Extended wet-spinning can modify spider silk properties

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Contrary to expectation, we demonstrate that spider dragline silk spun experimentally under water displays greater stiffness and higher resilience compared to silk spun "naturally" into air. We suggest that this consequence of extended wet-spinning is due to increased molecular orientation resulting from extension of the mobile phase.

There is a common – and wrong- understanding that spider silk solidifies upon contact with air[1-4] In fact, the 'typical' spider silk (such as the benchmark Major Ampullate dragline silk of the golden silk spider *Nephila* sp.) forms and solidifies deep inside the spider's spinning duct[5] due to the combination of acidification, phase-transition[6,7] and molecular flow orientation.[8] Thus spider spinning relies on internal rather than external solidification draw-down, which is the norm in all industrial fibre-spinning technology.[9] Nevertheless, spider silk seems to benefit from some degree of external, post-spigot draw-down, presumably because here the rest-water is 'squeezed out'[9] and the molecules are 'locked' into their final orientation.[7] The question arises whether the post-spigot draw-down is an integral part of the spider's natural spinning behavior – and thus necessary for the exquisite mechanical properties of spider silk – or whether it is a 'side-effect' of the spider living in air – with little relevance for silk mechanics. Since spider silk fibres are much less stiff in the wet and contracted state,[10,11] a quick loss of water was postulated to lead to a "dry" fibre with optimized mechanical strength.[12] Yet for polymers, the process of orientation takes time,[13] which implies a quick solidification is disadvantageous to the orientation of spider silk fibre due to the immobility of molecular chains therefore resulting in a fibre of sub-optimal mechanical properties. In order to test this question whether water has negative or positive effects on the mechanical properties of spider silk during the spinning process, we induced spiders to spin into water rather than air. Thus our experimental set-up essentially extended the spider's post-fibre-formation spinning-duct by 250 mm, *i.e.* two orders of magnitude from the natural (and internal) 2–4 mm.[14]

Our spinning setup is illustrated in Fig. 1. For the 'into-water' trials the spider's spigots were well submerged in a tank (with the lungs well above the water). A polytetrafluoroethylene (PTFE) stick guided the thread through the water bath; and the spinnerets were fixed into position to avoid the emergent thread touching either the spider or a hard surface. The spinning process was monitored under a microscope to ensure the silk thread was reeled out directly from the spigot of one major ampullate gland, without touching anything before it reached the guide PTFE stick. It is worth noting that a tension was maintained on the silk by the spinning motor during the 'into-water' trial. Therefore it is different from a supercontraction test in which the silk is relaxed. We reeled the silk at 20 mm s^{-1}, using 18 individual spiders for both the into-air (AS) and into-water (WS) samples. The mechanical tests were performed with our custom-built micro-scale materials testing machine.[11,15] Regular tests were performed on 4–7 samples of silks of different reeling conditions from each spider, and the corresponding loading-unloading tests were performed for 2–3 samples. We use "capacity to shrink" to quantify the maximum contraction ratio of a single thread in distilled water.[11] For the Raman test (Dilor LabRam-1B Raman microscope. A He–Ne laser was used to give 4.7 mW of energy at 632.81 nm) of each fibre, the long axis of the fibre was aligned either fully parallel (p) or fully orthogonal (o) to the direction of polarization of the laser beam. All the spectra were normalized to the intensity of the CH$_2$ bending peak at ~1450 cm^{-1} since it is

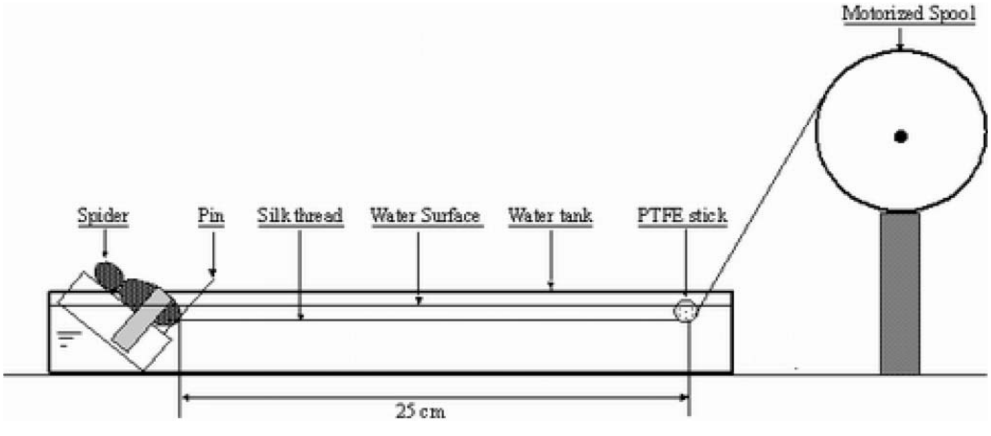

Fig. 1 The scheme of experimental set-up for forced spinning in water.

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insensitive to the conformation of the protein.[16,17] Reproducible results were obtained on 4 pairs of samples from two spiders.

When spun into the air at the 'normal' speed of 20 mm s^{-1}, (comparable to spiders' natural walking/spinning rate under room conditions) the silk of *Nephila edulis* is supposed to have optimized mechanical properties.[11,15] However, Fig. 2A shows that the silk can be even stronger if spinning is processed in water (as an example, the mechanical properties of the silk reeled from one spider in air and water are summarized in Table 1). For all silk samples from the 18 spiders we tested, samples of 13 spiders showed significant difference (two-tail independent t-test at a significance level of 0.05) between certain mechanical properties of WS and AS. In those samples, we observed a decrease of breaking strain but an increase of strain energy (the energy used to stretch the thread up to a particular strain value. In this paper, we investigated the strain energy whereby the strain is 10%), often accompanied by an increase of yield stress as well as increase of breaking stress and initial modulus. Meanwhile, the loading-unloading test results from those samples show that WS enjoys a smaller permanent setting than AS (Fig. 2B) and a better resilience (Table 2). In general, silks from 13 out of 18 spiders were stiffer when spun in water rather than in air. We may attribute the observed difference in mechanical properties to the better orientation of molecular chains in WS considering that the extended water-environment allowed more time for the molecules to align. This is implied by a greater capacity to shrink of WS[16,18–20] (Table 2). It is well known that the inter- and intra-molecule hydrogen bonds contribute to the orientation of molecular chains of silk protein.[11,21] The passing of the silk through the water bath undoubtedly changes the details of hydrogen bonding in silk, and thus affects its structure and properties (Liu, Shao and Vollrath, unpublished results).

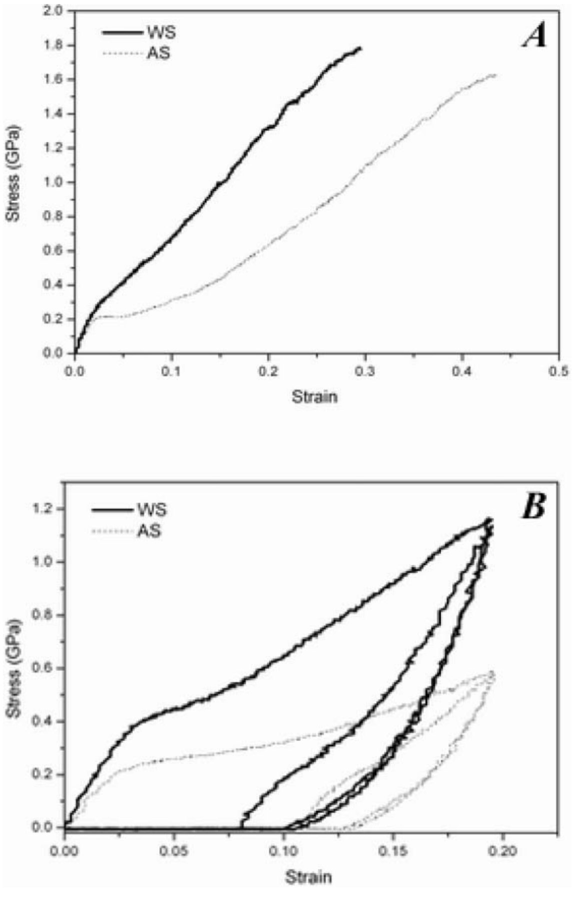

Fig. 2 Comparison of the typical tensile properties of spider silk spun at normal speed (20 mm s^{-1}) in air (AS) and in water (WS). (A) stress-strain curves; (B) loading-unloading cycles.

Considering silks from other 5 spiders (28% of our experimental subjects) showed no significant differences between the mechanical properties of WS and AS, we can infer the residual water in the silk to vary from spider to spider. For most spiders (72% of our experimental subjects), the molecular chains were not completely locked into place with fibre leaving the spigot and a consequent processing in water benefits the orientation of molecular chains.

Comparison of Raman spectra of silk reeled in air and water allows us to study the molecular order of a fibre. Fig. 3 illustrates the Raman spectra of single fibres. The Raman spectra of WS were quite similar to that of AS in both alignment directions (parallel and orthogonal to the direction of polarization of the laser beam). However, the significant difference of mechanical properties between the WS and AS implies that the orientation of polypeptide backbones inside these fibres might be different, which can be determined from the dichroism of Raman amide I and amide III bands. The increase of alignment of molecular chains along the fibre long axis has converse effects on the intensity of those two bands.[16,22] Hence the extent of backbone alignment along the fibre may be semi-quantitatively described using the intensity ratio of the orthogonal to the parallel direction of amide I (1667 cm^{-1}) or of the parallel to the orthogonal direction of amide III (1230 cm^{-1}).[23] The results are summarized in Table 3. The data suggest that the molecular chains of silk are more inclined to orient along the long axis of the fibre in the WS than the corresponding AS.

In summary, our experiments show that spider silk can be modified by extending the animal's natural extruder. For most

Table 2 The comparison of capacity to shrink and resilience between WS and AS, data from one spider and repeated by other spiders

Spinning environment	Capacity to shrink (%)	[a]Resilience (%)
Air	21 ± 1	20 ± 1
Water	30 ± 2	28 ± 1

[a] Resilience: The ratio between energy recovered and energy put in a loading–unloading cycle.

Table 1 The comparison of mechanical properties between AS and WS, data from one spider and repeated by other spiders

Spinning environment	Breaking strain (%)	Breaking stress (GPa)	Initial modulus (GPa)	Strain energy (kJ kg^{-1})	Yield strain (%)	Yield stress (GPa)
Air	42 ± 1	1.59 ± 0.08	11.80 ± 1.45	15.70 ± 1.28	2 ± 0	0.19 ± 0.02
Water	30 ± 2	1.73 ± 0.16	14.07 ± 1.85	26.04 ± 4.16	2 ± 0	0.22 ± 0.03

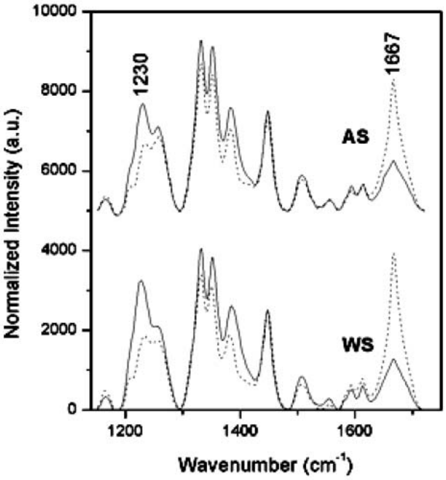

Fig. 3 The Raman spectra of AS and WS (reeled at 20 mm s^{-1}). A single fibre was aligned either parallel (solid line) or orthogonal (dash line) to the direction of polarization of the laser beam

Table 3 The intensity ratio of the orthogonal to parallel direction of the Raman band 1667 (amide I) and of the parallel to orthogonal direction of 1230 cm^{-1} (amide III)

Spinning environment	*I*[1667]o/*I*[1667]p	*I*[1230]p/*I*[1230]o
Air	2.63	1.59
Water	3.08	1.75

spiders, water-spinning did lead to higher molecular order, as our Raman measurements demonstrate, resulting in stronger spider silk. Clearly, in major ampullate dragline silk of *Nephila*, order is positively related to stiffness and negatively to extensibility.

Thus it appears from our experiments that we can industrially (as the spider could over evolutionary time) modify material properties by modifying the length of the aqueous bath in the duct following the internal draw-down (*i.e.* the length of the post-drawdown duct). It may be well worthwhile to compare the length of these duct sections in a range of spiders with silks of different mechanical properties. This would allow us to get some measure for the way evolution has dealt with the spinning aspects of modifying silk, as opposed to modifying the proteins of the feedstock.

Our experiments have important industrial implications as they demonstrate that in the design of an artificial spider silk-extruder the length of the aqueous post-draw duct will significantly affect the material properties of the fibre. These are engineering design criteria, which are independent of the feedstock composition or the conditions of the elongational flow that leads to the initial fibre formation. After all, these undoubtedly also important variables affecting silk properties were not affected by our experiment.

Finally, our experiments should once and for all put to rest the old belief that spider silk 'hardens' upon contact with air. Of course, drying out is necessary to give the dragline silk its typical stress–strain characteristics. This is shown by the increase in extensibility of silk plasticized by submersion in water or other suitable solvents.[11] But, as our experiments show, the process of drying does not in itself organize the molecular order, this organisation is in fact a matter of drawing-time as well as drawing-force (*i.e.* reeling speed) in the plasticized (*i.e.* wet) stage. Drying just 'locks' the order into place.

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