Tanks were sprayed with water every night and allowed to dry in the morning. Finally, tanks were wrapped in moist towels during the night to further maintain high humidity. *Cyrtarachne akirai* were fed by placing crickets in their webs if a web was found and the spider had not eaten within the last 7 days. Spider tanks were checked for fresh web construction every 3–4 h between 20.00 and 07.00 h. Fresh webs were most often found between 05.00 and 06.00 h. Most spiders spent 2–14 days in captivity before making their first web.

Eight *L. cornutus* were collected in the early evening from a bridge located in Akron, OH, USA, between July and September during 2017–2018 (41.1351682°N, −81.54862730000002°E). Specimens were placed in individual cages at ambient room humidity in a greenhouse that was partially open to the outside environment so that conditions varied based on weather conditions. Cages were sprayed nightly with water to maintain humidity. Crickets were placed in webs every 2–3 days once spiders had begun to eat. Webs were removed the next day to ensure the specimens created fresh webs for testing.

2.2. Collection of silk

Once *C. akirai* constructed a web, two adjacent samples of viscid thread were collected. One thread was adhered to paper cards across 12.58 mm gaps for tensile testing, while the other was collected onto a glass microscope slide for imaging. Between one and four samples were collected from each web, dependent on the size of the web and whether or not the spider was disturbed enough that it began to recycle its web during collection. Silk was glued to the cards using polyvinyl acetate, which dries relatively quickly but does not dehydrate the silk strand. For *L. cornutus*, capture threads were only collected on paper cards measuring 12.58 mm.

The difficulty of collecting silk, the tendency of spiders to quickly recycle their webs when disturbed and the relative rarity with which spiders constructed webs limited the total number of samples that we obtained. Thus, sample sizes represent individual threads pooled across all spiders, rather than individual webs.

2.3. Aggregate glue adhesion strength

Adhesion tests were conducted using the Nano Bionix® tensile tester (MTS Systems Corp., Oak Ridge, TN, USA). Tests were conducted as previously described [24,28]. Capture threads samples were 12.6 mm and were preloaded onto substrates until they registered 15 µN, held in place for 6 s, and were then pulled from the substrates at 0.1 mm s^{-1}, until release [28]. Pull-off speed and thread length were chosen to coincide with previous studies [24,28]. Fresh silk tests were conducted at the humidity giving maximum adhesion for each species, *C. akirai* were conducted at 92.3 ± 5% humidity ($N = 41$), *L. cornutus* at 60.8 ± 5% humidity ($N = 78$) using a plastic humidity chamber [24]. We previously hypothesized that the low viscosity of *C. akirai* aggregate glue facilitates adhesion on the moth cuticle by allowing the glue to rapidly spread and penetrate the scales [1,10]. Thus, *L. cornutus* was additionally tested at 83.8 ± 5% ($N = 24$) as this the humidity where *L. cornutus* silk has the same viscosity as *C. akirai* [24]. Owing to the lower humidity of the testing room, attention was paid to move samples between spider cages and the humidity chamber as fast as possible and, because of this, we were unable to photograph droplets to normalize for capture thread glue volume. Force as a function of displacement was measured until the glue completely detached from the surface. Peak forces were noted, and total energy dissipated during peeling, listed as work to release, was calculated from the force–displacement curve.

We measured adhesion on pristine scaled moth surfaces, 5.5 ± 0.4 mm measured using an electronic caliper, of the forewings of the wax moth, *Achroia grisella* (Order: Family). Though this moth species does not occur in the natural habitat of *Cyrtarachne*, it was chosen as it is easy to find and raise. The scale size of *A. grisella* should be similar to that of Cyrtarachne's natural prey as *A. grisella* is similar in size and moth scale size is proportionate to total wing length [31,32]. To prepare moth surfaces, forewings were adhered to cardboard strips using Cyanoacrylate glue with the wing's dorsal scales facing upwards. These strips were then adhered to stiff, L-shaped metal strips that could be mounted in Nano Bionix tester with the plane of the mothwing oriented perpendicular to the spider silk. Total sample sizes were *C. akirai* ($N = 17$), *L. cornutus* 60% ($N = 24$) and *L. cornutus* at 90% ($N = 24$).

We also performed a set of tests on bare mothwing cuticle (*C. akirai* ($N = 15$) and *L. cornutus* ($N = 30$)), where we removed the scales by rubbing the substrate with tweezers. 'Bare' cuticle substrates measured 5.8 ± 0.1 mm in width. These tests allowed us to partition the effects of substrate chemistry from topology. Samples for bare cuticle tests were attached as discussed previously and then manually rubbed until all scales were removed from the base cuticle. These surfaces were made to determine how much adhesion strength is lost due to scale detachment during peeling and correlate differences in adhesion strength between glass and hydrophobic scaled moth cuticle with substrate chemical difference and differences in hydrophilicity (*C. akirai* $N = 13$, *L. cornutus* $N = 24$) [1,2].

For each test, each surface sample was used a maximum of nine times—three separate samples for *L. cornutus* at both 60 and 90% RH and *C. akirai* at 90% RH. These samples were treated as independent replicates because natural variation in surface morphology was evenly distributed across species. The substrate was moved slightly between each of these tests to ensure that adhesion was being measured only on a fresh surface. Each capture thread was tested only once. Average work to release and peak force were calculated for each species and testing condition. Separate *t*-tests comparing work to release and peak force between *L. cornutus* and *C. akirai* were conducted interspecifically at each test condition and intraspecifically between test conditions.

2.4. Silk spreading behaviour

We compared the spreading rate of *C. akirai* aggregate glue on glass, pristine mothwing, hydrophilic mothwing and bare moth cuticle to determine if differences in adhesion strength correlated with variation in surface wetting and spreading speed. 0.5 mm long silk threads were collected onto a glass fork and inserted into a custom-made humidity chamber mounted on a Leica dm lb2 optical microscope. N_2 gas was pumped through water and into the chamber to reach 90% humidity [24]. Silk strands were given 5 min to acclimate to the new humidity. The spider silk thread was then raised into contact with the substrates at 0.1 mm s^{-1} using a motor-controlled manipulator followed by drying in N_2 gas [24]. Videos were filmed at 1000 frames per second (fps) under an Olympus BX53 light optical microscope. Videos were analysed using ProAnalyst Motion Analysis Software. Two droplets were chosen from each video depending on video clarity. Individual coordinate systems

were created for each droplet originating at their respective centers. X–Y coordinates were tracked on the positive y-direction edge of the droplet.

Since mothwings were opaque, samples for spreading videos for both bare ($N = 3$) and scaled moth forewings ($N = 6$) were lowered onto their test substrates instead of raised and lighted from above using a gooseneck microscope illuminator. Tests were filmed at 1000 fps and were brought into contact at 0.1 mm s^{-1} as described above [24]. Tracking of droplet spreading on scaled moth forewings was more difficult for *C. akirai*, as the glue spread underneath scales made exact tracking of the leading edge difficult. For these tests, the front of the droplet was estimated using a characteristic darkening of the scales as they were wetted. Samples of *L. cornutus* at 60% ($N = 3$) and *L. cornutus* at 90% ($N = 24$) capture silk were recorded on scaled cuticle as described for *C. akirai* [24]. Spreading behaviour for *L. cornutus* at 60% RH on glass is taken from Amarpuri *et al.* [24].

For each droplet, the spreading radius as a function of time (r) was normalized using an initial droplet radius (R), r/R. Normalized radii as a function of time were then averaged between droplets with the standard error calculated for each test condition. The maximum spreading radius was averaged for each spreading condition. Average curves were compared between each species on the scaled moth cuticle. To quantify the spreading rate, a capillary exponent was calculated, α. The radius of a spreading droplet on a surface is expected to follow the relation $R(t) \propto \sim Dt^{\alpha}$, where D is a constant and is related to the capillary number, surface roughness and equilibrium contact angle; α is a function of roughness and is proportional to the rate of spreading and t is time [33–37]. The value of the spreading coefficient D is related to the surface tension divided by the viscosity and surface roughness. In general, we expect much larger changes in viscosity rather than surface tension as humidity is changed; thus, changes in D should be attributed to a change in viscosity. Typical surfaces obey Tanner's Law where the rate of spreading is proportional to time raised to the 1/10 power [35–37]. Surface roughness can cause the spreading power law to be higher than this predicted value (r/R at t^{α}, $\alpha > 1/10$). The expected spreading exponent, α, will vary depending on the type of roughness, and is expected to be between 3/10 and 3/4 for moth scales.

2.5. Silk peeling behaviour

Differences in substrate surface structure can lead to different mechanisms of adhesive failure. To determine the mechanism of adhesive failure on mothwings, high-speed videos of aggregate glue peeling were recorded. Samples of silk were collected as mentioned above on glass forks measuring 5 mm. Strands of fresh viscid silk of *C. akirai* and *L. cornutus* were brought into contact and then recorded being pulled from hydrophobic scaled substrates. These videos were recorded at 60 fps, to maximize filming time, using the Leica dm lb2 optical microscope and humidity setup described previously for spreading tests. Peeling tests occurred at relative humidity for maximum adhesion for *C. akirai* at 90%, and *Larinioides* at 60%. Capture threads were photographed after peeling to qualitatively observe the amount and pattern of moth scales attached to the threads [3].

2.6. Infrared spectroscopy of *Cyrtarachne akirai* aggregate glue droplets

Previous experiments and observations show that the rapid decrease in adhesive strength of *C. akirai* aggregate glue through time is tied to the amount of water present within the glue droplets [28]. We hypothesized that free water is lost by *C. akirai* glue droplets as they spread and dry, leading to an increase in viscosity. To test this, we used IR spectroscopy to measure the ratio of free to bound water. Fresh *C. akirai* capture threads were collected onto a silicon crystal ($N = 3$) and analysed using a Thermo Nicolet 6700 Fourier transform IR spectrometer equipped with a mercury–cadmium–tellurium detector [38]. Spectra were taken from 1500 to 4500 nm. The intensity of the maximum peak in the ranges of 3250–3350 and 3350–3450 nm were measured because they corresponded to the amount of water present in the sample: the amount of bound water and loosely bound water present, respectively [39]. We measured the IR spectrum of single droplets immediately upon being placed into the chamber at 90% RH and then every 5 min for a total of 40 min. The ratio of these two peaks was compared over time to determine if there was a significant change in the relative amount of bound water within the glue droplets. We additionally measured the intensity of a common protein peak at approximately 1650 nm and calculated the ratio of heavily bound and loosely bound water to this peak to determine the relative loss of both types of water.

Additionally, we hypothesized that the rate at which lightly bound water transitioned to heavily bound water would increase because of drying of the glue droplet, explaining why the glue irreversibly loses adhesion when briefly exposed to even slightly 'dry' air (e.g. approx. 70% RH). To test this hypothesis, we used three test conditions. First, a single capture thread was placed into the chamber at 90% RH, an IR spectrum was taken and then repeated every 5 min for 40 min total. A second capture thread was placed into the chamber at 90% RH and then after the initial spectrum was taken the humidity was dropped to 70% RH for 2 min with readings taken every 5 min. Lastly, we tested a fresh thread after drying at 70% RH for 15 min prior to being tested as described above [39].

3. Results

3.1. Aggregate glue adhesion strength

Cyrtarachne akirai aggregate glue was confirmed to be significantly stronger on glass substrates than our model organism *L. cornutus*. The average work to release on glass was 7.27× higher for *C. akirai* compared to *L. cornutus* at glass 60% RH ($p = 0.05$) 16.5× for *L. cornutus* at 90% RH ($p = 0.03$). Adhesion for *L. cornutus* dropped by 2.28× ($p = 4.\ 63 \times 10^{-5}$) when humidity was increased ($p = 4.63 \times 10^{-5}$), table 1 [40].

When tested on natural substrates, adhesion strength dropped 1.7× for *L. cornutus* at 60% RH on scaled moth cuticle compared with bare cuticle, but this trend was not significant as the work to release was statistically equal for each surface ($p = 0.48$). As hypothesized, adhesion strength increased for *C. akirai* on scaled moth cuticle in both peak forces and work to release. Work to release for *C. akirai* was 1.6× ($p = 0.058$) stronger on scaled cuticle than on glass, table 1. This value is approximately 30× (*L. cornutus* at 60% RH $p = 9.06 \times 10^{-6}$) higher than the adhesion strength of *L. cornutus* on scaled cuticle, table 1.

Average work to release on bare cuticle was not statistically different from glass—1.34× ($p = 0.428$) for *C. akirai* but 2.52× ($p = 1.59 \times 10^{-7}$) lower for *L. cornutus* on bare cuticle compared with the glass substrates, table 1. Adhesion strength for *C. akirai* was 53% higher on scaled mothwings compared with bare cuticle. Work to release dropped 1.7× for *L. cornutus* at 60% RH on bare cuticle compared with scaled cuticle, table 1 [40]. Hydrophilic mothwings samples, CA < 0°, lead to a statistical decrease in work to release for *L. cornutus* compared with natural mothwing ($p = 0.01$) but

Table 1. Work to release and peak force on glass, bare cuticle, scaled cuticle and hydrophilic cuticle for *C. akirai*, *L. cornutus*. X's express tests we did not conduct.

	glass	bare cuticle	hydrophobic scaled cuticle	hydrophilic scaled cuticle
work to release (μJ)				
C. akirai	9.46 ± 3.61	7.04 ± 1.60	14.90 ± 3.01	8.9 ± 5.2
(RH = 90)	(*N* = 41)	(*N* = 15)	(*N* = 17)	(*N* = 13)
L. cornutus	1.3 ± 0.47	0.80 ± 0.08	0.47 ± 0.34	0.44 ± 0.27
(RH = 60)	(*N* = 20)	(*N* = 30)	(*N* = 24)	(*N* = 24)
L. cornutus	0.57 ± 0.5	X	0.48 ± 0.43	X
(RH = 90)	(*N* = 20)		(*N* = 24)	
peak force (μN)				
C. akirai	707.82 ± 154.04	711.67 ± 122.89	1619.42 ± 209.70	930 ± 156.29
(RH = 90)	(*N* = 41)	(*N* = 15)	(*N* = 17)	(*N* = 13)
L. cornutus	407.31 ± 25.3	357.29 ± 31.88	245.96 ± 29.27	206.97 ± 19.47
(RH = 60)	(*N* = 20)	(*N* = 30)	(*N* = 28)	(*N* = 24)
L. cornutus	201.06 ± 36.74	X	185.31 ± 23.53	X
(RH = 90)	(*N* = 20)		(*N* = 24)	

Table 2. α for *C. akirai* and *L. cornutus* on all test substrates.

test substrate	α	D
C. akirai—glass	0.09 ± 0.05	2.4 ± 0.67
(RH = 90)	(*N* = 7)	(*N* = 7)
C. akirai—bare mothwing	0.13 ± 0.03	3.75 ± 1.32
(RH = 90)	(*N* = 3)	(*N* = 3)
C. akirai—pristine mothwing	0.29 ± 0.16	9.81 ± 8.22
(RH = 90)	(*N* = 6)	(*N* = 6)
L. cornutus—glass	0.12	1.64
(RH = 60)		
L. cornutus—pristine mothwing	0.10 ± 0.01	1.41 ± 0.11
(RH = 60)	(*N* = 3)	(*N* = 3)
L. cornutus—pristine mothwing	0.25 ± 0.12	11.36 ± 11.8
(RH = 90)	(*N* = 6)	(*N* = 6)
L. cornutus—hydrophilic mothwing	0.1 ± 0.7	1.39 ± 0.33
(RH = 60)	(*N* = 10)	(*N* = 10)

no difference for *C. akirai* compared with natural mothwing or bare cuticle substrates ($p = 0.32$, 0.42).

3.2. Aggregate glue droplet spreading and peeling behaviour on moth forewing

The high-speed video showed that *C. akirai* glue droplets spread proportionately further, 6.5× their initial radius, within the same time period than *L. cornutus*, 60% RH, on the scaled mothwing surface, and further than on all other substrates, table 2 and figures 1 and 2 [40]. After initially spreading at a rate similar to other species glue up to approximately $t = 0.01$ s, *C. akirai* droplets began to accelerate, a novel behaviour. This change corresponded to when droplets met the grooves between scales and began to flow underneath the scales. Conversely, *L. cornutus* at 60% RH, spreading

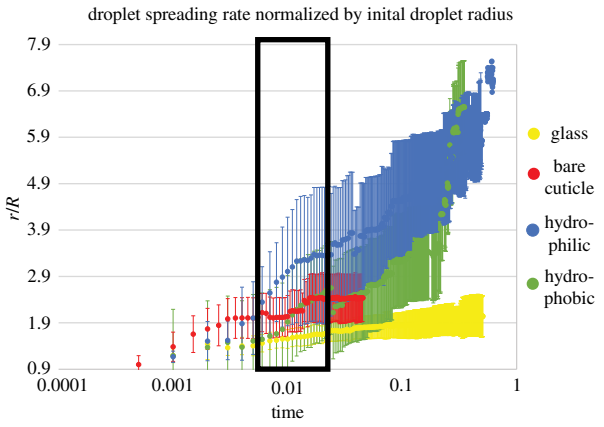

Figure 1. Average *C. akirai* aggregate glue droplet spreading on all test substrates: this graph shows the distance spread from initial droplet radius (R/r) versus time for *C. akirai* aggregate glue on glass (yellow), bare moth cuticle (red), natural mothwings (green) and hydrophilic mothwing (blue). Droplets spread further on bare hydrophobic prey cuticle than on glass substrates and spread even further on pristine hydrophobic moth cuticle with the scales still attached. Spreading behaviour on glass appears similar to other species of spiders except spreading 2.5× times faster [24]. Spreading behaviour on scaled surfaces differs qualitatively from other substrates at time approximately 0.01 s when the glue spreading rate increases. Owing to this acceleration only occurring on substrates with scales, we believe the low viscosity of *C. akirai* aggregate glue droplets seep below the scale line and spreading more readily due to capillary forces. Aggregate glue droplets spread further and faster on hydrophilic cuticle as predicted. (Online version in colour.)

was inhibited by the presence of scales spreading 1.3× less than on glass, table 2 and figure 4 [40]. Additionally, droplets on hydrophilic and hydrophobic cuticle at 60% RH failed to spread as much as at 90% RH, reaching only about 1.3× their initial diameter, figure 3 [40].

When tested at 90% RH, *L. cornutus* glue droplets showed two distinct spreading behaviours. When droplets came into contact with the area between scales, the ultimate spreading

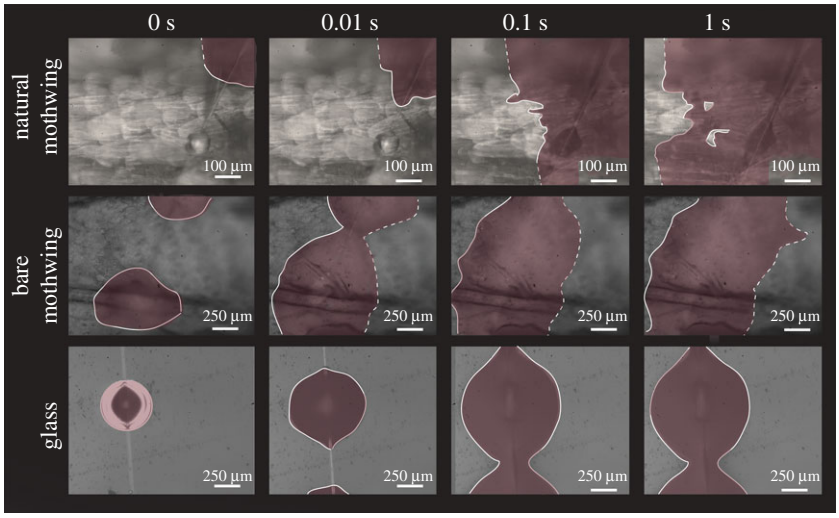

Figure 2. *Cyrtarachne akirai* aggregate glue droplet behaviour on glass, bare moth surfaces and natural moth surfaces: images of two *C. akirai* aggregate glue droplets are shown spreading on natural mothwing (20×), bare cuticle (50×) and glass (50×) at 90% RH. Solid lines track their spreading, while dashed lines estimate droplet spreading distance when out of focus. *Cyrtarachne akirai* glue droplets spread to approximately 2× their initial radius in 1 s on substrates without scales and spread upwards of approximately 5–6× their initial diameter when moth scales were present. Droplet spreading is primarily linear on unscaled substrates but irregular on scaled substrates as they flow under and over prey scales. Glue droplets have been false coloured using a transparent red layer to increase contrast relative to the substrates. A non-false-coloured figure can be viewed as electronic supplementary material. (Online version in colour.)

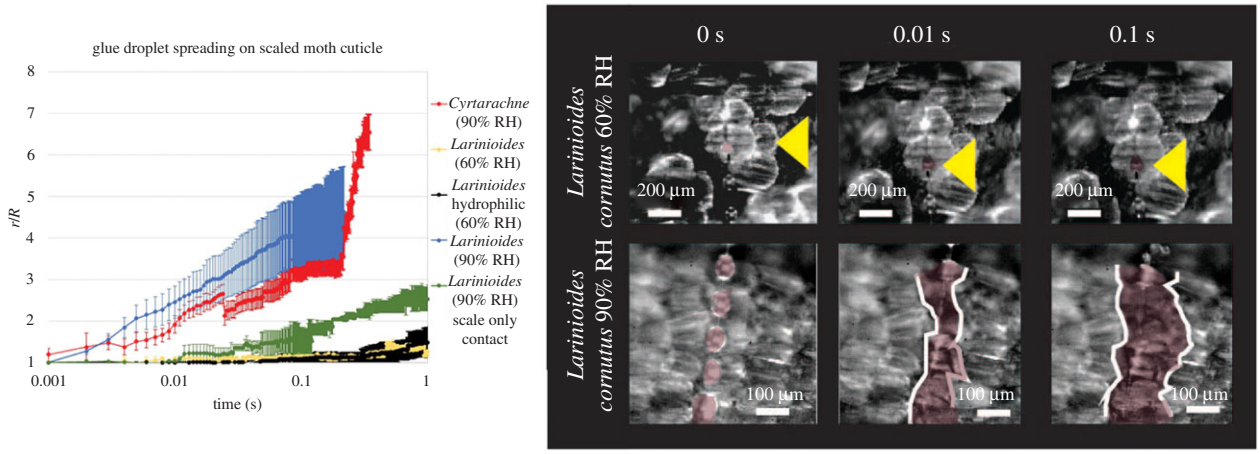

Figure 3. Comparison of average droplet spreading rate of *L. cornutus* aggregate glue droplets on moth surfaces: *L. cornutus* droplets on hydrophilic and hydrophobic cuticle at 60% RH failed to spread adequately reaching only approximately 1.3× their initial diameter. When tested at 90% RH, there were two distinct spreading behaviours observed. When *L. cornutus* droplets came into contact with the centre of a scale, green, they spread on average 2.5× their initial diameter due to the decrease in glue viscosity at 90% RH. When droplets were able to come into contact with an area between the scales of the cuticle, blue, spreading rate and distance greatly increased to approximately 4×. In this scenario, the spreading behaviour of *L. cornutus* glue was similar to *C. akirai,* red. On the right, images of aggregate glue droplets for *L. cornutus* at both 60% and 90% RH are shown during a collision, time 0–0.1 s. Owing to their small size and high viscosity, *L. cornutus*, at 60% RH, droplets spread upon the top of scales only. The yellow arrow shows a droplet which appears clear and then darkens as it comes into contact with the scale, though droplet spreading is almost unmeasurable. At 90% RH, the droplets of *L. cornutus* which come in contact with the sides of scales spread under the scale line similar to *C. akirai*. The white lines show the spreading front of the *L. cornutus* glue droplets. Glue droplets have been false coloured using a transparent red layer to increase contrast relative to the substrates. A non-false coloured figure can be viewed as electronic supplementary material.

distance of *L. cornutus* glue was similar to *C. akirai* though spreading was consistent, and they failed to show the same acceleration (figure 3) [40]. When not in contact with these grooves, the behaviour of *L. cornutus* glue was the same as at 60% RH.

The vastly different spreading behaviour of *C. akirai*, 90% RH, versus *L. cornutus*, 60% RH, droplets on natural mothwing served to create unique peeling behaviours. During failure on hydrophobic wings, *C. akirai* threads peeled away a large area of scales aggregated together, figure 4. When *L. cornutus*, 60%, threads failed on hydrophobic wings only 1–3 scales were generally removed by each glue droplet due to poor contact area achieved during spreading, figure 4 [40].

3.3. Infrared spectroscopy *Cyrtarachne akirai* aggregate glue

The total amount of both heavily bound and loosely bound water decreased during the course of all our trials when compared with the common protein peak, electronic supplementary material, table S1. The ratio of loosely bound to heavily bound water decreased during our trials

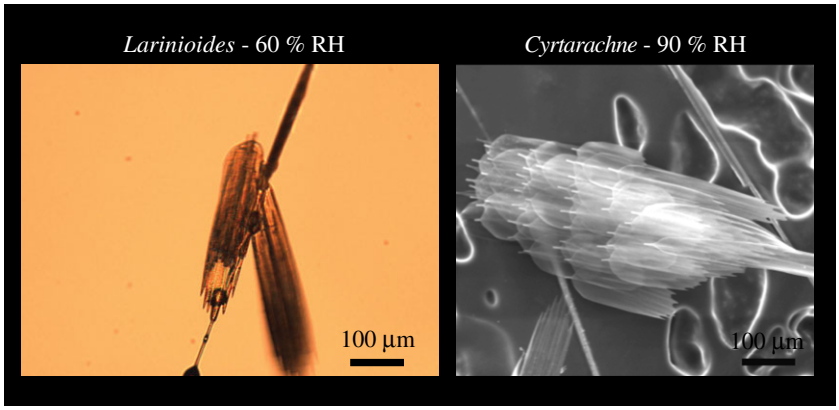

Figure 4. Peeling behaviour on scaled mothwing for *L. cornutus* and *C. akirai*: this shows examples of typical capture threads after peeling from natural hydrophobic mothwings for *L. cornutus*, optical 100×, and *C. akirai*, SEM 327×. *Larinioides cornutus* glue droplets often only attach to single scales. The large spreading radius of *C. akirai* glue droplets on natural mothwing is shown by the large area of scales, forming a metascale, that are typically left on the capture thread post peel. This leads to the higher energy absorption of *C. akirai* glue compared with other species.

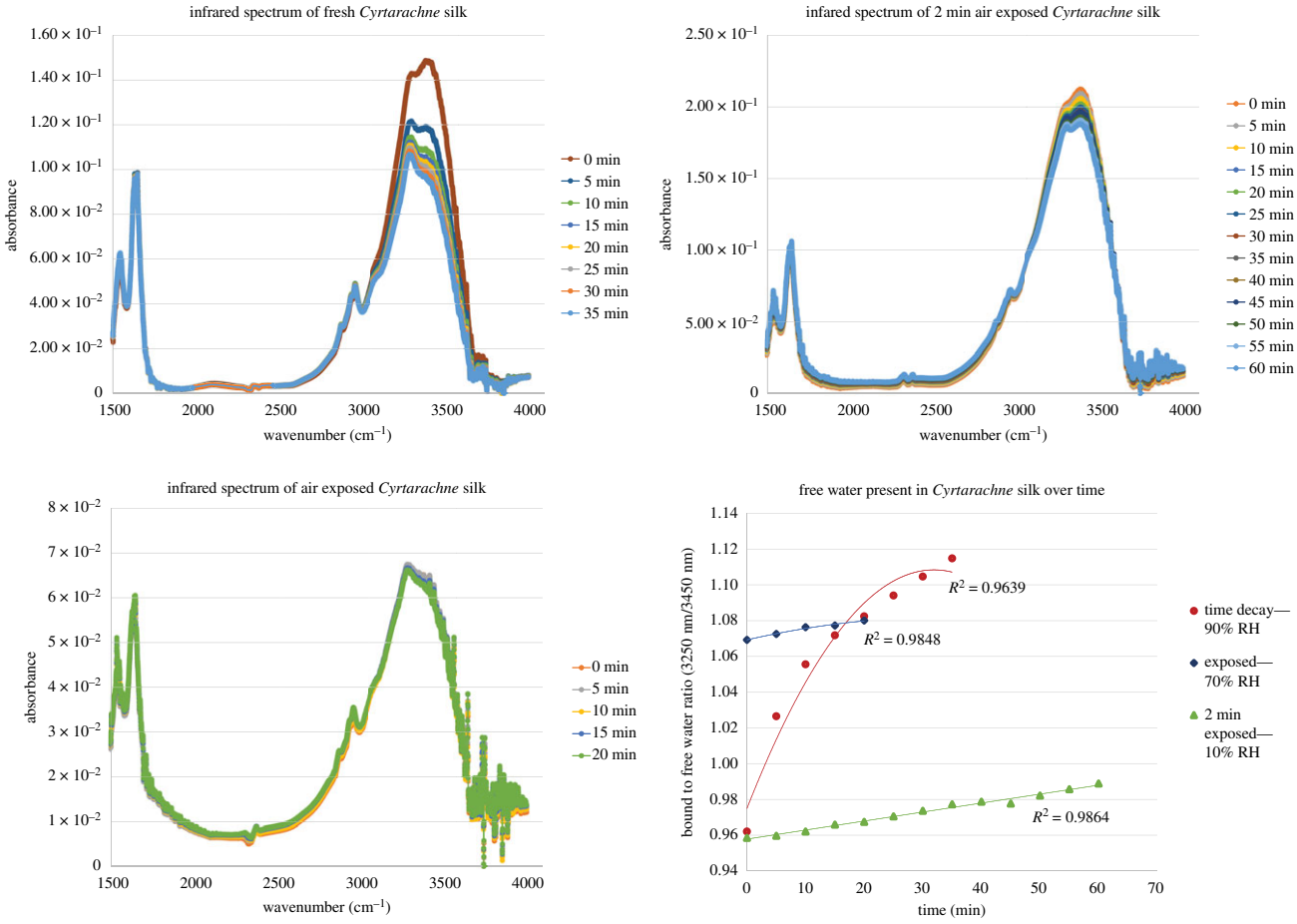

Figure 5. IR spectroscopy of *C. akirai* capture silk: graphs shows a preliminary IR spectrum of fresh, 70% RH shortly exposed and 70% long exposed capture threads over the course of 40–60 min. The graph on the right shows the ratio of the 3450 : 3250 peaks, signifying the amount of lightly bound versus heavily bound water for all tests. These tested decayed similarly though 70% RH began with less free water available at time = 0. Initial free water appears to be independent of exposure to low humidity. The discrepancy between tests is believed to be due to the silk age with less free water being available for older tests. Low humidity failed to increase water bounding speed. We believe this is due to it having a negligible effect relative to the bonding effect of droplets being spread upon the surface. This behaviour is once again unique to *C. akirai* and was not seen in the orb-weaver *L. cornutus* [38].

indicating that loosely bound water was being more readily lost, figure 5. The amount of loosely bound water is an indicator of the amount of water which is capable of flowing versus that which is stuck in an ice-like structure. Initial water binding was shown to be exponential for our fresh test and linear for our 70% RH shortly exposed and long exposed capture threads over the course of 40–60 min. Initial loosely bound water appears to be independent of exposure to low humidity, but rapid bounding of water seems to occur during this time, figure 5 [40]. This behaviour is once again unique to *C. akirai* and was not seen in the orb-weaver *L. cornutus* [38].

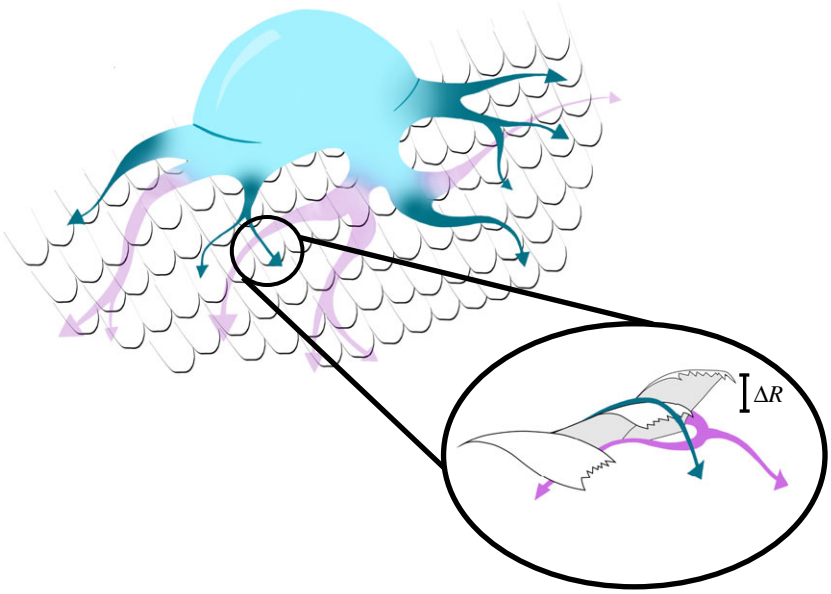

Figure 6. Flow of *C. akirai* aggregate glue over moth scales. In our model, aggregate glue droplets begin spreading on top of the scales, shown by the blue arrows, and upon reaching the edge of a scale seep below the top layer, shown in the close-up, attaching itself to the base cuticle. As droplets spread under the scale line, they flow between the base cuticle and scale, shown as purple arrows. While under the scale line, glue droplets come into contact with a 'tube' of scale and cuticle where the attraction between aggregate glue and the prey cuticle is higher than the cohesion of the aggregate glue; this creates capillary forces which pull the droplet forward. As they flow droplets meet junctions with which the capillary forces pull aggregate glue outwards, changing their direct, akin to water flowing through tributaries, as it is nonlinear. Additionally, the flow of glue pulls scales downwards, flattening the substrate and lowering the total space that may be occupied by the adhesive. This minimizes the amount necessary to properly anchor the scale to the base cuticle further increasing droplet flow distance. We have labelled this change in height ΔR. (Online version in colour.)

4. Discussion

We found that *C. akirai* capture threads adhere to mothwings through an interaction between the exceptionally large size of the glue droplets, which ensures contact of the glue and the space between scales, and usually low viscosity, which allows the glue to flow around and underneath the moth's detachable scales, figures 1 and 6 [40]. The glue thus adheres moth scales firmly to the cuticle and forms a composite adhesive structure that is actually stronger than the glue itself on clean insect cuticle [40]. *Cyrtarachne akirai* is essentially using a key defensive feature of moth prey—their sacrificial scales—against themselves (table 1; [40]). After *C. akirai* aggregate glue penetrates beneath the top layer of the scales, the glue continues to spread at an accelerated rate so that it covers a larger surface area than if the scales were not present. We hypothesize that the acceleration in spreading is due to capillary forces interacting with the low viscosity of *C. akirai* glue. Our question then becomes 'what prevents cohesive failure of the low viscosity glue?' IR spectroscopy shows a rapid decrease in the proportion of loosely bound water within *C. akirai* glue droplets, as seen in suspended droplets over longer time scales when exposed to low humidity [28,40]. We therefore hypothesize that the water-soluble components in the glue spread further and faster than the adhesive glycoproteins. This leads to a micro-drying event which collapses the glycoproteins, increasing bulk cohesion and adhesive strength.

Cyrtarachne akirai capture threads required approximately 30× more work to detach from scaled cuticle than *L. cornutus*. This behaviour can be attributed to the larger spreading radius of *C. akirai* glue droplets and their ability to maintain bulk cohesive forces. *Cyrtarachne akirai* capture thread adhesion primarily failed when the glue detached from the base cuticle so that large numbers of scales were embedded in the glue (figure 4). By contrast, *L. cornutus* threads at 90% RH peeled without detaching many scales, hinting this glue type continues to fail due to low bulk forces and interfacial attachments. Thus, we believe *C. akirai* droplets must be increasing in cohesive strength during prey capture, as water is removed through spreading.

We have created a model to explain why *C. akirai* glue spreads further on scaled mothwings than on any other substrate and why the glue accelerates once it seeps under the scales (figure 6). We use three key observations to help guide our model. First, spreading accelerates only once the droplet has seeped beneath the scale line (figure 1). At this point, the aggregate glue greatly increases its contact area with the moth cuticle as it is sandwiched between the base cuticle and scales [36,41–44]. Second, the flow of the glue beneath the scales does not follow a linear pathway; rather the glue changes direction as it radiates outwards, pulling down even more scales (figure 2 [40]). At each junction between scales, the glue is pulled perpendicularly, filling the space beneath the scales and then flowing forward again, continuing the process as it spreads outwards. Third, we observed as the glue spreads, scales were pulled down against the base cuticle, figure 6. This promotes the spreading of the glue as less material is needed to cover the same area of scales. These observations support the *post hoc* hypothesis that low droplet viscosity allows capillary forces to increase the spreading rate once the droplet has seeped beneath moth scales.

As moth scales are incorporated into *C. akirai* glue, a composite material is created which increases the normally weak attachment between scales and cuticle. The work required to remove a given area of scales from the cuticle, therefore,

becomes greater than the summation of the individual scales [1]. The function of this composite material is likely to be enhanced by the dehydration of the droplet during spreading, lowering water concentrations and increasing bulk cohesive forces.

IR spectroscopy shows a rapid decrease in the amount of loosely bound water present in the glue over several minutes, both when briefly exposed to low humidity or when spread upon a substrate. The variation between tests is likely to be due to minor differences in the silk age as the process of dehydration begins as soon as the silk is spun. When compared with a common protein peak, 1650 nm, the total amount of loosely and heavily bound water both decreased over time, with loosely bound water decaying faster, even when humidity was kept constant (electronic supplementary material, table S1). We hypothesize that during spreading capillary forces will act on *C. akirai* glue droplets to pull the water-soluble components away from the centre of the glue droplet, removing free water from the glycoprotein solution even faster. *Cyrtarachne akirai* thus uses moth scales to create a transition in its glue from low to high viscosity, allowing the glue to both spread readily and maintain high bulk forces during peeling. Thus, *C. akirai* aggregate glue balances the competing roles of spreading and bulk cohesion for adhesion by using defensive features of its prey to promote both spreading of the glue and rapid changes in viscosity.

Data accessibility. Raw data presented in tables 1 and 2 and used to create figures 1 and 3 are all available as electronic supplementary material. Raw data used to create figure 5, IR interpretation, are available as its own set of excel workbooks (rar file) as electronic supplementary material.

Authors' contributions. C.D. is responsible for writing the manuscript and conducting all experiments unless otherwise noted. A.T. collected our *Cyrtarachne* specimens and designed the tanks used to house them. He also provided information on proper spider husbandry. T.M. helped edit the manuscript and additionally provide appropriate citations and background information on the ecology of these spiders. G.A. helped carry out spreading experiments and helped in data analysis/interpretation. D.M. helped in the analysis and quantification of spreading data listed in table 2 and helped in the writing of the initial manuscript. A.D. and T.B. provided heavy manuscript editing and draft writing. They also helped check figures for accuracy and helped with data presentation.

Competing interests. We declare we have no competing interests.

Funding. Funding was provided by grants from the Japanese Society for the Promotion of Science, C.D. (grant no. IIA-1414839/SP14013) and The National Science Foundation, T.B. (grant no. NSF IOS 1257809).

Acknowledgements. C.D. would like to thank all his fellow students at the University of Akron for all their help throughout his dissertation and this project. This paper could not have been done without all their support, incite, expertise and friendship.

References

1. Bikales NM. 1971 *Adhesion and bonding*. New York, NY: Wiley–Interscience.
2. De Gennes PG, Brochard-Wyart F, Quéré D. 2004 Capillarity and gravity. In *Capillarity and wetting phenomena*. New York, NY: Springer.
3. Eisner T, Alsop R, Ettershank G. 1964 Adhesiveness of spider silk. *Science* **146**, 1058–1061. (doi:10.1126/science.146.3647.1058)
4. Xie J, Powers JM, McGuckin RS. 1993 In vitro bond strength of two adhesives to enamel and dentin under normal and contaminated conditions. *Dent. Mater.* **9**, 295–299. (doi:10.1016/0109-5641(93)90046-S)
5. Hansen WR, Autumn K. 2005 Evidence for self-cleaning in gecko setae. *Proc. Natl Acad. Sci. USA* **102**, 385–389. (doi:10.1073/pnas.0408304102)
6. Silverman HG, Roberto FF. 2007 Understanding marine mussel adhesion. *Mar. Biotechnol.* **9**, 661–681. (doi:10.1007/s10126-007-9053-x)
7. Stowe MK. 1986 Prey specialization in the Araneidae. In *Spider-webs, behavior, and evolution* (ed. W Shear), pp. 101–131. Stanford, CA: Stanford University Press.
8. Zheng Y, Gao X, Jiang L. 2007 Directional adhesion of superhydrophobic butterfly wings. *Soft Matter.* **3**, 178–182. (doi:10.1039/B612667G)
9. Wagner T, Neinhuis C, Barthlott W. 1996 Wettability and contaminability of insect wings as a function of their surface sculptures. *Acta Zool. Stockholm.* **77**, 213–225. (doi:10.1111/j.1463-6395.1996.tb01265.x)
10. Opell BD. 1994 The ability of spider cribellar prey capture thread to hold insects with different surface features. *Funct. Ecol.* **8**, 145–150. (doi:10.2307/2389897)
11. Eberhard WG. 1980 The natural history and behavior of the bolas spider, *Mastophora dizzydeani* sp. n.(Araneae). *Psyche* **87**, 143–170. (doi:10.1155/1980/81062)
12. Cartan CK, Miyashita T. 2000 Extraordinary web and silk properties of *Cyrtarachne* (Araneae, Araneidae): a possible link between orb-webs and bolas. *Biol. J. Linn. Soc.* **71**, 219–235. (doi:10.1111/j.1095-8312.2000.tb01255.x)
13. Vincent JF, Wegst UG. 2004 Design and mechanical properties of insect cuticle. *Arthropod. Struct. Dev.* **33**, 187–199. (doi:10.1016/j.asd.2004.05.006)
14. Triplehorn CA, Johnson NF. 2005 *Study of insects*. Boston, MA: Thomson Brooks.
15. Yeargan KV. 1994 Biology of bolas spiders. *Annu. Rev. Entomol.* **39**, 81–99. (doi:10.1146/annurev.en.39.010194.000501)
16. Eberhard WG. 1990 Function and phylogeny of spider webs. *Annu. Rev. Ecol. Syst.* **21**, 341–372. (doi:10.1146/annurev.es.21.110190.002013)
17. Baba YG, Kusahara M, Maezono Y, Miyashita T. 2014 Adjustment of web-building initiation to high humidity: a constraint by humidity-dependent thread stickiness in the spider *Cyrtarachne*. *Naturwissenschaften* **101**, 587–593. (doi:10.1007/s00114-014-1196-9)
18. Robinson MH, Robinson B. 1975 Evolution beyond the orb web: the web of the araneid spider Pasilobus sp., its structure, operation and construction. *Zool. J. Linn. Soc.* **56**, 301–313. (doi:10.1111/j.1096-3642.1975.tb00272.x)
19. Boutry C, Blackledge TA. 2010 Evolution of supercontraction in spider silk: structure–function relationship from tarantulas to orb-weavers. *J. Exp. Biol.* **213**, 3505–3514. (doi:10.1242/jeb.046110)
20. Foelix R. 2011 *Biology of spiders*. New York, NY: OUP.
21. Sahni V, Blackledge TA, Dhinojwala A. 2011 A review on spider silk adhesion. *J. Adhes.* **87**, 595–614. (doi:10.1080/00218464.2011.583588)
22. Opell BD, Karinshak SE, Sigler MA. 2013 Environmental response and adaptation of glycoprotein glue within the droplets of viscous prey capture threads from araneoid spider orb-webs. *J. Exp. Biol.* **216**, 3023–3034. (doi:10.1242/jeb.084822)
23. Amarpuri G, Zhang C, Blackledge TA, Dhinojwala A. 2017 Adhesion modulation using glue droplet spreading in spider capture silk. *J. R. Soc. Interface.* **14**, 20170228. (doi:10.1098/rsif.2017.0228)
24. Amarpuri G, Zhang C, Diaz C, Opell BD, Blackledge TA, Dhinojwala A. 2015 Spiders tune glue viscosity to maximize adhesion. *ACS Nano* **9**, 11 472–11 478. (doi:10.1021/acsnano.5b05658)
25. Townley MA, Pu Q, Zercher CK, Neefus CD, Tillinghast EK. 2012 Small organic solutes in sticky droplets from orb webs of the spider *Zygiella atrica* (Araneae; Araneidae): β-alaninamide is a novel and abundant component. *Chem. Biodivers.* **9**, 2159–2174. (doi:10.1002/cbdv.201200077)
26. Townley MA, Tillinghast EK. 2013 Aggregate silk gland secretions of araneoid spiders. In *Spider ecophysiology*. Berlin, Germany: Springer.
27. Guo Y, Chang Z, Guo HY, Fang W, Li Q, Zhao HP, Feng XQ, Gao H. 2018 Synergistic adhesion

mechanisms of spider capture silk. *J. R. Soc. Interface.* **15**, 20170894. (doi:10.1098/rsif.2017.0894)

28. Diaz C, Tanikawa A, Miyashita T, Amarpuri G, Jain D, Dhinojwala A, Blackledge TA. 2018 Supersaturation with water explains the unusual adhesion of aggregate glue in the webs of the moth-specialist spider, *Cyrtarachne akirai*. *R. Soc. open sci.* **5**, 181296. (doi:10.1098/rsos.181296)
29. HARRICK PLASMA. 2017 *Overview | Harrick Plasma*. Harrick Plasma. Web. 9 February 2017. (https://harrickplasma.com/technical-library)
30. Tanikawa A. 2013 Two new species of the genus *Cyrtarachne* (Araneae: Araneidae) from Japan hitherto identified as *C. inaequalis*. *Acta Arachnol.* **62**, 95–101. (doi:10.2476/asjaa.62.95)
31. Simonsen TJ, Kristensen NP. 2007 Scale length/wing length correlation in Lepidoptera (Insecta). *J. Nat. Hist.* **37**, 673–679. (doi:10.1080/00222930110096735)
32. Miyashita T, Sakamaki Y, Shinkai A. 2001 Evidence against moth attraction by *Cyrtarachne*, a genus related to bolas spiders. *Acta Arachnol.* **50**, 1–4. (doi:10.2476/asjaa.50.1)
33. McHale G, Shirtcliffe NJ, Aqil S, Perry CC, Newton MI. 2004 Topography driven spreading. *Phys. Rev. Lett.* **93**, 036102. (doi:10.1103/PhysRevLett.93.036102)
34. Shirtcliffe NJ, McHale G, Newton MI, Zhang Y. 2009 Superhydrophobic copper tubes with possible flow enhancement and drag reduction. *ACS Appl. Mater. Inter.* **1**, 1316–1323. (doi:10.1021/am9001937)
35. Cazabat AM, Stuart MC. 1986 Dynamics of wetting: effects of surface roughness. *J. Phys. Chem. US.* **90**, 5845–5849. (doi:10.1021/j100280a075)
36. Eddi A, Winkels KG, Snoeijer JH. 2013 Short time dynamics of viscous drop spreading. *Phys. Fluids* **25**, 013102. (doi:10.1063/1.4788693)
37. Jung S, Hutchings IM. 2012 The impact and spreading of a small liquid drop on a non-porous substrate over an extended time scale. *Soft Matter.* **8**, 2686–2696. (doi:10.1039/c2sm06565g)
38. Singla S, Amarpuri G, Dhopatkar N, Blackledge TA, Dhinojwala A. 2018 Hygroscopic compounds in spider aggregate glue remove interfacial water to maintain adhesion in humid conditions. *Nat. Commun.* **9**, 1890. (doi:10.1038/s41467-018-04263-z)
39. Amarpuri G, Chaurasia V, Jain D, Blackledge TA, Dhinojwala A. 2015 Ubiquitous distribution of salts and proteins in spider glue enhances spider silk adhesion. *Sci. Rep. UK.* **5**, 9030. (doi:10.1038/srep09030)
40. Diaz C, Tanikawa A, Miyashita T, Maksuta, D, Amarpuri G, Blackledge T. 2018 Data from: The moth-specialist spider *Cyrtarachne akirai* uses prey scales to increase adhesion. Dryad Digital Repository.
41. Chen L, Bonaccurso E. 2013 Effects of surface wettability and liquid viscosity on the dynamic wetting of individual drops. *Phys. Rev. E* **90**, 022401. (doi:10.1103/PhysRevE.90.022401)
42. Fratzl P, Gupta HS, Fischer FD, Kolednik O. 2007 Hindered crack propagation in materials with periodically varying Young's modulus—lessons from biological materials. *Adv. Mater.* **19**, 2657–2661. (doi:10.1002/adma.200602394)
43. Pearson RA, Yee AF. 1991 Influence of particle size and particle size distribution on toughening mechanisms in rubber-modified epoxies. *J. Mater. Sci.* **26**, 3828–3844. (doi:10.1007/BF01184979)
44. Ayyaswamy PS, Catton I, Edwards DK. 1974 Capillary flow in triangular grooves. *J. Appl. Mech.* **41**, 332–336. (doi:10.1115/1.3423288)