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Gumfooted lines in black widow cobwebs and the mechanical properties of spider capture silk

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Abstract

Orb-weaving spiders produce webs using two types of silk that have radically different mechanical properties. The dragline silk used to construct the supporting frame and radii of the web is stiff and as strong as steel, while the capture spiral is much weaker but more than ten times as extensible. This remarkable divergence in mechanical properties has been attributed to the aqueous glue that coats the capture spiral, which is thought to decrease capture spiral stiffness and increase its extensibility. However, discerning the effect of the aqueous glue on fiber performance is complicated because dragline silk and the capture spiral are assembled from different proteins, which may also affect mechanical performance. Here, we use the sticky gumfooted lines of black widow cobwebs to test the effect of the addition of aqueous glue on the mechanical properties of dragline silk. We also surveyed orb-webs spun by a broad range of species for bundles of looped silk. Such bundles, termed windlasses, have been thought to increase capture spiral extensibility by "paying out" additional lengths of silk. Our results suggest that neither plasticization of silk by aqueous glue nor excess silk in windlasses can by themselves account for the remarkable extensibility of orb-weaver capture silk compared to other spider silks. This argues that the unique amino acid motifs of the flagelliform fibroins that constitute the core of the capture spiral play an essential role in capture silk's extreme extensibility.
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Keywords: Major ampullate silk; Flagelliform fibroin; Orb-web; *Latrodectus hesperus*; Dragline silk; Capture spiral silk

Introduction

Orb-weaving spiders in the taxon Araneoidea produce webs using two types of fibrous silks that have radically different material properties (Fig. 1). Dragline silk is spun from major ampullate glands and is used to construct the frame and supporting radii of webs. Dragline silk is renowned for its unique combination of high tensile strength, stiffness, and extensibility that makes dragline silk exceptionally tough. This allows dragline silk to absorb far more kinetic energy without breaking than do manmade high-performance fibers such as Kevlar (Gosline et al., 1986). In contrast, the sticky capture spiral of the orb-web is composed of silk fibers produced by the flagelliform glands and coated with aqueous glue from the aggregate silk glands. Capture spiral threads are over ten times more extensible than dragline silk, but are neither as stiff nor as strong (Denny, 1976). The low stiffness of capture

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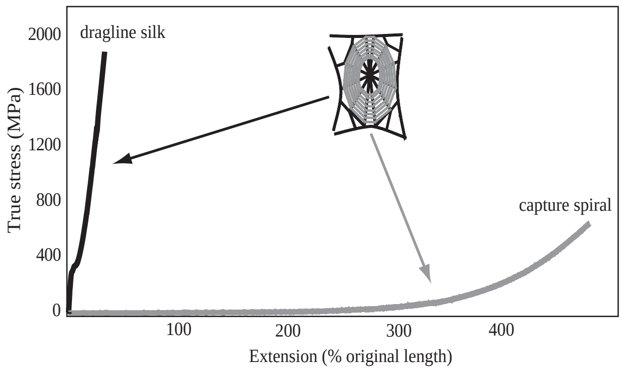

Fig. 1. Representative stress–strain curves for dragline silk (black) and capture spiral (gray) from the orb-weaver *A. argentata*. Major ampullate dragline silk provides a strong and stiff framework that supports the highly extensible, energy absorbing capture spiral. Note the one order of magnitude greater extensibility and the three orders of magnitude reduction in stiffness that characterize capture spiral relative to dragline silk.

spiral silk means that very little force is required to extend the silk 200–300% of its original length until the fiber gradually stiffens under the increasing strain. This results in a distinctive "j" shaped stress–strain curve, in contrast to dragline silk which is characterized by a stress–strain curve that is similar to the behavior of typical viscoelastic fibers in that it is initially a very stiff material and that it has a distinct yield region around 2–3% strain (Fig. 1).

The low stiffness and amazing extensibility of capture spiral relative to dragline silk is likely due to their different molecular structures. Though dragline and capture spiral silks are composed primarily of proteins from a single gene family, the genes coding for their silk fibroins are very divergent from one another (Gatesy et al., 2001). Therefore, the amino acid sequence of the flagelliform silk protein within the core of the capture spiral could result in a functionally unique molecular structure that increases extensibility. In particular, lengthy tandem arrays of $GPGGX_n$ amino acid subrepeats within flagelliform silk fibroins are hypothesized to form β-spirals that act as highly extensible molecular "nanosprings" (Hayashi and Lewis, 1998; Becker et al., 2003).

While the capture spiral and dragline silk do differ in protein sequence, it is possible that factors besides amino acid sequence may also explain the dramatic differences in mechanical properties between the two types of silk. At least two other hypotheses have been proposed to account for the unusual properties of capture spiral silk: (1) loosely coiled bundles of silk within glue droplets of the capture spiral, termed "windlasses", that unravel when fibers are stressed; and (2) hydration of the capture silk fibroins. These three explanations act at very different levels of organization and are not necessarily mutually exclusive.

The windlass hypothesis originated from a study by Vollrath and Edmonds (1989), where they coined the term "windlass" to describe the coiling of slackened flagelliform fibers within glue droplets that they observed when capture spirals of *Araneus diadematus* were relaxed to approximately 50% or less of their original length. While Vollrath and Edmonds originally proposed windlasses as a mechanism that maintained tension during *relaxation* of capture threads, windlasses have since been interpreted in the literature as structures that facilitate the extensibility of capture silks (e.g. arguments of Schneider, 1995 vs. Vollrath and Edmonds, 1995; see also Becker et al., 2003). Under this hypothesis, it is the paying out of excess silk from these windlasses that makes capture spiral threads so extensible. We test the windlass hypothesis by examining the webs of a phylogenetically diverse sampling of araneoid orb-weavers for the presence of windlasses in capture spiral. If windlasses are not present in capture spiral at native tension then they cannot function to enhance capture spiral extensibility.

The hydration hypothesis suggests that the difference in properties between capture spiral and dragline silks is caused by the hydration of the flagelliform core fibers of the capture spiral by water from the surrounding glue. Hydration is thought to alter the molecular bonding of silk fibroins to one another, causing the silk to behave as a rubber with a particularly low modulus of elasticity (Gosline et al., 1984). Thus, the aqueous coating of glue that makes capture spiral sticky may also plasticize the silk, thereby increasing capture spiral extensibility (Vollrath and Edmonds, 1989). This hypothesis is best tested by directly comparing the mechanical performance of the two types of silk with and without aggregate glue added to the fibers. Unfortunately, this test is difficult because normally an aqueous aggregate secretion coats the flagelliform core fiber of the capture spiral and never coats dragline silk. Thus, it is difficult to separate the effects of hydration by this gluey coating from the material properties of the flagelliform fibers themselves. However, the sticky gumfooted lines of cobwebs provide a unique opportunity to test the hypothesis that the extreme extensibility of araneoid orb-weaver capture silk is due to plasticization of the silk by aqueous glue. Black widows, *Latrodectus* spp. (Theridiidae), evolved from orb-weaving spiders, but now construct cobwebs rather than orbs (Griswold et al., 1998). Instead of capture spirals, gumfooted lines extend downward to the substrate from the supporting scaffolding of the cobweb, and only the bottom 5–15 mm are coated with aqueous glue (Fig. 2A; Benjamin and Zschokke, 2002, 2003). The aggregate glands that produce the sticky droplets of the gluey "foot" region are homologous to the aggregate glands

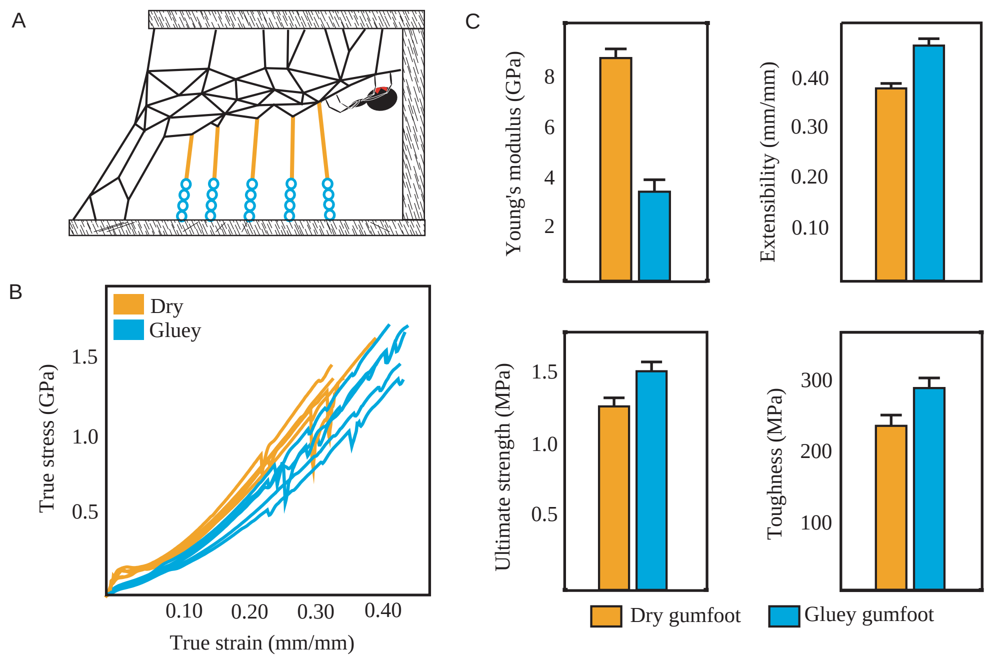

Fig. 2. (A) Cobwebs are evolutionarily derived from orb-webs. The gluey capture spiral of the orb-web has been transformed into functionally analogous sticky gumfooted lines in cobwebs. Gumfooted lines are continuous silk threads that are coated with glue at their base (in blue) but are dry (in orange) where they attach to the rest of the web. (B) Representative stress–strain curves for five paired samples of dry and glue gumfooted silk from the web of a single black widow spider. (C) Mechanical properties of gumfooted threads compared across the transition from dry silk to regions coated with aqueous glue ($N = 25$). Values are mean $\pm$ SE.

used by orb-weaving spiders to coat capture spirals (Coddington, 1989). We used this unique web architecture to test the effect of aqueous glue on silk performance by comparing the mechanical properties of gluey and dry regions along continuous gumfooted lines. If hydration is the primary mechanism accounting for capture spiral's performance, then we would expect that the gluey portion of the gumfooted lines should behave more like capture spiral than like dragline silk.

Materials and methods

We collected 25 samples of paired gluey and dry threads from gumfooted lines of the cobwebs of seven western black widow spiders (*Latrodectus hesperus* Chamberlin and Ivie 1935). Five mm long silk samples were mounted onto "c" shaped cardboard supports directly from the web using cyanoacrylate glue. For each paired sample, no more than 5 mm separated the sample of dry silk from the sample of gluey silk. We generated force-extension data for each silk sample using a Nano Bionix UTM tensile tester (MTS Systems Corporation, Oak Ridge, TN). The Nano Bionix is capable of generating load–displacement data from very fine silk fibers, with a load resolution of 50 nN and an extension resolution of 35 nm. Fibers were extended at a constant rate of 1% engineering strain/s (0.05 mm/s) until the samples failed. Using similar methods, we also generated comparative data for five samples of radial thread (major ampullate silk) and five capture threads (flagelliform silk with aggregate glue) from two orb-webs built by the silver garden spider *Argiope argentata* (Fabricius 1775).

To determine the cross-sectional area of the fibers that were tested, we used polarized light microscopy to obtain three digital images of the four silk fibers within the dry sample and then measured the diameters of each of the four fibers using NIH Image 1.63 (US National Institutes of Health). This allowed us to compute the total cross-sectional area of silk that was tested mechanically by summing the areas of each of the four individual fibers that comprised each sample. This method produces highly repeatable measurements that are similar in accuracy to measurements obtained through scanning electron microscopy, but it also accounts for variation in cross-sectional area from sample to sample (Blackledge et al., in press). The cross-sectional area computed from these dry samples was also used for the gluey sample within each pair because it was difficult to visualize the fibers within the glue droplets of the wet portion of the gumfooted lines. Note that the fibers of the gluey portion of the gumfooted lines were continuous with the dry portion.

We used Testworks 4.0 software (MTS Systems Corp.) to calculate true stress:

$$\sigma_t = F/A,$$

where F is the force applied to the sample and A is the instantaneous cross-sectional area of the sample calculated assuming an isometric volume, and true strain:

$$\varepsilon_t = \ln(L/L_0),$$

where L is the actual length of the sample and L_0 is the original length of the sample. We then recorded the ultimate strength and extensibility of each sample as the true stress and true strain values at failure. We calculated the initial stiffness (i.e. Young's modulus) for each sample by measuring the slope of the initial elastic region of the stress–strain curve. Finally, we calculated the toughness of each sample as the total area under the stress–strain curve.

We examined 276 rows of capture spiral from orb-webs for windlasses by adhering 20 mm long samples of capture spiral, at their native tension, to glass slides and then examining the flagelliform core fibers along the entire sample using the same polarized light microscope setup as described above. A sample was scored as lacking windlasses only if the flagelliform core fibers ran straight through all of the sticky droplets in that sample. Three to ten rows of capture spiral were examined per web for six genera of orb-weaving spiders, including *Araneus gemmoides* Chamberlin and Ivie 1935 ($N = 1$), *Araneus* sp. ($N = 2$), *Argiope argentata* ($N = 21$), *Gasteracantha cancriformis* (Linnaeus 1758) ($N = 2$), *Neoscona domiciliorum* (Hentz 1847) ($N = 1$), *Nephila clavipes* (Linnaeus 1767) ($N = 1$), and *Wagneriana tauricornis* (O.P. Cambridge 1889) ($N = 1$).

Results

Gluey regions of the gumfoot had 60% reduced stiffness compared to dry regions (3.7 ± 0.5 vs. 8.9 ± 0.4 GPa [mean $\pm$ SE], respectively; paired t-test; $t = 9.0$, df = 24, $P < 0.000001$), but 16% greater ultimate strength (1529 ± 86 vs. 1316 ± 45 MPa; $t = 2.4$, df = 24, $P < 0.05$), 18% greater extensibility (0.46 ± 0.02 vs. 0.39 ± 0.01; $t = 5.4$, df = 24, $P < 0.00001$), and 22% greater toughness (281 ± 22 vs. 231 ± 10 MPa; $t = 2.4$, df = 24, $P < 0.025$; Figs. 2B and C).

Examination of capture spiral silk revealed that fewer than 2% of samples had "windlasses" present at native tensions. The extensibility of the capture spirals from all of these webs was qualitatively high and we found that the extensibility of 18 samples of *A. argentata* capture spirals, relative to original length, was $501 \pm 13\%$ (mean $\pm$ SE), which is similar to published values for the extensibility of capture spiral silk for other species of araneids (Denny, 1976; Gosline et al., 1986; Vollrath and Edmonds, 1989). In addition, there were no "windlasses" in any of the gumfooted lines that we tested or examined.

Discussion

The mechanical properties of the sticky gumfooted lines appear to be affected by the presence of aqueous glue. The gluey regions had reduced stiffness and increased extensibility, strength, and toughness compared to adjacent dry regions (Fig. 2C). These differences are solely attributable to changes induced by the aqueous glue because the core fiber of the gumfoot was contiguous within each paired sample and therefore presumably had the same fibroin composition. Although the glandular origin of sticky gumfooted lines has been controversial (Benjamin and Zschokke, 2002), evidence suggests that sticky gumfooted lines are composed of the same major ampullate dragline silk that orb-weavers use for the frames and radii of their webs. Individual gumfooted lines consist of four fibers, one pair of which is produced as the spider moves down to the substrate and the other pair of which is produced as the spider moves back up to the supporting scaffolding. The pairs of fibers are similar in diameter to one another, to the major ampullate scaffolding threads within the cobweb, and to the diameter of major ampullate silk fibers pulled from anaesthetized black widows (Blackledge et al., unpublished). Furthermore, the major ampullate core fibers of the gumfooted line have mechanical properties similar to orb-weaver major ampullate dragline silk rather than flagelliform silk.

Vollrath and Edmonds (1989) used two lines of evidence to argue that the difference in mechanical performance of orb-weaver dragline and capture spiral silk was due primarily to the water coating of the capture spiral. First, they compared the mechanical performance of normal capture spiral to capture spiral produced by senescent spiders that failed to coat their flagelliform fibers with glue. They found that uncoated spiral had a stiffness and hysteresis more like that of dragline silk rather than coated capture spiral. Vollrath and Edmonds therefore concluded that the difference in mechanical performance between dragline and capture spiral was due to the water coating in the glue of the capture spiral. However, it is highly aberrant for spiders to fail to coat their capture spirals with glue, leaving it unknown whether or not these senescent spiders were also producing atypical or irregular flagelliform core fibers. Second, Vollrath and Edmonds used an analogy to supercontracted dragline silk. When dragline silk is hydrated, it undergoes supercontraction, a phenomenon during which unrestrained fibers can shrink by up to 45% in length while increasing in volume (Work, 1977). Supercontracted major ampullate silk behaves as a rubber with a greatly reduced initial stiffness, and the mechanical properties qualitatively begin to resemble those of capture spiral silk (Work, 1977; Gosline et al., 1984). Yet, these changes in material properties due to hydration by water, as well as the alteration in mechanical performance of sticky gumfooted lines by glue that we found, pale in comparison to the dramatic differences in performance between the dragline and capture spiral silk of orb-webs. Capture spiral is ten times more extensible than dragline silk, over one thousand times less stiff than dragline silk, and has a lower ultimate strength (Fig. 1), which together result in capture spiral and dragline silk having similar values for toughness (Denny, 1976). In contrast, we found that the gluey coating of gumfooted lines increased extensibility, ultimate strength, and toughness by only 16–22% compared to adjacent dry regions of the exact same fibers (Fig. 3). The aggregate glue used by black widows to coat gumfooted lines is produced from aggregate glands homologous to those used to coat the capture spirals of orb-webs (Coddington, 1989). Therefore, it seems unlikely that modulation of the mechanical properties of flagelliform silk by aggregate secretions is by itself the key to the remarkable extensibility of capture spiral.

The accumulation of slack flagelliform fibers into stretchable windlasses within glue droplets also does not explain capture silk extensibility. Windlasses have been interpreted in the literature as a mechanism that increases the extensibility of capture silks by paying out excess fiber (e.g. arguments of Schneider, 1995; see also Becker et al., 2003). However, windlasses were originally proposed as a mechanism for maintaining tension during *relaxation* of capture threads and formed only when fibers under strain were relaxed (Vollrath and Edmonds, 1995). For this reason, it is not surprising that our examination of capture spiral from the webs of seven different species of spiders failed to find significant numbers of windlasses in the silk at native tension.

Our study has thus largely excluded two hypothesized mechanisms by which extrinsic factors could account for the remarkable extensibility and reduced stiffness of orb-weaver capture silk relative to dragline silk. First, we found that hydration of dragline silk by aqueous glue does result in plasticization of silk, but that this plasticization results in only a minor change in mechanical properties compared to the one order of magnitude difference in extensibility and three orders of magnitude difference in stiffness of capture spiral vs. dragline silk (Fig. 3). Second, we found that windlasses do not enhance capture thread extensibility because windlasses are not found in capture threads at native tension. This argues that the striking difference in the amino acid sequences of flagelliform silk proteins compared to major ampullate silk proteins likely plays a critical role in explaining the impressive extensibility of the orb's capture spiral, either by itself or as an essential factor that interacts with and greatly enhances the effects of plasticization of capture spiral by aggregate glue. In particular, the nucleotide sequence of flagelliform silk genes suggests that up to 80% of the fibroin is composed of $GPGGX_n$ amino acid subrepeats. These $GPGGX_n$ repeat elements are predicted to result in the formation of helical molecular nanosprings, rather than the rigid β-pleated sheets that form the stiff crystalline regions of dragline silk, thereby increasing the extensibility of flagelliform silk relative to other types of silk that either have shorter $GPGGX_n$ repeats or lack them altogether (Becker et al., 2003; Hayashi and Lewis, 1998).

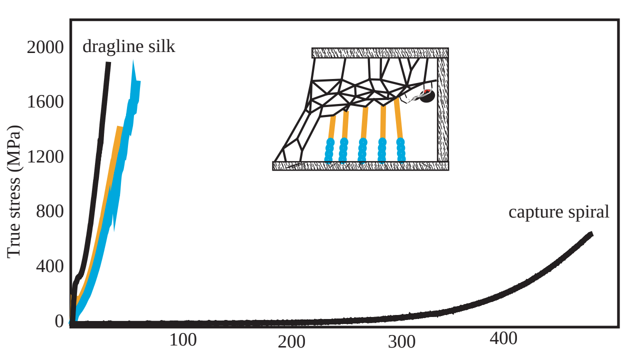

Fig. 3. Plasticization of black widow dragline silk by aqueous glue does not alter the mechanical performance of dragline silk to resemble the mechanical performance of orb-weaver capture spiral. Black curves show the mechanical characteristics of dry dragline and glue-coated capture spiral silks from an orb-web. Representative curves for both dry (orange) and wet (blue) sticky gumfooted silk are shown.

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