

Unraveling the mechanical properties of composite silk threads spun by cribellate orb-weaving spiders

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Summary

Orb-web weaving spiders depend upon the mechanical performance of capture threads to absorb the energy of flying prey. Most orb-weavers spin wet capture threads with core fibers of flagelliform silk. These threads are extremely compliant and extensible due to the folding of their constituent proteins into molecular nanosprings and hydration by a surrounding coating of aqueous glue. In contrast, other orb-weavers use cribellate capture threads, which are composite structures consisting of core fibers of pseudoflagelliform silk surrounded by a matrix of fine dry cribellar fibrils. Based on phylogenetic evidence, cribellate capture threads predate the use of viscid capture threads. To better characterize how pseudoflagelliform and cribellar fibrils function, we investigated the mechanical performance of cribellate capture threads for three genera of spiders (*Deinopis*, *Hyptiotes* and *Uloborus*). These taxa

spin very diverse web architectures, ranging from complete orbs to evolutionarily reduced triangle webs and cast nets. We found that the pseudoflagelliform core fibers of these webs were stiffer and stronger, but also less extensible, than flagelliform silk. However, cribellate capture threads achieved overall high extensibilities because the surrounding cribellar fibrils contributed substantially to the tensile performance of threads long after the core pseudoflagelliform fibers ruptured. In the case of *Deinopis* capture threads, up to 90% of the total work performed could be attributed to these fibrils. These findings yield insight into the evolutionary transition from cribellate to viscid capture threads.

Key words: biomechanics, Deinopidae, flagelliform silk, major ampullate silk, orb web, pseudoflagelliform silk, Uloboridae.

Introduction

Orb-weaving spiders within the Araneoidea are some of the most diverse and abundant predators of flying insects (Foelix, 1996; Wise, 1993). As such, orb-weaving spiders depend upon their webs to stop the massive kinetic energy of flying insects and retain those insects long enough for the spiders to attack and subdue them (Eberhard, 1990). An orb web consists of a framework of stiff and strong radial threads that supports a spiral of sticky capture silk, the primary means by which prey adhere to the web. In addition to being covered with viscous glue, capture silk is also highly extensible, which allows the silk to gradually decelerate intercepted insects, thereby preventing prey from ricocheting out of webs (Denny, 1976; Gosline et al., 1999; Lin et al., 1995). Thus, the potential for an orb web to retain prey long enough to be captured by the spider depends intimately upon the mechanical properties of these capture threads. Araneoid capture threads are composite structures that consist of two parts: a core pair of axial fibers spun from flagelliform silk and a surrounding coating of aqueous glue spun from aggregate silk glands (Peters, 1986). The aggregate silk secretions make capture threads sticky and

can modulate the mechanics of the flagelliform axial fibers (Gosline et al., 1984; Vollrath and Edmonds, 1989; Vollrath et al., 1990). However, it is the core axial fibers that provide the primary tensile mechanics of araneoid capture threads (Becker et al., 2003; Blackledge et al., 2005b; Craig, 1987; Opell and Bond, 2000; Opell and Bond, 2001).

Flagelliform silk achieves its high extensibility largely due to the interactions of its constituent protein molecules (Hayashi and Lewis, 1998; Hayashi et al., 1999). cDNA derived from the flagelliform silk gland reveal that this silk is predominantly made from tandemly arrayed repeats of glycine-proline-glycine [GPG(X)_n] (Hayashi and Lewis, 2000). These GPG(X)_n motifs explain the high extensibility and low stiffness of flagelliform silk by functioning as molecular ‘nanosprings’ (Becker et al., 2003; Hayashi and Lewis, 2001; Zhou et al., 2001).

The supporting scaffold for the capture spiral is spun from major ampullate silk, which is also used by spiders for non-prey capture functions such as trailing draglines. This silk is composed primarily of two different proteins, major ampullate spidroins 1 and 2 (MaSp1 and MaSp2). Both proteins contain

substantial amounts of alanine-rich motifs that form crystalline regions that provide dragline silk with its remarkable strength and stiffness (Simmons et al., 1996; Termonia, 1994). MaSp2 also includes GPG(X)_n repeats similar to those in the flagelliform fibroin (Gatesy et al., 2001; Guerette et al., 1996). However, MaSp2 contains substantially fewer GPG(X)_n repeats than flagelliform fibroin and this significant difference correlates with major ampullate silk being an order of magnitude less stretchy and several orders of magnitude stiffer than flagelliform silk (Denny, 1976).

The orb web architecture is thought to have evolved prior to the origin of the Araneioidea because spiders within the sister lineage Deinopoidea also spin orb webs (Coddington, 1986a; Griswold et al., 1998). However, in contrast to araneoids, deinopoids utilize an ancestral type of adhesive capture threads called cribellate silk (Lubin, 1986; Peters, 1984; Peters, 1992). Because outgroups to the orb-weaving spiders (Deinopoidea + Araneioidea) also spin cribellate capture threads, this type of adhesive silk is likely ancestral for all orb-weaving spiders (Coddington, 1986a; Griswold et al., 1999; Opell and Bond, 2000). Like viscid silk, cribellar capture threads are composite structures, consisting of core fibers and a surrounding sticky matrix. The pair of axial core fibers is produced by the pseudoflagelliform gland (Peters, 1984; Peters, 1992) and, because the Araneioidea and Deinopoidea are sister-groups, these glands are likely homologous to the araneoid flagelliform glands that are used to spin the axial fibers of viscid silk (Coddington, 1989; Opell and Bond, 2001; Platnick et al., 1991). Instead of aqueous glue, the axial fibers of cribellate capture silk are surrounded by puffs of tiny cribellar fibrils that can be as thin as 10 nm in diameter (Peters, 1984; Peters, 1992). Although cribellar fibrils are dry, they achieve stickiness through a combination of van der Waals and hygroscopic forces (Hawthorn and Opell, 2002; Hawthorn and Opell, 2003) that allows cribellar fibrils to adhere to even very smooth surfaces (Opell, 1994a).

Comparing extant Araneioidea and Deinopoidea, the evolutionary transition from cribellar to viscid capture threads is associated with a 95% increase in species diversity. Thus, this shift has been identified as a likely key innovation in the diversification of spiders (Bond and Opell, 1998; Coddington and Levi, 1991; Opell and Bond, 2001). Viscid capture threads may offer several advantages over cribellate capture threads. The aggregate silk coating of viscid threads spontaneously forms droplets as it is spun, making these capture threads less expensive to produce than cribellate threads because araneoid spiders do not have to spend the time and energy that deinopoids do to comb cribellar fibrils into adhesive puffs (Townley et al., 1991; Vollrath et al., 1990; Vollrath and Tillinghast, 1991). Viscid silk also allows spiders to achieve a greater stickiness per unit area in their webs, which enhances retention of prey (Opell, 1998; Opell, 1999). Furthermore, viscid silk has reduced UV reflectance compared to cribellar capture threads and this decrease in visibility may make araneoid webs more difficult for insects to avoid (Craig et al., 1994). Finally, Köhler and Vollrath proposed that a specific

key innovation in the diversification of araneoid spiders was a dramatic increase in the extensibility of viscid capture silk relative to cribellate capture threads (Köhler and Vollrath, 1995). This mechanical difference allows webs spun by the Araneioidea to more effectively dissipate the kinetic energy of flying insects, thereby broadening their range of potential prey.

An alternative hypothesis has been offered to explain the differences in the mechanical performance of viscid and cribellate capture spiral silks (Opell and Bond, 2000). Opell and Bond proposed that differences in thread extensibility were attributable to the variation between araneoid and deinopoid spiders in the details of web architecture and spider size rather than to a punctuated increase in the extensibility of flagelliform silk in araneoids. However, once the confounding influences of spider size and web architecture were removed, they found evidence for only a gradual increase in capture thread extensibility between deinopoids and araneoids.

Understanding the functional differences between viscid and cribellate capture spiral silks requires a detailed mechanical analysis of how capture threads absorb energy as they are stretched. While viscid capture silks have been mechanically characterized in a number of studies (Blackledge and Hayashi, 2006; Denny, 1976; Köhler and Vollrath, 1995; Opell and Bond, 2001; Vollrath and Edmonds, 1989), analyses of cribellate capture spiral silks are complicated by the fact that the puffs of cribellar threads that surround the axial fibers are themselves fibrous and may exhibit tensile properties of their own. Here, we provide a detailed study of the mechanical performance of the cribellate capture threads spun by several deinopoid spiders. We examine species of spiders from both extant families within the Deinopoidea: Deinopidae and Uloboridae. The taxa that we study display a variety of web shapes, from the complete orb webs of *Uloborus* to the specialized triangular webs of *Hyptiotes* and the cast nets of *Deinopis*, thereby representing a broad range of selective forces that could act upon the mechanical performance of silk. For the first time, we are able to attribute individual aspects of mechanical performance to different structural components of these composite threads. We also compare the properties of capture silk to those of major ampullate silk from the same species. Finally, we discuss the implications of these findings for understanding the evolutionary origin of the viscid capture threads of araneoid spiders.

Materials and methods

Spiders

We examined the capture silk spun by spiders from three genera within the Deinopoidea. These genera included representatives from both extant families of deinopoids: Deinopidae and Uloboridae, and each genus constructed webs with very different architectures from one another. Because relatively little is known about the tensile properties of cribellate threads, sampling more than one genus allowed us to draw some general conclusions about the mechanical performance of cribellate silk while also allowing us to explore

how these properties differ among diverse web types. *Uloborus diversus* Marx 1898 spins small horizontal orb webs in shrubs and bushes in the southwestern USA and captures predominately small flying insects (Eberhard, 1971; Eberhard, 1972). We collected *Uloborus* from areas adjacent to the campus of the University of California, Riverside, CA, USA. *Hyptiotes cavatus* (Hentz 1847) and *Hyptiotes gertschi* (Chamberlin and Ivie 1935) construct triangular shaped webs that consist of modified orbs where only three adjacent sectors of the web have sticky silk, while all other sectors are so highly reduced that they lack adhesive silk and only the central hub remains (Opell et al., 1990). Triangle webs are held under tension by *Hyptiotes*, which release the webs when flying insects strike them, causing the silk to relax slightly and further entangle the prey (Lubin, 1986; Opell, 1988). *Hyptiotes cavatus* was collected in Gainesville, FL, USA and *H. gertschi* was collected from the University of California's James San Jacinto Mountain Natural Reserve, CA, USA. *Deinopis spinosa* Marx 1889 constructs a net-like web that is held by the front legs of the spider (Coddington, 1986b). *Deinopis* actively lunges at flying or pedestrian arthropod prey, stretching the net as the web is pressed down onto the prey (Coddington and Sobrevila, 1987). In contrast to *Hyptiotes*, this attack does not decrease strain within the web, but rather strain increases as *Deinopis* uses its legs to expand the web area up to ten times while lunging at prey (Coddington and Sobrevila, 1987). *Deinopis* were collected in Gainesville, FL, USA.

Silk

We collected silk from webs constructed in the laboratory by adult or penultimate female spiders. Spiders had been maintained in captivity for 1–3 weeks prior to testing. All spiders were misted with tapwater every 1–2 days and fed a variety of small insects, mostly small house crickets *Acheta domesticus* L., once or twice a week. Because of the different behaviors of these spiders, we employed a variety of strategies to obtain usable webs. Like other orb-weavers, *Uloborus* can be easily induced to spin webs within small, box-like cages (e.g. Watanabe, 2000). We constructed cages from 20 cm×20 cm cardboard boxes where the top had been cut away and covered with clear plastic wrap (SaranWrap®). Individual samples of capture silk were collected directly from these webs onto 'C'-shaped cardboard mounts, across 10 mm gaps, and secured with cyanoacrylate glue (SuperGlue®). *Hyptiotes* typically spin their webs near the ends of bare tree branches and were induced to spin webs in the laboratory across a collection of sticks that were glued onto a board. A 19 liter glass aquarium was placed upside down over the sticks to confine the spiders to the space during the night, when they spun webs. The aquarium could then be removed and whole webs collected onto 10 cm×15 cm cardboard frames that were coated with double-sided tape. Securing the entire web prevented the spider from collapsing the web and potentially damaging the silk. We then collected individual samples of silk from these frames as described above for *Uloborus*. Finally,

Deinopis were housed in plastic terrariums that contained bare tree branches as substrate. *Deinopis* typically spun webs soon after the lights were turned out. We then collected the webs onto ~4 cm diameter rings that were constructed from pipe cleaners coated with double sided sticky tape. By quickly moving the ring up toward the spider from underneath the web, we could usually startle the spider into dropping its web without striking. This allowed us to capture the web by its outer supporting frame threads without the web having been stretched by the spider.

In addition to capture silk, we also collected samples of major ampullate silk from *Deinopis* and *Uloborus* by manually pulling dragline from spiders that had been secured to the stage of a stereo microscope (see Blackledge et al., 2005a; Blackledge et al., 2005c). These samples of silk were 21 mm in gage length. We also collected samples of major ampullate silk from the webs of *Hyptiotes* along regions of the radial threads outside of the capture area. These samples were 10 mm in gage length.

Mechanical analysis of silk

We used polarized light microscopy to measure the sizes of threads. This method produces repeatable and accurate measurements of the diameters of many silk fibers (Blackledge et al., 2005a). Cribellate sticky silk consists of a pair of core axial fibers encircled by extensive puffs of ultrathin (10–100 nm) cribellar fibrils (Peters, 1984). These haloes of fibrils firmly adhered to the microscope slides used during measurement, ruining the samples for testing. Therefore, for each web we measured the diameters of the axial fibers for one subset of 5–10 capture threads and mechanically tested a second set of threads. We computed the total cross-sectional areas of the axial fibers of each sample as the sum of the areas of the two fibers that constitute the strand. We then used the average cross-sectional area of capture threads from a web as the cross-sectional area for all capture threads from that same web that were tested mechanically. The diameters of the axial threads of uloborid webs are homogeneous within individual webs (Opell, 1994b). Therefore, there should have been little variation in diameter among our samples from any one spider's web.

We used a Nano Bionix® tensile tester (MTS Systems Corp., Oak Ridge, TN, USA) to generate load–extension data. All tests were performed with a load resolution of 50 nN and an extension resolution of 35 nm, at a constant extension rate of 1% strain s⁻¹ until the fibers failed. This strain rate was chosen because it was also within the range of many other studies on spider silk mechanics (e.g. Blackledge et al., 2005b; Blackledge et al., 2005c; Swanson et al., 2006), maximizing comparability of results.

We transformed raw load-extension data into stress and strain values to normalize data across fibers of different sizes. We calculated true stress (σ_t), where load is normalized to the instantaneous cross-sectional area of a fiber, as: $\sigma_t = F/A$, where F is the force applied to the specimen and A is the instantaneous cross-sectional area of the fiber calculated from

the original cross-sectional area under an assumption of constant volume.

Some of the literature on spider silk mechanics uses engineering stress where force measurements at all extension values are normalized to the initial cross-sectional areas of fibers. However, engineering stress can greatly underestimate the stress experienced by fibers at high extensions. Using true stress values facilitates comparison of the mechanical properties of different silks that vary in extensibility (Blackledge et al., 2005c).

Strain measures the extension of a fiber relative to its length. We calculated true strain rather than engineering strain values because true strain provides a more realistic value of the stretchiness of highly extensible fibers. True strain (ϵ_t) was calculated as: $\epsilon_t = \log_e(L/L_0)$, where L is the instantaneous length of the fiber at each extension value and L_0 is the original gage length of the fiber.

We used our true stress and true strain measurements to calculate six variables of interest. Young's modulus measures the stiffness, or ability of fibers to resist deformation, and is calculated as the slope of the linear region of the stress-strain curve prior to the yield point. The yield strain and yield stress measure the point at which the mechanical behavior of fibers changes from elastic to viscous. Extensibility is the true strain at the point of failure of the fiber. Ultimate strength is the true stress at the point of failure of the fiber. Toughness (i.e. work of extension or work to fracture) is a measure of the energy necessary to rupture a fiber of a given volume and was calculated as the area under the true stress-true strain curve. The calculation of toughness is unaffected by the use of engineering *versus* true stress-strain curves because it is a measure of the energy absorbed by a given volume of fiber.

Data analysis

We used MANOVA to test for differences among the four species of spiders in the thread diameter, Young's modulus, yield stress and strain, extensibility, ultimate strength, and toughness for the axial fibers of capture threads. ANOVAs and Tukey's Honest Significant Difference tests were then used to

explore univariate differences among species. We also used ANOVAs and Tukey's Honest Significant Difference tests to compare the total extensibility of capture threads and the percent of work done by the cribellar fibrils among species (see below).

Results

The qualitative stress-strain behavior of cribellate capture threads differed from that of previously described major ampullate silk and the viscid capture threads of araneoid orbweavers. Cribellate capture threads exhibited an initial elastic behavior and defined yield region, as does major ampullate silk (but not viscid capture silk). However, the stiffness of most cribellate capture threads (~1 GPa in Table 1) was almost an order of magnitude lower than the stiffness of major ampullate threads (~10 GPa) (Blackledge et al., 2005c; Swanson et al., 2006). After fiber yield, tensile behavior became more rubber-like, resembling that of viscid capture fibers. Cribellate capture threads were initially very compliant after fiber yield, but then exhibited an exponential rise in stress until failure of the axial fiber. In contrast to viscid capture silk, the region of high compliance was very short (<20% strain compared to ~100% for viscid capture silk) and the axial fibers broke after being stretched ~50–100% of their original length instead of the ~300–400% that viscid capture silk stretches. Viscid capture silk was under substantial tension in webs (strain, mean \pm s.e.m. = $35 \pm 1.6\%$ for *Argiope*). In comparison, cribellate capture threads were remarkably relaxed in webs (strain, mean \pm s.e.m. = $2.3 \pm 0.6\%$ for both *Hyptiotes* and *Uloborus*).

The mechanical behavior of cribellate capture threads was distinct from all other types of silks that have currently been characterized because of the significant amount of energy absorbed by the surrounding cribellar fibrils after the core axial fibers failed. The axial fibers ruptured after reaching peak stresses at strains of 0.3–0.6 (Fig. 1). At this point, failure of the core fibers was easily verified by shining a light on the sample in the tensile tester or by examining the samples under

Table 1. Mechanical properties of the core axial fibers of capture threads spun by three genera of cribellate spiders

Species	Single axial fiber diameter (μm)	Young's modulus (GPa)	Stress at yield (MPa)	Strain at yield	Extensibility	Ultimate strength (MPa)	Toughness (J m^{-3})
<i>Deinopis spinosa</i> ($N=5, n=27$)	1.03 ± 0.10^a	1.15 ± 0.31^a	55 ± 23^a	0.023 ± 0.007^a	0.73 ± 0.14^a	404 ± 65^a	92 ± 19^a
<i>Hyptiotes cavatus</i> ($N=3, n=22$)	0.51 ± 0.01^{bc}	7.04 ± 0.37	121 ± 5^a	0.019 ± 0.002^a	$0.44 \pm 0.04^{a,b}$	1439 ± 15^b	206 ± 13^b
<i>Hyptiotes gertschi</i> ($N=2, n=24$)	$0.78 \pm 0.11^{a,c}$	1.06 ± 0.33^a	129 ± 113^a	0.058 ± 0.035^a	$0.41 \pm 0.04^{a,b}$	$1171 \pm 227^{a,b}$	$120 \pm 17^{a,b}$
<i>Uloborus diversus</i> ($N=11, n=81$)	0.40 ± 0.02^b	1.05 ± 0.33^a	39 ± 4^a	0.026 ± 0.001^a	0.32 ± 0.01^b	1379 ± 105^b	$118 \pm 11^{a,b}$

All values are mean \pm s.e.m.; N is the number of spiders for which silk was sampled. n is the total number of silk threads tested. s.e.m. was calculated using individual spiders (N) as the sampling unit.

Values that share superscripts within a column indicate lack of significant pairwise differences using Tukey's Honest Significant Difference tests at $P < 0.05$.

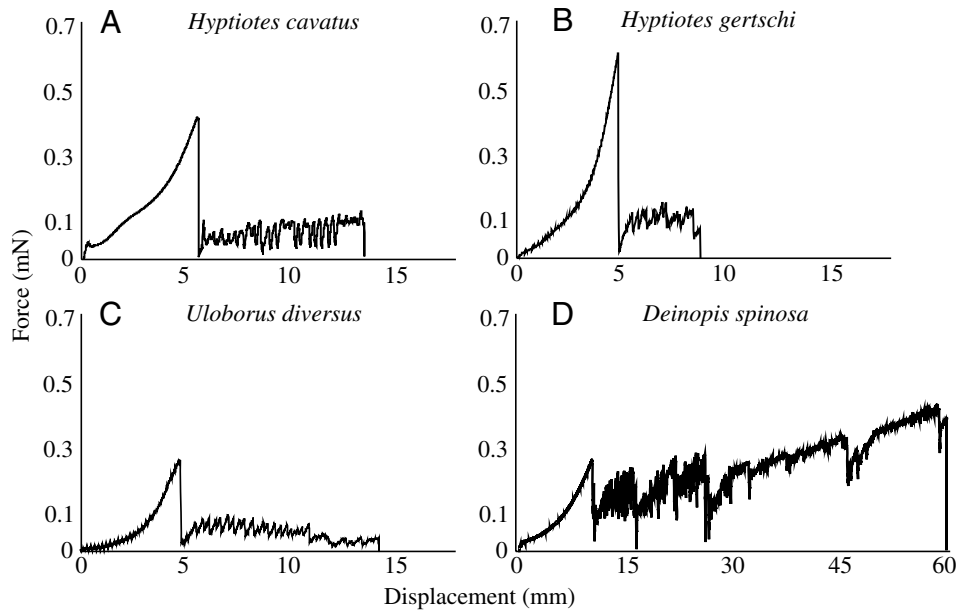


Fig. 1. Exemplar force–displacement curves for cribellate capture threads. All of the curves were qualitatively similar in the extending and breaking of axial fibers followed by the gradual breaking of cribellar fibrils. All threads were initially 10 mm in length. Note that the magnitude of the x -axis for *Deinopis spinosa* is three times that of the other species.

a stereomicroscope. However, cribellate capture threads maintained their integrity due to the sheath of cribellar fibrils surrounding the axial fibers. This allowed the threads to continue to stretch to as much as 500% of their original length before failure. During this post-axial failure extension, loads generated by these fibrils ranged from ~10–60% of the maximal force exerted by the axial fibers themselves for *Uloborus* and *Hyptiotes* (Fig. 1).

Cribellar fibrils are too small to resolve using light microscopy. Therefore, we did not know the total cross-sectional area of these very fine fibrils for our samples and could not compute stress values post-axial failure. However, we could calculate the relative amount of work done by the capture threads prior to and after the axial fibers ruptured by comparing the respective areas under the force–displacement curves (i.e. calculating work rather than toughness). The amount of work done by the fibers post-axial failure ranged among species from 24% to 92% of the total work of the thread. For *Hyptiotes* and *Uloborus*, the force generated by these cribellar fibrils tended to fluctuate within a constant range that rarely exceeded more than 30–50% of the peak force of the axial fibers. In contrast, the force generated by the cribellar fibrils of the capture threads of *Deinopis* also fluctuated, but continuously increased with strain until it exceeded the force exerted on the specimen at axial failure. This pattern, combined with the long post-axial failure extension of *Deinopis* capture threads, resulted in more than 90% of the total energy absorption occurring after the core axial fibers failed (Table 2).

The properties of the axial fibers differed significantly among taxa (MANOVA, $F_{21,7}=10.8$, $P<0.00001$). Univariate ANOVAs indicated significant differences in all parameters except stress and strain at yield (all P at least <0.01 ; Table 1). We used Tukey's Honest Significant Difference tests to make *post-hoc* pairwise comparisons among species. *Deinopis* axial fibers had the largest mean diameter and were significantly

thicker than *Uloborus* and *Hyptiotes cavatus* axial fibers. *Uloborus* axial fibers had the smallest mean diameter and were significantly thinner than *Hyptiotes gertschi* axial fibers. The axial fibers of *Hyptiotes cavatus* were stiffer than those of other species. *Deinopis* axial fibers were stretchier but weaker than those of *Uloborus*. *Hyptiotes cavatus* also spun axial threads that were stronger than *Deinopis*. Finally, *Hyptiotes cavatus* spun axial threads that were tougher than those of *Deinopis*.

The properties of the capture threads post-axial failure also differed significantly among species (MANOVA, $F_{6,2}=10.5$, $P<0.00001$). Both the total extensibility of the capture threads and the percent of work done/accomplished post-axial failure differed among species (Table 2; ANOVAs, $P<0.0001$).

Table 2. Comparison of the energy absorption of capture threads pre- and post-failure of the axial fibers

Species	Axial extensibility	Total extensibility	% work by cribellar fibrils
<i>Deinopis spinosa</i> (N=5)	0.73±0.14 ^a	1.81±0.13	92±1 ^a
<i>Hyptiotes cavatus</i> (N=3)	0.44±0.04 ^{a,b}	0.78±0.13 ^a	36±12 ^b
<i>Hyptiotes gertschi</i> (N=2)	0.41±0.04 ^{a,b}	0.63±0.09 ^a	24±15 ^b
<i>Uloborus diversus</i> (N=11)	0.32±0.01 ^b	0.95±0.08 ^a	71±4 ^a

% work by cribellar fibrils compares the area under the force displacement curve post failure of the axial fiber to the area under the total force-displacement curve.

All values are mean ± s.e.m.; N is the number of spiders for which silk was sampled.

Values that share superscripts within a column indicate lack of significant pairwise differences using Tukey's Honest Significant Difference tests at $P<0.05$.

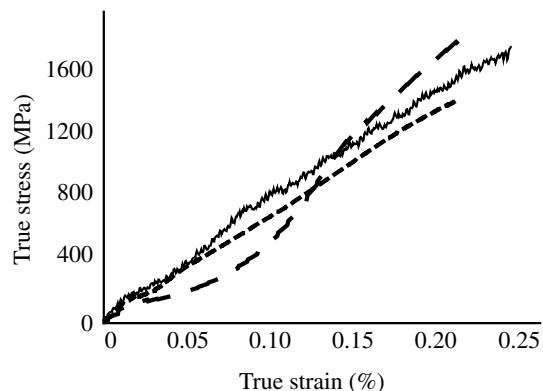


Fig. 2. Comparison of the mechanical performance of major ampullate dragline spun by three genera of cribellate orb-weavers. Solid line, *Uloborus diversus*; thick broken line, *Hyptiotes cavatus*; thin broken line, *Deinopis spinosa*.

Capture threads spun by *Deinopis* stretched significantly further than threads spun by all other taxa (all $P < 0.005$). More than half of all of the work done by the capture threads was done by the cribellar fibrils for *Deinopis* and *Uloborus*, but less than half for both species of *Hyptiotes* ($P < 0.05$).

Major ampullate dragline silk spun by *Deinopis*, *Hyptiotes cavatus* and *Uloborus* exhibited mechanical behavior similar to that described for other web-building spiders (Blackledge et al., 2005c; Swanson et al., 2006). The fibers exhibited a stiff elastic region of ~ 10 GPa, followed by fiber yield and subsequent extension until failure that demonstrated a more or less linear relationship (Fig. 2, Table 3). In contrast to the capture threads, we found no significant difference among species in the mechanical performance of their major ampullate silks (MANOVA, $F_{1,4} = 3.6$, $P > 0.10$).

Discussion

Our study quantifies for the first time how energy is absorbed in mechanically distinct ways by separate components of cribellate capture threads. At the outset, most energy is absorbed through the extension of the axial core fibers of the capture threads. However, the capture threads continue to absorb energy long after the axial fibers have

ruptured through the extension of the surrounding mass of cribellar fibrils. In some cases, such as with *Deinopis* threads, the load carried by these fibrils can exceed that of the core axial fibers (Table 2). This composite nature means that the mechanical performance initially depends upon the molecular interactions of proteins within the core axial fiber spun from the pseudoflagelliform glands, but subsequently is dictated by the performance of hundreds of thinner fibrils spun from numerous cribellar glands.

The mechanical behavior of the axial fibers of cribellate capture threads is qualitatively similar to other types of dry silk, such as major ampullate silk. At the start of the tensile test, cribellate capture threads exhibit a small, but detectable, elastic response that was followed by fiber yield and a subsequent increase in stress until failure. For major ampullate silk, the initial elastic response and fiber yield are attributed to hydrogen bonding between molecules within the amorphous region. These bonds are ruptured during extension of the fibers, resulting in a sudden shift and realignment of molecules at fiber yield. The similarity in mechanical performance between pseudoflagelliform and major ampullate silks suggests that hydrogen bonding may also play an important role in the organization of molecules within pseudoflagelliform silk.

The similarities between pseudoflagelliform and major ampullate silks are limited to the elastic region. Thereafter, pseudoflagelliform silk is readily distinguished from major ampullate silk by its higher extensibility and a reduced stiffness that is almost an order of magnitude lower than that of major ampullate silk (~ 1 GPa in Table 1 versus ~ 10 GPa for major ampullate silk in Table 3) (Blackledge and Hayashi, 2006; Blackledge et al., 2005c). The pseudoflagelliform axial fibers also continue to stiffen post-yield in contrast to major ampullate silk, which has a relatively constant post-yield stiffness. This exponential increase in stress as cribellate threads are stretched is qualitatively similar to the mechanical behavior of flagelliform silk, which forms the axial fibers of viscid capture threads spun by araneoid orbweavers. This mechanical behavior suggests that molecules within the pseudoflagelliform fibers are substantially less organized than in major ampullate silk and that the strain-hardening of the fibers is caused by an increase in the alignment of molecules within the fibers. Such 'j' shaped stress-strain curves may be important for the biological function of both cribellate and

Table 3. Mechanical properties of major ampullate silk fibers

Species	Single fiber diameter (μm)	Young's modulus (GPa)	Stress at yield (MPa)	Strain at yield	Extensibility	Ultimate strength (MPa)	Toughness (J m^{-3})
<i>Deinopis spinosa</i> ($N=4$, $n=42$)	0.50 ± 0.16	13.6 ± 0.9	273.7 ± 31.5	0.024 ± 0.004	0.22 ± 0.02	1264 ± 163	138 ± 7
<i>Hyptiotes cavatus</i> ($N=2$, $n=13$)	0.92 ± 0.01	10.7 ± 0.5	215.5 ± 8.1	0.022 ± 0.001	0.20 ± 0.01	1837 ± 3	164 ± 1
<i>Uloborus diversus</i> ($N=6$, $n=39$)	0.59 ± 0.06	7.8 ± 1.6	145 ± 33	0.017 ± 0.004	0.22 ± 0.02	1418 ± 222	158 ± 27

For this study, s.e.m. was calculated using individual spiders (N) as the sampling unit rather than the total number of fibers tested (n).

viscid capture silk in two ways. First, the relatively high initial compliance allows the silks to stretch under load to dissipate the kinetic energy of prey and align the angle of force applied to the fibers along the longitudinal axis (Gosline et al., 1999). Second, the rapid increase in stiffness of the fibers near failure provides an added safety factor. Fibers are stressed near failure only at extreme strains, which reduces the likelihood of material flaws inducing a very premature failure of the fiber.

The 'j'-shaped stress-strain curve of flagelliform silk is thought to result in part from the folding of individual molecules into molecular nanosprings that increasingly stiffen as fibers are stretched (Becker et al., 2003; Hayashi and Lewis, 1998). These molecular nanosprings are formed by the long, tandem arrays of the GPG(X)_n amino acid sequence motif (Hayashi and Lewis, 1998; Hayashi and Lewis, 2000). This motif is also found in MaSp2, one of the two components of major ampullate silk, but in much smaller proportion and with only a few repeats per tandem array compared to the flagelliform silk protein (Gatesy et al., 2001). If the extensibilities of major ampullate and flagelliform silks are related to the prevalence of GPG(X)_n motifs, then the intermediate extensibility of pseudoflagelliform silk suggests that it is composed of proteins with more GPG(X)_n motifs than in major ampullate silk but less than in flagelliform silk. However, among the first cDNAs that were recently reported for cribellate deinopoid silks was a flagelliform silk-like transcript from *Deinopis spinosa* that encoded forty consecutive glycine-proline containing motifs [mostly GPQ(X)_n] (Garb et al., 2006). Forty tandem glycine-proline containing motifs is greater than what is found in MaSp2 but within the range of what is found in araneoid flagelliform silk proteins (Gatesy et al., 2001). Assuming that the transcript from *Deinopis* is a major component of the pseudoflagelliform core fiber, this finding indicates that the intermediate mechanical properties of cribellate capture silk may result from the substitution of glutamine for glycine in GPQ(X)_n motifs compared to GPG(X)_n motifs.

An alternative explanation for the hybrid-like performance of pseudoflagelliform silk is that the axial fibers of cribellate capture threads may be composed of multiple types of fibroins, with each type possessing distinctive amino acid sequence motifs that impart different functional properties. A precedent for this hypothesis is the dual protein composition (MaSp1 and MaSp2) of araneoid major ampullate silk (Hinman and Lewis, 1992; Gatesy et al., 2001). Because MaSp1 and MaSp2 are hypothesized to contribute different properties to major ampullate silk (Sponner et al., 2005), pseudoflagelliform silk glands may synthesize a set of fibroins that interact to produce a fiber with properties intermediate between flagelliform and major ampullate silks. One additional factor that has yet to be considered is the role played by the aqueous glue coating of viscid capture threads, which modulates the performance of the flagelliform core fibers by decreasing their stiffness and increasing elasticity (Blackledge et al., 2005b; Vollrath and Edmonds, 1989). Currently, comparative data are limited for the quantitative

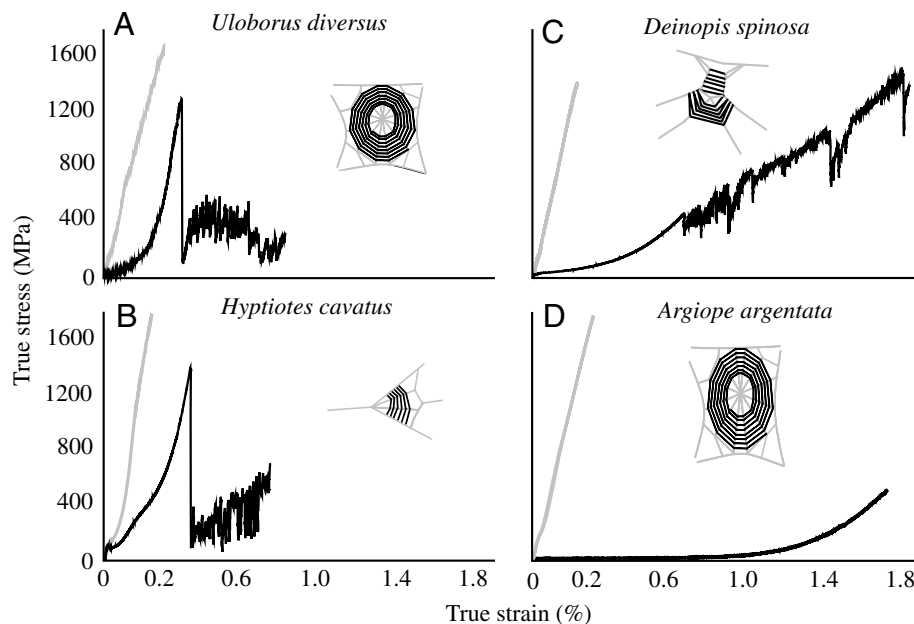
performance of flagelliform silk that has been manipulated to be dry and for pseudoflagelliform silk that has been experimentally hydrated so that the importance of this additional factor cannot easily be assessed (see Vollrath and Edmonds, 1989; Blackledge et al., 2005b).

Cribellate threads continue to absorb energy long after the axial fibers fail through the extension and rupturing of numerous puffs of tiny (10–50 nm) fibrils. While it has long been recognized that cribellar fibrils make capture threads sticky (Opell, 1994c; Peters, 1986), the contribution of these fibrils to the mechanical absorption of energy has not been quantified in detail. Our study demonstrates that cribellar fibrils contribute substantially to both the overall extensibility and the work performed by capture threads (Table 2). For some species, such as *Deinopis*, 90% of the total work done by the capture thread occurs after the axial fibers have ruptured (Table 2). A possible mechanism for this post-axial failure extension of the fiber is that the cribellar fibrils may themselves be extending as a tangled mass and, causing the rapid increases and decreases in force (Fig. 1) as different groups of fibrils stretch and fail.

Deinopis, *Hyptiotes* and *Uloborus* construct webs that look remarkably different from one another and function in ways that likely subject the silks to different selective pressures. However, these spiders produce major ampullate silks with similar mechanical performances to one another (Figs 2, 3 and Table 3). This similarity is not surprising given that major ampullate silk is used for other critical functions besides support of capture webs (Foelix, 1996). In particular, major ampullate silk is used as a lifeline by many orb-weaving spiders, and this may have provided the selective forces that have shaped the material performance of major ampullate silk prior to its incorporation into orb webs (Blackledge et al., 2005c; Osaki, 1996; Swanson et al., 2006). In contrast, cribellate capture silk is used exclusively in prey-capture webs and we find strong differences in the performance of capture threads spun by these three genera. In particular, capture threads spun by *Deinopis* are weaker but more extensible than those spun by *Hyptiotes* and *Uloborus*. *Deinopis* capture threads also have greater post-axial failure extensibility and work capacity (Tables 1 and 2).

The webs spun by *Deinopis* are unusual in two ways. The webs themselves consist of nets that the spiders hold by their first three pairs of legs (Coddington, 1986b). The spiders actively push these nets onto passing insect prey as the spiders spread their legs apart, thereby stretching the net (Coddington and Sobrevila, 1987). Any force generated by this stretching and by the prey itself must be absorbed primarily by the capture threads alone because these webs lack many of the major ampullate radii that support the capture silk in typical orb webs (Coddington and Sobrevila, 1987; Coddington, 1986b). *Deinopid* capture threads also have an additional coiled thread that runs parallel to the axial fibers (Peters, 1984; Peters, 1992). Although difficult to measure accurately using light microscopy, the diameters of the coiled threads were quite large, approaching the diameters of the axial fibers. Given its

Fig. 3. Comparison of the biomechanical performance of the capture threads (in black) and the supporting major ampullate threads (in gray) for three genera of cribellate (deinopoid) spiders and one ecribellate (araneoid) spider. Major ampullate silk performs similarly across all four genera. In contrast, the strength, stiffness and extensibility of capture threads can vary greatly among taxa with diverse web architectures. (A) *Uloborus diversus* spins a complete orb web. (B) *Hyptiotes cavatus* spins a reduced triangle web that is held under tension by the spider. (C) *Deinopis spinosa* spins a specialized net that is held between the front six legs and pressed on top of prey, thereby stretching the web greatly during prey capture. (D) *Argiope argentata* (Fabricius 1775) spins a complete orb web but uses a capture spiral that is composed of glue-coated flagelliform fibers (see Blackledge and Hayashi, 2006).



The capture threads in A–C exhibit behavior typical of cribellate silk where most of the stress is generated within a pair of core axial fibers that fail at high peak stresses and moderate strains. The thread then continues to strain and absorb force through the extension and failure of hundreds of surrounding fibrils until final failure. Because we could not measure the total cross-sectional area of these fibrils, stress values after failure of the axial fibers should be interpreted only as a relative indication of qualitative changes in force generated by the fibrils as the entire structure is strained.

relative size and length, the coiled thread may account for the general increase in load that is generated during post-axial failure extension of *Deinopis* capture threads as it does not rupture until long after the axial fibers have failed (Coddington and Sobrevila, 1987). However, this auxiliary fiber cannot account for any of the post-axial failure extension of *Hyptiotes* and *Uloborus* capture threads because it is lacking in uloborid webs (Peters, 1992).

The partitioning of the mechanical performance of cribellate threads into separate axial core and cribellar components has important implications for understanding the evolution of capture silk in orb-weaving spiders. Araneoid spiders are the sister taxon to the Deinopoidea and evolved from cribellate orb-weaving ancestors (Griswold et al., 1999). Thus, the ancestor of all orb-weaving spiders also spun cribellate silk. We can therefore gain insight into the evolutionary origin of viscid capture silk under the assumption that the cribellate silk spun by modern deinopoid spiders still retains plesiomorphic characteristics of this ancestor. Köhler and Vollrath proposed that a key innovation in the origin of araneoid spiders was an abrupt increase in the extensibility of viscid capture silk relative to cribellate orb-weaving ancestors (Köhler and Vollrath, 1995). Opell and Bond tested this hypothesis by comparing the extensibilities of capture threads spun by diverse cribellate orbweavers in the Uloboridae and several araneoid orbweavers (Opell and Bond, 2000). Using independent contrast analysis, Opell and Bond found evidence for a gradual increase in thread extensibility during the evolutionary transition from cribellate to viscid capture threads. Furthermore, differences in extensibility were

primarily related to changes in spider size and web architecture rather than to the type of silk composing the core of the capture threads. Opell and Bond therefore concluded that there was no absolute difference in the extensibility of viscid and cribellate capture threads (Opell and Bond, 2000). Our data agree with Opell and Bond from the perspective of the structural performance of the entire capture thread. We found that the total extensibilities of cribellate capture threads varied from true strains of 0.6 to 1.8 (Table 2), which are equivalent to engineering strains of 100–500% and are in the range of values that Opell and Bond found for other species of uloborids. However, our study reveals that the pseudoflagelliform axial fiber itself was much less extensible than the entire capture thread, failing at an engineering strain of 40–90%. This range of extensibilities for the axial fibers is significantly less than the whole capture thread extensibilities that Opell and Bond reported in their analysis, and is also significantly less than the extensibility of flagelliform silk (Fig. 3D). Therefore, from a perspective focusing on the evolution of the material properties of axial core fibers of capture threads, our data are consistent with an increase in extensibility that is associated with the origin of flagelliform silk. In other words, the salient evolutionary change in the mechanical performance of capture threads was not in the overall extensibility of capture threads, but rather a change in how that extensibility was achieved. Finally, the importance of the cribellar fibrils for the mechanical absorption of energy during prey capture suggests that this silk has evolved under significant selection for its tensile properties in addition to its better-studied adhesive properties.

List of symbols

A	cross-sectional area of fibers
F	force
L	instantaneous fiber length
L_0	fiber gage length
ϵ_t	true strain
σ_t	true stress

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