The Journal of Experimental Biology 212, 1981-1989
Published by The Company of Biologists 2009
doi:10.1242/jeb.028944

# How super is supercontraction? Persistent *versus* cyclic responses to humidity in spider dragline silk

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Accepted 15 April 2009

## SUMMARY

**Spider dragline silk has enormous potential for the development of biomimetic fibers that combine strength and elasticity in low density polymers. These applications necessitate understanding how silk reacts to different environmental conditions. For instance, spider dragline silk 'supercontracts' in high humidity. During supercontraction, unrestrained dragline silk contracts up to 50% of its original length and restrained fibers generate substantial stress. Here we characterize the response of dragline silk to changes in humidity before, during and after supercontraction. Our findings demonstrate that dragline silk exhibits two qualitatively different responses to humidity. First, silk undergoes a previously unknown cyclic relaxation–contraction response to wetting and drying. The direction and magnitude of this cyclic response is identical both before and after supercontraction. By contrast, supercontraction is a 'permanent' tensioning of restrained silk in response to high humidity. Here, water induces stress, rather than relaxation and the uptake of water molecules results in a permanent change in molecular composition of the silk, as demonstrated by thermogravimetric analysis (TGA). Even after drying, silk mass increased by ~1% after supercontraction. By contrast, the cyclic response to humidity involves a reversible uptake of water. Dried, post-supercontraction silk also differs mechanically from virgin silk. Post-supercontraction silk exhibits reduced stiffness and stress at yield, as well as changes in dynamic energy storage and dissipation. In addition to advancing understanding supercontraction, our findings open up new applications for synthetic silk analogs. For example, dragline silk emerges as a model for a biomimetic muscle, the contraction of which is precisely controlled by humidity alone.**

Key words: biomimetic muscle, humidity, major ampullate fibroin, spider silk, supercontraction.

## INTRODUCTION

Most spiders spin a variety of different silks that are utilized for diverse ecological functions (Blackledge and Hayashi, 2006). Dragline silk, produced from major ampullate silk glands, evolved early in the diversification of spiders, perhaps in the Jurassic (Vollrath and Selden, 2007), and it was the first silk to be spun as discrete structural threads, rather than sheets of fibers. The origin of dragline silk is a hypothesized key innovation in spiders' evolutionary success (Bond and Opell, 1998). More than 40,000 species of spiders now use dragline silk for a variety of functions such as lifelines and the frames of webs.

In addition to its clear evolutionary importance, dragline silk exhibits many desirable qualities that make it a focus of biomimetic research (Hakimi et al., 2007; Vollrath and Porter, 2006b). Dragline silk is mechanically impressive. It combines high tensile strength and elasticity in a low density fiber, achieving a strength to weight ratio ~5× greater than steel and a toughness ~3× greater than Kevlar (Booth and Price, 1989; Gosline et al., 1986; Guan, 2007; Vollrath, 2000; Vollrath and Knight, 2001; Vollrath and Porter, 2006b). Dragline silk is also spun under environmentally benign conditions and is immunologically compatible with living tissue (Hakimi et al., 2007; Vadlamudi, 1995; Vollrath and Porter, 2006a). Finally, spider dragline silk 'supercontracts' (Work, 1981). The silk absorbs water at high humidity, altering its material properties and shrinking up to 50% of its original length, if unrestrained, while increasing in overall volume. This process generates substantial stress in silk when it is restrained and the potential to perform work. Supercontraction may provide a mechanism that tensions webs as they become loaded with dew or rain (Elices et al., 2004; Guinea et al., 2003). Potentially more important, supercontraction probably plays an essential role in determining the molecular orientation of silk during the spinning process as the still wet fiber is extruded through the spinning duct, thereby increasing the alignment of silk molecules along the fiber axis (Pérez-Rigueiro et al., 2003). But, supercontraction may also be exploited by materials scientists to tailor silk's already impressive properties (Guinea et al., 2005).

Our understanding of the mechanics of supercontraction is growing (Guinea et al., 2003; Guinea et al., 2005; Pérez-Rigueiro et al., 2003; Pérez-Rigueiro et al., 2005; Savage et al., 2004; Work, 1981; Yang et al., 2000). Spider dragline silk is a hierarchically structured material composed of a blend of multiple types of proteins (Hinman and Lewis, 1992; Xu and Lewis, 1990). Among species spanning millions of years of evolutionary history, the amino acid sequences of dragline silk proteins, called major ampullate spidroins (MaSp), are highly conserved, so that they can be classified into two groups often termed MaSp1 and MaSp2 (Gatesy et al., 2001). Both MaSp1 and MaSp2 contain numerous poly-alanine repeat blocks that are hypothesized to fold into β-sheet crystals during the spinning process, thereby locking together individual proteins and stiffening the overall fiber (Grubb and Jelinski, 1997). The regular

spacing between amino acids within these crystals is such that multiple strong hydrogen bonds are maintained between silk molecules even as silk fibers are extended to failure (Grubb and Jelinski, 1997). The poly-alanine repeats are interspersed between glycine-rich blocks (Simmons et al., 1996), particularly glycine–glycine–X in MaSp1 and glycine–proline–glycine–$X_n$ in MaSp2, where X represent any one of a limited number of amino acids, as well as a small proportion of more variable spacer regions (Gatesy et al., 2001). Together, these form the 'amorphous' network of the silk proteins, which, although overall softer and less organized than the β-sheet crystals, is itself divided into regions of variable structural organization (Grubb and Jelinski, 1997). The glycine-rich blocks form linker regions, possibly either $3_1$ helices (Kummerlen et al., 1996) or non-periodic β-sheet lattice crystals (Thiel et al., 1997), which are immediately adjacent to the β-sheet crystals. They maintain high degrees of secondary structure through strong hydrogen bonding (Savage and Gosline, 2008a). By contrast, the random-coil region of the amorphous network is quite malleable, structurally isotropic, and its organization is determined in large part by the degree of shear force applied to the liquid silk as it is spun into a solid fiber (Ortlepp and Gosline, 2004; Pérez-Rigueiro et al., 2005; Vollrath and Knight, 2001). Again, hydrogen bonding maintains this structural organization, but the strength of those bonds is significantly lower because of the relatively poor orientation of the molecules.

During supercontraction, water is hypothesized to plasticize silk fibers by breaking hydrogen bonds between proteins thereby allowing re-orientation of silk molecules to lower energy levels (Guinea et al., 2003; Guinea et al., 2005; Jelinski et al., 1999; Savage et al., 2004; Schafer et al., 2008; Yang et al., 2000). Recent studies have focused on the importance of disrupting secondary structure in the glycine-rich blocks for mobilization of proteins within the amorphous network (Savage and Gosline, 2008a; Savage and Gosline, 2008b; van Beek et al., 2002). This allows the random-coil regions to move rapidly to more disordered, higher entropy configurations, driving the contraction of the silk. However, the degree of water uptake during supercontraction, how water affects fiber performance, and the permanence of the whole process are poorly understood (Agnarsson et al., 2009a). Furthermore, we lack a general understanding of how silk responds to water outside supercontraction *per se*.

Here, we characterize both the static and dynamic mechanics of supercontraction when silk is restrained such that it cannot shrink, we demonstrate permanent water uptake during supercontraction, and we quantify the permanent change in both mechanical and thermodynamic properties of silk that results from supercontraction. Furthermore, we show that dragline silk exhibits a cyclic response to changes in relative humidity that is both qualitatively and quantitatively distinct from supercontraction. This cyclic response produces high forces that can be precisely controlled through humidity alone. Thus, spider silk emerges as an attractive model for biomimetic muscle fibers (Agnarsson et al., 2009b).

The interactions between silk and water are highly complex. Although 'supercontraction' is broadly applied in the literature as a term describing the changes that water induces in the molecular structure of dragline silk and hence its performance, the outcomes of those interactions depend in part on the initial state of the silk. Originally, supercontraction referred to the physical shrinking of unrestrained silk fibers upon wetting (Work, 1977; Work and Morosoff, 1982). However, the term is also used to describe the substantial forces that develop when restrained fibers are wetted (Bell et al., 2002; Guinea et al., 2003; Savage et al., 2004). Because supercontraction is sometimes used to refer simply to the wetting of silk and at other times to the behavior of wetted silk under specific conditions, we employ the term 'supercontraction' here in a generic sense to refer to changes induced in silk by initial exposure to humidity. We define the process of wetting (W) independent of the silk itself. During wetting (W), the silk may be unrestrained (U), restrained at a constant length (S), or held under a constant load (L), each of which results in different responses. Thus, the earliest descriptions of supercontraction, observing the shrinking of fibers in water droplets refer to wetted, unrestrained silk (WU), while we term the more recent approach of measuring stress generated during supercontraction of silk restrained at a constant length as ($WS_x$) where $x$ refers to the amount of strain, and under constant load as (WL).

## MATERIALS AND METHODS

We sampled major ampullate dragline silk from five specimens of the golden silk orbweaver *Nephila clavipes* (Linnaeus 1776) from FL, USA and Costa Rica. Spiders were housed individually in cages at room temperature (~23°C), fed crickets two to three times per week, and misted daily with water. To examine the effect of relative humidity on dragline silk, we collected fibers by forcible silking and glued them across 21 mm gaps in paper slides using Superglue® (cyanoacrylate), as described by Blackledge et al. (Blackledge et al., 2005c). This procedure allowed us to collect samples consisting purely of major ampullate dragline silk. The diameter of each silk sample was measured at six points along the length of the fiber using polarized light microscopy (Blackledge et al., 2005a). We also collected bulk samples of 2–4 mg of silk by spooling silk fibers onto plastic test tubes mounted on a rotating mandrel. These bulk samples were used to examine changes in silk mass in response to humidity and for thermogravimetric analysis (TGA).

### Quasi-static and dynamic stress response of restrained silk to humidity

We used a Nano Bionix tensile tester (Agilent Technologies, Oakridge, TN, USA) to examine how humidity affects the stress generated in restrained fibers of dragline silk [see Blackledge et al. (Blackledge et al., 2005c) for details of the tester]. The tensile tester was equipped with an environmental chamber that allowed precise and rapid control of humidity from ~1–95% at a constant temperature (±0.2°C). We mounted silk at ambient humidity (13% for most of the experiments, range 13–20%) and a standard 0.5% strain (these conditions are termed $WS_{0.5\%}$). We utilized two different protocols to manipulate humidity. For some samples, we rapidly cycled from ambient to ~90% humidity as fast as the environmental chamber allowed, achieving the complete range within 60–120 s. We also performed stepwise tests in which humidity was increased slowly in 10% intervals that each lasted 5–10 min. This allowed us to investigate the effects of absolute humidity *versus* rate of change in humidity. Humidity in the environmental chamber was regulated by dividing the flow of dry gaseous $N_2$ into channels, one of which flowed through a 1 m high water column and the other which remained dry. The controller then mixed the two flows before they entered the chamber to achieve the desired humidity. The feedback between the hygrometer on the chamber and the controller was slow enough that an initial switch from wetting to drying was usually accomplished through a brief burst of completely dry $N_2$ that was then rapidly mixed with a small amount of moist gas. This resulted in a brief 'undershooting' of the targeted dryness that normally occurred too quickly to register on the hygrometer, but which did manifest itself in some tests (see below). The opposite effect

sometimes occurred during wetting, but never to a large enough degree that the critical humidity causing supercontraction was reached unintentionally.

We continuously measured the force generated by restrained silk to an accuracy of ±2 μN and calculated stress by normalizing to the original cross-sectional area of each fiber. Increased stress indicated that the fiber was pulling (contracting) and decreased stress indicated that the fiber was relaxing, although we held the gage length of samples constant throughout the test. For some tests, we also measured the dynamic properties of the silk, storage and loss moduli, to better understand how storage and dissipation of energy were affected by water, using techniques described by Blackledge et al. (Blackledge et al., 2005c). During testing, silk was vibrated at 20 Hz with a force amplitude of 4.5 mN.

### Tensile mechanics of virgin *versus* supercontracted silks

We also examined how supercontraction affected the tensile properties of spider silk. We compared the mechanical performance of post-supercontraction ($WS_{0.5\%}$) dried fibers with virgin silk fibers that were treated identically, except that they were never exposed to high humidity.

Stress generated during collection of silk through forcible silking affects the tensile properties of spider silk (Elices et al., 2006). To minimize this bias, silk samples from a single dragline from an individual spider were collected in sequence and then alternate samples were used for virgin and supercontracted tests (Pérez-Rigueiro et al., 2005). Tensile testing followed the method described by Blackledge et al. (Blackledge et al., 2005b; Blackledge et al., 2005c). We calculated eight mechanical properties. (1) Ultimate strength, or true breaking stress, measured the force required to break a fiber relative to its instantaneous cross-sectional area, which was calculated assuming constant volume during extension (Vollrath et al., 2001). (2) Extensibility, or true breaking strain, measured the extension of a fiber at failure and was calculated as the natural log of the breaking length divided by original length, using the standard isovolumetric assumption (Guinea et al., 2006). (3) Young's modulus measured the stiffness of the silk as the slope of the stress–strain curve within the initial elastic region. (4) Toughness measured the energy required to rupture a fiber and was calculated from the area under the stress–strain curve. (5) Yield stress indicated the transition from elastic behavior to permanent deformation of the fiber. (6) Storage modulus was the energy stored elastically in the fiber through entropic interactions, reversible bonding and deformation of covalent bonds. (7) Loss modulus was the energy dissipated in the fiber, i.e. energy lost as heat. (8) Tan δ, or loss tangent, was the ratio of loss to storage modulus (tan δ=loss modulus/storage modulus) and measured relative viscoelasticity (Vogel, 2003). We used paired *t*-tests to compare the mechanical performance of adjacent samples of virgin and supercontracted fibers.

### Water uptake by dragline silk

To measure water uptake by dragline silk we used two complementary approaches. We affixed the 2–4 mg bundles of silk to the NMAT (nanomechanical actuating transducer) head of the Nano Bionix tensile tester (Agilent Technologies, Oakridge, TN, USA). The ends of the fibers were loose such that this protocol approximates a WU test. We then exposed the silk bundles to two to four cycles of humidity high enough to induce supercontraction. Subsequently, we dried the silk by returning the chamber to room humidity (13%) for 5–10 min. Before and after testing, we weighed each bundle of silk to the nearest 1 μg using a Cahn25 Automatic Electrobalance (Cahn Instruments Co., Cerritos, CA, USA). This allowed us to determine the total permanent change in mass of silk that occurred during exposure to water.

The Nano Bionix also provided a continuous, relative measure of mass change throughout the test. The machine measures the amount of electromagnetic force needed to maintain the position of the NMAT head such that small changes in the mass of any material attached to it could be measured in real time as a change in load. The load resolution of the NMAT head has a lower limit of 50 nN and displacement resolution of 0.1 nm, although in practice these relatively noisy tests resulted in resolutions of ~1 μN. Cyclical changes in load exerted on the NMAT head indicated temporary movement of water into or out of the silk, while the difference between the load exerted on the NMAT head by the dry fibers pre- and post-supercontraction indicated permanent absorption of water during supercontraction. We then expressed the continuous measure of the relative changing load measured on the NMAT head as a change in the absolute silk mass by normalizing it to the difference in mass of silk at the beginning *versus* the end of the test, measured on the electrobalance.

### Thermogravimetric analysis

We performed thermogravimetric analysis (TGA) on bundles of virgin and dried supercontracted (WU) silk to determine if supercontraction permanently altered the material within silk fibers. TGA exposes materials to gradual increase in temperature (from ambient to ~500°C) and measures the relative mass lost from the samples as individual molecular compounds boil off at different critical temperatures. This provides a highly sensitive mechanism to detect whether the molecular compounds differ among material samples. All tests were conducted in a $N_2$ atmosphere.

### Repeatability of supercontraction

If entropy drives the shrinking of dragline silk during supercontraction then restoring order to the silk molecules might recover the ability of silk to again supercontract after it is dried. To test this hypothesis we examined the restrained supercontraction response of silk ($WS_{0\%}$) on the Nano Bionix after fibers were physically shrunken and then re-extended. Before the first supercontraction test, we strained the virgin silk to ~0.5% and allowed it to relax at 0.1% $s^{-1}$, recording the extension at which it first relaxed to 0 MPa stress. After each supercontraction test, we allowed the silk to fully contract in length to a relaxed state while still wet, shrinking by ~30% of its length and held it in place for 5 min. We then pulled the wet silk back to its original starting length (i.e. to within ±1 μm of the length at which the virgin silk relaxed to 0 MPa stress) before drying the sample for 10 min. We then exposed the silk to a rapid increase in humidity, measuring the stress response of the fiber ($WS_{0\%}$). The entire process was repeated 10 times.

## RESULTS

### Response of silk to changes in relative humidity

*Nephila* dragline silk supercontracted at ~70% humidity and generated stress of ~40–80 MPa ($WS_{0.5\%}$), similar to previously reported research. However, we also found that the silk exhibited a novel, cyclic response to changes in humidity that was distinct from supercontraction. In contrast to supercontraction, stress was generated as fibers dried during the cyclic response, and water instead induced relaxation. Furthermore, supercontraction occurred only once in restrained silk fibers, whereas the cyclic response was highly repeatable across many cycles of humidity. Surprisingly, the

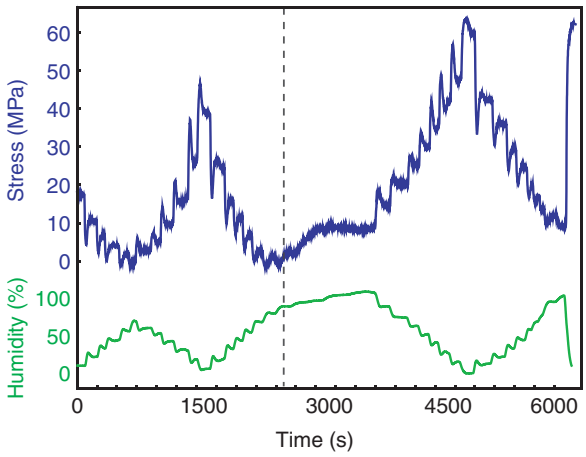


Fig. 1. Response of spider silk ($WS_{0.5\%}$) to gradual changes in humidity. Initially, as the dry air is moistened the silk relaxes until humidity approaches 50%. The fiber then tenses in response to drying and relaxes in response to wetting. The dashed line indicates the onset of supercontraction when humidity reaches 70%, the only time that the fiber tenses in response to increasing humidity. The slow increase in humidity results in relatively low supercontraction stress (Agnarsson et al., 2009a). The spikes in stress at the onset of each stepped change in humidity are caused by the slow response time of the hygrometer, which allows a brief burst of 'overly' dry or moist air to flood the chamber when first decreasing or increasing humidity respectively. Note the rapid increase in stress when dry air was allowed to quickly flood the chamber at the end of the test.

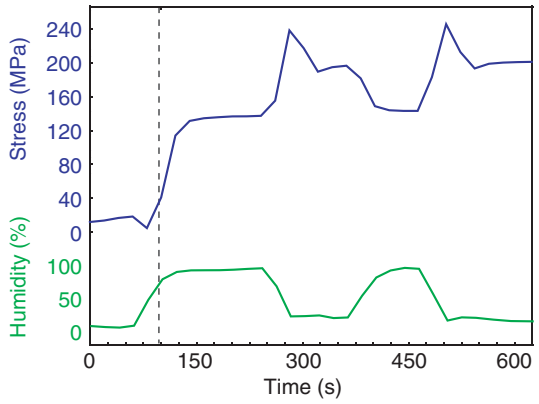


Fig. 2. Response of spider silk ($WS_{0.5\%}$) to rapid cyclical changes in humidity. When humidity first increases, the silk briefly begins to relax as in Fig 1. The dashed line indicates the onset of supercontraction at 70% humidity. The spikes in stress at ~280 and 480 s result from an initial burst of dry air entering the chamber during drying because of the response time of the hygrometer. Notice that the supercontraction stress is relatively high compared with Fig. 1 because of the rapid wetting of the silk (Agnarsson et al., 2009a).

stress generated by the cyclic response exceeded that of supercontraction in some circumstances. Fig. 1 illustrates both the cyclic and supercontraction response of spider silk to humidity for a single 5 µm diameter fiber mounted at a low stress of 20 MPa ($WS_{0.5\%}$). As humidity slowly increases in a stepwise manner to 60%, stress reduces to zero and the fiber completely relaxes. The fiber then tenses well beyond mounting stress when it is dried. The fiber again relaxes as humidity increases until a critical level of ~70% RH, which causes supercontraction, thereby resulting in a sudden tensioning of the fiber (dashed line in Fig. 1). After supercontraction, the silk continues to react cyclically to changes in humidity – tensing as it dries and relaxing as humidity increases, but the silk never again supercontracts. Throughout the test, small spikes in stress are evident at the beginning of each stepped transition to drier humidity and small drops in stress are seen at each increase in humidity. These 'artifacts' are caused the by a brief initial under- or overshooting of the target humidity, but illustrate the very rapid and reversible nature of the cyclic response.

Fig. 2 shows the response of a 5 µm silk thread ($WS_{0.5\%}$) to rapid increase in humidity to nearly 90% over ~60 s. The fiber initially supercontracts to a stress of 100 MPa. The substantially larger stress here than that shown in Fig. 1 results from the faster rate of hydration during supercontraction (Agnarsson et al., 2009a). When dried, the fiber further tenses and generates an additional stress of nearly 100 MPa. Again, a brief spike in stress is seen at the onset of drying until the environmental chamber stabilizes. Subsequent cycling of humidity results in a second relaxation-contraction cycle as before. However, tensioning never occurs in response to increasing humidity after supercontraction. The cyclic response of silk to humidity is highly repeatable and precisely controllable. We sometimes find a slight increase in both the minimum and maximum stress across cycles (Fig. 3), but it is not yet clear why this occurs. Overall, the silk displays high resilience during cyclic contraction and a general lack of fatigue even after eight cycles run for nearly 100 min (Fig. 3).

Fig. 4 documents changes in the dynamic properties of restrained ($WS_{0.5\%}$) dragline silk in response to humidity. Supercontraction differs fundamentally from cyclic contraction. Storage and loss modulus both increase during supercontraction and are accompanied by an increase in the loss tangent. Thus, the silk becomes stiffer. Past studies identified that the stiffness of unrestrained silk decreases during supercontraction, which agrees with the hypothesized decrease in orientation of silk molecules within fibers (Shao and Vollrath, 1999; Work, 1985). However, a key difference for our study is that we restrained the virgin fibers at ~0.5% strain. This held the absolute length of the silk constant when exposed to water so that the fiber effectively became highly strained during supercontraction (i.e. its length was equivalent to that of a supercontracted fiber that was allowed to relax and then stretched close to failure). This resulted in an overall increase in stiffness, as

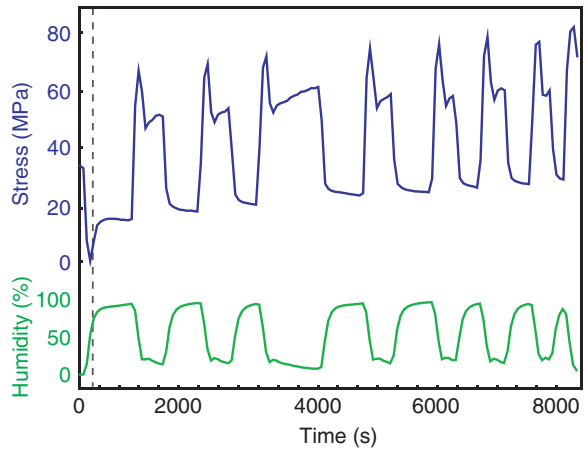


Fig. 3. Response of spider silk ($WS_{0.5\%}$) to eight cycles of rapid change in humidity. The dashed line indicates the only occurrence of supercontraction. The spikes in stress at the onset of each drying cycle result from the response time of the hygrometer allowing initial bursts of very dry air to enter the chamber.

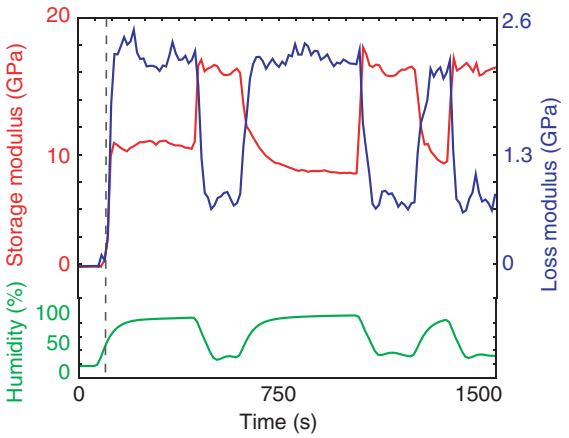


Fig. 4. Effect of humidity on the dynamic properties of spider silk. The silk was initially strained to ~0.5% and humidity was cycled as rapidly as possible. The dashed line indicates supercontraction, the only time that the storage modulus and loss modulus increase simultaneously.

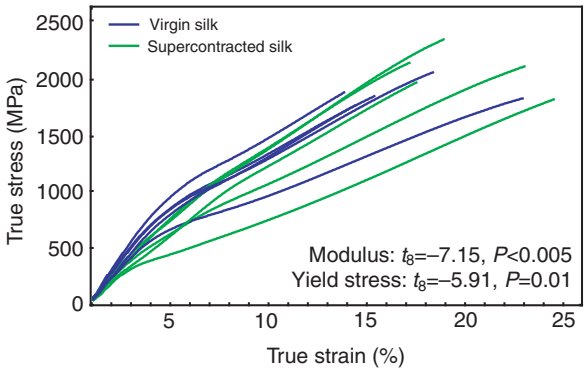


Fig. 5. Tensile performance of virgin silk compared with dry $WS_{0.5\%}$ silk (restrained virgin silk that was wetted, dried, and then tested at ambient humidity). Statistics indicate significant differences based upon *t*-tests.

would occur if a previously relaxed and supercontracted thread were stretched. Most importantly, the cyclic contraction of the silk resulted in increased storage modulus and decreased loss modulus and tan δ during drying. These changes reversed when the fibers were exposed to high humidity.

### Mechanical properties of virgin *versus* supercontracted silks

We found no difference in the ultimate strength, elasticity or toughness of virgin silk compared with dry, post-supercontraction silk (Table 1). However, initial stiffness (Young's modulus) and stress at yield were both higher in virgin silk (Fig. 5; modulus: $t_8$=–7.15, $P$<0.005; yield stress: $t_8$=–5.19, $P$=0.01). Storage modulus and tan δ at yield also were both higher in virgin than post-supercontracted fibers (Table 2, Fig. 6; storage modulus at initial tan δ: $t_8$=20.8, $P$<0.001). Together, these differences suggest a greater level of organization within the amorphous regions of proteins in virgin silk.

### Uptake of water by dragline fibers

The four bundles of silk permanently gained mass during supercontraction (1.6±0.5%; mean ± s.e.m.), when comparing the dry pre- and post-supercontraction mass on a microbalance at an ambient room humidity of ~15%. We also continuously observed the relative change in load generated by silk, using the Nano Bionix, and normalized it to the actual mass of the silk as measured on the microbalance (Fig. 7). These data corroborated our observation that at least some of the mass gained during supercontraction was never lost, even when humidity was lower than the initial (room) humidity at which samples were weighed on the microbalance. Furthermore, they demonstrated that, subsequent to supercontraction, the silk increased in mass as humidity increased and decreased in mass as humidity decreased. This change was reversible and highly proportional to the change in humidity (Fig. 7).

### Thermogravimetric analysis

The TGA analysis confirmed that supercontraction altered the silk material. The thermal stability of supercontracted fibers differs distinctly from that of virgin fiber, especially from 350–450°C (Fig. 8).

### Repeatability of supercontraction

Fig. 9 shows that supercontraction is repeatable if fully relaxed wet silk is physically extended and then dried. In this case, the supercontraction stress of the virgin silk was slightly greater than in later repetitions, probably because the molecular order imposed by the spider during spinning differed somewhat from the more repeatable effects of our extending of the silk.

## DISCUSSION

*Nephila* dragline silk responds to humidity in a highly predictable manner that can be clearly divided into two distinct processes (Figs 1–3). First, supercontraction occurs through an irreversible uptake of water when silk first encounters humidity above a critical value of ~70%. Supercontraction permanently alters the molecular organization of restrained silk, even after it is dried, as demonstrated

Table 1. Tensile properties of virgin silk and previously wetted dry silk compared with values from other studies

| | Young's modulus (GPa) | Yield stress (MPa) | Ultimate strength (MPa) | Extensibility [ln(%)] | Toughness (MPa) |
|---|---|---|---|---|---|
| Virgin silk | 21.3±1.2* | 565±28* | 1583±46 | 0.20±0.02 | 208±20 |
| Dry, post SC | 15.7±1.0* | 420±34* | 1693±85 | 0.24±0.02 | 251±15 |
| Other studies | | | | | |
| Work, 1976 | 11± 5 | – | 870±350 | 0.17±0.05 | – |
| Swanson et al., 2006 | 13.8±0.9 | – | 1215±58 | 0.172±0.009 | 111±30 |
| Cunniff et al., 1994 | 22 | – | 1300 | 0.12 | 80 |
| Putthanarat et al., 2004 | 7±2 | 220±70 | 1100±300 | 0.15±0.04 | |

Values are mean ± s.e.m.
SC, supercontraction.
*Statistically significant difference between virgin and post-wetting restrained dry silk.

Table 2. Dynamic properties of *Nephila clavipes* silk before and after exposure to water, and compared with those of other spiders

| | Initial storage modulus (GPa) | Storage modulus at peak tan δ (GPa) | Storage modulus at break (GPa) | Initial tan δ | Peak tan δ | Tan δ at break | Source |
|---|---|---|---|---|---|---|---|
| *Nephila clavipes* | | | | | | | |
| Virgin silk | 20.3±0.4* | 28.4±1.6 | 48.0±1.1 | 0.012±0.020 | 0.157±0.009* | 0.138±0.003 | This study |
| Dry, post $WS_{0.5\%}$ | 16.5±1.6* | 28.1±3.8 | 51.7±2.8 | 0.040±0.027 | 0.129±0.009* | 0.130±0.004 | This study |
| *Argiope argentata* | 7.8±0.7 | – | 38.1±1.9 | 0.065±0.004 | – | 0.057±0.002 | Blackledge and Hayashi, 2006 |
| *Latrodectus hesperus* | 10 | – | – | 0.1 | – | – | Blackledge et al., 2005c |

Values are mean ± s.e.m.
*Statistically significant difference between virgin and post-wetting restrained dry silk.

by the differences in thermal stability and mechanical performance of dry supercontracted ($WS_{0.5\%}$) silk compared with dry virgin silk (Figs 5–8) (Guinea et al., 2003; Guinea et al., 2005; Jelinski et al., 1999; Savage et al., 2004; Schafer et al., 2008; Vollrath and Porter, 2006b; Yang et al., 2000). Second, cyclic contraction occurs reversibly and in proportion to drying or wetting of silk, both before and after supercontraction.

Although previous studies characterized basic aspects of supercontraction (Bell et al., 2002; Elices et al., 2006; Guinea et al., 2005; Liu et al., 2008; Pérez-Rigueiro et al., 2003; Pérez-Rigueiro et al., 2005; Pérez-Rigueiro et al., 2007; Plaza et al., 2006; van Beek et al., 1999; Vollrath and Porter, 2006b), the cyclic response of silk to relative humidity is novel. This cyclic response is unique in that it can potentially generate more stress than supercontraction, but does so as humidity decreases. It is also reversible and highly repeatable. Even after eight cycles of contraction and relaxation over 100 min, there is no sign of fatigue (Fig. 3). This novel property of dragline silk can be exploited to do work and generate energy, offering potential for the development of biomimetic muscle fibers, sensors and other applications (Agnarsson et al., 2009b).

Supercontraction results from water interacting with molecular bonding between protein molecules in silk (Guinea et al., 2003; Schafer et al., 2008; van Beek et al., 1999). To our knowledge, this is the first time that water absorption has been quantified (Fig. 7; ~1–2% of original specimen mass), and shown to permanently alter the material within silk (Fig. 8). Thus, water permanently binds to silk during supercontraction, rather than simply increasing the mobility of silk proteins. This contrasts with the hypothesis that supercontraction is a reversible phenomenon, which predicts that water is lost from silk upon drying, and is supported by the similarity in mechanical properties of virgin and dried supercontracted fibers (Shao et al., 1999). Here, we found that supercontraction does alter tensile mechanics of restrained silk ($WS_{0.5\%}$). Even after it is completely dried, $WS_{0.5\%}$ supercontracted silk is more compliant and yields more easily than virgin silk tested at the same humidity (Fig. 5). This is consistent with the findings of Guinea at al. (Guinea et al., 2005) who found that forcibly silked draglines become more compliant after supercontraction and hence more similar to naturally spun silk (Pérez-Rigueiro et al., 2005). Furthermore, less energy is stored during fiber extension within the elastic region (Fig. 6). One explanation for these differences is that we constrained fibers to a constant length (e.g. $WS_{0.5\%}$). If silk is allowed to supercontract unrestrained (i.e. physically shorten; WU) and then the silk is physically stretched before drying it can again undergo supercontraction (Fig. 9). This process can be repeated many times with almost no change. This recovery of 'supercontrability' is consistent with the hypothesis that physical stretching adds energy to the silk, thereby reconfiguring the molecules in the random-coil region of the amorphous network back to a more ordered state that possesses higher free energy as a result of its decreased entropy. Once dried, the reformation of hydrogen bonds maintains the

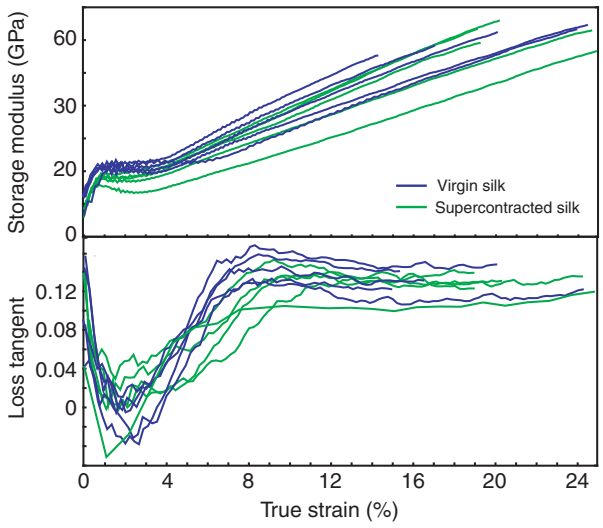


Fig. 6. Comparison of dynamic properties between tensile tests of virgin and dried ($WS_{0\%}$) silk (restrained virgin silk that was wetted, dried, and then tested at ambient humidity). Storage modulus at initial tan δ differs significantly between virgin and supercontracted silks ($t_8$=20.8, $P$<0.001).

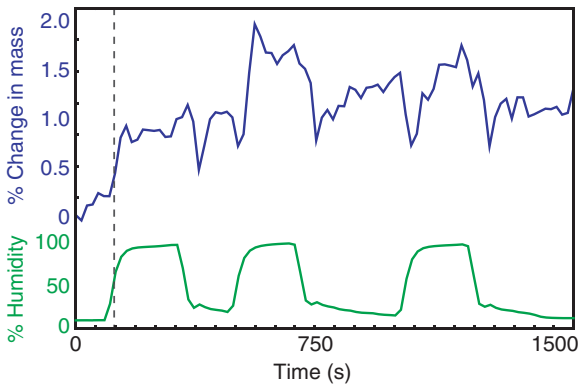


Fig. 7. Mass gained by bundles of silk (WU) as a function of rapid changes in humidity. The dashed line indicates the onset of supercontraction in the unrestrained fibers. Note that the ~1% of mass gained during supercontraction is never lost, even at humidity lower than ambient.

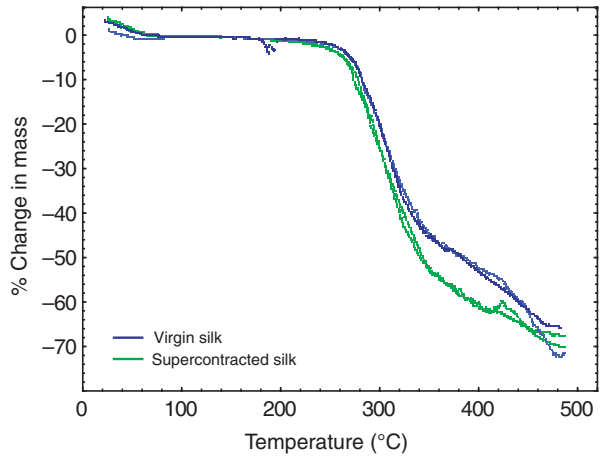


Fig. 8. Thermogravimetric analysis (TGA) of virgin and supercontracted silk (WU). All curves are normalized to their mass at 100°C. (*N*=2 for each type of silk).

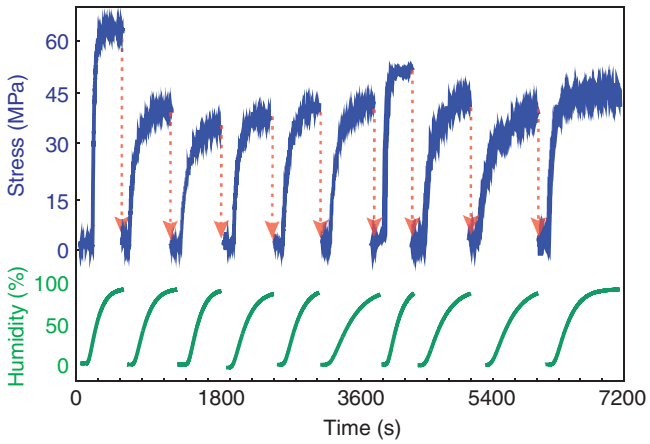


Fig. 9. Repeatability of supercontraction when silk is re-extended and dried before wetting. The individual supercontraction tests are of the same fiber mounted at ~0% strain. After each repetition, the silk was re-extended to the same physical length and then dried for 5 min before being wetted again. This 'resetting' of the fiber is indicated by the red dashed arrows. The slightly larger response during supercontraction of the virgin fiber probably reflects a somewhat greater initial organization of the amorphous network.

organization of the silk proteins. Subsequently, the increased mobility of silk proteins when again wetted allows the silk to supercontract back to a higher entropic state.

Cyclic contraction results from a reversible loss of water during drying, in contrast to supercontraction (Fig. 10). Furthermore, the force and shrinking generated during cyclic contraction are themselves completely reversible. The molecular model developed to explain supercontraction (Eles and Michal, 2004; Gosline et al., 1984; Savage and Gosline, 2008a; Yang et al., 2000), does not account for this pattern. Dragline silk consists of multiple fibroins linked by poly-alanine β-sheet crystals embedded in an amorphous network. This amorphous network consists of relatively ordered glycine-rich linker regions and proline-containing random-coils. These two secondary structures are formed by different major ampullate spidroins that may phase separate during fiber formation such that they are dispersed heterogeneously throughout the silk (Sponner et al., 2005). Although hydrogen bonding within the random-coils is relatively weak and disrupted by even small amounts of water, the stronger hydrogen bonds of the glycine-rich linker regions are only partially disrupted at high humidity (~70%). This disruption in secondary structure is sufficient to alter the random-coil network so that it is no longer held in place. This allows the fibroins to reconfigure toward higher entropy and cause the entire silk fiber to suddenly contract in length while expanding in overall volume (Fig. 10C). The silk now behaves like a filled rubber with a relatively low modulus.

During cyclic contraction, we propose that swelling is induced by the uptake of water molecules associating with hydrophilic amino acids, possibly within the random-coil region. When water first penetrates silk, it interacts primarily with hydrophilic amino acids in the random-coil networks. The breaking of these relatively weak hydrogen bonds allows the silk to relax slightly, but the glycine-rich linker regions still maintain overall orientation such that the random-coil network remains mostly oriented. Thus, only a small displacement can occur. Upon drying, the water molecules are removed, which allows reformation of hydrogen bonding within the random-coils and stiffens the silk (Fig. 10D). The key here is that disruption of hydrogen bonding in the amino acids within the random-coil region does not itself provide enough mobility within the silk for entropy to drive supercontraction. Rather, the mobility necessary to induce supercontraction depends upon both the disruption of these bonds and the much stronger hydrogen bonding within the glycine-rich linker regions. Therefore, the swelling that occurs during water uptake causes the silk to relax, reducing stress in $WS_{0.5\%}$ tests and lengthening silk in WL tests. This association is reversible and, upon drying, the water molecules leave the silk, thereby causing the silk to contract. This process occurs independently of water associating with the hydrogen bonds that maintain order within the amorphous regions of proteins. Thus, cyclic contraction can occur prior to supercontraction or after enough water has been absorbed to disrupt bonding within the glycine-rich linkers to cause supercontraction. Moreover, cyclic contraction is a phenomenon that occurs in other hydrophilic biological materials, with the magnitude of the response scaling directly with the stiffness of each material (Agnarsson et al., 2009b).

Our model proposes independent roles of water-induced mobility for supercontraction and cyclic contraction (Fig. 10). The glycine-rich linker regions that maintain fibroin orientation before supercontraction mostly occur in MaSp1, whereas the random-coils are formed by the greater abundance of proline in MaSp2 (Gatesy et al., 2001). These two proteins are partially phase-separated within silk threads, with MaSp2 occurring largely in the interiors of threads and MaSp1 dispersed throughout (Sponner et al., 2005). The partial separation of these proteins and the differences in their proposed roles in supercontraction and cyclic contraction, suggest that they involve mobilization of different regions of the silk.

Supercontraction differs fundamentally from cyclic contraction, although both can generate large, comparable stresses. When restrained dragline silk supercontracts ($WS_{0.5\%}$), storage modulus, loss modulus and tan δ all increase (Fig. 4). By contrast, during cyclic contraction, storage modulus increases while both loss modulus and tan δ decrease in response to drying. One probable explanation is that supercontraction effectively 'stretches' silk during WS tests, when the fiber is held at a constant length, and that this stiffens the silk relative to a WU test. Although water normally plasticizes silk and reduces its stiffness during the cyclic response, the permanent binding of water to the silk during supercontraction instead alters the energetic equilibrium of the material in a way that effectively

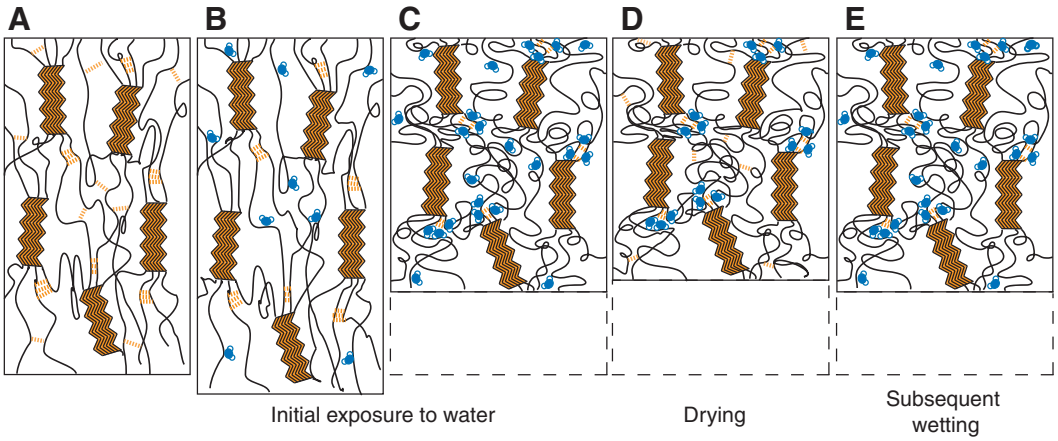


Fig. 10. Hypothesized model of interaction of water molecules with spider silk proteins. The silk fiber is oriented vertically in the cartoon and structures are greatly exaggerated in size for clarity. (A) Virgin silk consists of multiple fibroins linked by crystals embedded in an amorphous network. The poly-alanine β-sheet crystals are represented by the parallel zig-zag lines and the strong hydrogen bonding between individual fibroins within the crystals is indicated by the solid orange background. The orientation of the crystals along the axis of the fiber is maintained by hydrogen bonding within the amorphous network. Two levels of structural organization are indicated. First, glycine-rich regions form secondary structures (either $3_1$ helices or non-periodic lattice crystals) that are relatively stable, as indicated by three parallel hydrogen bonds (the gold lines). These linker regions occur at the interface between poly-alanine β-sheet crystals. Much of the amorphous region consists of random-coil networks the orientation of which is largely determined during the spinning process and is maintained by relatively weak hydrogen bonds indicated by single gold lines. Some research suggests a phase separation leads to spatial segregation of the glycine-rich and random-coil regions, which we have not attempted to depict here. (B) When water first penetrates silk (as indicated by the individual blue molecules) it interacts primarily with hydrophilic amino acids in the random-coils. The breaking of these relatively weak hydrogen bonds allows the silk to relax slightly, but the glycine-rich linker regions still maintain overall orientation such that the random-coil network remains mostly oriented. (C) Once humidity exceeds a critical threshold of ~70%, water penetrates the glycine-rich linker regions (as indicated by the triads of blue molecules) and disrupts hydrogen bonding within them. Structural studies demonstrate that these linker regions still maintain most of their secondary structure (as indicated by the two remaining hydrogen bonds). However, the disruption is sufficient to allow the fibroins to reconfigure toward higher entropy and cause the entire silk fiber to suddenly contract in length while expanding in overall volume. The silk now behaves like a filled rubber with a relatively low modulus. If the silk was instead restrained, as typically done in the current study, the 'supercontraction' response instead manifests as a large increase in stress, but only minor increase in entropy of the random-coil network. (D) Upon drying, some water molecules remain bound in the linker regions, but those within the random-coils are removed. This allows the reformation of hydrogen bonding, stiffening and shrinking the fiber. (E) Subsequent wetting again allows water molecules to infiltrate the random-coil network, breaking hydrogen bonds, thereby relaxing the silk and lowering its modulus, but has little effect on the linker regions. Our data indicate that the change shown in A *vs* B and C *vs* D *vs* E is a highly reversible phenomenon that we term cyclic contraction. By contrast, the change shown in B *vs* C, termed supercontraction, is a persistent phenomenon unless outside forces are imposed on the fiber to stretch it and restore orientation within the random chain network (not shown). In this circumstance, we currently do not know what happens to the water molecules that are 'permanently' bound during supercontraction.

increases its strain, thereby stiffening the silk. Thus, supercontraction can play an important role in keeping orb webs under tension when wetted (Savage et al., 2004), even though water normally increases the compliance of materials.

The relationship between humidity and contraction of dragline silk is clearly more complicated than previously described. Bell et al. (Bell et al., 2002) suggested that supercontraction stress could not maintain tension in wet webs because they found that supercontraction stress was transient and that fibers relaxed within 5 min. They argued that this stress relaxation is a major impediment to technological applications of spider silk. Subsequently, Savage et al. (Savage et al., 2004) replied that the stress–relaxation was largely an artifact of Bell et al. using forcibly silked, rather than naturally spun silk. However, we used forcibly silked fibers from the same species as Bell et al. Clearly, we did not find evidence for substantial creep over relatively similar time periods (Fig. 2). Therefore, the tension produced by supercontraction can indeed compensate for loads applied to a web by water. Any role of cyclic contraction for web function is unknown, but may be important. For instance, the tensioning of silk upon drying after supercontraction would probably more than compensate for any creep that occurred in wetted silk.

Spider dragline silk is a blend of two different proteins that form a fiber with a complex structure of β-sheet crystals and an amorphous network divided into organized linker regions and random-coils. Water can quickly enter silk and associate with amino acids in the amorphous network, altering their molecular bonding. We have shown that this occurs through two very different processes. Water binds to silk during supercontraction, disrupting bonding within glycine-rich linker regions and increasing local mobility of molecules (Liu et al., 2008; van Beek et al., 1999). This allows the molecules to reconfigure into a less organized state that shrinks the fiber. Reconfiguration is largely driven by the higher entropy of the molecules in the random-coil region when supercontracted compared with their oriented arrangement in virgin silk. Increased mobility of molecules may also explain the relaxation phase of the cyclic response to humidity. But, a key difference is that increased humidity results in reduced tension. This could result if the mobilization of silk molecules enabled by water is limited to local regions within the silk, probably the random-coils. The low orientation of molecules within the random-coils results in relatively weaker hydrogen bonds thereby explaining why even small increases in humidity cause silk to relax cyclically. Moreover, our hypothesized model suggests the intriguing possibility that supercontraction in fact depends upon water-induced mobilization occurring in two different regions of the silk, both the oriented linker region and the random-coil region, and that neither alone is sufficient.

We are grateful to Taylor Gondek for conducting the supercontraction repeatability test. We thank Linden Higgins and Claire Ritschoff for providing some of the spiders used in this study. John Gosline provided especially insightful comments on the manuscript. This work was supported by National Science Foundation

awards DBI-0521261, DEB-0516038 and IOS-0745379 to T.A.B., award DMR-0512156 to A.D., and by awards DMI-0520967 and CMMI-0746703 to S.C.W. Additional funding was provided by a Slovenian Research Agency research fellowship ARRS Z1-9799-0618-07 to I.A., and from the Integrated Bioscience Program at University of Akron.

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