Toughness-Enhancing Metastructure in the Recluse Spider's Looped Ribbon Silk

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Supplementary Information

Supplementary Text

Silk tensile testing

The cross-sectional area of each individual's silk was measured from a looped strand deposited onto mica (Table S1). To encourage the silk to adhere flat to the substrate, a 20 μL water droplet was applied to each mica sample and spin-coated. This treatment was not found to substantially affect the calculated silk dimensions (Fig. S3). To find cross-sectional area, AFM contact mode scanning was conducted with a 0.27 N/m nominal spring constant tip (BudgetSensors) in an NTEGRA Scanning Probe Laboratory (NT-MDT). AFM scans were flattened and analyzed using Gwyddion (www.gwyddion.org).

Individual loop lengths (L_i) were measured with Fiji/ImageJ (http://fiji.sc/) by inspecting a looped strand deposited onto mica. Loop size was quite consistent for each individual during a single spinning session: on average, the loop size standard deviation was 4% of the mean (Table S1). The total loop length for each sample was found by multiplying the number of loops for the sample by the mean loop length for the individual.

A power analysis was conducted to determine the sample size necessary to detect the desired effect. Since looped silk was compared to non-looped silk from each individual in a single spinning event, a paired analysis was deemed most appropriate. The following two-tailed equation was used to perform the power analysis:[1]

$$n = 2\left(\frac{s \cdot \left(Z(\alpha_s/2) + Z(\beta)\right)}{w}\right)^2$$

where n is the number of samples (rounded to the nearest integer), Z is the normal inverse cumulative distribution function, α_s is the significance level of the test (type I error), β is the type II error (power = $1 - \beta$), s is the sample standard deviation, and w is the effect that is desired to be detected. For all power analyses and other tests, we used $\alpha_s = 0.05$ and $\beta = 0.1$ (90% power).

For the purpose of power analysis, we assumed a mean strength of 1 GPa and standard deviation of 0.25 GPa as estimates of *Loxosceles* silk properties since these values roughly match those of prior findings for spider silk[2] and the stiffness and extensibility of *Loxosceles* silk has been found to reflect those of other silks.[3] Passieux et al. observed a ≈50% decrease in a looped fiber's strength relative to that of a non-looped fiber,[4] so we conservatively opted to test for a 40% strength decrease in *Loxosceles* silk; we thus let w = 0.4 GPa. The power analysis calculation yielded n = 8.

To test if the pairing between looped and non-looped samples was effective, looped strength was plotted vs. non-looped strength and the correlation coefficient was calculated to be 0.886, with a corresponding P-value of 0.003. This strong correlation and significant result led us to reject the null hypothesis that there is no relationship between non-looped and looped strength and justified the use of a paired analysis.[1]

Tests for normality using the D'Agostino-Pearson omnibus K2 normality test[5] yielded a P-value of 0.85 for the strength data and 0.40 for the toughness data, indicating that neither data set is inconsistent with a Gaussian distribution.

Paired student's t-tests of the paired strength and toughness data were conducted using MATLAB ('ttest' function, version R2015b, MathWorks) to test the null hypotheses that there was no difference in strength and toughness between non-looped and looped strands. The 95% confidence interval (CI) was calculated using the equation:

$$c = \frac{T(1 - \alpha_s/2, n - 1) \cdot s}{\sqrt{n}}$$

where c is half of the CI and T gives the Student's t-test inverse cumulative distribution function for the inputs of cumulative probability and degrees of freedom, respectively.[1]

The strength test resulted in a P-value of 0.53, leading us to fail to reject the strength null hypothesis at a significance level of 0.05. In addition, the entire 95% CI of the strength differences fell within the 25% zone of equivalence relative to non-looped strength,[1] which has an upper bound equal to 125% of the mean non-looped strength and a lower bound equal to 75% of the mean non-looped strength (Fig. 2b, black dotted lines). Naturally, the CI also falls within a 40% zone of relative equivalence—the target effect in our power analysis.

The toughness test yielded a P-value of <0.001, leading us to reject the toughness null hypothesis (Fig. 2c).

Tape tensile testing

For tensile tests of looped strapping tape, we assumed 5% standard deviation in tape toughness and 22% toughness increase predicted by the model for a single loop of size α = 1.5, we conservatively desired to detect a 10% increase in toughness. A two-sided, two-sample pooled t-test power analysis conducted using MATLAB ('sampsizepwr' function, 't2' test type) yielded n = 7. We opted for 8 samples due to the ease of testing. D'Agostino-Pearson normality tests did not reveal a significant deviation from normality in any of the samples: P = 0.22 for non-looped strength, P = 0.44 for looped strength, P = 0.52 for non-looped toughness, and P = 0.54 for looped toughness. F-tests for equal variance also failed to reject the null hypothesis of no significant difference between the variances of looped and non-looped samples, with P = 0.28 returned in a comparison of looped and non-looped strength data and P = 0.26 in a comparison of toughness (MATLAB, 'vartest2' function). A two-sided, two-sample Student's t-test of the strength data resulted in a P-value of 0.25, leading us to fail to reject the null hypothesis of no difference between looped and non-looped tape strength; also, the 95% CI computed using MATLAB ('ttest2' function) fell within a 25% relative zone of equivalence (Fig. S2a). A two-sided, two-sample Student's t-test of the toughness data yielded a P-value of 0.005, leading us to reject the null hypothesis of no difference in the toughness of looped and non-looped tape.

For tests of folded masking tape, we again assumed 5% standard deviation in tape toughness. Because preliminary testing indicated a three or four-fold increase in toughness due to folding, we conservatively desired to detect an effect of 20% toughness increase. We conducted a two-sided, two-sample pooled t-test power analysis using MATLAB to yield $n = 3$. With only three samples, evaluated metrics of normality and equivalency of variance are unhelpful;[1] however, the plotted distribution of data (Fig. 4f) does not appear to indicate a severe deviation from the assumptions of normality or equal variance. A two-sided, two-sample Student's t-test conducted on folded and straight tape samples with a null hypothesis of no difference in strength yielded a significant result (P=0.043), yet the 95% CI fell within the 25% relative zone of equivalency (Fig. S2b). This result indicates that folding induces a significant decrease in strength, and an effect of less than 25% of the non-folded mean strength can be predicted in 95% of cases. Another two-sided, two-sample Student's t-test with a null hypothesis of no difference in folded v. straight strand toughness yielded P<0.001, leading us to conclude a significant increase in toughness due to folding (Fig. 4f).

Looped fiber modeling

To model the tensile behaviour of a looped fiber, we first considered an elastic material. The behaviour of the strand was iteratively described, with the total length of the strand recalculated after each loop opening.

In the model, the loaded length L_0 of the strand is first strained up to the loop breaking stress σ_ℓ, resulting in an initial strain $\varepsilon_0 = \sigma_\ell / E$, where E is the elastic modulus of the material. At ε_0, a loop is released, immediately adding length αL_0 to the strained fiber. The change in strain due to the ith loop opening, $\Delta\varepsilon_i$, is equal to $\Delta\varepsilon_i = \alpha - \Delta\varepsilon_{\mathrm{recov}}{}^i$, where α reflects the slack added by a loop opening and $\Delta\varepsilon_{\mathrm{recov}}{}^i = (\sigma_\ell / E)*(1 + \alpha(i - 1))$ is the elastic strain recovery. The $(1 + \alpha(i - 1))$ term reflects the strained length $L_0(1 + \alpha(i - 1))$ that relaxes when the ith loop opens. $\Delta\varepsilon_i$ can be positive or negative. If positive, the length added from a loop opening is greater than the elastic strain recovery, and the stress drops to 0 (Fig. 3a,d). If $\Delta\varepsilon_i$ is negative, the loop length is less than the strand's elastic recovery, and the stress does not reach 0 (Fig. 3b,e). After the ith loop opens, the new strand length equals the sum of L_0 and $i\alpha$. Once all N loops have opened, the fiber is strained until failure at σ_{u}.

To gauge the toughness gain due to looping, it was also necessary to model the expected stress-strain curve of a non-looped strand whose length is equivalent to the total length of the looped strand, $L_0(1 + N\alpha)$. In this curve, the initial slack length of $L_0 N\alpha$ is first exhausted. Then, additional strain $\Delta\varepsilon_n = (\sigma_{\mathrm{u}} / E) * (1 + N\alpha)$ is required to reach σ_{u} (Fig. 3, red curves).

In our model of a plastic looped fiber, we considered a strain-hardening plastic material since silk and many other materials are strain-hardening.[6,7] To simplify the strain-hardening plastic model, the plastic region of the stress-strain curve was approximated to have some constant linear slope less than E (Fig. 3d). Upon loop opening, only the elastic behaviour of the curve contributes to

$\Delta\varepsilon_{recov}{}^{i}$. To model the stress response in the plastic region after one or more loops have opened, the strand must be considered a heterogeneous system: a portion of the strand has been plastically deformed and work-hardened, i.e. it has a stiffness of E, while the newly opened loop length behaves plastically. These two heterogeneous components were modelled as springs in series according to $k_{\mathrm{eff}}^{\,i} = \dfrac{k_{\mathrm{w}} k_{l}}{k_{\mathrm{w}} + k_{l}}$, where k_{w} is the spring constant of the work-hardened strand section containing $i - 1$ loops, k_{ℓ} is the spring constant of the ith looped section, and $k_{\mathrm{eff}}^{\,i}$ is the effective spring constant of the aggregate strand.

Supplementary Tables

Indiv	Sex	Age (yrs)	A (μm^2)	ℓ or n	L_i (μm)	n_ℓ	n_s	$\bar{\sigma}_u$ (GPa)	$\bar{W}$ (J/g)	$\bar{\varepsilon}_{max}$
A	m	2	0.405	n	-	-	3	0.72	91	0.32
				ℓ	893±16	4	2	0.63	24	–0.46
B	f	2	0.639	n	-	-	3	0.70	82	0.29
				ℓ	765±41	2	3	0.67	52	–0.10
C	f	2	0.620	n	-	-	3	0.70	90	0.31
				ℓ	973±21	5	4	0.79	47	–0.28
D	f	2	0.297	n	-	-	3	0.67	76	0.30
				ℓ	861±21	5	3	0.55	38	–0.15
E	f	2	0.596	n	-	-	3	0.52	69	0.33
				ℓ	904±9	3	3	0.49	24	–0.37
F	m	2	0.525	n	-	-	3	0.43	55	0.31
				ℓ	729±71	3	4	0.62	32	–0.47
G	m	2	0.455	n	-	-	2	0.43	58	0.32
				ℓ	782±64	2	4	0.54	36	–0.17
H	f	2	0.554	n	-	-	3	0.57	71	0.31
				ℓ	902±31	3	3	0.57	28	–0.43

Table S1. Silk tensile test data for all eight individuals tested, where m indicates a male individual, f indicates a female individual, A is the cross-sectional area, ℓ indicates a looped sample, n indicates a non-looped sample, L_i is the average (and standard deviation) single loop length, n_ℓ is the number of loops measured to determine L_i, n_s is the number of silk tensile samples for the given individual and silk type, $\bar{\sigma}_u$ is the mean ultimate strength, $\bar{W}$ is the mean effective toughness, and $\bar{\varepsilon}_{max}$ is the mean maximum true extensibility. Since $\bar{W}$ and $\bar{\varepsilon}_{max}$ are calculated from the total length of the fiber (initially loaded length plus total loop length), a negative $\bar{\varepsilon}_{max}$ indicates that the looped strand fractured before an extension equaling the total length of the strand was reached.

ID	w (mm)	ℓ or n	α	σ_u (MPa)	W (J/g)
A	12.2	n	-	354	3.10
B	12.5	n	-	390	3.80
C	13.0	n	-	409	4.27
D	12.3	n	-	359	3.45
E	10.1	n	-	344	2.87
F	14.6	n	-	404	4.01
G	14.3	n	-	411	4.18
H	9.8	n	-	415	4.01
I	14.9	ℓ	1.52	408	5.40
J	9.7	ℓ	1.46	340	3.93
K	12.7	ℓ	1.56	346	4.76
L	11.0	ℓ	1.43	374	4.90
M	13.7	ℓ	1.90	304	3.73
N	10.6	ℓ	1.75	314	4.32
O	13.3	ℓ	1.35	422	5.75
P	10.3	ℓ	1.39	400	5.90

Table S2. Strapping tape tensile test data, where w is the tape width, ℓ indicates a looped sample, n indicates a non-looped sample, α is the normalized loop size, σ_u is the ultimate strength, and W is the toughness.

ID	f or n	α	σ_u (MPa)	W (J/g)
A	n	-	39	0.68
B	n	-	40	0.77
C	n	-	42	0.84
D	f	0.49	33	2.57
E	f	0.50	38	2.38
F	f	0.50	36	3.08

Table S3. Label tape tensile test data, where f indicates a folded sample, n indicates a non-looped sample, α is the normalized loop size, σ_u is the ultimate strength, and W is the toughness.

Supplementary Figures

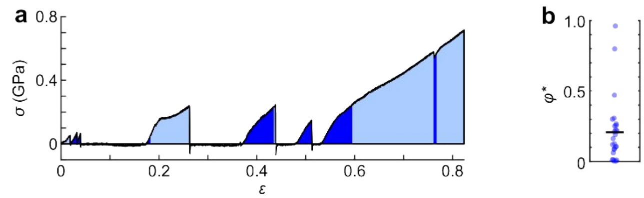

Fig. S1. Estimated relative enhancement due to looping in *Loxosceles* silk. (a) Representative looped stress-strain curve from Fig. 2a. The dark blue areas are only present due to "strain cycling" after loop opening events. The light blue areas represent regions of the curve where the strand first encountered a given stress, which would also be present in a non-looped system. (b) Estimated relative enhancement $\varphi^* = (W_\ell - W_\ell^*)/W_\ell^*$ of the looped toughness due to strain cycling in each test (n=26, circles) and the average (horizontal bar), where W_ℓ^* is the toughness of the unravelled portion of the strand (light blue areas, a) and W_ℓ is the toughness of the entire looped strand (all blue areas, a).

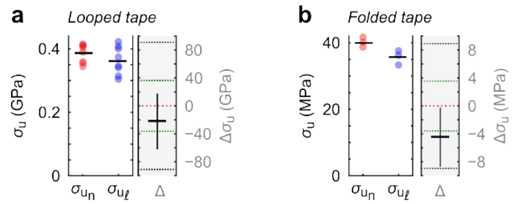

Fig. S2. Ultimate strength of (a) looped strapping tape and (b) folded label tape. Left frames (white background) give the strength of each sample (circles) and mean (horizontal bars), and right frames (grey background) show the mean difference between hidden length and non-hidden length samples (horizontal bar), 95% CI (vertical bar), zero difference (red dotted line), 10% relative zone of equivalence (green dotted lines), and 25% zone of equivalence (black dotted lines). In (a), the 95% CI intersects the zero difference line, indicating a non-significant result (two-tailed two-sample *t*-test, P=0.25, n=8), while the 95% CI below the zero line in (b) reflects a significant decrease in strength (two-tailed two-sample *t*-test, P=0.043, n=3). The length of the 95% CIs, as well as the 10% and 25% zones of equivalence, give a sense of the relative scale of the effect of introducing hidden length: all hidden length groups can be considered equivalent to the control at a level of 25% relative equivalency (since the 95% CIs lie completely within that zone), while at 10% relative equivalency, the looped tape data (a) is ambiguous and the folded tape (b) would be considered not equivalent.

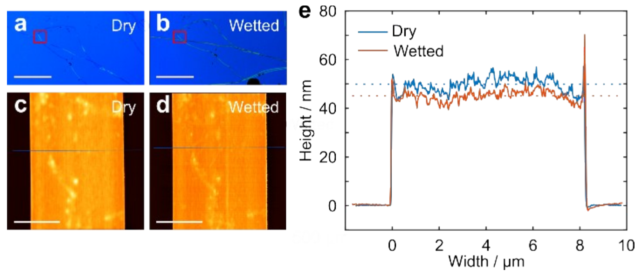

Fig. S3. Comparison of *Loxosceles* silk deposited on a Si substrate before and after wetting, with wetting accomplished by deposition of a water droplet and spin-coating to dry. (a,b) Optical microscopy images of dry and wetted silk, respectively, with approximate scanned areas indicated by red boxes. (c,d) AFM scans of the same section of silk before and after wetting, with sampled cross-sectional profiles indicated by blue lines. (e) Comparison of cross-sectional profiles of the silk strand before and after wetting, with dashed lines indicating the median height of each silk surface. Calculated cross-sectional areas: 0.407 μm^2 dry, 0.372 μm^2 wet.

Supplementary Videos

Video S1. *Loxosceles* spinning behavior and resulting silk structure observed while roaming unrestrained. Filmed at 60 fps, shown at 30 fps (0.5x speed).

Video S2. Angled view of *Loxosceles* spinneret behavior. Filmed at 1000 fps, shown at 25 fps (1/40th speed).

Video S3. Angled view of *Loxosceles* spinneret behavior, with only the right anterior lateral spinneret and associated posterior spinnerets active. Filmed at 1000 fps, shown at 25 fps (1/40th speed).

Supplementary References

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