A BIOLOGICAL AND EVOLUTIONARY APPROACH TO THE STUDY OF SPIDER SILK MATERIAL PROPERTIES

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A BIOLOGICAL AND EVOLUTIONARY APPROACH TO THE STUDY OF SPIDER SILK MATERIAL PROPERTIES

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Dissertation

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Spider silk is a biomaterial that combines high strength and extensibility. Because of these exceptional material properties, silk could be used for many applications. However, before we can mass-produce spider silk analogs for these applications, we need to better understand the relation between silk molecular structure and its properties. Furthermore, the whole range of properties that can be achieved by silks may not have been gauged yet, as most studies focus on a few selected species.

The first part of my research focused on silk plasticity. I found that common house spiders (*Achaearanea tepidariorum*) change their silk properties in function of their prey type (cricket or pillbug). Silk properties also differ between different regions of the cobweb spun by a common house spider. However, silk properties did not differ for other species (black widows and bridge spiders). Major ampullate silk plasticity increased during spider evolution. Silk plasticity may be mediated by a valve present in the spinning duct of Orbicularia, which allows them to apply shear forces during forcible silking and control their speed during falls. Silk plasticity may have been selected for as spiders make more diverse uses of their major ampullate silk.

The second part of my research dealt with supercontraction. Supercontraction refers to the shrinking of silk exposed to high humidities. Several hypotheses on its mechanisms and functions have been proposed, but seldom tested. By measuring supercontraction in
many different spider species, with various silk composition, web type and silk uses, I tested three of these hypotheses. GPGXX amino acid motif are likely involved in supercontraction. Furthermore, supercontraction probably allows spiders to better tailor their silk properties, but, contrary to an early idea, it may not help protect webs from water drops. Supercontraction may affect how whole webs function too. Supercontracted webs were found to absorb more kinetic energy and deform more when hit by a projectile (mimicking a flying prey). Thus, another potential function of supercontraction is to improve web performance.

To conclude, my research shed light on various aspects of silk material properties by taking an evolutionary approach. This approach may be expanded further in order to better understand silk evolution and estimate the range of silk properties. This study also helps better understand how silk properties and web architecture interact within spider webs.
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CHAPTER I
INTRODUCTION

Spider silk has received a lot of interest lately because of its combination of high strength and extensibility. These properties together make it a high-performance material, as tough as man-made materials like Kevlar, but also biodegradable and biocompatible. There are many potential applications for spider silk, especially in the medical field (Hardy et al., 2008; Kluge et al., 2008). However, mass production of “natural” silk spun by spiders is impossible. Therefore, researchers are trying to produce silk proteins through bacteria or create synthetic polymers that mimic silk properties (Lewis, 1992; Vendrely & Scheibel, 2007). Artificial production of silk requires a complete understanding of silk behavior in relation to its structure. Much research is carried on silk molecular structure, focusing on both silk biochemical composition and effects of spinning condition. Different protein motifs, i.e. short repeated amino acid sequences, are thought to give silk its properties (Gosline et al., 1984; Gosline et al., 1999; Hayashi et al., 1999). External conditions, such as temperature, speed of reeling, etc., are also known to influence silk material properties (Dicko et al., 2004a; Liu et al., 2005a). Thus, our understanding of the relation between silk properties and silk molecular structure is progressing steadily.

However, most of these studies focus on two orb-weaving spiders genera: Araneus and Nephila. Yet, there are over 3500 spider genera known (Platnick, 2000-2010), with...
different silk proteins sequences and ecologies. Silk material properties also differ between spider taxa (Swanson et al., 2006a; Swanson et al., 2007). Evolution has shaped silk properties in spiders for over 400 million years. What make silk properties adaptive for spiders? Only a more extensive sampling of the diverse spider taxa can allow us to understand the evolutionary history of silk. This would point to silk characteristics associated to specific ecologies and behaviors.

Studying diversity of silk may also open new possibilities in terms of materials. Since silk diversification and evolution has lasted for hundreds of million years, by studying a wide range of spider silk, we can identify constraints in material properties (e.g. a trade-off between strength and extensibility). We can also understand how spiders adapted their silk to their environment and ecology. For example, we can find how silk properties change between spiders spinning complete orb-webs and derived spiders that use simpler webs to catch prey.

My PhD research focused on evolution and plasticity of silk properties in a variety of spider taxa. It can be divided into two main parts:

1) Plasticity of spider silk: I studied how silk material properties vary with spider condition (e.g. prey type) and silk's intended use (e.g. different parts of the web or silk laid while walking vs. while dropping). I compared this plasticity between spider taxa, in relation to spider's morphology, in particular, the presence of a valve within the spinning duct that could serve as a clamp and brake. After establishing that silk properties differed between the various elements of complex webs, I also tested for convergence of silk properties between elements of different web types (orb-web vs. cobweb) that served similar functions (prey capture or support).
2) Evolution and mechanisms of supercontraction: Supercontraction refers to the strong shrinking of materials (up to half the thread original length in spider silk). Although it exists in other materials, what is unique about spider silk supercontraction is that it happens under mild conditions (when the humidity rises above ~70%) (Work, 1977). Supercontraction has potentially important consequences, both for the spider's ecology and for industrial applications of spider silk. Yet, we have limited knowledge of its occurrence or function, and mainly theoretical knowledge of its mechanisms. I surveyed supercontraction in various spider taxa, both to reconstitute supercontraction evolutionary history, and to test current hypotheses on supercontraction's mechanisms and function. I also investigated the effects of supercontraction on webs experimentally, focusing mainly on prey capture, in order to study ecological consequences of supercontraction.
CHAPTER II

THE COMMON HOUSE SPIDER ALTERS THE MATERIAL AND MECHANICAL PROPERTIES OF COBWEB SILK IN RESPONSE TO DIFFERENT PREY

Introduction

For many spiders, how efficiently webs function has major consequences for prey capture. Web function is determined both by overall architecture and by the mechanical performance of individual silk threads. Mechanical performance of silk threads results from the inherent material properties of the silk and the thread’s overall structure. Material properties, such as ultimate strength and extensibility, are in turn determined by the molecular organization of silk proteins. In contrast, structural properties describe the overall shape of threads and include variation in the number or diameter of the individual strands that compose a discrete thread. Thus, spiders could potentially alter web function through several different mechanisms. For instance, a spider could spin webs that support more weight by attaching more threads together, by producing silk with superior ultimate strength and stiffness, or simply by spinning thicker threads.

Web architecture is a plastic trait that responds to prey type and availability (ap Rhisiart & Vollrath, 1994; Sandoval, 1994; Schneider & Vollrath, 1998). However, the functional implications of this variation are poorly understood, and these studies largely focus on only a single type of web, the orb-web. Most spiders spin other web
architectures but little is known about functional variation in them (e.g. Blackledge & Zevenbergen, 2007; Zevenbergen et al., 2008).

The mechanical performance of spider silk also plays an important role in web function (Craig, 1987a; Denny, 1976; Gosline et al., 1986). However, the degree to which spiders control the structural or material properties of silk in webs is even less understood than architectural plasticity. Silk material properties vary among individual spiders within a species (Köhler & Vollrath, 1995; Madsen et al., 1999) and within single spiders (Garrido et al., 2002a; Madsen et al., 1999). Although some of this variation may appear random, several consistent effects of environmental conditions on silk properties have been identified. Garrido et al. (2002b) showed that the orb-weaving spider Argiope trifasciata produced silk that resisted higher load when climbing compared to silk spun while walking on a horizontal surface. Pan et al. (2004) showed that the orb-weaving spider Araneus ventricosus spun stronger dragline silk, with a higher ultimate strength, when housed in taller cages. The authors hypothesized that the spider assessed the risk of a fall from a higher height and spun stronger silk in response. Tso et al. (2007) studied the effect of prey type on the material properties of dragline silk of another orb-weaver, Nephila pilipes. They observed that spiders fed flies increased the stiffness of dragline silk compared to those fed crickets. These different studies indicate that spiders can control the mechanical performance and material properties of their silk. However, they primarily involve either silk forcibly drawn from restrained spiders or silk spun by walking spiders. Whether spiders can tune the material properties of silk used for prey capture in webs is largely unknown.
In this study, we tested whether the cobweb-weaving common house spider *Achaearanea tepidariorum* (Araneae: Theridiidae) responds to different prey by altering the structural or material properties of silk in its web. Spiders were fed either large, fast prey (late instar crickets) or small, slow prey (pillbugs). The two prey differed in kinetic energy as well as nutritionally, either of which could affect silk production.

*A. tepidariorum* cobwebs consist of two regions (Fig. 2.1): an array of supporting threads and sticky gumfooted threads (Benjamin & Zschokke, 2003). The supporting threads are all dry dragline silk spun from the major ampullate silk gland. Sticky gumfooted threads are spun vertically from the substrate to the supporting threads of the web and are also composed of major ampullate silk (Benjamin & Zschokke, 2002; Blackledge *et al.*, 2005c). Most of the sticky gumfooted threads are dry, but their lower 1-2cm are covered with glue secretions, produced from aggregate silk glands. These droplets adhere to insects during prey capture. Once a sticky gumfooted thread intercepts a prey, the thread detaches from the ground and tension in the web then pulls small insects off of the ground or restrains larger prey (Argintean *et al.*, 2006; Szlep, 1965). The spiders then quickly attack the prey and bring it to the retreat where it is consumed.

In our experiment, we investigated silk from both supporting threads and sticky gumfooted threads. Because these threads have different functions in the web, we expected them to potentially exhibit different material and structural properties that may relate to how spiders or their prey interact with the web.
Fig. 2.1: *Achaearanea tepidariorum* cobweb. The supporting threads form a network in the upper region of the web and connect to near-vertical sticky gumfooted threads. These sticky gumfooted threads are gluey in their lower portion and adhere directly to prey during capture. (Note: the glue droplets are enlarged in the figure.)

**Methods**

**Spiders**

We collected adult *A. tepidariorum* at the University of Akron’s Bath Field Station (Bath, OH) and local homes in the surrounding area (Akron, OH). We weighed the animals to the nearest mg and measured their right femurs to the nearest 0.1mm. We then calculated body condition as the residual of a regression of mass versus femur length (Jakob *et al.*, 1996). We ranked spiders by condition and allocated them to two different feeding regimes (n = 14 for cricket-fed spiders and n = 13 for pillbug-fed spiders) as follows: the spider in highest condition was allocated to the pillbug feeding regime, the second highest to the cricket feeding regime, the third highest to the pillbug feeding regime, etc. As a result, there was no difference between treatments in the initial condition or body mass of the spiders.
Spiders were housed in 36.8 x 21.8 x 24.4 cm clear plastic cages (Kritter Keepers®, San Marcos, CA), with cardboard frames to support webs (Fig. 2.1). The frames consisted of two 28 x 20 cm cardboard sheets, on top and bottom, which were joined by 34 cm high wooden sticks (two on back and one on front). This design allowed the spiders to spin webs between the top and bottom cardboard sheets, while assuring easy access to all regions of webs for silk collection. Borders, 2.5 cm wide, were added to the top and bottom to provide enclosed “corners” that encouraged spiders to build webs within the frames.

Spiders were housed at a constant temperature of 24°C under a 15 : 9 hr light / dark cycle. The spiders were acclimated to the cages for two days before the feeding regimes started.

Feeding regime

We fed the spiders for one week a diet of either crickets (purchased from Fluker's Farm, Port Allen, LA) or pillbugs (collected from the University of Akron’s Bath Field Station, Bath, OH and residences, Akron, OH). Crickets weighed (mean ± SE) 230 ± 40 mg while the pillbugs weighed 49 ± 5 mg. Spiders received either one cricket every two days (three total over the week) or two pillbugs each day (14 total), so that the overall quantity of food was similar (98.6 mg per day of crickets versus 98 mg per day of pillbugs). However, ingested biomass differed between treatments, due to spiders’ inability to extract as much material from pillbugs as from crickets (see below).

Silk collection

After one week of feeding, we destroyed the webs and gave the spiders two days to build new webs. This ensured that all silk threads were spun only after spiders
experienced the full feeding regime. We then collected samples of silk for each web from the sticky gumfooted threads (n=3) and from the uppermost supporting threads (n=3). The silk was secured across 10.7 mm holes in cardboard mounts, fastened on either end of the gap with cyanoacrylate glue, and then cut free from the web with a soldering iron.

We only collected the dry region of sticky gumfooted threads because the glue droplets alter mechanical performance of the silk (Blackledge et al., 2005b). We collected supporting threads from the top five cm of the web. For both types of silk, most threads were double-stranded, but we were occasionally unable to find enough double-stranded samples and therefore collected some three- or four-stranded samples. Spiders normally spin threads consisting of two strands because of their paired spinning organs, while four stranded threads are actually two separate threads spun at different times. Therefore, we rationalized that double stranded threads best standardized material testing because the two strands were produced simultaneously and thus were probably more similar to each other in tension and material flaws compared to four-stranded threads.

**Diameter measurements**

We used polarized light microscopy to take two digital photographs of each sample at 1000X. We measured the diameter of each strand in a sample twice from each photograph using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2007) and counted the number of strands per thread. The multiple measurements accounted for the slight shape anisotropy of silk so that cross-sectional area could be approximated by a single average diameter (Blackledge et al., 2005a). From these measurements, we calculated both the
total cross-sectional area of the silk sample and the average diameter of single strands within the sample.

**Mechanical and material properties of the silk**

We tested the mechanical performance of silk threads on a Nano Bionix UTM (MTS Corp.) using established protocols. All fibers were extended at 1% strain/s and the resulting force values measured. We first considered raw failure load, which represents the force supported by the thread before breaking, without normalizing to thread diameter. Failure load thus gives us information about the capacity of a thread to support forces and is a direct determinant of how threads interact with prey and spiders. We also measured the material properties of threads. The material properties describe the intrinsic quality of the silk spun by spiders and combine with the structural aspects of threads (size and number of strands) to determine how threads interact with prey. We recorded six aspects of material properties for each sample (Fig. 2.2): Young's modulus, yield stress, yield strain, extensibility (breaking strain), ultimate strength (breaking stress) and toughness (breaking energy). Young's modulus measures the initial stiffness of the material. The higher Young's modulus is, the more the silk resists deformation under a given load. The yield point measures the transition of the material from elastic to plastic behavior. Before yield, the material is perfectly elastic, which means that when a force applied to the material is removed, the material returns to its original shape and retains its original mechanical performance. After yield, the material permanently deforms and its future mechanical performance is permanently altered. In general, silk extends more easily after yielding. Yield stress and yield strain are, respectively, the true stress and true
strain at yield. Similarly, ultimate strength and extensibility are the true stress and true strain at the breaking point.

We used true stress and true strain, rather than engineering stress and engineering strain, because these are more reliable for very elastic materials such as spider silk (Blackledge et al., 2005c). True stress ($\sigma$) measures the force supported per area of thread and was calculated as:

$$\sigma = \frac{F}{A}$$

where $F$ is the force exerted on the material, and $A$ is the instantaneous cross sectional area of the silk fiber at time $t$. We computed the cross-sectional area at time $t$ by assuming a constant volume for the fiber (Vollrath et al., 2001). $A$ was calculated as:

$$A = \frac{V}{l} = \frac{A_0 l_0}{l}$$

where $V$ is the volume of the fiber, $A_0$ is the original cross-sectional area, $l$ is the length of the fiber at time $t$ and $l_0$ is the original length of the fiber.

![Stress–strain curve](image)

Fig. 2.2. Stress–strain curve for spider dragline silk. The yield point is indicated by the initial change in the slope of the curve. The breaking point appears as a second, final peak. The area under the stress–strain curve measures the toughness, or ability to absorb kinetic energy, of the silk.
True strain ($\varepsilon$) measures the relative extension of the fiber and was calculated as:

$$\varepsilon = \ln \left( \frac{l}{l_0} \right)$$

where $l$ is the instantaneous length of the fiber at time $t$ and $l_0$ is the original length of the fiber.

Toughness, or breaking energy, represents the total energy that can be absorbed by the silk before breaking. It was calculated as the area under the stress-strain curve.

**Statistical analysis**

We tested whether the average single strand diameter differed between treatments using t-tests because data were normally distributed (Shapiro Wilks normality tests, $P = 0.0560$ for pillbug-fed spiders' support threads, $P = 0.3838$ for pillbug-fed spiders' gumfooted threads, $P = 0.3763$ for cricket-fed spiders' support threads and $P = 0.0981$ for cricket-fed spiders' gumfooted threads,). However, spider mass was correlated with diameter (Griffith & Salanitri, 1980; Osaki, 2003; Vollrath & Köhler, 1996). Therefore, we removed the effect of spider mass by using an ANCOVA with mass and treatment as covariates.

We tested for normality using Shapiro-Wilks normality tests. For sticky gumfooted threads, the failure loads were not normally distributed ($P = 0.6082$ for pillbug-fed spiders but $P < 0.0001$ for cricket-fed spiders), so we compared the failure load of silk from cricket-fed spiders and silk from pillbug-fed spiders using a Mann-Whitney U test. For supporting threads, the data were normal ($P = 0.1254$ for pillbug-fed spiders and $P = 0.5563$ for cricket-fed spiders), so we instead used a t-test. To test for a potential influence of spider mass on failure load, we ran an ANCOVA with mass and treatment as
covariates. Since the data for sticky gumfooted threads were not normally distributed, we transformed them by taking the inverse (Shapiro-Wilks normality test after transformation, $P = 0.9100$ for pillbug-fed spiders and $P = 0.4231$ for cricket-fed spiders).

We tested the effect of prey type on all six aspects of material properties using a Hotelling T$^2$ (Johnson & Wichern, 2002). According to chi-plots, all samples were multivariate normal. We ran post-hoc Tukey's HSD tests to determine which of the variables were affected by the treatment. To test for a potential effect of spider mass on material properties, we ran a MANCOVA with mass and treatment as covariates.

*Forces exerted by spiders and prey on the web*

To better understand web performance, we calculated the forces exerted by spiders and prey on the web and compared them to the mechanical performance of silk threads. A prey initially exerts a force on a sticky gumfooted thread that is proportional to its kinetic energy. We computed the average kinetic energy ($KE$) of jumping crickets and crawling pillbugs as:

$$KE = \frac{1}{2}mv^2$$

where $m$ is the mass of the prey and $v$ its speed.

We measured the average mass of each prey type for 6 crickets and 13 pillbugs and calculated the average speed of each prey type by racing 4 crickets and 12 pillbugs on a 40-cm race track, twice for each individual.

We also calculated the energy $E_f$ that can be absorbed by a single sticky gumfooted thread before failure as:

$$E_f = TAh$$
where $T$ is the toughness of the silk (as measured during the tensile test), $A$ is the average cross-sectional area of the thread and $h$ is the average height of the thread (~8 cm in our experiment).

We determined $h$ by averaging the height of 10 randomly chosen sticky gumfooted threads measured on pictures of five webs.

In order to better understand the effects of small prey on silk, we computed the energy absorbed by sticky gumfooted threads before yielding. The yield point measures when a viscoelastic material starts showing plastic behavior and deforms permanently. A silk thread that passed yield may not function optimally during subsequent prey capture events. In contrast, silk threads that function exclusively within their elastic region, *i.e.* before yield, maintain the original performance of the web in terms of stopping power, structural tension and transmission of vibrations. Thus, producing silk that does not yield in response to prey may be as important as producing silk that does not break. Toughness (energy absorbed upon failure) can be calculated from a stress-strain curve by integrating stress to the point of failure. Thus, we computed the energy that can be absorbed upon yielding by integrating stress to the yield point.

We also considered how the static loading of spiders or prey suspended in webs might interact with supporting threads. We computed the average static load generated by the weight $w$ of spiders, pillbugs, and crickets, as:

$$w = mg$$

where $m$ is the average mass and $g$ is the acceleration due to gravity.

However, supporting threads must maintain their functional integrity through repeated bouts of prey capture. Thus, the silk should not yield during prey capture and it

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is important to consider whether spiders tune their silk to prevent yielding. If prey apply large loads to webs then a high yield stress may prevent supporting threads from permanently deforming during prey capture. We computed the load at yield ($L_y$) using the average yield stress ($\varepsilon_y$) and the average thread cross-sectional area ($A$) for supporting threads as:

$$L_y = \varepsilon_y A.$$

Results

Spider mass

Cricket-fed spiders were heavier than pillbug-fed spiders (t-test, $P = 0.0009$; mean ± SE = 91.9 ± 8.8 mg and 49.8 ± 2.9 mg respectively). Cricket-fed spiders were also in better body condition than pillbug-fed spiders (t-test, $P < 0.0001$).

Thread diameter

For both regions of the web, cricket-fed spiders spun thicker single strands of silk compared to pill-bug fed spiders (t-test, $P = 0.0008$ for sticky gumfooted threads and $P = 0.0006$ for supporting threads). Single strand diameter for sticky gumfooted threads was (mean ± SE) 2.408 ± 0.136 µm for cricket-fed spiders and 2.034 ± 0.088 µm for pillbug-fed spiders. For supporting threads, single strand diameter was (mean ± SE) 2.682 ± 0.182 µm for cricket-fed spiders and 2.113 ± 0.091 µm for pillbug-fed spiders (Fig. 2.3).

For both types of threads, spider mass was positively correlated with single-strand diameter when included in an ANCOVA with prey type ($P = 0.00488$ for sticky gumfooted threads and $P < 0.0001$ for supporting threads). However, there was no effect of prey type on single thread diameter for either sticky gumfooted threads ($P = 0.0769$) or...
for supporting threads (P = 0.1828) once spider mass was included. This suggested that the difference in diameter between the two feeding regimes revealed by the t-test was explained primarily by differences in spider mass rather than prey per se.

**Mechanical properties**

Silk from cricket-fed spiders broke at higher load, for both sticky gumfooted threads (P = 0.00004) and supporting threads (P = 0.00227) (Fig. 2.4).

Spider mass was positively correlated with failure load of both types of threads when included in an ANCOVA with prey type (P = 0.0008 for sticky gumfooted threads (transformed data) and P < 0.0001 for supporting threads). However, there was no effect of prey type on failure load for either sticky gumfooted threads (P = 0.0884) or for supporting threads (P = 0.1897) once spider mass was included. This suggested that the difference in failure load between the two feeding regimes revealed by the univariate tests was, like the difference in diameters, explained by difference in spider mass rather than prey per se.
Material properties

The type of prey captured by spiders had an effect on the material properties of supporting thread silk (Hotelling $T^2$, $P = 0.0073$), but not on the material properties of sticky gumfooted thread silk (Hotelling $T^2$, $P = 0.1514$) (Fig. 2.5). For supporting threads, cricket-fed spiders spun silk with higher Young's modulus (Tukey's HSD, $P = 0.0068$) and toughness (Tukey's HSD, $P = 0.0448$) than pillbug-fed spiders.

For supporting threads, Young's modulus was significantly correlated with both prey type ($P = 0.0163$) and spider mass ($P = 0.0422$) in a MANCOVA. Toughness was correlated with prey type ($P = 0.0476$) but not spider mass.

Supporting thread silk differed from sticky gumfooted thread silk for pillbug-fed spiders (Hotelling $T^2$, $P = 0.0388$), but not for cricket-fed spiders. For pillbug-fed spiders, supporting threads silk was stiffer than sticky gumfooted thread silk (Tukey's HSD, $P = 0.0421$). In contrast, sticky gumfooted thread silk was more extensible than supporting thread silk (Tukey's HSD, $P = 0.0012$).

Forces exerted by spiders and prey on the web

The average kinetic energy of a cricket was over 550 $\mu$J while for a pillbug it was 2.5 $\mu$J (Table 2.1). The energy that can be absorbed before failure of sticky gumfooted threads was ~110 $\mu$J for cricket-fed spiders but only 50 $\mu$J for pillbug-fed spiders. The energy absorbed before yielding was 1.8 $\mu$J for cricket-fed spiders and 1.3 $\mu$J for pillbug-fed spiders.

The average weight of a cricket was 2.25 mN while the average weight of a cricket-fed spider was slightly under 1 mN (Table 2.2). Both pillbugs and pillbug-fed spiders
Table 2.1: Kinetic energy of prey compared to energy absorbed by sticky gumfooted threads spun by spiders fed each type of prey. Silk values were calculated for 8 cm long sticky gumfooted threads.

<table>
<thead>
<tr>
<th></th>
<th>Cricket</th>
<th>Pillbug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prey kinetic energy (µJ)</td>
<td>579.72</td>
<td>2.61</td>
</tr>
<tr>
<td>Energy absorbed upon yield by the silk (µJ)</td>
<td>1.84</td>
<td>1.31</td>
</tr>
<tr>
<td>Energy absorbed upon failure by the silk (µJ)</td>
<td>112.74</td>
<td>50.78</td>
</tr>
</tbody>
</table>

Fig. 2.5. Material properties of silk spun by spiders fed different prey (mean ± SE). Dark bars represent pillbug-fed spiders and open bars represent cricket-fed spiders. Asterisks indicate a significant difference.
Table 2.2: Average mass of spiders and their prey compared to maximum sustainable load of individual supporting threads upon failure and upon yield.

<table>
<thead>
<tr>
<th></th>
<th>Cricket-fed spiders</th>
<th>Pillbug-fed spiders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spider weight (mN)</td>
<td>0.901</td>
<td>0.488</td>
</tr>
<tr>
<td>Prey weight (mN)</td>
<td>2.254</td>
<td>0.480</td>
</tr>
<tr>
<td>Load at yield of the silk (mN)</td>
<td>2.187</td>
<td>1.156</td>
</tr>
<tr>
<td>Load at failure of the silk (mN)</td>
<td>6.150</td>
<td>3.433</td>
</tr>
</tbody>
</table>

weighed on average 0.5 mN. The load at yield of supporting threads was slightly under 2.2 mN for cricket-fed spiders and over 1 mN for pillbug-fed spiders.

Discussion

Silk threads spun by spiders fed faster, heavier prey (crickets) are thicker and absorb more force before breaking than threads spun by spiders fed lighter, slower prey (pillbugs). Supporting thread silk is also stiffer and tougher for spiders fed faster, heavier prey. This suggests that spiders alter silk properties in response to different prey and that this response may tune silk to improve prey capture. However, alternative explanations, involving intrinsic effects of spider condition or mass on silk production are important and are also discussed below.

*Nutritional effects on spider mass and condition*

When confronted by long-term differences in prey, spiders may alter their silk either in response to how prey interact physically with webs or in response to differences in nutritional value of prey. Spiders fed heavier prey tended to be heavier themselves, even though we offered the same total amount of food to both groups. This difference was probably due to the heavy exoskeletons of pillbugs and their higher surface to volume ratios resulting in less ingestible material. We estimated the proportion of wet biomass extracted from crickets and pillbugs by weighing each prey just before it was released in
the spider's cage and just after the spider finished feeding on it. Spiders extracted 69.1 ± 6.1% of crickets but only 53.5 ± 6.4% of pillbugs (mean ± SE). The 16% less ingestible biomass available to pillbug-fed spiders likely explains why their body masses and body conditions were lower than those of cricket-fed spiders.

Thus, the difference in the material and mechanical properties of the silk between treatments might be a response to different levels of starvation rather than a response to the physical challenges presented by different prey during capture. For instance, the strong, elastic silk spun by cricket-fed spiders may be metabolically expensive and starved spiders simply do not have the energy to produce it.

Both thread diameter and failure load increased proportional to spider mass. However, prey type did not influence either. Some material properties (e.g. Young's modulus) were influenced by both spider mass and prey type. Other material properties (e.g. toughness) only depended on prey type. This demonstrates that spider body mass clearly plays an important role in determining variation in silk but that prey type was also important.

This suggests that spiders alter different aspects of silk production in response to internal versus external stimuli. For instance, both structural and mechanical properties of silk varied in response to changes in spider body condition and mass but not in response to prey type. In contrast, the material properties of the silk were influenced by both spider body characteristics and the specific type of prey captured. However, further research is needed to fully explore this hypothesis.
Effects of prey interacting with webs

Nutritional quality of prey can affect silk by changing spider condition, but spiders may also alter their silk threads in response to how prey physically interact with webs. Prey present different physical challenges to different regions of the cobweb. Prey initially impact the sticky gumfooted threads with a force proportional to their speed and body mass. Later, while hanging in webs, captured prey apply static loads to the supporting threads proportional to their mass. Spiders also exert static loads in the supporting thread region of webs that are proportional to their mass. How do these physical challenges relate to silk performance?

The total energy that a moving prey exerts upon a single sticky gumfooted thread is equal to its kinetic energy. This means that a larger, faster prey transfers more energy to the sticky gumfooted threads of a web. Sticky gumfooted threads spun by pillbug-fed spiders can absorb 20 times the kinetic energy exerted by a pillbug before breaking (Table 2.1). In contrast, a single sticky gumfooted thread spun by a cricket-fed spider is unable to absorb the kinetic energy of a jumping cricket before breaking. Thus, their webs likely require crickets to contact multiple threads before the crickets are effectively stopped or require the supporting threads to absorb some of the prey energy transmitted from the sticky gumfooted threads. This suggests that sticky gumfooted threads are over-engineered for small prey (pillbugs) but under-engineered for large, fast prey (cricket).

Sticky gumfooted threads spun by pillbug-fed spiders can only absorb half of the kinetic energy of a pillbug before yielding. However, sticky gumfooted threads function only once because they detach from the substrate when contacted by prey. Thus, maintaining future performance by spinning sticky gumfooted threads that can absorb the
energy of a prey without yielding may be irrelevant. In contrast, supporting threads in the upper regions of cobwebs need to function during multiple prey capture events such that preventing yield could be an important constraint on their performance.

At rest, the spider stays within the supporting region of the web. This is also where it consumes prey. Therefore, a web spun in response to heavier prey may need supporting threads that resist higher loads and absorb more energy than threads spun by spiders capturing lighter prey. Heavier spiders also need stronger threads that will support their own weight. In our experiment, cricket-fed spiders gained more mass than pillbug-fed spiders. Thus, both supporting larger prey and spiders growing heavier when consuming larger prey should place increased demands on silk performance by cricket-fed spiders.

In order to investigate whether spider weight or prey weight affects silk strength, we computed the average static load generated by spiders, pillbugs, and crickets (Table 2.2). Both spiders and dead prey hanging in the supporting region of webs apply loads equal to their weight. The failure load for supporting threads spun by cricket-fed spiders is three times the average weight of a cricket and six times the average weight of the spider. For supporting threads spun by pillbug-fed spiders, the failure load is six times the average weight of both pillbugs and spiders. This large difference suggests that spiders do not tune threads in webs to prevent the threads from breaking under the static loads of prey or their own bodies.

In contrast, load at yield for supporting threads spun by cricket-fed spiders is twice the weight of a spider, but only slightly less than the average weight of a cricket (Table 2.2). Thus, a single supporting thread could not hold a large prey, such as a cricket, without deforming permanently. The load at yield for supporting threads spun by pillbug-
fed spiders is twice the weight of either spiders or pillbugs, so that a single thread could easily support them. In conclusion, while single threads in webs usually function below yield, large prey generate enough load to permanently deform individual threads, impairing their future performance. Moreover, the importance of preventing fiber yield may also explain the observed increase in stiffness and toughness of cricket-fed spider silk because any increase in fiber yield would be accompanied by increases in both of these performance parameters (Fig. 2.6).

**Potential mechanisms for control of silk material properties**

Regardless of why spiders respond to different prey by altering the material properties of silk, the physiological mechanisms by which those changes occur are poorly understood. We examine three potential hypotheses.

a) *Modification of the amino acid composition of silk*

Variation in amino acid sequence confers different properties to spider silk by affecting the structural conformation of silk proteins (Hayashi & Lewis, 1998; Hinman et al., 2000). In particular, proline gives more extensibility to the silk while alanine provides strength and stiffness (Hayashi & Lewis, 2001; Hayashi et al., 1999).

![Fig. 2.6. Effect of increase in fiber yield stress on material properties.](image)

*Fig. 2.6. Effect of increase in fiber yield stress on material properties. The dotted line indicates a material with a higher yield stress compared with a second material of equivalent breaking point (solid line). This increase makes the material both stiffer (by increasing pre-yield slope as indicated by the curved arrow) and tougher (the extra gray area).*
Spiders could assemble the same silk from different proportions of amino acids when consuming varied prey, thereby affecting silk performance. Indeed, differences in spider's diet are reflected in changes in the amino acid composition of the silk (Craig et al., 2000; Tso et al., 2005) and this can be accompanied by differences in silk material properties (Tso et al., 2007). Thus, variation of silk material properties in *Achaearanea* could potentially be a response to amino acid differences between crickets and pillbugs, but this remains to be tested.

*b) Modification of the ratio of different proteins in silk*

Orbicularian dragline silk is a blend of two proteins named spidroin 1 and spidroin 2 (Ayoub et al., 2007; Gatesy et al., 2001). These proteins likely confer different material properties to dragline silk (Rising et al., 2005). Spidroin 1 is rich in alanine that is hypothesized to confer strength and stiffness to silk (Xu & Lewis, 1990). Spidroin 2 is rich in proline that is hypothesized to confer extensibility to silk (Hinman & Lewis, 1992). Therefore, spiders could alter the material properties of their silk by changing the ratio of these two proteins.

In our experiment, cricket-fed spiders may have produced silk richer in spidroin 1 thereby making it stiffer than pillbug-fed spiders’ silk. This could result directly from more amino acids that used in the synthesis of spidroin 1 being available when consuming crickets. Alternatively, it could result from cricket-fed spider incorporating relatively more spidroin 1 in their supporting threads silk in response to their heavier cricket prey.
c) Modification of spinning effects

Spider silk is stored inside the silk glands as a liquid dope. As the dope passes through the spider's spinning duct, it increases in crystal orientation and solidifies under the influence of several physico-chemical processes, including the application of shear forces that align the protein molecules along the axis of the fiber (Knight & Vollrath, 1999). If spiders are able to control this process, they could manipulate the physical properties of their silk. In particular, spiders uses a sphincter muscle to apply force to the silk as it is extruded, altering its material properties by affecting its molecular orientation (Ortlepp & Gosline, 2004). This provides a potential mechanism for cricket-fed spiders to spin stiffer silk in our experiment without necessitating a change in amino acid composition or protein expression.

Conclusion

We found that spiders modified silk in response to different prey and to changes in their internal condition. Spider body mass and condition affected mechanical and structural properties of whole threads while body mass and prey type induced changes in the silk material itself. Spiders fed large, fast prey spun thicker silk able to resist higher forces. The silk from their supporting threads was also stiffer and tougher. These modifications could function to allow the web to support the weight of heavy prey or spiders without deforming permanently or to stop prey with higher kinetic energy. However, because of a confounding effect of spider condition, this variation may also result from potential metabolic costs of spinning stiffer and tougher silk. We currently cannot separate these two hypotheses, but future experiments could better manipulate the
physical interaction of different prey with webs while maintaining spider condition or nutritional status. Thus, this system provides an opportunity to explore why and how spiders tailor the performance of silk to different ecological conditions. Understanding the mechanisms of variation of silk material properties will also help develop processes that replicate the extraordinary performance of spider silk in synthetic fibers.

Acknowledgments

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CHAPTER III
BIOMECHANICAL VARIATION OF SILK LINKS SPINNING PLASTICITY TO
SPIDER WEB FUNCTION

Introduction

Spider silk is an outstanding material. Silk can be almost as strong as steel but also as much as thirty times more extensible (Gosline et al., 1986). The combination of strength and extensibility allows spider silk to absorb more kinetic energy without breaking than most known materials. Scientists are therefore trying to understand how silk achieves these properties in order to replicate them while spiders depend upon them for survival. Spiders can spin silks from up to seven different types of glands and each type of silk possesses unique material properties (Blackledge and Hayashi, 2006). However, silk spun from the same gland can also exhibit extreme variability, even at the intra-individual level (Madsen et al., 1999). Both the potential adaptive value and the mechanisms causing this variation are unclear (but see Boutry & Blackledge, 2008; Tso et al., 2007). Physiological mechanisms by which spiders might control silk variation include modulation of the forces applied on the silk and changes in pH, water and ion content within the spinning duct as well as variation of the ratio of the different proteins composing silk. Understanding how spiders control silk properties through spinning is critical for industrial production of silk. Moreover, understanding how spiders control
silk properties for different uses also provides insight into how evolutionary forces have shaped silk performance.

Spiders modify their silk when spinning under a variety of different conditions. For instance, producing silk that can bear higher loads (improved mechanical performance) may allow webs to sustain the spiders’ own weight better as they capture larger prey (Boutry & Blackledge, 2008). Spiders can improve the load bearing capacity of silk by increasing either ultimate strength (an intrinsic material property) or thread diameter (a structural property). For example, silk spun by spiders housed in taller cages exhibits greater tensile strength than silk spun by spiders housed in shorter cages (Pan et al., 2004). Silk spun by spiders climbing vertical walls is thicker than silk spun by spiders walking on horizontal surfaces, again allowing the threads to sustain higher loads (Garrido et al., 2002b). However, most of these studies focus on silk spun as a safety line or as a way to mark paths, rather than on silk used to construct webs. Even though webs are critical for survival of many spiders, the potential for spiders to alter the mechanical properties of silk while spinning webs remains poorly investigated.

Cobwebs are three-dimensional networks of silk composed of several architecturally and functionally distinct regions (Eberhard et al., 2008). The common house spider *Achaearanea tepidariorum* (Araneae: Theridiidae) spins a cobweb consisting of a retreat suspended within a series of tangled supporting threads from which project near-vertical sticky gumfooted threads (Benjamin & Zschokke, 2003). The spider rests and consumes prey within the supporting threads. Therefore, supporting threads must be able to bear the weight of both the spider and prey (Boutry & Blackledge, 2008). In contrast, sticky gumfooted threads are gluey along their lower 1-2 cm and are used solely in prey capture.
Prey stick to the glue and are held by the threads, which resist the prey’s struggles. Sticky gumfooted threads function only once because they detach from the substrate when contacted by prey. In contrast, supporting threads remain in the web through many days and must maintain their function nearly continuously. Both supporting threads and sticky gumfooted threads are likely spun using the same type of dragline silk. Evidence for their homology comes from previous studies showing similarities in morphology (Benjamin & Zschokke, 2002) and mechanical performance (Blackledge et al., 2005b; Blackledge et al., 2005c). Thus, any differences in performance related to the structures’ divergent functions should result from spiders actively modifying the silk while it is spun.

We tested whether the mechanical performance, in terms of both structural and material properties, of *Achaearanea* silk differed between supporting threads and sticky gumfooted threads. We expected supporting threads to break at higher loads than sticky gumfooted threads, allowing them to resist the weights of both spiders and prey. In contrast, we predicted that sticky gumfooted threads would absorb more energy before breaking, allowing them to better stop moving prey. We also expected supporting threads to resist yielding (*i.e.* permanently deforming) better than sticky gumfooted threads because supporting threads are longer-lasting and function repeatedly in webs.

**Material and methods**

**Spiders**

Adult *A. tepidariorum* (*n* = 26) were collected at the University of Akron’s Bath Field Station (Bath, OH) and surrounding homes (Akron, OH). Spiders were housed in 38 x 20 x 23 cm clear plastic cages (Kritter Keepers®, San Marcos, CA), with cardboard frames to support webs (Fig. 2.1). The frames consisted of two 18 x 20 cm cardboard sheets, on
top and bottom, joined by 34 cm high wooden sticks (two on back and one on front). This design allowed the spiders to spin webs between the top and bottom cardboard sheets, while assuring easy access to silk. Spiders were housed at a constant temperature of 24°C under a 15 : 9 light / dark cycle. The spiders were fed one cricket (purchased from Fluker's Farm) every two days.

*Silk collection*

Samples of silk were collected from two-day old webs. Three samples of sticky gumfooted threads and three samples of the uppermost supporting threads were collected for each web. Silk was secured on both ends of a 10.7 mm hole in cardboard mounts, using cyanoacrylate glue, and then cut free from the web with a hot soldering iron. Only the dry upper regions of sticky gumfooted threads were collected because the glue droplets alter mechanical performance of the silk (Blackledge *et al.*, 2005b). Supporting threads were collected from the top 1-5 cm of the web.

*Diameter measurements*

Polarized light microscopy was used to take two digital photographs of each sample at 1000X. The diameter of each strand in a sample was measured three times from each photograph using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2007). The multiple measurements accounted for the slight shape anisotropy of silk so that cross-sectional area could be approximated by a single average diameter (Blackledge *et al.*, 2005a). From these measurements, both the total cross-sectional area of the silk sample and the average diameter of single strands within the sample were calculated.
Mechanical and material properties of the silk

The mechanical performance of silk threads was then tested on a Nano Bionix UTM (MTS Corp.) using established protocols (Blackledge et al., 2005c). All fibers were extended at 1% strain/sec and the resulting force values measured. We recorded raw failure load, which represents the force resisted by the thread upon breaking without correction for thread diameter. Failure load thus gave us information about the capacity of a thread to support forces, which directly determines how threads interact with prey and spiders.

We also measured the material properties of threads. The material properties describe the intrinsic quality of the silk spun by spiders, and interact with the structural aspects of threads (such as diameter and number of strands) to determine how threads interact with prey. We recorded six material properties for each sample (Fig. 2.2): Young's modulus, yield stress, yield strain, extensibility (breaking strain), ultimate strength (breaking stress) and toughness (breaking energy). Young's modulus measures the initial stiffness of the material. Silk with a higher Young's modulus better resists deformation under a given load. The yield point measures the transition of the material from elastic to plastic behavior. After yield, the material deformation is irreversible and its future mechanical performance is permanently altered. Yield stress and yield strain are, respectively, the true stress and true strain at yield. Similarly, ultimate strength and extensibility are the true stress and true strain at failure.

We used true stress and true strain, rather than engineering stress and engineering strain, because these are more reliable for very elastic materials such as spider silk (Blackledge et al., 2005c). True stress ($\sigma$) measures the force supported per area of thread
and is calculated as:

\[ \sigma = \frac{F}{A} \]

where \( F \) is the force exerted on the material, and \( A \) is the instantaneous cross sectional area of the silk fiber at time \( t \). The cross-sectional area at time \( t \) is computed by assuming a constant volume for the fiber (Vollrath et al., 2001).

True strain (\( \varepsilon \)) measures the relative extension of the fiber and is calculated as:

\[ \varepsilon = \ln \left( \frac{l}{l_0} \right) \]

where \( l \) is the instantaneous length of the fiber at time \( t \) and \( l_0 \) is the original length of the fiber. Toughness, or breaking energy, represents the total energy that can be absorbed by the silk before breaking. It is calculated as the area under the stress-strain curve.

**Statistics**

Mean single-strand diameter was compared between supporting threads and sticky gumfooted threads collected from the same web using a paired t-test (the data were normally distributed, Shapiro-Wilks tests, \( P = 0.3102 \) for supporting threads, \( P = 0.4727 \) for sticky gumfooted threads). Failure load was not normally distributed for sticky gumfooted threads because of a single outlier. After this outlier was removed, the data were normal (Shapiro-Wilks test, \( P = 0.1529 \) for supporting threads and \( P = 0.8867 \) for sticky gumfooted threads). Therefore, failure load was compared between the two regions of the web using a paired t-test, which controlled for the strong effect that spider size has on thread diameter (Osaki, 2003). Material properties were compared among supporting threads and sticky gumfooted threads using a MANOVA and post-hoc Tukey's HSD tests were used to identify specific differences.
Results

Supporting threads broke at higher loads than sticky gumfooted threads (mean ± SE, 8.25 ± 0.89 and 3.81 ± 0.21 mN respectively, t = -5.66, df = 24, P < 0.0001) (Fig. 3.1).

Supporting threads were also thicker than sticky gumfooted threads (single-strand diameter, mean ± SE, 1.885 ± 0.091 and 1.571 ± 0.059 μm respectively, t = 4.26, df = 25, P = 0.0003) (Fig. 3.2).

Material properties differed between supporting threads and sticky gumfooted threads (MANOVA, Wilk's Lambda = 0.0053, n = 24, 22, P = 0.0025). Post hoc Tukey’s HSD tests showed that sticky gumfooted threads were more elastic (mean ± SE, 0.4359 ± 0.0152 and 0.3498 ± 0.0106 mm/mm respectively, p < 0.0001), less stiff (Young's modulus, mean ± SE, 9.86 ± 0.37 and 11.41 ± 0.57 GPa respectively, p = 0.0239) and yielded more easily (yield stress, mean ± SE, 226 ± 9 and 280± 13 MPa respectively, p = 0.0013) than supporting threads (Fig. 3.3). In contrast, ultimate strength, toughness and yield strain did not differ between supporting threads and sticky gumfooted threads.
Discussion

We found that the mechanical performance of major ampullate silk varied in different regions of cobwebs spun by the spider *Achaearanea tepidariorum*. This implied that the spiders could actively modulate the properties of a single type of silk while spinning different regions of webs. We predicted that variation in silk performance should relate to
the different functions of each region of the cobweb. For instance, supporting threads resist the static weight of both spiders and subdued prey, and should therefore bear higher loads while sticky gumfooted threads function primarily to absorb kinetic energy of prey during capture, suggesting that they should be tougher. We found that supporting threads from *Achaearanea* cobwebs were stiffer and broke under higher loads than sticky gumfooted threads, but we did not find a difference in silk toughness between the two regions of cobwebs. Thus, spiders can control the properties of their silk in ways that fit web function.

Table 3.1 compares the mechanical performance of *Achaearanea* silk to cobweb silk of another theridiid, the western black widow *Latrodectus hesperus*. The silk of these two cobweb-weaving spiders is remarkably similar, even though the performance of major ampullate silk can vary among other species of spiders by more than 100% (Swanson *et al.*, 2006a). Moore and Tran (1999) report significantly lower values for Young’s modulus, extensibility and toughness, about half that of our own study or Blackledge *et al.*’s (2005b; 2005c). This may in part be attributed to differences in methods. Moore and

<table>
<thead>
<tr>
<th>silk</th>
<th>Young’s modulus (GPa)</th>
<th>ultimate strength (MPa)</th>
<th>extensibility (mm/mm)</th>
<th>toughness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sticky gumfooted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. tepidariorum</em></td>
<td>9.9</td>
<td>1569</td>
<td>0.44</td>
<td>271</td>
</tr>
<tr>
<td><em>L. hesperus</em></td>
<td>8.9</td>
<td>1316</td>
<td>0.39</td>
<td>231</td>
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<tr>
<td><em>L. hesperus</em></td>
<td>9.2</td>
<td>957</td>
<td>0.47</td>
<td>236</td>
</tr>
<tr>
<td>Supporting threads</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. tepidariorum</em></td>
<td>11.4</td>
<td>1638</td>
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<td>285</td>
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<tr>
<td><em>L. hesperus</em></td>
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<td>1100</td>
<td>0.22</td>
<td>136</td>
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<tr>
<td><em>L. hesperus</em></td>
<td>10.7</td>
<td>1069</td>
<td>0.42</td>
<td>238</td>
</tr>
</tbody>
</table>

Table 3.1: Comparison of silk material properties from different regions of the cobwebs of *Achaearanea tepidariorum* and the western black widow *Latrodectus hesperus*. All of these threads are produced from the same type of silk gland.
Tran assumed constant diameter instead of constant volume (as we did), which results in underestimated stress values for stretchy materials.

Sticky gumfooted threads and supporting threads exhibit different material properties, but we argue that both still consist of major ampullate silk because these differences are subtle compared to the divergence in material properties of silks spun from different glands. For instance, flagelliform silk extends to 1.50 times its original length (Swanson et al., 2007) while tubuliform silk’s strength is only 0.5 GPa (Blackledge & Hayashi, 2006), compared to cobweb silk’s extensibility of ~0.4 and strength of 1.5 GPa. Minor ampullate silk is closest in performance to major ampullate silk (Blackledge & Hayashi, 2006). However, minor ampullate threads are ~50% thinner, such that silk from these two different glands are easily distinguished. We observed minor ampullate silk in a few samples (7 out of 140), but the inclusion / exclusion of these samples in the statistical analysis did not affect our results. Sticky gumfooted threads and supporting threads are often spun nearly simultaneously (Benjamin & Zschokke, 2003). Thus, the differences we found in mechanical performance of sticky gumfooted threads and supporting threads resulted primarily from effects of how spiders assembled the silk threads rather than variation in chemical composition of the silk.

The higher failure load of supporting threads was not achieved through increased ultimate strength, an intrinsic material property of the silk, but rather through structural change in thread diameter. Structural changes, such as spinning thicker threads, are likely more costly to spiders because they require more silk proteins to spin equivalent lengths of threads. On the other hand, altering materials properties could potentially adjust silk’s performance without increasing production costs. Thus, it is surprising that spiders favor
adjusting structural, rather than material, properties of silk. However, this pattern appears to be common. The orb-weaving spider *Nephipa clavata* increases the failure load of its dragline safety threads by enlarging thread diameter rather than improving tensile properties (Osaki, 1996; Osaki, 1999; Osaki, 2003). Furthermore, throughout the orb-weaving Araneidae, larger species of spiders spin threads that absorb more energy primarily because they are thicker, with only minimal variation in material properties of the silk (Craig, 1987a; Craig, 1987b). *Achaearanea tepidariorum* itself produces stronger silk when exposed to heavier prey by spinning thicker threads without changing tensile strength (Boutry & Blackledge, 2008). These studies suggest that spiders control the mechanical performance of silk primarily by altering structural properties, both at the intra- and inter-specific levels. Denny (1976) suggested that orb-webs use the minimum possible amount of silk necessary to function. This predicts that, in active web-spinners, there has been strong selection historically to maximize the material properties of silk.

Quite simply, there may be few physiological mechanisms left by which spiders can further increase silk tensile strength, thereby explaining why structural properties are commonly so much more variable.

Supporting threads are not removed or destroyed during prey capture, and therefore must remain functional through several capture events. In contrast, sticky gumfooted threads detach from the substrate when adhering to prey, and are thus used only once. Therefore, in contrast to sticky gumfooted threads, supporting threads must support the weight of both spider and prey without deforming permanently. This may explain why the stiffness and yield stress are higher for supporting threads than sticky gumfooted threads. Measurements on western black widow cobweb show that supporting threads do
not pass their yield point during prey capture, contrary to sticky gumfooted threads (Argintean et al., 2006).

As predicted, sticky gumfooted threads are less stiff and more elastic than supporting threads, but not tougher. This pattern is actually not restricted to Achaearanea. Another theridiid, the western black widow, also spins sticky gumfooted threads that are less stiff and more extensible, but equally tough as supporting threads (Blackledge et al., 2005c). This suggests that this pattern may result from selective forces or constraints. In particular, toughness directly depends on extensibility. Yet, more extensible silk sticky gumfooted threads are not tougher, a quality that we predicted to be important for stopping moving prey. Is there any advantage to spinning flexible, elastic sticky gumfooted threads other than increasing toughness? Could increased extensibility itself have a functional advantage? We suggest that extensibility plays an important role in determining the forces that sticky gumfooted threads and supporting threads experience during prey capture. Figure 3.4 illustrates a simple model force exerted by prey on sticky gumfooted threads and supporting threads.

As the crawling prey pulls on the sticky gumfooted thread, it exerts a roughly horizontal force, $F$. This force creates a reaction force, $R_g$, in the sticky gumfooted thread. $R_g$ depends on the angle $\alpha_g$ between the sticky gumfooted thread and the horizontal plane on which the insect is crawling:

$$R_g = \frac{F}{\cos(180 - \alpha_g)}$$

$\alpha_g$ can vary between 90° and 180°. $R_g$ is greatest at $\alpha_g = 90°$ while at $\alpha_g = 180°$, $R_g$ reaches its minimum and is equal to $F$ (Fig. 3.5.A). More compliant silk allows the thread
Fig. 3.4. Model of force transmission in the cobweb. F (in red) is the force exerted by the prey pulling on the gumfooted thread. F creates a reaction force which is projected on the sticky gumfooted thread (Rg, in blue). The resulting tension generates additional reaction forces that are projected on the supporting threads (Rs1 and Rs2, in green). The values of Rs1 and Rs2 depend on the angles between the threads, $\alpha_g$, $\alpha_s1$ and $\alpha_s2$. More compliant sticky gumfooted threads increase $\alpha_g$ and ultimately result in less force on the supporting threads.

Fig. 3.5. Effect of angles on the forces experienced by the sticky gumfooted threads and supporting threads: (A) Change in the reaction force in the gumfooted thread, Rg, in function of angle $\alpha_g$. The force F exerted by the prey is indicated by a thin horizontal line. (B) Change in the reaction forces in the supporting threads, Rs1, Rs2 and total Rs (Rs1+Rs2), in function of angle $\alpha_s1$. The tension in the gumfooted thread, Rg, is indicated by a thin horizontal line. The reaction force in the gumfooted thread (Rg) and the total reaction force in the supporting threads (total Rs) decrease as $\alpha_g$ and $\alpha_s1$ increase, i.e. as the gumfooted thread aligns with the prey direction, and as one of the supporting threads aligns with the gumfooted thread.

to extend and the angle $\alpha_g$ to reach 180° more easily, thereby reducing the reaction force in the sticky gumfooted thread (Fig. 3.6).

The tension in the sticky gumfooted thread is proportional to Rg, and is opposed by reaction forces in each of the two supporting threads attached to the sticky gumfooted thread (R$s1$ and R$s2$). R$s1$ and R$s2$ depend on the angle between the sticky gumfooted thread and each supporting thread:
Fig 3.6. Example of forces transmitted in the web for different silk compliances. In both cases, the prey applies a force $F$ of 5mN, which displaces the gumfooted thread from its original position (dashed grey line). In (A), the silk is very compliant and the gumfooted thread displaces more, so that the angle $\alpha_g = 120^\circ$, while in (B), the silk is stiffer and $\alpha_g = 100^\circ$.

$$R_{s1} = \frac{R_g \sin(180 - \alpha_{s2})}{\sin(\alpha_{s1} + \alpha_{s2} - 180)}$$

and

$$R_{s2} = \frac{R_g \sin(180 - \alpha_{s1})}{\sin(\alpha_{s1} + \alpha_{s2} - 180)}$$

Figure 3.5.B shows how $R_{s1}$ and $R_{s2}$ vary as a function of $\alpha_{s1}$ (up to a maximum of $180^\circ$). We assume that the angle $\beta$ between the two supporting threads remains constant, and therefore, $\alpha_{s2}$ is negatively correlated with $\alpha_{s1}$. As $\alpha_{s1}$ increases, $R_{s1}$ increases while $R_{s2}$ decreases. The sum of $R_{s1}$ and $R_{s2}$ (total $R_s$) reaches its minimum when $\alpha_{s1} = 180^\circ$, $R_{s1} = F$ and $R_{s2} = 0$. This occurs when the sticky gumfooted thread is aligned with one of the supporting threads, which is facilitated by reduced silk stiffness. In reality though, $\beta$ does not remain constant. Instead, the angle decreases as the prey pulls on the sticky
gumfooted thread. Total $R_s$ decreases as $\beta$ decreases. Thus, if a sticky gumfooted thread stretches further under a given force from prey then the total force supported by the sticky gumfooted thread decreases both because $\beta$ is reduced and because $\alpha_{s1}$ tends to $180^\circ$.

More compliant silk allows $\alpha_{s1}$ to approach $180^\circ$ and $\beta$ to decrease under lower forces, thereby reducing the total reaction force in the supporting threads. To conclude, the more the sticky gumfooted thread silk extends under a given force generated by prey, the lower the tensions in the sticky gumfooted thread and supporting threads. Thus, compliant sticky gumfooted thread silk may allow the cobweb to resist higher forces exerted by stronger, heavier prey.

Different species of cobweb spiders spin sticky gumfooted threads of greatly varying lengths (Eberhard et al., 2008). Assuming identical silk stiffness, longer sticky gumfooted threads allow the angle $\alpha_g$ of our model to increase, when a prey stuck to the thread crawls the same distance. In this case, the forces supported by the sticky gumfooted threads and supporting threads ($R_g$ and $R_s$) will be smaller, which should allow the web to resist stronger, heavier prey. Other factors come into play, though. For instance, webs with longer sticky gumfooted threads may not be able to catch heavier prey if the silk is weaker or if the angle between the two supporting threads ($\beta$ in our model) is closer to $180^\circ$. The combination of these factors may explain the large differences in web architecture among species.

Insects typically escape quickly from webs, before they are attacked by spiders (Rypstra, 1982). In orb-webs, even a small increase in the number of sticky threads contacted by prey can make escape significantly less likely (Blackledge & Zevenbergen, 2006). The high extensibility of sticky gumfooted threads may facilitate prey movement
within webs, allowing them to contact multiple sticky gumfooted threads, causing the prey to become even more entrapped. Longer sticky gumfooted threads will allow the prey to move further, which would increase the probability that it gets more entangled in the web. Once again, other factors are involved, for example sticky gumfooted thread density. Thus, spiders may be able to manipulate the retention times of prey in their webs by altering these architectural properties, much as orb-weaving spiders can by altering the spacing between sticky spirals.

We argue that spiders may have limited mechanisms for improving silk tensile strength due to potentially strong selective pressures on this property in the past. However, other material properties (Young’s modulus, extensibility and yield stress) are variable at the intra-individual level. While it seems likely that high tensile strength is always desirable for silk performance, these other properties may in fact experience selection for both “increased” and “decreased” performance, depending on the function of the silk. For instance, we previously showed how compliant sticky gumfooted threads may allow the web to bear higher forces from prey. Yet, high compliance in supporting threads may result in a web that deforms easily.

We found strong support for intra-individual variation in silk properties, where spiders consistently varied the structural and material properties of their silk when spinning different regions of the same web. While other studies documented intra-individual variation in the dragline silk of spiders spun across several days (Madsen et al., 1999), we examined silk from two day-old webs. This means that spiders could vary the material properties of their silk in the very short time interval that it took them to spin different regions of the web (e.g. within seconds, Szlep, 1965). This argues persuasively
that the intra-individual variation we found is not due to changes in internal body condition or amino acid intake, as suggested in previous studies (see Tso et al., 2007 for a discussion of how prey intake relates to changes in silk amino acid composition and material properties). Instead, Achaearanea seems to control silk material properties during web spinning using nearly instantaneous physiological mechanisms, such as modulating the pressure applied in the spinning duct and “internal drawdown” mechanism (Ortlepp & Gosline, 2004; Perez-Rigueiro et al., 2005; Vollrath & Knight, 2001). Learning how spiders control silk properties could allow material scientists and polymer scientists to replicate those mechanisms to fine-tune silk production, broadening the range of potential applications for synthetic silk. Moreover, it is clearly essential for understanding the function of the rich diversity of different types of webs spun by spiders.

Conclusion:

Sticky gumfooted threads and supporting threads from Achaearanea tepidariorum's cobweb exhibit different mechanical, structural and material properties even though they are spun within the same web and are produced from the same major ampullate silk gland. For example, the extensibility of major ampullate silk can vary by 20% within an individual cobweb. Supporting threads bear higher loads before failure and resist higher stresses without deforming permanently. This allows the supporting threads of cobwebs to resist loading by spiders and prey while maintaining the silk’s performance through time. The spiders increase failure and yield load by spinning thicker threads, rather than improving tensile strength, suggesting that spiders may have relatively few physiological mechanisms by which to alter at least some material properties of silk. In contrast,
stiffness and extensibility are more variable within webs. Sticky gumfooted threads are more compliant than supporting threads. This may decrease the force that sticky gumfooted threads transmit to supporting threads during prey capture and facilitate prey entangling themselves further in the cobweb. Our study shows that material properties of spider silk can vary at the intra-individual level, in accordance to previous research, but it also demonstrates that this occurs in the very short time interval over which spiders spin webs. More important, we argue that the variation in the mechanical properties of *A. tepidariorum* major ampullate silk relates to its different functions in varied regions of the cobweb. Thus, spiders can control silk production during web-spinning in ways that may enhance web performance.

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CHAPTER IV
SILK PLASTICITY WITHIN ORB-WEBS AND COBWEBS

Introduction

As stated in the previous chapters, spider major ampullate silk exhibits non-negligible intra-individual variability both in terms of silk intrinsic material properties (Boutry & Blackledge, 2008; Pan et al., 2004; Madsen et al., 1999) and in terms of overall load that threads can resist (Garrido et al., 2002b). Our own previous research found that silk's mechanical (load that can be supported by threads) and material properties (intrinsic quality of the silk) differed among regions of the cobweb of Achaearanea tepidariorum (Theridiidae) (Chapter III).

Cobwebs are composed of two main regions in A. tepidariorum: a tangle of supporting threads that maintain web shape and support a series of vertical sticky gumfooted threads that directly adhere to prey. All parts of the web are likely made of the same silk: major ampullate silk. Sticky gumfooted threads function by “popping up” once they are hit by a prey and can only be used once. Therefore, we hypothesized that these threads must be able to absorb energy better than supporting threads, possibly through high compliance and extensibility. Since they are used only once, they do not need to keep their integrity through repeated capture bouts, and may thus have a low yield point (point at which threads are permanently deformed). In contrast, supporting threads must have higher yield, since they stay functional for weeks. They also must be stiff in order to
keep the web's shape, and strong to support the weight of the spider and its prey.

Supporting threads were able to resist higher loads than gumfooted threads, and did not deform as easily. In contrast, sticky gumfooted threads were more compliant and extensible, which could facilitate their “pop up” behavior during prey capture.

Orb-webs are also composed of different regions made of major ampullate silk. Among them, the frame and mooring guys support the web and maintain its shape. Thus, their function is close to that of the supporting threads from cobwebs. In contrast, radii are attached to the sticky spiral that adheres to the prey, and they deform along with the spiral during prey capture. The radii are elements of the orb-web directly involved in prey capture, and can thus be compared to the sticky gumfooted threads from cobwebs.

Therefore, we can make similar predictions for the elements of the orb-webs as the ones we made for cobwebs. Frame threads and mooring guys should be stiff to maintain the integrity of the web, and strong to resist the pressures applied on them. Radii should be compliant to deform during prey capture and be able to absorb the energy of prey.

We tested this idea by measuring and comparing the mechanical and material properties of silk samples from radii and frame threads of orb-webs of the bridge spider, as well as silk from sticky gumfooted threads and supporting threads from the cobweb of the western black widow. We compared our results to what we found on the common house spider, *Achaearanea tepidariorum*'s cobweb (see Chapter III).

**Material and Methods**

We obtained western black widows, *Latrodectus hesperus* (Theridiidae), and bridge spiders, *Larinioides sclopetarius* (Araneidae) from Riverside, CA and Akron, OH,
respectively. The spiders were kept in the lab in cages appropriate to their web type, at 24°C under a 15:9 hr light/dark cycle. Black widows were kept in cardboard 17 x 15 x 29 cm frames, composed of a “retreat” portion (an open box ~ 6 cm high) attached to two 23 x 15 platforms, one on top and one on bottom, held apart by a vertical 17cm high balsa stick attached at the distal edge of each platform. The frames were placed inside 20 x 20 x 30 cm plastic terrariums (KritterKeepers, Medium size). The widow stayed in the retreat portion and built a cobweb from there. The open area that extends from the retreat gave us easy access to the web for silk collection. The bridge spiders were kept in 11.5 x 40 x 40 cm cages made of a mesh frame closed by two removable 40 x 40 cm plexiglass sheets. The spider built an orb-web attached to the mesh frame. The removable plexiglass sides ensured easy access to the web for silk collection (Fig. 4.1).

We collected four silk samples each from sticky gumfooted threads and supporting threads for black widows, and from radii and frame threads for bridge spiders. Cobwebs last many days, and black widows add new elements regularly. In contrast, orb-webs are destroyed and rebuilt every day. Therefore, we used two-day old webs for black widows and one-day old webs for bridge spiders. We collected the samples on cut-out cards with a 15.33 mm gap. We glued the threads on each side of the gap with cyanoacrylate glue and cut them free from the web with a soldering iron. Only the dry portions of the sticky gumfooted threads were collected. Most samples were double-stranded, although a few support threads and frame threads were single-stranded.
We calculated the diameter of each silk sample as detailed in Chapter III (Methods, Diameter measurements). We measured the mechanical and material properties of the threads as detailed in Chapter III (Methods, Mechanical and material properties of the silk).

We considered supporting threads from cobwebs and frame threads from orb-webs to be “support” elements while sticky gumfooted threads from cobwebs and radii from orb-webs were “capture” elements. We compared the mechanical performance and average single-strand diameter of support and capture threads using a paired t-test (all samples were normal). We compared the material properties of support and capture elements using a one-way MANOVA.

Fig. 4.1. Webs and cages of *Latrodectus hesperus* (A) and *Larinioides sclopetarius* (B). In (A), the plastic cage enclosing the frame is not depicted. *Latrodectus* cobweb is similar to *Achaearanea*’s (Fig. 2.1) except for the presence of a "sheet" of silk between the support threads and gumfooted threads. (B): *Larinioides* orb-web is composed of a capture area of sticky silk (in blue) enclosed by a series of dry, frame threads (in red). The capture area is held by dry radii that join at the center of the web and deform with the capture area during prey capture (in red).
Results

For *L. sclopetarius*, support elements resisted higher loads and were thicker than capture elements (failure load (mN), mean ± SE, 13 ± 3 for support threads and 9 ± 1 for capture threads, paired t-test, *P* = 0.0387; single strand diameter (um), mean ± SE, 2.175 ± 0.214 for support threads and 1.952 ± 0.148 for capture threads, paired t-test, *P* = 0.0465, *n*=11). This was not the case for *L. hesperus* (failure load (mN), mean ± SE, 15 ± 1 for support threads and 13 ± 2 for capture threads, paired t-test, *P* = 0.1573; single strand diameter (um), mean ± SE, 2.305 ± 0.114 for support threads and 2.155 ± 0.196 for capture threads, paired t-test, *P* = 0.0990, *n*=8) (Fig. 4.2). The single-strand diameter of support elements was also larger than that of capture threads (single-strand diameter (um), mean ± SE, 2.229 ± 0.128 for support threads and 2.037 ± 0.115 for capture threads, paired t-test, *P* = 0.0287, *n*=19) (Fig. 4.3). When only *L. sclopetarius* was considered, capture elements yielded at a slightly higher strain than support elements (yield strain (mm/mm), mean ± SE, 0.0408 ± 0.0054 for capture threads and 0.0298 ± 0.0010 for support threads, MANOVA, Wilk's lambda, *P* = 0.0584, *n*=19, Tukey's post-

![Fig. 4.2. Failure load of silk from different web elements. Bars represent mean ± SE. Support elements are in dark grey and capture elements in white.](image1)

![Fig. 4.3. Thread diameter of different web elements. Bars represent mean ± SE. Support elements are in dark grey and capture elements in white.](image2)
hoc HSD, P < 0.1). There was no difference in material properties of silk from different regions of *L. hesperus'* cobweb (MANOVA, Wilk's lambda, P = 0.2674, n=15).

**Discussion**

Both orb-weaving and cobweb-weaving species studied spun support elements with higher load-bearing capacities than capture elements. However, especially for the cobweb-weaver *L. hesperus*, this increased ability to bear high loads resulted from changes in the structure of the thread (diameter) rather than changes in the material properties of the silk. This suggests that spiders cannot modulate silk material properties much. This lack of plasticity could result from a strong past selection on silk material properties.

Previous research from our lab had found that the cobweb-weaver *Achaearanea tepidariorum* spun support threads with higher failure load than capture threads, due to thicker diameter. The support threads were also stiffer, less extensible and with a higher yield point than capture threads. Although one of the two species investigated in this study (*L. sclopetarius*) had support threads with a slightly higher yield point than capture threads, the other differences in material properties found in *A. tepidariorum* were not found in either species. *A. tepidariorum'*s silk may be more plastic than other species, but since *Achaearanea* and *Latrodectus* are closely related, this is a surprising finding.

Differences in silk plasticity may be related to supercontraction ability and silk proline content according to Guinea *et al.*'s tailoring hypothetical function of supercontraction (see Chapter VI for a more detailed explanation). Briefly, higher supercontraction ability may allow spiders to modulate their silk material properties.
better (Guinea et al., 2005a). This higher supercontraction ability may result from a higher proportion of proline, an amino acid involved in motifs forming beta-turns, in the silk (Liu et al., 2008a; Liu et al., 2008b). L. sclopetarius presented slightly higher plasticity in web silk material properties compared to L. hesperus. The exact proline content of L. sclopetarius silk is unknown. However, orb-weaving spiders from the family Araneidae, which L. sclopetarius belongs to, tend to have ~10% proline in their silk (Liu et al., 2008b) (range: 8.8% to 14.3%). In contrast, L. hesperus silk contains ~2% proline. The fact that L. sclopetarius silk is more plastic and higher in proline than L. hesperus silk agrees with Guinea et al.'s tailoring hypothesis. Proline content of A. tepidariorum silk, which is more plastic than either L. hesperus or L. sclopetarius silk, is unknown. However, A. tepidariorum supercontracts three times as much as L. hesperus, suggesting that A. tepidariorum's silk contains more proline than L. hesperus's (see Chapter VI).

Radii in orb-webs (capture elements) dissipate prey kinetic energy through plastic deformation (Gosline et al., 1986; Ko & Jovicic, 2004). Therefore, for radii to absorb prey energy, they have to be fairly compliant and easily pass the yield point. In contrast, frame threads (support elements) have to be stiff enough to hold the web in shape and resist forces due to prey impact. Hence, by spinning radii that have a lower yield than frame threads, L. sclopetarius may improve energy dissipation by the web.

Unlike cobwebs though, orb-webs are spun anew every day. Therefore, even if radii pass their yield point and lose some of their functionality, new radii will be spun the next day. In this respect, radii are somewhat similar to sticky gumfooted threads from cobwebs, which function only once (see Chapter III). However, frame threads are also
renewed every day. For *A. tepidariorum*, it is important that support threads maintain their function over time, hence they should have a high enough yield that they do not deform permanently under the weight of the spider and prey. For *L. sclopetarius*, it may be less critical for frame threads to maintain their function through time since they are renewed each day. Therefore, the spider may not need to spin threads with a very high yield. The fact that orb-webs last only one day while cobwebs last for weeks may explain why there are fewer differences in material properties of radii and frame threads compared to sticky gumfooted threads and support threads of *A. tepidariorum*.

It is surprising that supporting threads and sticky gumfooted threads do not differ in material properties in cobwebs of *L. hesperus*, unlike cobwebs of *A. tepidariorum*, as the two species are closely related. Furthermore, previous research had found differences in the material properties of sticky gumfooted threads and supporting threads of *L. hesperus* (Blackledge et al., 2005c). It is possible that the prey offered to *Latrodectus* were too small for her to increase the yield of its support threads. We previously demonstrated that prey type can influence silk properties, with spiders producing stronger support threads that do not deform easily when given heavy prey (see Chapter II). If the prey offered was too small compared to the spider, *L. hesperus* may not have increase the stiffness and yield point of its support threads. Because of this, there may have been no differences in the material properties of support threads and sticky gumfooted threads.

It is striking that *L. sclopetarius* also increases the diameter of its frame threads compared to its radii. If Guinea *et al.*'s hypothesis is correct, *Larinioides*, with their high silk supercontraction ability, should have high silk plasticity. Therefore, it would be possible for them to increase silk strength or stiffness in order to increase the load the
thread can bear. A change in material properties should also be less costly than a change in thread diameter.

Conclusion

The differences in material properties of silk forming different regions of the cobweb of *A. tepidariorum* were not found in the cobweb of *L. hesperus*, and barely in the orb-web of *L. sclopetarius*. However, *L. sclopetarius* spun support threads that were thicker and able to resist higher loads than their capture threads. Support threads that can bear higher loads may allow the orb-web to keep its shape and improve its performance. However, since this increase in mechanical performance is achieved through changes in thread structure (thicker threads), material properties of *Larinioides' silk may have limited variability.
CHAPTER V
PLASTICITY IN MAJOR AMPULLATE SILK PRODUCTION
INCREASED DURING SPIDER EVOLUTION

Introduction

Spider silk is a biopolymer with strong biomimetic potential. It is also central to many aspects of spider ecology, from communication to prey capture. Because silk is so important to spiders, it has probably been subjected to strong selective pressures during the ~400 million years of spider evolution. Although modern spiders spin up to seven different types of silk (Blackledge & Hayashi, 2006; Hinman et al., 2000), most research focuses on major ampullate silk, particularly major ampullate silk forcibly obtained (i.e. manually reeled by a human experimenter from an immobilized spider under controlled conditions). However, this method neglects the potential importance of variation in major ampullate silk properties when spun under different conditions, especially intra-specific differences. Structural properties (e.g. diameters of silk threads) and mechanical performance (failure load) depend upon spinning conditions (Garrido et al., 2002b). Moreover, material properties (i.e. the intrinsic qualities of silk) also vary at the inter- and intra-individual levels (Madsen et al., 1999; Boutry & Blackledge, 2009). Such differences may result in part from variation in amino acid intake (Zax et al., 2004) or enhance web performance in prey capture (Boutry & Blackledge, 2008; Tso et al., 2005). A clear understanding of silk variability and its mechanisms in a phylogenetic context
would indicate how spider ecology has shaped the evolution of silk production and suggest the range of properties that might be achieved in synthetic analogs of spider silk.

Several main mechanisms are proposed to explain how spiders might control silk properties: changes in the chemical composition of the liquid silk dope or variation in the external or internal conditions under which silk dopes are spun into fibers (see Boutry & Blackledge, 2008 for more details). In orb-weaving spiders, silk is spun from a dope composed of two proteins called MaSp1 and MaSp2 that differ in amino acid sequence (Xu & Lewis, 1990; Hinman & Lewis). The two proteins likely form different secondary structures (Hayashi et al., 1999; Liivak et al., 1997; Kümerlen et al., 1996; Rising et al., 2005; van Beek et al., 2002), which should result in different material properties: β-sheets formed by MaSp1 improve silk strength and stiffness while glycine-proline-glycine motifs present in MaSp2 provide elasticity (Hayashi et al., 1999; Guerette et al., 1996; Gosline et al., 1999; Gosline et al., 1986; Savage & Gosline, 2008a; Simmons et al., 1996). Thus, spiders could potentially control the strength and extensibility of major ampullate silk by varying the ratio of MaSp1 to MaSp2 in their glands.

The liquid silk dope stored in the ampulla of the gland then passes through the spinning duct before exiting as a solid fiber through the spigot. Various physical and biochemical processes transform the liquid dope into a solid fiber, in particular pH changes (Dicko et al., 2004b; Vollrath & Knight, 2001), ions re-absorption (Vollrath & Knight, 2001) and shear forces (Knight & Vollrath, 2001). Variation in any of these spinning effects could also modulate the material properties of the resulting silk, independently of protein composition.
External spinning conditions also influence silk properties. For instance, spinning speed influences molecular orientation within fibers (Eles & Michal, 2004a) and thereby material properties, with silk spun at higher rates being stiffer, stronger and less extensible (Chen et al., 2006; Vollrath et al., 2001). Friction forces applied when silk is forcibly collected may also increase silk molecular orientation (Vollrath & Knight, 2001) and thereby, silk stiffness (Perez-Rigueiro et al., 2005) and toughness (Vollrath & Knight, 2001).

Thus, we identified three mechanisms by which spiders can modulate silk properties: one biochemical mechanism, and two “spinning effects”. In terms of biochemical changes, alteration of the ratio of the two proteins, MaSp1 and MaSp2, that comprise dragline silk, may change silk properties. The first “spinning effect” correspond to changes in silk spinning speed, whether due to spider moving speed or to the speed at which a human experimenter reels dragline. The second “spinning effect” refers to variation in the amount of friction forces applied by the “brake” in the spinning duct.

However, these hypotheses focus on evolutionarily derived orb-weaving spiders (Orbiculariae), neglecting the rich evolutionary history of silk production within spiders. In this paper, we consider three major spider clades: haplogynes, RTA clade spiders and Orbiculariae. Haplogynes are among the most basal araneomorph (non-tarantula) spiders and include commonly recognized families such as pholcids (daddy-long-leg spiders), which spin aerial tangle webs. In contrast, entelegynes are far more diverse and abundant. Entelegyne diversity is dominated by two main evolutionary clades, RTA clade spiders and Orbiculariae (Blackledge et al., 2009b). Orbiculariae include all orb-weaving species (e.g. garden spiders, barn spiders, golden orb-weavers) as well as many species derived
from orb-weaving ancestors, such as the cobweb spinning Theridiidae (widow spiders and their relatives). Finally, the RTA clade includes many different families of spiders that have largely lost the use of silk during prey capture (e.g. lynx spiders, Oxyopidae and jumping spiders, Salticidae), but also some lineages with unique webs such as the terrestrial funnel-web weaving Agelenidae (Fig. 5.1).

Spiders utilize silk from their major ampullate glands to spin draglines in several ecological contexts. When spiders drop from high positions, they spin a safety line of silk, which prevents them from falling and allows them to climb back to their original position. We refer to it as dropping dragline (DDL). As they walk, spiders also lay a trail of silk, which is used, among other things, for intra-specific communication (Tietjen & Rovner, 1983; Pollard et al., 1987). We refer to it as walking dragline (WDL). A third type of spinning condition, and the most common type of collection for silk studies, is forcible silking. Forcibly obtained silk (which we will call forcible dragline, FDL) is gathered as a human experimenter pulls on silk from the spider's spinnerets.
We define silk plasticity as variation in the material properties of major ampullate silk spun by a single spider under different conditions. To use the terminology defined previously, silk plasticity represents how much the WDL, DDL and FDL spun by a single spider differs in terms of material properties (e.g. strength, stiffness, extensibility, etc.) Our current knowledge of silk plasticity in different spider clades is very limited. Previous studies on plasticity have focused on Orbiculariae only. Orbiculariae use major ampullate silk in many situations, for instance, in web-building, as a safety line when dropping and as a trail when walking. In contrast, many RTA clade spiders do not build webs, and many of them are terrestrial (so they do not use dropping dragline). Because Orbiculariae make more varied uses of major ampullate silk than RTA clade spiders, higher silk plasticity may be expected in Orbiculariae. However, this has not been tested.

Here, we compare the properties of silk spun under three different conditions (WDL, DDL and FDL) by six species belonging to three diverse evolutionary lineages of spiders (Fig. 5.1): three Orbiculariae species, the cobweb spiders *Achaearanea tepidariorum* (Theridiidae) and *Latrodectus hesperus* (Theridiidae) and the orb-weaving *Larinioides sclopetarius* (Araneidae); two RTA clade species, the Agelenidae *Hololena adnexa* and the Oxyopidae *Peucetia viridans*; and finally, one haplogyne species, the Pholcidae *Pholcus phalangioides*.

**Material and Methods**

**Spiders**

Five *P. viridans* were purchased from SpiderPharm (Yarnell, AZ). Nine *L. hesperus* were collected from Riverside (CA) and nine *H. adnexa* from Berkeley (CA). Four *P.*
*phalangioides* came from Akron (OH) and four more from Prague (Czech Republic). All other species (five *A. tepidariorum* and twelve *L. sclopetarius*) were collected from Akron and Bath (OH). All individuals were female, usually adult or subadult. The spiders were housed in the laboratory under a 15:9 light/dark cycle, at 24°C and fed one to two crickets a week.

**Silk collection**

We collected silk under three different conditions: FDL, DDL and WDL. These three spinning conditions correspond to different pressures applied on the silk and different spinning speeds, maximizing the potential to discover variation in material properties due to spinning effects. We collected four to five samples per individual for each spinning condition. The values of the material properties of these four to five samples were tested and then averaged for each individual within spinning condition.

To collect FDL, spiders were anesthetized with CO₂ and restrained on Petri dishes using tape. Silk was manually pulled from the major ampullate spigots at a speed of ~0.01 m/s and suspended across 15.3 mm gaps on cardboard mounts. The silk was secured to the mount using cyanoacrylate glue. The spider’s spinnerets were observed under a dissecting microscope to ensure that the silk collected was produced from the major ampullate spigots. Spiders were awake during silk collection.

To obtain WDL, spiders were placed in a tank lined with a piece of black cardboard folded into fluted 2.5 cm high ridges. The spiders then voluntarily laid dragline silk from ridge to ridge as they walked around the enclosure. The silk was collected on cardboard mounts across a 15.3 mm gap, secured with cyanoacrylate glue, and then cut free from the substrate using a hot soldering iron. All samples were composed of two strands of silk.
of equal diameter, which was indicative of them containing only major ampullate threads. Spiders sometimes lay minor ampullate silk along the major ampullate silk as they walk, but these threads are thinner and therefore easily identified.

To obtain DDL, spiders were placed on the border of a ~1 m high table and gently pushed off the edge after securing a dragline. Dropping spiders typically spun a silk safety line, but were unharmed in the rare instances when they did not. The silk was collected onto a “comb” made of a 75 cm-long strip of balsa wood with ~10 cm-long “teeth” of balsa glued perpendicularly to it and covered in double-sided tape. This device allowed us to collect many samples of silk, in sequential order, from a single safety line. Once again, silk was collected from the comb onto cardboard mounts across 15.3 mm gaps, secured with cyanoacrylate glue, and then cut free with a soldering iron. Silk may vary along the thread, because the velocity of falling spiders increases and how much they brake changes from the beginning to the end of the fall (Ortlepp & Gosline, 2004). Therefore, samples were collected at the beginning, middle and end of the 75 cm-long thread. *P. phalangioides* rarely produced safety lines for the entire 1 m drop. Instead, we pushed these spiders from a height of ~20-30 cm, and collected only one sample per fall.

**Tensile tests and material properties measurements**

Silk was placed under a polarized light microscope at 1000x magnification (Blackledge *et al.*, 2005a) and three pictures of each sample were taken using an Olympus® Q Color5 camera and ImagePro software (Media Cybernetics, Inc., Bethesda, MD). The diameter of each strand was measured three times for each picture using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland,
USA, http://rsb.info.nih.gov/ij/, 1997-2007). From the diameter of each strand, we calculated the total cross-sectional area to determine the stress during the tensile tests.

The tensile tests were run on a Nano Bionix (MTS Systems Corp., Oakridge, TN). True stress ($\sigma$) represents the force per area exerted on the sample and was calculated assuming constant volume (Guinea et al., 2006) as:

$$\sigma = \frac{F}{A}$$

where $F$ is the force exerted on the material, and $A$ is the instantaneous cross-sectional area of the silk fiber at time $t$.

True strain ($\varepsilon$) is the relative extension of the sample and was calculated as:

$$\varepsilon = \ln\left(\frac{l}{l_0}\right)$$

where $l$ is the instantaneous length of the fiber at time $t$ and $l_0$ is the original length of the fiber. True strain was used instead of engineering strain, since it gives more accurate results for viscoelastic materials (Blackledge et al., 2005c).

From the stress-strain curves obtained, six material properties were calculated:

- Young's modulus measures material stiffness and was calculated as the slope of the stress-strain curve in the elastic, pre-yield region. Stiffness represents how much the material extends if subjected to a given force (pull). Stiffness can have important consequences for silk function. For instance, if spiders spin silk while dropping, the less stiff the silk, the more the thread will extend under a given load.
- Yield stress and yield strain were calculated as the stress and strain at yield, respectively. Yield is the transition from elastic to plastic behavior, marking the point
after which the material deforms permanently. Thus, yield stress and yield strain represent how much force per area and extension the material can sustain before deforming permanently.

- Ultimate strength and extensibility were calculated as the stress and strain at failure, respectively. They represent how much force per area and extension the material can sustain before breaking.

- Toughness measures the amount of energy that can be absorbed by the silk before breaking and was calculated as the area under the stress-strain curve. Toughness typically increases with ultimate strength and extensibility. Toughness is critical for silk function because threads are often subjected to high energy impacts during prey capture or when acting as safety lines.

Statistics

We compared the actual average silk performance per spider between clades and spinning conditions using a full factorial design nested MANOVA with the six material properties as dependent variables and silk type (FDL, DDL or WDL), spider clade and species as covariates, with species nested within the clade - silk type interaction. The use of a nested MANOVA with species allowed us to identify possible differences in plasticity between species belonging to the same clade. The MANOVA also identified which properties varied with spinning conditions differently for the three clades. For the material properties whose plasticity differed among clades, we assessed how each property differed with spinning condition through ANOVAs. We ran an ANOVA for each material property and each spider clade (e.g. strength of Orbicularia silk), with the material property as the dependent variable and spinning condition as the independent
variable. This series of analyses identified which material properties differed between each pair of spinning conditions, and whether these differences were similar between orbicularian, RTA clade and haplogyne spiders. For this series of analyses, once again, we did not average per species and considered each spider and spinning condition as an observation. For instance, DDL from *Latrodectus* #6 is considered as an observation. For each material property, the values for the 4-5 samples of *Latrodectus* #6 dropping dragline were averaged.

**Results**

3.1. *Do silk properties vary with spinning conditions, independently of spider clade?*

Silk properties differed between FDL, DDL and WDL when the results from all species were pooled (MANOVA on material properties, effect of silk type, Wilk's lambda, P < 0.0001, n= 134). In particular, compared to WDL, FDL had higher yield stress (mean ± SE, 566 ± 59 for FDL and 350 ± 21 MPa for WDL, Tukey's HSD, P = 0.0028) and yield strain (mean ± SE, 0.038 ± 0.002 for FDL and 0.029 ± 0.001 mm/mm for WDL, Tukey's HSD, P = 0.0090) but lower extensibility (mean ± SE, 0.240 ± 0.014 for FDL and 0.310 ± 0.011 mm/mm for WDL, Tukey's HSD, P < 0.0001) and toughness (mean ± SE, 202 ± 15 for FDL and 238 ± 13 MPa for WDL, Tukey's HSD, P = 0.0022). DDL had intermediate properties: compared to FDL, it had lower yield strain (mean ± SE, 0.030 ± 0.001 for DDL and 0.038 ± 0.002 mm/mm for FDL, Tukey's HSD, P = 0.0102), and compared to WDL, it had lower extensibility (mean ± SE, 0.254 ± 0.008 for DDL and 0.310 ± 0.011 mm/mm for WDL, Tukey's HSD, P < 0.0001). Table 5.1 summarizes the average material properties of all three types of silk.
Table 5.1: Material properties of silk obtained under different spinning conditions from six spider species (average ± SE). Silk was collected forcibly (FDL), by letting a spider spin while dropping (DDL) and by letting a spider spin while walking (WDL).

<table>
<thead>
<tr>
<th>Material</th>
<th>Young's Modulus (GPa)</th>
<th>Yield Stress (MPa)</th>
<th>Yield Strain (mm/mm)</th>
<th>Ultimate Strength (MPa)</th>
<th>Extensibility (mm/mm)</th>
<th>Toughness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pholcus phalangioides</em></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FDL</td>
<td>10.5 ± 2.8</td>
<td>344 ± 67</td>
<td>0.036 ± 0.002</td>
<td>1107 ± 215</td>
<td>0.238 ± 0.022</td>
<td>144 ± 29</td>
</tr>
<tr>
<td>DDL</td>
<td>11.2 ± 1.0</td>
<td>354 ± 41</td>
<td>0.036 ± 0.003</td>
<td>1054 ± 102</td>
<td>0.199 ± 0.013</td>
<td>117 ± 19</td>
</tr>
<tr>
<td>WDL</td>
<td>6.4 ± 0.9</td>
<td>224 ± 25</td>
<td>0.039 ± 0.004</td>
<td>875 ± 60</td>
<td>0.260 ± 0.008</td>
<td>119 ± 8</td>
</tr>
<tr>
<td><em>Hololena adnexa</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FDL</td>
<td>10.4 ± 1.7</td>
<td>275 ± 67</td>
<td>0.037 ± 0.007</td>
<td>954 ± 133</td>
<td>0.223 ± 0.032</td>
<td>111 ± 23</td>
</tr>
<tr>
<td>DDL</td>
<td>18.9 ± 3.3</td>
<td>367 ± 87</td>
<td>0.026 ± 0.003</td>
<td>1439 ± 124</td>
<td>0.215 ± 0.012</td>
<td>198 ± 34</td>
</tr>
<tr>
<td>WDL</td>
<td>20.2 ± 2.1</td>
<td>428 ± 58</td>
<td>0.024 ± 0.003</td>
<td>1906 ± 102</td>
<td>0.245 ± 0.012</td>
<td>260 ± 20</td>
</tr>
<tr>
<td><em>Peucetia viridans</em></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>FDL</td>
<td>32.1 ± 1.1</td>
<td>1283 ± 105</td>
<td>0.043 ± 0.003</td>
<td>1738 ± 162</td>
<td>0.126 ± 0.027</td>
<td>157 ± 47</td>
</tr>
<tr>
<td>DDL</td>
<td>22.8 ± 2.8</td>
<td>516 ± 18</td>
<td>0.029 ± 0.001</td>
<td>1450 ± 153</td>
<td>0.218 ± 0.021</td>
<td>208 ± 25</td>
</tr>
<tr>
<td>WDL</td>
<td>18.9 ± 2.2</td>
<td>497 ± 56</td>
<td>0.030 ± 0.002</td>
<td>1268 ± 86</td>
<td>0.325 ± 0.018</td>
<td>283 ± 43</td>
</tr>
<tr>
<td><em>Achaearanea tepidariorum</em></td>
<td></td>
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</tr>
<tr>
<td>DDL</td>
<td>12.1 ± 0.3</td>
<td>314 ± 19</td>
<td>0.029 ± 0.001</td>
<td>1062 ± 144</td>
<td>0.308 ± 0.025</td>
<td>183 ± 42</td>
</tr>
<tr>
<td>WDL</td>
<td>9.3 ± 1.0</td>
<td>245 ± 35</td>
<td>0.030 ± 0.001</td>
<td>937 ± 144</td>
<td>0.380 ± 0.027</td>
<td>204 ± 25</td>
</tr>
<tr>
<td><em>Latrodectus hesperus</em></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>FDL</td>
<td>18.4 ± 0.9</td>
<td>518 ± 60</td>
<td>0.028 ± 0.001</td>
<td>1552 ± 156</td>
<td>0.340 ± 0.023</td>
<td>293 ± 27</td>
</tr>
<tr>
<td>DDL</td>
<td>23.0 ± 1.2</td>
<td>601 ± 33</td>
<td>0.030 ± 0.001</td>
<td>1667 ± 141</td>
<td>0.268 ± 0.019</td>
<td>284 ± 36</td>
</tr>
<tr>
<td>WDL</td>
<td>17.3 ± 1.2</td>
<td>449 ± 31</td>
<td>0.030 ± 0.002</td>
<td>1198 ± 144</td>
<td>0.353 ± 0.022</td>
<td>257 ± 18</td>
</tr>
<tr>
<td><em>Larinioides sclopetarius</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FDL</td>
<td>14.8 ± 1.0</td>
<td>516 ± 69</td>
<td>0.040 ± 0.005</td>
<td>1972 ± 65</td>
<td>0.207 ± 0.009</td>
<td>222 ± 13</td>
</tr>
<tr>
<td>DDL</td>
<td>17.3 ± 1.6</td>
<td>392 ± 29</td>
<td>0.029 ± 0.002</td>
<td>1768 ± 137</td>
<td>0.293 ± 0.011</td>
<td>278 ± 27</td>
</tr>
<tr>
<td>WDL</td>
<td>13.5 ± 0.8</td>
<td>319 ± 19</td>
<td>0.029 ± 0.001</td>
<td>1759 ± 102</td>
<td>0.346 ± 0.010</td>
<td>293 ± 20</td>
</tr>
<tr>
<td>All species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDL</td>
<td>16.9 ± 1.3</td>
<td>566 ± 59</td>
<td>0.038 ± 0.002</td>
<td>1555 ± 87</td>
<td>0.240 ± 0.014</td>
<td>202 ± 15</td>
</tr>
<tr>
<td>DDL</td>
<td>18.0 ± 1.0</td>
<td>433 ± 22</td>
<td>0.030 ± 0.001</td>
<td>1476 ± 67</td>
<td>0.254 ± 0.008</td>
<td>225 ± 15</td>
</tr>
<tr>
<td>WDL</td>
<td>14.0 ± 0.9</td>
<td>350 ± 21</td>
<td>0.029 ± 0.001</td>
<td>1370 ± 78</td>
<td>0.310 ± 0.011</td>
<td>238 ± 13</td>
</tr>
</tbody>
</table>
3.2 How do silk properties change with spinning conditions across different spider clades?

The three clades did not show the same silk plasticity (MANOVA, effect of the interaction of silk type and valve presence, Wilk's lambda, $P = 0.0355$, $n= 134$).

According to the nested MANOVA, different species from the same clade varied in their plasticity for Young's modulus, yield stress, strength, extensibility (MANOVA, effect of species, all $P < 0.0001$, $n= 134$) and toughness ($P = 0.0128$). However, despite variability within clades, silk plasticity still differed among the three clades (Orbiculariae, RTA clade and haplogyne) for strength (MANOVA, effect of the interaction of silk type and valve type, $P = 0.0052$, $n= 134$) and toughness ($P = 0.0014$).

Both RTA clade spiders and Orbiculariae showed a difference between FDL and WDL, but Orbiculariae had stronger FDL (mean $\pm$ SE, $1762 \pm 86$ for FDL versus $1499 \pm 95$ for DDL and $1298 \pm 97$ MPa for WDL, ANOVA, Wilk's lambda, $P = 0.0202$, $n= 74$) while RTA clade spiders had weaker FDL (mean $\pm$ SE, $1346 \pm 145$ for FDL versus $1444 \pm 88$ for DDL and $17586 \pm 113$ MPa for WDL, ANOVA, Wilk's lambda, $P = 0.0437$, $n= 39$) (Figure 5.2 and Table 5.1). Haplogynes showed no difference in strength between silk spun under different conditions (ANOVA, Wilk's lambda, $P= 0.3794$, $n= 21$). Toughness was not affected by spinning conditions for Orbiculariae (ANOVA, Wilk's lambda, $P = 0.8746$, $n= 74$) or haplogynes (ANOVA, Wilk's lambda, $P = 0.5364$, $n= 21$) but it differed strongly for RTA clade spiders, with both types of naturally spun silk being tougher than FDL (mean $\pm$ SE, $134 \pm 21$ for FDL versus $203 \pm 21$ for DDL and $272 \pm 19$ MPa for WDL, ANOVA, Wilk's lambda, $P < 0.0001$, $n= 39$) (Figure 5.2 and Table 5.1).
Discussion

Phylogenetic variation and mechanisms of silk plasticity

The material properties of spider silk we recorded (Table 5.1) are within the range of values reported in other studies (Lawrence et al., 2004; Shao & Vollrath, 1999; Swanson et al., 2006a), although *P. viridans* individuals consistently exhibit surprisingly high silk stiffness and yield stress.

Silk plasticity differed between the three clades. These differences between clades could be related to variation in silk biochemical composition and anatomy between haplogyne, RTA clade and orbicularian spiders.

The spinning duct of Orbiculariae includes a well-developed, muscled valve (Vollrath, 2000; Vollrath & Knight, 1999), whose role is still debated. The valve is
composed of “lips” formed by a thickening of the duct cuticle and is operated by a series of muscles (Wilson, 1962). The valve may act as a clamp that allows spiders to brake when dropping on draglines (Vollrath & Knight, 1999). This valve may also be the “friction brake” that applies forces during forcible silking, according to Ortlepp and Gosline (2004).

The spinning duct valve became more complex through spider evolution (Wilson, 1969). If complexity is an indication of efficiency, then derived spiders should have a better functioning valve. Thus, compared to haplogyne and RTA clade spiders, Orbiculariae would be better at resisting forcible silking, thereby applying high friction forces (Ortlepp & Gosline, 2004) that increase molecular orientation of the silk fiber, increasing its strength (Perez-Rigueiro et al., 2005). This is congruent with what we observed.

The valve also allow the spiders to “clamp down” on the thread and control spinning speed during falls. During forcible silking, strength and stiffness of silk increase with spinning speed (Chen et al., 2006; Vollrath et al., 2001). However, this may be due to the spider resisting silking more as reeling speed increase. When dissolved silk proteins are spun in vitro, stiffness and ultimate strength also increase with drawing speed (Lazaris et al., 2002; Seidel et al., 2000), similarly to what happens with silkworm silk (Capello & McGrawth, 1994). This suggests that, even during natural spinning, higher spinning speed results in stronger and stiffer silk.

In that case, Orbiculariae's ability to control spinning speed during falls would allow them to spin DDL and WDL at similar speed, resulting in similar properties, as observed here. Friction forces are also applied when the spider brakes during a fall, but the forces
applied during falls are much smaller than those applied during forcible silking, and
probably do not increase silk molecular orientation (Ortlepp & Gosline, 2004). The fact
that spiders brake as they fall is supported by high-speed videos (500 frames/s) of two
Larinioides sclopetarius: during the fall, the speed decreased, sometimes after a short
plateau (Fig. 5.3).

We hypothesized that the reduced valve of RTA clade and haplogyne spiders would
not allow them to resist forcible silking or control speed during falls as much as
Orbiculariae. In that case, we should see no differences in the properties of FDL and
WDL, as was the case here. But RTA clade and haplogyne spiders should also spin DDL
at higher speed than WDL, if they cannot control their speed during drops. This should
result in DDL that is stiffer and stronger than WDL. This was not the case in our
experiment. This suggests these clades may somehow control their dropping speed,
possibly using their hindlegs to slow down (pers. obs.). It is also possible that even a
reduced spinning duct valve functions as a brake during falls, but not as a friction brake
to resist forcible silking. High-speed videos also showed that average dropping speeds of

![Figure 5.3: Speed of dropping Larinioides sclopetarius (Orbiculariae, in red) and Peucetia viridanis (RTA clade spider, in blue).]
RTA clade and orbicularian spiders is identical (0.5 m/s for the RTA clade *Peucetia viridans* vs. 0.6 m/s for the orbicularian *Larinioides sclopetarius*, n=2 per species) (Fig. 5.3).

Nevertheless, Orbiculariae, RTA clade and haplogyne spiders differ in much more than just the complexity of their spinning duct valves. For instance, Orbiculariae express two different silk proteins (MaSp1 and MaSp2) in their major ampullate glands (Xu & Lewis, 1990; Hinman & Lewis). Although data are sparse, haplogyne and RTA clade spiders probably lack the two well-differentiated proteins, MaSp1 and MaSp2, found in Orbiculariae major ampullate silk, and instead produce only MaSp1-like proteins (Gatesy *et al.*, 2001; Pouchkina-Stantcheva & McQueen-Mason, 2004; Tian *et al.*, 2004). The histology of the glands' epithelium and the general anatomy of the spinning apparatus also differ between clades (Kovoor, 1977). Therefore, variation in the material properties of silks spun under different conditions may also result from changes in silk protein composition or aspects of the morphology of the spinning apparatus other than the valve itself. In particular, if changes in the proportion of the two silk proteins, MaSp1 and MaSp2, determine differences in silk properties, then RTA clade spiders should not exhibit any variability since they only possess proteins that are quite close in structure. Yet, RTA clade spiders do show silk plasticity (FDL differed from WDL in RTA clade species). This supports the idea that silk plasticity is due to “spinning effects” more than biochemical changes.
4.2. Functional consequences of silk plasticity: Relation between silk control and silk uses

Our results show that Orbiculariae can control silk properties better than RTA clade and haplogyne spiders, since they can resist forcible silking and control spinning speed during falls. However, RTA clade spiders also display a reduced but detectable ability to control spinning speed during falls, maybe through their rudimentary spinning duct valve in conjunction with the use of hindlegs to slow down.

Major ampullate silk is used by spiders to perform many behaviors, but derived spiders, such as Orbiculariae, use major ampullate silk for a greater diversity of functions than other clades, such as RTA clade or haplogyne spiders. For instance, many RTA clades species do not spin webs, contrary to Orbiculariae. Even when RTA clade or haplogyne spiders spin webs, these webs are less complex and stereotyped than those of Orbiculariae (Blackledge et al., 2009b). Orbiculariae webs are made of clearly different elements composed of major ampullate silk, unlike webs from other clades. Therefore, we argue that Orbiculariae make more complex use of their major ampullate silk than RTA clade or haplogyne spiders. Therefore, we argue that Orbiculariae make more complex use of their major ampullate silk than RTA clade or haplogyne spiders.

Better control of silk properties may have been selected for as spiders made more diverse uses of their silk. Being able to tune silk properties to silk intended use, rather than having silk properties determined simply by spinning conditions, would certainly be an advantage. As an example, silk from different regions of A. tepidariorum cobweb exhibit different silk properties (Boutry & Blackledge, 2009). It is possible that the spider actively controls silk properties so that silk from each web region performs better.
Forcibly obtained silk properties differ from naturally spun silk in both RTA clade spiders and Orbiculariae. This is consistent with other studies (Madsen & Vollrath, 1999; Perez-Rigueiro et al., 2001; Guinea et al., 2005b). Therefore, when forcible dragline is used to characterize the properties of a species silk, it may not be representative of silk spun in natura by the spider, except maybe if the silk is reeled very slowly. If silk is collected while the spider is under anesthesia, the animal cannot brake and apply friction forces to the silk. Thus silk collected from anesthetized spiders may not differ from naturally spun silk, because anesthetized spiders may not be able to resist forcible silking. On the other hand, acidification due to the CO₂ used in anesthesia may also affects silk properties (Madsen & Vollrath, 1999). Therefore, studies interested in relating silk properties to spider ecology (for instance, how silk is used in webs as in Craig, 1987a; Ko & Jovicic, 2004; Opell, 1994; or how silk varies across species, as in Swanson et al., 2006a), collecting silk spun by walking spiders may be a better option than collecting silk by forcible silking.

In conclusion, we showed that silk plasticity, measured as variability in the material properties of silk spun under different conditions (FDL, DDL and WDL), exist in all Entelegyne spiders. We strongly believe that silk plasticity is associated primarily with forces applied on the silk within the duct, and not to biochemical changes, since biochemical changes do not agree with our observation of plasticity in RTA clade spiders. Thus, spinning conditions are critical determinants of silk material properties. In particular, the presence of a complex spinning duct valve in Orbiculariae seems related to the ability of spiders to control silk properties. This ability to control silk properties has increased through spider evolution, possibly in relation to increased number of silk uses.
Acknowledgments

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CHAPTER VI

EVOLUTION OF SUPERCONTRACTION IN SPIDER SILK:
STRUCTURE-FUNCTION RELATIONSHIP FROM TARANTULAS
TO ORB-WEAVERS

Introduction

Spider major ampullate silk is a promising biomaterial, combining high strength and
elasticity (Gosline et al., 1986). Furthermore, silk is biocompatible (Allmeling et al.,
2006; Gellynck et al., 2006). Potential applications range from artificial tendons and
ligaments (Kluge et al., 2008) to microspheres for drug delivery (Lammel et al., 2008).
However, large amounts of spider silk are hard to obtain. Researchers are therefore
working to produce synthetics fibers based on spider major ampullate silk (Vendrely &
Scheibel, 2007). However, in contrast to most known materials, silk supercontracts under
mild conditions (when humidity rises above ~70%, Work, 1977). During
supercontraction, water infiltrates the silk and causes it to shrink, up to half its dry length
(Work, 1977). This process also generates high stresses if the fiber is restrained.

Supercontraction could play a critical role in the production of dragline silk by spiders by
allowing spiders to “tailor” silk properties (Guinea et al., 2005a). Although it can hinder
certain applications of silk, it can also lead to new uses that involve silk moving objects
rather than simply resisting loads (Agnarsson et al., 2009b). Thus, there is a critical need
to understand the mechanisms causing supercontraction. Supercontraction is relatively
well documented among orb-weaving spiders such as Araneidae and Nephilidae (Grubb & Ji, 1999; Savage et al., 2004; van Beek et al., 2002; Work, 1981), and was also found in Pisauridae (Shao & Vollrath, 1999) and Theridiidae (Shao & Vollrath, 1999; Work, 1981). Whether silk from other taxa supercontracts, in particular silk from “basal” taxa such as tarantulas and haplogynes (e.g. daddy-long leg and spitting spiders), remains uninvestigated. The current molecular model for supercontraction (Eles & Michal, 2004b; Termonia, 1994) and the possible functions proposed for supercontraction (Guinea et al., 2003; Guinea et al., 2005a; Lewis, 1992; Work, 1981) are largely based on our knowledge of silk composition and web ecology of Araneidae and Nephilidae, a small fraction of all existing spiders (~10% of spiders species). Understanding the supercontraction behavior of silk from other taxa, with different silk composition and ecologies, provides a critical test of the proposed mechanisms and functions for supercontraction. Here, we present the first comprehensive study of supercontraction among all spiders and use a phylogenetic perspective to understand the origin and function of supercontraction in spider major ampullate silk.

Spider major ampullate silk is composed of proteins containing repeated amino acid motifs, i.e. short, stereotyped amino acid sequences that form specific secondary structures. Orbiculariae's major ampullate silk contains poly-alanine and glycine-alanine motifs that form beta-sheet crystals (Jelinski et al., 1999; Kümerlen et al., 1996; Simmons et al., 1994; Xu & Lewis, 1990), glycine-glycine-X motifs that form 310 helices (Bram et al., 1997; van Beek et al., 2002) and glycine-proline-glycine motifs (Ayoub et al., 2007; Hayashi & Lewis, 1998; Hayashi et al., 1999; Hinman et al., 2000; Hinman & Lewis, 1992). There is no consensus as to what structures are formed by the glycine-
proline-glycine motifs. They have been described as helical fractions (Vollrath & Porter, 2009), proline-rich network chains (Savage & Gosline, 2008a), beta-spirals (Hayashi & Lewis, 1998; Hayashi et al., 1999) and various types of beta-turns (Ohgo et al., 2006). In this paper, we will simply refer to these motifs as GPGXX motifs, where G represents glycine, P proline and X any one of a small subset of amino acids.

Currently, supercontraction is hypothesized to result from rearrangements of the non-crystalline fractions formed by the GPGXX motifs and the 3_10 helices within the silk fiber (Blackledge et al., 2009a; Eles & Michal, 2004b; Termonia, 1994). When the silk is dry, these non-crystalline regions are maintained parallel to the fiber axis by hydrogen bonds. However, when the humidity rises, water disrupts these hydrogen bonds, allowing the non-crystalline regions to rearrange to lower energetic configurations and driving supercontraction (Eles & Michal, 2004b; Savage & Gosline, 2008b; Yang et al., 2000). This rearrangement leads to the shrinking and thickening of the fiber and, at the molecular level, to an observed loss of orientation (Grubb & Ji, 1999; Parkhe et al., 1997).

If supercontraction is induced by a rearrangement of the glycine-glycine X or GPGXX motifs, then there should be a positive relationship between abundance of these motifs in the silk and strength of supercontraction. Major ampullate silk contains one or two types of proteins, both termed major ampullate spidroins or MaSp for short (Hinman & Lewis, 1992; Xu & Lewis, 1990). Mygalomorphs (tarantulas) lack clearly differentiated silk glands (Palmer, 1985; Palmer et al., 1982). Their silk proteins contain long repeats, rich in alanine and serine (Garb et al., 2007). Major ampullate glands appeared with Araneomorphae spiders, which include haplogyne spiders such as daddy long leg spiders,
and entelegyne spiders. Haplogyne major ampullate silk is composed of long repeat units rich in alanine, serine and glycine (Gatesy et al., 2001). These proteins differ from the major ampullate spidroins found in haplogyne's sister taxon, entelegyne spiders, which include most common spiders, such as orb-weavers and wolf spiders. Entelegynes possess a MaSp1-like protein, rich in poly-alanine and glycine-alanine repeats that form beta-sheets, as well as glycine-glycine-X helices (Gatesy et al., 2001; Pouchkina-Stantcheva & McQueen-Mason, 2004 but also see Tian et al., 2004). The second protein, MaSp2, includes GPGXX motifs (Hinman & Lewis, 1992) acting as molecular nanosprings (Becker et al., 2003). MaSp2 is known from Orbiculariae (orb-weaving spiders and their relatives) but is likely absent from all other taxa (see Methods). Therefore, if supercontraction is due to the rearrangement of GPGXX motifs, silk containing MaSp2 proteins (i.e. Orbiculariae silk) should supercontract more than silk lacking MaSp2.

Such a phylogenetically-based approach may also provide insight into the two hypothesized functions proposed for supercontraction: tailoring of silk properties during fiber spinning and tightening of orb-webs loaded with water.

According to the tailoring hypothesis, silk is in a supercontracted state at the beginning of the spinning process, when it is first drawn from a liquid solution. The extent to which the supercontracted silk is stretched during spinning determines molecular alignment, and thereby, the properties of the fiber after extrusion and drying (Guinea et al., 2005a). The tailoring hypothesis predicts that supercontraction was selected for in spiders that use major ampullate silk in diverse ecological contexts. For instance, basal spider taxa, such as tarantulas, largely use sheets of silk to line burrows or
to construct “simple” brushed sheet webs on the substrate. Discrete major ampullate silk threads are first used in webs within haplogyne spiders. However, their webs tend to be relatively simple and constructed close to the substrate. Examples include the “lampshade” web of *Hypochilus* and the disorganized sheet webs of *Kukulcania*. Entelegynes, the sister taxa of haplogynes, include, among others, two clades that dramatically shifted how they use dragline silk. Most RTA clade species, such as jumping spiders and wolf spiders, do not spin capture webs and only lay a trail of dragline silk as they walk. In contrast, Orbiculariae not only use draglines, but also spin a diversity of complex webs composed of distinct architectural elements, such as orb-webs and cobwebs. These webs are suspended in the air and have multiple discrete elements made of major ampullate silk (e.g. radii, frame and mooring guys in orb-webs). These elements serve distinct functions that place different demands on the threads in terms of mechanical performance. Orbicularian spiders may thus need to spin silk threads with different material properties based on the threads' function. Therefore, the ability to tailor silk properties may have been selected for in Orbiculariae. On the other hand, species that do not use silk in webs (many tarantulas and RTA clade spiders) may have less need to modulate silk properties. If tailoring of silk is achieved through supercontraction (Guinea *et al.*, 2005a), then higher supercontraction shrink and stress should have been selected for in Orbiculariae compared to other taxa.

The second hypothesized function of supercontraction is to prevent orb-webs from sagging under the weight of dew drops by tensing threads (Guinea *et al.*, 2003; Lewis, 1992; Work, 1981). This hypothesis predicts that supercontraction has been selected for in species that spin aerial orb-webs in contrast to non-orb-weaving species. Orb-webs are
spun only by Orbiculariae. Furthermore, several derived families of Orbiculariae now
spin novel web types, such as the cobwebs of Theridiidae (Coddington & Levi, 1991;
Eberhard et al., 2008). Planar orb-webs contain major ampullate radii that only are in
contact at the center of the web. In contrast, in cobwebs, each major ampullate support
thread contacts many other threads, forming a complex, seemingly disorganized,
network. Since cobweb threads connect with many other threads, loads may be better
dealt between threads in cobwebs than they are in orb-webs, which may allow cobwebs
to resist loads better than orb-webs. Therefore, unlike orb-webs, cobwebs may not need
high tension to resist the load of dew drops. Hence, if supercontraction has been selected
for web tightening, supercontraction may have secondarily been lost in Orbiculariae that
lost the orb-web.

To summarize, if supercontraction is caused by GPGXX motifs, then all spiders
producing silk rich in MaSp2 should spin major ampullate silk that supercontracts more,
resulting in all Orbiculariae exhibiting higher supercontraction than all other taxa. The
same pattern is predicted if supercontraction evolved under selection for tailoring silk
properties. In contrast, if supercontraction functions to tighten wet orb-webs, then orb-
weaving species within the Orbiculariae should spin silk that supercontracts more than
non-orb-weaving species, whether these are Orbiculariae or not (Table 6.1). Under this
hypothesis, we predict that non-orb-weaving Orbiculariae would lose supercontraction as
they switched to three-dimensional webs because supercontraction did not yield any
advantage for web protection from water drops in these species, thereby relaxing
selection for it. However, it is also possible that supercontraction was somewhat
maintained as it is associated to a desirable property of silk. We tested these hypotheses
by investigating supercontraction in 28 species from 21 families spanning the order Araneae.

Finally, we examine two different aspects of supercontraction across spiders. Unrestrained fibers shrink as they contract while restrained fibers instead develop tension. These two aspects of supercontraction may have evolved under different selective forces. By measuring supercontraction in many diverse taxa, this study can begin to separate the different evolutionary pressures that shaped both aspects of supercontraction.

Material and Methods

Spider maintenance and silk collection

Most spiders were wild caught but some were purchased from either SpiderPharm (Yarnell, AZ) or TarantulaSpiders.com (FL). Spiders were housed in a variety of cages, depending upon their web spinning behaviors, and maintained in the laboratory at 24°C under a 15 : 9 light / dark cycle. Spiders were silked within a week after entering the laboratory. Table 6.2 presents the taxa used in this study, as well as their origin, silk collection method and numbers of individuals and thread samples used.

Table 6.1: Expected level of supercontraction in different spider taxa in function of proposed hypothesis on supercontraction mechanisms and function. +, high supercontraction; --, low supercontraction.

<table>
<thead>
<tr>
<th>Orbiculariae mechanism: β-spirals</th>
<th>Orbiculariae function: silk tailoring</th>
<th>Orbiculariae function: web tightening</th>
<th>other spiders</th>
</tr>
</thead>
<tbody>
<tr>
<td>orb-weaving</td>
<td>non-orb-weaving</td>
<td>other spiders</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 6.2: Taxa used in this study with indications of the spidroins present in the silk and the type of web. If the presence of MaSp2 had not been investigated in the spider’s family and we inferred presence / absence of MaSp2 from the phylogeny, a (I) was added on column #3. The silk was collected either by forcible silking (FS) or was naturally spun (NS). The last column (“# spiders”) indicates the number of individual spiders per species and the total number of silk samples tested in this study.

<table>
<thead>
<tr>
<th>species</th>
<th>family</th>
<th>MaSp2 present</th>
<th>web type</th>
<th>silk collection</th>
<th>origin</th>
<th># spiders/samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephebopus uatuman (Lucas et al.)</td>
<td>Theraphosidae</td>
<td>No (I)</td>
<td>no web</td>
<td>NS</td>
<td>TarantulaSpiders</td>
<td>3 / 8</td>
</tr>
<tr>
<td>Grammostola rosea (Walckenaer)</td>
<td>Theraphosidae</td>
<td>No (I)</td>
<td>no web</td>
<td>NS</td>
<td>TarantulaSpiders</td>
<td>5 / 13</td>
</tr>
<tr>
<td>Aphonopelma seemani (F.O.P. Cambridge)</td>
<td>Theraphosidae</td>
<td>No (I)</td>
<td>no web</td>
<td>NS</td>
<td>TarantulaSpiders</td>
<td>2 / 4</td>
</tr>
<tr>
<td>Hypochilus thorelli (Marx)</td>
<td>Hypochilidae</td>
<td>No (I)</td>
<td>lampshade</td>
<td>NS</td>
<td>USA, TN</td>
<td>4 / 19</td>
</tr>
<tr>
<td>Kukulcania hibernalis (Hentz)</td>
<td>Filistatidae</td>
<td>No</td>
<td>sheet</td>
<td>NS</td>
<td>SpiderPharm</td>
<td>7 / 34</td>
</tr>
<tr>
<td>Dziguetia canities (McCook)</td>
<td>Diguetidae</td>
<td>No (I)</td>
<td>tentweb</td>
<td>FS</td>
<td>SpiderPharm</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Pholcus phalangioides (Fuesslin)</td>
<td>Pholcidae</td>
<td>No (I)</td>
<td>tangle</td>
<td>FS</td>
<td>Czech Republic</td>
<td>4 / 8</td>
</tr>
<tr>
<td>Scytoes sp. (Latreille)</td>
<td>Scytodidae</td>
<td>No (I)</td>
<td>no web</td>
<td>NS</td>
<td>Costa Rica</td>
<td>4 / 29</td>
</tr>
<tr>
<td>Eresus kollari (Rossi)</td>
<td>Eresidae</td>
<td>No (I)</td>
<td>tube</td>
<td>NS</td>
<td>Czech Republic</td>
<td>3 / 7</td>
</tr>
<tr>
<td>Hololena adnexa (Chamberlin &amp; Gertsch)</td>
<td>Agelenidae</td>
<td>No</td>
<td>funnel</td>
<td>FS</td>
<td>USA, CA</td>
<td>5 / 16</td>
</tr>
<tr>
<td>Hogna helluo (Walckenaer)</td>
<td>Lycosidae</td>
<td>No (I)</td>
<td>no web</td>
<td>FS</td>
<td>USA, OH</td>
<td>2 / 16</td>
</tr>
<tr>
<td>Amaurobius ferox (Walckenaer)</td>
<td>Amaurobiidae</td>
<td>No (I)</td>
<td>funnel</td>
<td>FS</td>
<td>USA, VA</td>
<td>3 / 12</td>
</tr>
<tr>
<td>Salticus scencius (Clerck)</td>
<td>Salticidae</td>
<td>No (I)</td>
<td>no web</td>
<td>FS</td>
<td>USA, OH</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Tengella radiata (Kulczynski)</td>
<td>Tengellidae</td>
<td>No (I)</td>
<td>funnel</td>
<td>FS</td>
<td>Costa Rica</td>
<td>4 / 14</td>
</tr>
<tr>
<td>Dolomedes tenebrosus (Hentz)</td>
<td>Pisauridae</td>
<td>No</td>
<td>no web</td>
<td>FS</td>
<td>USA, OH</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Peucetia viridans (Hentz)</td>
<td>Oxyopidae</td>
<td>No (I)</td>
<td>no web</td>
<td>FS</td>
<td>SpiderPharm</td>
<td>8 / 36</td>
</tr>
<tr>
<td>Uloborus diversus (Marx)</td>
<td>Uloboridae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>USA, CA</td>
<td>8 / 30</td>
</tr>
<tr>
<td>Pityohyphantes costatus (Hentz)</td>
<td>Linyphiidae</td>
<td>Yes (I)</td>
<td>sheet</td>
<td>FS</td>
<td>USA, OH</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Tetragnatha sp. (Latreille)</td>
<td>Tetragnathidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>USA, OH</td>
<td>2 / 8</td>
</tr>
<tr>
<td>Latrodectus hesperus (Chamberlin &amp; Ivie)</td>
<td>Therididae</td>
<td>Yes</td>
<td>cobweb</td>
<td>FS</td>
<td>USA, CA</td>
<td>9 / 44</td>
</tr>
<tr>
<td>Achaearanea tepidiorum (Koch)</td>
<td>Therididae</td>
<td>Yes</td>
<td>cobweb</td>
<td>FS</td>
<td>USA, OH</td>
<td>9 / 34</td>
</tr>
<tr>
<td>Synoctaxus sp. (Simon)</td>
<td>Therididae</td>
<td>Yes</td>
<td>“mesh”</td>
<td>FS</td>
<td>Costa Rica</td>
<td>2 / 8</td>
</tr>
<tr>
<td>Nephiela clavipes (Linnaeus)</td>
<td>Nephiilidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>USA, FL</td>
<td>6 / 24</td>
</tr>
<tr>
<td>Zygiella x-notata (Clerck)</td>
<td>Araneidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>Slovenia</td>
<td>8 / 32</td>
</tr>
<tr>
<td>Araneus diadematus (Clerck)</td>
<td>Araneidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>USA, OH</td>
<td>3 / 12</td>
</tr>
<tr>
<td>Verrucosa arenata (Walckenaer)</td>
<td>Araneidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>USA, OH</td>
<td>2 / 15</td>
</tr>
<tr>
<td>Larintoides sclopeterius (Clerck)</td>
<td>Araneidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>USA, OH</td>
<td>4 / 19</td>
</tr>
<tr>
<td>Nuctenea umbratica (Clerck)</td>
<td>Araneidae</td>
<td>Yes</td>
<td>orb</td>
<td>FS</td>
<td>Slovenia</td>
<td>6 / 22</td>
</tr>
</tbody>
</table>
Silk was mainly collected using forcible silking. The spider was anesthetized with carbon dioxide and taped down, ventral side facing up, on a Petri dish. Major ampullate silk was manually reeled off the spinnerets at ~10 cm/s, and collected on cut-out cards across 15.3 mm gaps. The silk was glued on either side of the gap using cyanoacrylate glue (Superglue®) (Blackledge et al., 2005b). During the process, the spinnerets and silk threads were observed under a stereomicroscope, to ensure the silk collected came from the major ampullate spigot. Three to four samples were collected and tested for each individual spider.

For a few taxa, it was impossible to collect silk by forcible silking. In this case, naturally spun silk was collected. The spider was allowed to run across a fan-shaped piece of cardboard. As it ran, the spider laid a trail of dragline silk, across the peaks of the cardboard, which was collected onto cut-out cards. As with forcibly obtained silk, the threads were glued on each side of the 15.3 mm gap with cyanoacrylate glue, and three to four samples were collected per spider. Dragline silk is composed of major ampullate silk strands, sometimes accompanied by thinner minor ampullate silk strands. The samples were observed under a microscope, and all samples that contained thin, minor ampullate, strands were discarded. Thus, the samples we used were made of one or two strands of major ampullate silk only.

Naturally-spun silk tends to be more compliant and weaker than forcibly-obtained silk, probably because of its decreased molecular orientation (Guinea et al., 2005b; Madsen & Vollrath, 1999; Perez-Rigueiro et al., 2001). However, the silks that were naturally-spun did not drastically differ in their supercontraction behavior from the silks
that were forcibly-obtained from related species (see below). Therefore, we think that differences in collection methods *per se* had only minor effects on our results.

Tarantulas lack well-differentiated silk glands and therefore do not produce major ampullate silk. However, tarantula use their silk for functions analogous to major ampullate silk's, such as lining burrows. Furthermore, as tarantulas belong to Mygalomorphae, the sister group to the clade of spiders producing major ampullate silk, their silk is ideal for an outgroup comparison.

*Silk diameter measurements and supercontraction tests*

Three pictures were taken of each sample using polarized light microscopy at 1000X (Blackledge *et al.*, 2005a). Each strand diameter was measured using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2007) and the total cross-sectional area calculated.

We measured two different aspects of supercontraction: the stress generated in restrained fibers and the degree to which unrestrained silk shrank when exposed to water. Previous studies measured supercontraction as the degree of fiber shrinking (e.g. Work, 1981). However, the stress generated during supercontraction by the fiber is also important, as it will affect the performance of structures made of silk (webs or potentially, man-made silk structures). Supercontraction tests were carried on a Nano Bionix tensile tester (MTS Corp., Oakridge, TN) equipped with a humidity chamber, as described in Agnarsson *et al.* (2009a). The relative humidity inside the chamber could be set to any value between ~1% and ~95%. Silk samples were mounted at room humidity (5-15%) and pulled on at 0.1% strain, until just taut (Savage *et al.*, 2004). Following the terminology adopted by Blackledge *et al.* (2009a), the tests performed were WS0.1%
tests (strained at 0.1% then wetted). Figure 6.1 is a diagram of the supercontraction test. Humidity was ramped up from ambient humidity to over 75% within 2 min. When supercontraction critical humidity was reached, the hydrogen bonds were disrupted, freeing the molecules to move to lower energy states. However, the fiber was unable to shrink because it was held by the grips. Thus, stress instead developed within the fiber. We refer to this as supercontraction stress (SS) and calculated it using engineering stress as:

\[ SS = \frac{F}{A} \]

where \( F \) is the force generated by the sample and \( A \) is the area of the sample.

---

**Figure 6.1:** Method used to measure supercontraction stress and percentage of shrink. The upper part of the figure describes the tensile testing device while the lower part is a typical curve of stress through time. A silk thread of length \( l_0 \) (black line) is mounted between the grips of a tensile tester (grey rectangles) at low humidity and 0.1% strain. As the humidity rises to ~70%, silk supercontracts but the thread is held at constant length, which results in the apparition of a supercontraction stress. The thread is relaxed, at which point the stress goes back to zero. The thread is then slowly extended. Once the thread length passes the post-supercontraction length \( l_1 \), stress rises again. Supercontraction percentage of shrink is calculated as the difference between the original length \( l_0 \) and the final length \( l_1 \).
The fiber was then relaxed to half its original length ($l_0$) so that it was completely slacked, and immediately pulled at 0.01 mm/s to twice its original length, while the load was recorded. If the fiber had been unrestrained when we increased humidity, it would have shrunk from $l_0$ to a post-supercontraction length $l_1$. When the slacked fibers were stretched to this post-supercontraction length $l_1$, a stress developed within the fiber. This allowed us to measure $l_1$. Percentage of shrink (PS), which is the proportion by which the fiber shrinks when supercontracting, was then calculated as:

$$PS = \frac{l_0 - l_1}{l_0}$$

For certain silks, fibers were still under tension after relaxing to half their original length. In these cases, the fibers were relaxed further. The samples that still presented a stress before the beginning of the pull were discarded.

*Correlation between SS, PS and preload tension*

Samples with a high preload tension, that is samples with a high tension within the sample prior to supercontraction, exhibited no SS even though they supercontracted, as evidenced by their positive PS. This suggested that preload tension influenced SS. This was a particularly important issue because supercontraction tests were performed at constant 0.1% strain, which could result in variable preload tensions across samples. To test for a correlation between preload tension and supercontraction, 15 silk samples each from two *L. hesperus* individuals were collected. These samples were mounted at different preload tensions, ranging from 0 to 170 MPa. Supercontraction tests were then run as described above, and SS and PS were recorded. For each individual, SS and PS were regressed versus preload tension.
Spider phylogeny

Phylogenetic relationships may influence supercontraction of silk. For instance, the level of supercontraction from silk of closely related taxa may be more similar than that of distant taxa simply due to phylogenetic inertia. Independent contrasts (IC) were used to correct for the non-independence of related species (see Statistical analysis). No existing phylogeny includes all of the species in our study, but we estimated species relationships using Coddington's Araneae phylogeny (Coddington, 2005) with additions from Raven (1985) for tarantulas and Blackledge et al. (2009b) for apical relationships within Orbiculariae (Fig. 6.2).

Web ecology and silk proteins

This study tried to relate supercontraction to the spinning of orb-webs and the presence of MaSp2 silk proteins. Table 6.1 describes the type of webs spun by each taxon and the presence / absence of MaSp2 in each taxon's silk. The presence / absence of MaSp2 in silk was inferred from cDNA data from Garb et al., 2007 and Gatesy et al., 2001 for mygalomorphs; Tian et al., 2004 for Kukulcania sp.; Gatesy et al., 2001; Pouchkina-Stantcheva & McQueen-Mason, 2004; Rising et al., 2007; Tian et al., 2004 for RTA clade species (Hololena, Amaurobius, Hogna, Dolomedes and Tengella); Gatesy et al., 2001 for Tetragnathidae; Hinman & Lewis, 1992; Sponner et al., 2005; Xu & Lewis, 1990 for Nephilidae; Gatesy et al., 2001; Guerette et al., 1996 for Araneidae; and Ayoub et al., 2007; Ayoub & Hayashi, 2008; Gatesy et al., 2001 for Theridiidae. For RTA clade spiders, Rising et al. (2007) suggested the presence of a protein somewhat similar to MaSp2, but much poorer in GPGXX motifs. Additionally, Gatesy et al. (2001) did not find any MaSp2-like sequence in RTA clade spiders. Therefore, we considered
the taxa from the RTA clade to lack MaSp2. The silk proteins of many of the taxa used here have not yet been characterized. In this case, the phylogeny was used to infer whether their silk likely contained MaSp2. MaSp2 is known in several Orbiculariae, including Nephilidae, Uloboridae and Araneidae, but is not found in the RTA clade. RTA clade spiders and Orbiculariae are all higher Entelegyne *sensu* Coddington and Levi (1991). Therefore, we considered all RTA clade spiders and all sister taxa to the higher Entelegyne to be lacking MaSp2, and all taxa derived from the RTA clade to have MaSp2.

Among the taxa that possess MaSp2, the proportion of MaSp2 in the silk may affect silk properties and behavior (Liu *et al*., 2008b; Savage & Gosline, 2008a). However, data

Figure 6.2: Phylogeny of the taxa used in this study. Major clades are indicated by different colors.
on the percentage of MaSp2 in various species' silk are generally lacking. Therefore, we only used presence / absence of MaSp2 as a criterion in this study.

Statistical analysis

The average SS and PS per species were used in all the analyses. The analyses compared supercontraction between species with or without MaSp2 in their silk, and between species that spin or do not spin orb-webs. A series of standard ANOVAs, with either SS or PS as the dependent variable, and either presence of MaSp2 or type of web (orb-web or non-orb-web / no web) as the independent variable, were conducted. When testing the effect of web type, analyses were conducted both with all taxa and only within Orbiculariae species. The non-independence of phylogenetically related taxa was accounted for by following Garland et al.’s independent contrasts method (Garland et al., 1993). Using PDSIMUL and PDANOVA from the PDAP package, F distributions were created, taking into account the phylogeny and assuming no relation between SS/ PS and presence of MaSp2 / web type. ANOVAs were run using PDSINGLE, with either SS or PS as the dependent variable, and either presence of MaSp2 or type of web (orb-web or non-orb-web / no web) as the independent variable. The F statistics from the ANOVA were compared to the critical values obtained from the simulated F distributions. *Hogna helluo* was removed from our data set for SS since stress data could not be collected for this species.
Results

Correlation between SS, PS and preload tension

Supercontraction stress was strongly correlated with preload tension in both individuals of *L. hesperus* (linear regression, 1st individual, P = 0.0206, 2nd individual, P < 0.0001), but percentage of shrink was independent of preload tension (linear regression, 1st individual, P = 0.2073, 2nd individual, P = 0.2602) (Fig. 6.3). A similar pattern was observed in *Nephila clavipes* and *Peucetia viridans* (data not shown).

![Figure 6.3: Relation between preload tension and supercontraction stress (A) or percentage of shrink (B). Blue squares and regression line represent the first *Latrodectus* individual while red diamonds and regression line correspond to the second.](image-url)
Evolution of supercontraction in spiders in relation to protein composition and web type

Nearly all spiders species investigated presented some form of supercontraction (Fig. 6.4). We considered that silk supercontracted when the 95% confidence interval for the mean for both SS and PS did not include zero. For both *Ephebopus uatuman* and *Aphonopelma seemani* (Theraphosidae), the average SS and PS 95% confidence interval included zero. Therefore, we consider that they do not exhibit any supercontraction. All the other species showed both a SS and PS greater than zero, therefore exhibiting some level of supercontraction.

Across all species, PS was greater for species possessing MaSp2 in their silk (standard ANOVA, $P < 0.0001$, n=28, IC ANOVA, $P = 0.0118$, n=27) and also for species that spin orb-webs (standard ANOVA, $P < 0.0001$, n=28, IC ANOVA, $P = 0.0014$, n=27). Within the Orbiculariae though, PS was not different between orb-weaving species and derived taxa that lost the orb-web (standard ANOVA, $P = 0.0750$, n=12, IC ANOVA, $P = 0.1991$, n=11). After accounting for phylogeny, supercontraction stress was independent of the presence of MaSp2 (IC ANOVA, $P = 0.4187$, n=28) and the type of web spun (IC ANOVA, $P = 0.2020$, n=28). However, if phylogenetic relations were ignored, supercontraction stress was greater in species whose silk contains MaSp2 (standard ANOVA, $P = 0.0224$, n=28) and who spin orb-webs (standard ANOVA, $P = 0.0136$, n=28). Because SS is strongly correlated with the preload tension in the sample at the beginning of the test, we consider PS to be a more reliable indicator of supercontraction capacity.
Figure 6.4: Supercontraction shrink (panel A) and stress (panel B) for 28 spider taxa (mean + SE) with phylogeny of the taxa. Yellow bars represent non-orb-weaving species while green bars represent orb-weaving species. The red branches of the phylogeny correspond to species whose silk contains MaSp2 (Orbiculariae) while the blue branches are species whose silk lacks a well-differentiated MaSp2 (non-Orbiculariae). The species name is abbreviated as follow: Eu = E. uatum, Gr = G. rosea, As = A. seemani, Ht = H. thorelli, Kh = K. hibernalis, Pp = Pholcus phalangioides, Dc = D. canities, Sc = Scytodes sp., Ek = E. kollari, Ha = H. adnexas, Hh = H. helluo, Af = A. ferox, Ss = S. scenicus, Tr = T. radiata, Dt = D. tenebrosus, Pv = P. viridans, Ud = U. diversus, Pc = P. costatus, Te = Tetragnatha sp., Lh = L. hesperus, At = A. tepidariorum, Sy = Synotaxus sp., Nc = N. clavipes, Zx = Z. x-notata, Ad = A. diadematus, Va = V. arenata, Ls = L. sclopetarius, Nu = N. umbratica.
Discussion

Evolutionary history of supercontraction in spiders

The major ampullate silk of nearly all the species investigated supercontracts to some degree. However, the degree of supercontraction varies greatly. For instance, major ampullate silk from the zebra jumper *Salticus scenicus* shrank by only 1.2% when wetted while silk from the bridge orb-weaver *Larinioides sclopetarius* shrank by 53%. Supercontraction stress varied from 3 MPa for the ladybird spider *Eresus kollari* to 145 MPa for the green lynx spider *Peucetia viridans*. Moreover, we found no direct correlation between SS and PS. These two responses can therefore be considered as distinct components of supercontraction.

There is no consensus on what constitutes a threshold response for supercontraction. For instance, Work (1981) observed that dragline silk from RTA clade species shrank by about 5% when exposed to humidity. Yet, he decided that this was too low and that this silk did not supercontract. In contrast, Shao and Vollrath (1999) found that silk from the RTA clade Pisauridae shrank by as much as 15% and considered that their silk did supercontract. We used 95% confidence intervals to identify non-null PS and SS, thus providing an unbiased definition of supercontraction. If we follow this method, silk from only two tarantulas (Mygalomorphae) does not supercontract. This suggests that supercontraction evolved very early in the evolutionary history of spiders. Since supercontraction exists in all Araneomorphae, it must have appeared at least 225 million years ago, with the origin of Araneomorphae (Selden et al., 1999). Supercontraction seems rare in Mygalomorphae, and may have appeared later in certain species.

Furthermore, non-orbicularian silk typically shrink by less than 20% while orbicularian
silk contracts by 30-50%. However, in contrast to PS, there was no obvious difference in SS between non-Orbiculariae and Orbiculariae.

In general, silk from Orbiculariae supercontracted more than silk from non-Orbiculariae. Within Orbiculariae, non-orb-weaving species did not statistically differ from orb-weaving species in term of supercontraction ability. These results support the idea that supercontraction is due to GPGXX motifs and serves a tailoring function.

Selective pressure on supercontraction stress

Although both PS and SS originate with basal araneomorph spiders, the evolutionary pathways of these two aspects of supercontraction differ. While PS increased in Orbiculariae, changes in SS were associated neither with the presence of MaSp2 nor the spinning of orb-webs. Supercontraction stress was not correlated with percentage of shrink but depended upon preload tension prior to supercontraction.

In addition, SS correlates with PS within RTA clade spiders (multiple linear regression, PS: P= 0.0030, n= 7) but not in Orbiculariae (linear regression, PS: P= 0.3865, n= 10) (Fig. 6.5). Why is the relation between SS and PS clade-specific? RTA clade spiders spin stiffer silk, in general, than Orbiculariae (Swanson et al., 2006b). This difference may result from the high proportion of GPGXX motifs in the MaSp2-rich silk of Orbiculariae (Liu et al., 2008b). Regardless, silks that are stiff should produce stronger stresses when restrained than silks that are compliant since, by definition, stiffer silks produce higher stresses for a given strain, or shrink in our case. This explains why RTA clade spiders' silks produce strong SS even though they do not contract much. In contrast, orbicularian silk produces fairly low SS despite considerable shrinking, such that no correlation between SS and PS was found.
It is worth noting that silks from *Nephila* and *Latrodectus*, which have a low proportion of MaSp2 (Liu *et al.*, 2008b), behave similarly to RTA clade spiders, with high SS for relatively low PS.

If SS depends in part on silk stiffness, which itself may come from silk molecular orientation, selection may not have been able to act on SS as much as it has acted on PS. However, it is also possible that PS has been under stronger selection than SS because it is more important. For instance, if supercontraction's function is to modulate silk properties, what really matters are wet silk properties, such as extensibility, which correlate with PS (multiple linear regression, extensibility: $P < 0.0001$, $n=23$) but not with SS (multiple linear regression, extensibility: $P = 0.5265$, $n=23$).

**Molecular mechanism of supercontraction**

The presence of MaSp2, rich in GPGXX motifs, is associated with an increased capacity of major ampullate silk to supercontract. This is congruent with Eleš and Michal's model for supercontraction (Eleš & Michal, 2004b), which states that, as relative

![Figure 6.5: Relation between supercontraction stress and percentage of shrink for RTA clade spiders (blue diamonds) and Orbiculariae (red diamonds).](image)
humidity increases, water disrupts the hydrogen bonds that hold the GPGXX motifs and $3_{10}$ helices within the silk parallel. The GPGXX motifs and helices then rearrange to a lower energetic state and the fiber loses its orientation. According to this hypothesis, silk containing GPGXX motifs should supercontract more.

Liu et al. (2005b; 2008a) found that, at the intraspecific level, silk that shrinks more when exposed to water is also stronger, stiffer, less extensible and better able to recover after being stretched, all characteristics generally associated to a more oriented fiber. This also supports Eleas and Michal's model, in which the loss of orientation of GPGXX motifs causes supercontraction. However, at the interspecific level, there is, if anything, a negative correlation between strength or stiffness of the silk and supercontraction, although silk that supercontracts more still recover better after stretching. For instance, RTA clade spiders exhibit lower supercontraction than Araneidae, and also have stiffer silk (Swanson et al., 2006b). This is because interspecific variation in supercontraction is driven primarily by the amount of GPGXX motifs in silk while intraspecific variations likely result from differences in the orientation of relatively similar proportions of GPGXX chains. Since GPGXX motifs are also thought to be involved in silk compliance and extensibility, fewer GPGXX motifs result in stiffer and less extensible silk, in addition to silk that contracts less (Fig. 6.6).

It is worth noting that all spiders spun major ampullate silk that supercontracted, except for some tarantulas. Although thorough research on silk molecular structure outside Orbicularia is lacking, the few data available suggest that silks from non-Orbicularia nearly completely lack GPGXX motifs. Thus, although our study suggests a clear association between presence of GPGXX motifs and enhanced supercontraction, it
is clearly possible for silk with few or no GPGXX motifs to still supercontract. In this case, other molecular structures, such as $3_{10}$ helices (Kümmerlen et al., 1996) formed by glycine-glycine-X motifs that are present in MaSp1 proteins, may be involved in supercontraction (Blackledge et al., 2009a). These structures may represent the molecular basis upon which natural selection operated upon during the evolutionary increase in the supercontraction capacity of orb-weaving spider silk.

**Selective pressure on supercontraction shrink: function of supercontraction**

Supercontraction is hypothesized to tense the orb-web thereby preventing it from sagging under the weight of dew drops (Guinea et al., 2003; Lewis, 1992; Work, 1981). When Orbiculariae that spun orb-webs were compared to all taxa, including some derived

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**Figure 6.6:** Hypothetical mechanisms explaining differences in supercontraction and material properties at the intra and interspecific levels. Red arrows with "+" represent an increase in the property, while blue arrows with "-" represent a decrease in the property.
Orbiculariae that did not spin orb-webs, orb-weaving species spun silk that supercontracted more, consistent with the “tightening” hypothesis. Non-orb-weaving Orbiculariae exhibit reduced supercontraction compared to orb-weaving Orbiculariae, but higher supercontraction than non-Orbiculariae (Table 6.3). Non-Orbiculariae do not spin webs (e.g. *P. viridans*, *Scytodes* sp.), spin “loose” webs (e.g. *P. phalangioides*), or spin webs attached to the substrate (e.g. *K. hibernalis*, *H. adnexa*). Therefore, supercontraction's web tightening function may not be needed in these species.

Supercontraction may have been selected for in Orbiculariae as they started spinning planar orb-webs, which explains why non-orbicularian silk supercontracts less than orbicularian silk. When some Orbiculariae switched from orb-webs to three-dimensional webs, silk's supercontraction capacity decreased, but partially remained as an ancestral character.

Recently, Guinea *et al.* (2005a) proposed that supercontraction facilitates tailoring of silk properties during fiber spinning. Within the spider's spinning duct, silk is initially in a supercontracted state and is thus as compliant as possible. Stretching of the silk thread during spinning controls molecular orientation and hence, the material properties of the fiber. Such fine control may not be needed by non-Orbiculariae. In effect, these species'
capture webs, if spun at all, are poorly differentiated and lack distinct web elements. In the case of tarantulas, webs are not even composed of distinct threads, while non-Orbiculariae araneomorph species use discrete threads within their webs (Blackledge et al., 2009b). In contrast, Orbiculariae's webs are composed of multiples elements, each spun from major ampullate silk, which meet different mechanical challenges. For instance, orb-webs contain radii that absorb energy during prey capture, frame threads that support the orb and mooring guys that attach the web to the substrate. Cobwebs contain supporting threads that maintain web architecture and hold sticky gumfooted threads that are used for prey capture. The silk from these different elements may have varied material properties (Boutry & Blackledge, 2009). This could lead to selection for increased plasticity of major ampullate silk properties in Orbiculariae, in contrast to other taxa. If supercontraction serves a tailoring function, then we could expect supercontraction to be higher in Orbiculariae than in non-Orbiculariae araneomorph, and to be even lower in mygalomorph than in any araneomorph. This is exactly what is seen in our data set.

If supercontraction allows spiders to better modulate silk properties (i.e. the tailoring hypothesis), then species characterized by high supercontraction should exhibit greater intra-individual variability in material properties of major ampullate silk. This agrees with our observation that Orbiculariae (whose silk supercontracts strongly) exhibit higher major ampullate silk plasticity than RTA clade spiders (whose silk supercontracts less) (Chapter V). Guinea et al's tailoring hypothesis and Work's web tightening hypothesis are not mutually exclusive. Supercontraction may have been selected for in Orbiculariae because of both its web tightening and its silk tailoring functions.
Within Orbiculariae, high PS is not confined solely to taxa that spin orb-webs, even though non-orb-weaving Orbiculariae exhibit slightly reduced supercontraction. Yet, non-orb-weaving Orbiculariae do not need their webs to remain tight under humid condition (web tightening function of supercontraction). Supercontraction may remain in non-orb-weaving species because it serves a tailoring function, which is needed to spin different elements within cobwebs, for instance. Still, because web tightening is not needed in these species, selection for high supercontraction may be lower.

Supercontraction may also be associated to a desirable property. The same GPGXX motifs that allow supercontraction through their rearrangement, according to Eles and Michal's model, are thought to enhance silk extensibility (Gosline et al., 1986; Hayashi et al., 1999; Termonia, 1994). Thus, since supercontraction and extensibility are likely affected by the same molecular structure (GPGXX motifs), supercontraction may have been preserved in non-orb-weaving Orbiculariae as a byproduct of selection on silk extensibility. In fact, within Orbiculariae, there is a positive relation between supercontraction PS and extensibility (linear regression, P < 0.0001, n=11) (Fig. 6.7). It is possible that the appearance of complex, planar orb-webs in Orbiculariae created the selective pressures for initial increase in supercontraction, and the origin of MaSp2 and GPGXX motifs in silk is the mechanism that allowed this higher supercontraction. In other words, MaSp2 may have been selected for in Orbiculariae because MaSp2 enhances supercontraction, which itself allows better tailoring of silk properties for complex web building and tightening of orb-webs.
Conclusion

Supercontraction is widespread among spiders and evolved early in their evolutionary history, likely with the origin of araneomorph spiders, 225 million years ago. However, the degree to which silk supercontracts varies strongly among species. Supercontraction includes two aspects: shrinking of unrestrained fibers (PS) and development of stress within restrained fibers (SS). These two different responses to water evolved independently. Variation in supercontraction stress (SS) is randomly distributed with respect to spider phylogeny, unlike shrinking (PS), which is higher in Orbiculariae. Supercontraction stress' measurement depends upon preload tension. Increased supercontraction in Orbiculariae agrees with Eles and Michal's model, which states that GPGXX motifs play an essential role in supercontraction. However, the presence of supercontraction in non-Orbiculariae, which lack GPGXX motifs, suggests that other

Figure 6.7: Relation between supercontraction shrink and wet silk extensibility. Each point corresponds to one species. Orbiculariae are represented by the red diamonds and non-Orbiculariae by the blue squares.
molecular structures, such as glycine-glycine-X$_{310}$ helices, are also involved in supercontraction.

Our results are congruent with Guinea et al.'s functional hypothesis that supercontraction helps spiders tailor silk properties during fiber spinning, although we cannot discard Work's web tightening functional hypothesis. Finally, supercontraction shrink may also have been selected for because of its association with desirable material properties such as extensibility.

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CHAPTER VII

SPIDER SILK SUPERCONTRACTION AND

THE PERFORMANCE OF ORB-WEBS

Introduction

Water plays a critical role in shaping the material properties of silk. In particular, the sticky silk used in the capture spiral of orb-webs depends on high humidity for stickiness and compliance. Even major ampullate silk, which forms the dry backbone of the web, responds to water by supercontracting when exposed to high humidity (> 70%), shrinking by up to half its length if unrestrained (Work, 1977). It is probably common for spider webs to be exposed to conditions inducing supercontraction, such that spider webs would have to function with supercontracted silk.

It has been proposed that supercontraction allows orb-webs to maintain tension and shape under humid conditions (Work, 1977) and facilitates silk recovery after stretching. However, there is some debate concerning the effect of supercontraction on web function. Bell *et al.* (2002) found that supercontraction induced tensions of 300 MPa in the web. They argued that such tension would reduce the web's ability to resist further loads and absorb energy, for instance during prey capture. In contrast, Savage *et al.* (2004) found that tension due to supercontraction is barely 50 MPa, such that supercontraction should not limit web performance. Nevertheless, these studies all focused on single thread measurements. This is a critical limitation as webs are composite architectures of
multiple threads that interact during prey capture. Thus, in this study, we focus on the behavior of whole webs after supercontraction.

We focus only on orb-webs, because their behavior, particularly during prey capture, is better understood than other web types. Furthermore, the major ampullate silk spun by orb-weaving spiders supercontracts more than that of other species (Boutry & Blackledge, 2010; Shao & Vollrath, 1999). Orb-webs are made of different structural elements, mainly mooring guys that attach the web to the substrate, frame threads that surround the capture area, which is itself composed of radii supporting a sticky spiral. Except for the sticky spiral, all the structural elements of the orb-web are made of major ampullate silk, which supercontracts at high humidity.

The properties of supercontracted silk and dry silk (in our case, silk that is not, and has never been, supercontracted) are quite different. At low strains, supercontracted silk is more compliant, more extensible, and therefore, tougher (Guinea et al., 2005a; Perez-Rigueiro et al., 2003; Shao et al., 1999). Because of these differences in silk material properties, one may expect webs whose silk is supercontracted to perform differently from webs whose silk is not supercontracted.

In particular, supercontracted webs should deform more easily, and extend more overall when hit with the same energy. They should also be able to absorb more energy as supercontracted silk is tougher than dry silk. This would allow supercontracted webs to stop heavier, faster compared to dry webs. Prey may also bounce against stiff webs, a phenomenon known as “trampoline effect”. As supercontracted silk is more compliant than dry silk, trampoline effects may be less important in supercontracted webs, and the risk of prey bouncing off the web may decrease. Other consequences of supercontraction
for web function may include less prey escape, as suggested by Denny (1976), and lower vibration transmission, as stiffer webs may transmit vibrations better (Walcott, 1963; Watanabe, 2000).

However, in webs, differences in the properties of supercontracted and dry silk may be balanced by thread tension. In effect, silk threads in the web are not free, but are attached to other threads, which means they are held on both ends. Therefore, when a thread in a web supercontracts, instead of shrinking, it develops a tension. Some properties of silk, such as compliance, change with silk tension: for instance, supercontracted silk fibers are more compliant at zero tension, and they become stiffer as the tension in the fiber increases. Thus, within webs, supercontracted silk threads may be stiffer than expected for instance.

We compared the prey capture behavior of supercontracted and dry orb-webs from two spider genera: Argiope and Nephila. Using balsa wood blocks as prey, we measured web deflection and energy absorption of webs held at different humidity.

Material and Methods

Two genera of orb-weaving spiders were used: Argiope (Araneidae, 6 individuals) and Nephila (Nepilidae, 4 individuals). Argiope spiders were collected from Bath and Akron (OH, USA) and Nephila spiders were purchased from Tarantulaspiders.com (FL, USA). Although both are orb-weavers, the properties of their silk differ, with Nephila silk being stiffer than Argiope's (Swanson et al., 2006a; Sensenig et al.). Nephila silk also shrinks less during supercontraction (Work, 1981; Liu et al., 2008b). Furthermore, web architecture differs between Nephila and Argiope, with Nephila webs having more radii
and a slightly larger capture area (Sensenig et al., 2010), and keeping its auxiliary spiral even after the web is completed (Foelix, 1987). The spiders were housed in cages composed of a meshed frame 10-cm wide, closed by two removable 40 x 40 cm plexiglass squares. This design allowed the spider to walk on and attach its web to the mesh, while still making the web visible. Furthermore, the removable sides allowed us to throw prey at the web. The webs were misted every day, but only after the trials took place. Thus, the webs used in the study had not experienced high humidity and supercontraction before the actual trial.

The webs were placed in a chamber with controlled humidity. Prey impact trials took place at less than 35% humidity for dry webs, and over 70% (and in most cases, over 80%) for supercontracted webs. All webs were exposed to the humidity at which the trial took place for at least 15 minutes before the trial started.

To ensure that the silk from webs kept at high humidity was indeed supercontracted, radii were collected from *Argiope* webs after the video trials (12 samples from 4 webs at high humidity and 9 samples from 4 webs at low humidity) and used for supercontraction tests as described in Boutry & Blackledge (2010). Supercontraction stress was used as a measure of supercontraction. In that specific case, supercontraction shrink is not a good measure of supercontraction, as shrink will not differ between samples of the same silk type after relaxation and humidity increase. Supercontraction stress, on the other hand, will be much smaller for samples that are already supercontracted compared to dry samples, as the supercontracted samples have already developed a supercontraction stress before our supercontraction test starts. Supercontraction stress of silk from webs kept at
high and low humidity was compared using a Mann-Whitney U test, as the data were not normally distributed.

To mimic spider's prey, we used balsa blocks, ranging in weight from 90 mg to 300 mg. The weights reached velocities of 2 to 4 m/s, with one block thrown at 4.5 m/s, and kinetic energies between 0.2 and 2 mJ. These weights and the kinetic energies used in this study correspond to relatively large, fast prey, such as grasshoppers (Blackledge & Zevenbergen, 2006) or honeybees (Denny, 1976). A variety of blocks were thrown by hand perpendicular to each web, and each web received three to five different weights. As webs were hit multiple times, during the later hits, the web may have been already stretched and may not function as well as during the first hit. To minimize this problem, we made sure that the weights hit relatively undamaged area of the web. Orb-webs seem able to function even with a few radii broken and undamaged webs are very rare in nature (Alam & Jenkins, 2005).

The behavior of the web was recorded from profile, with a Fastech camera (San Diego, CA, USA) at 500 frames/second. An 8 x 8 cm cardboard square was used for calibration. It was also noted whether the block bounced off the web, was caught, or broke through the web.

The high-speed videos were analyzed using ProAnalyst Motion Analysis software (Xcitex, Inc., Cambridge, MA, USA). The top and bottom of the block were tracked before and after the block impacted the web, and its displacement between two frames was calculated as:

$$\delta_x = \left( \frac{x_{top}^{t} + x_{bot}^{t}}{2} \right) - \left( \frac{x_{top}^{t-1} + x_{bot}^{t-1}}{2} \right)$$
\[ \delta_y = \left( \frac{y_{t \text{top}} + y_{t \text{bot}}}{2} \right) - \left( \frac{y_{t-1 \text{top}} + y_{t-1 \text{bot}}}{2} \right) \]

where \( x \) are the coordinates on the x-axis and \( y \) are the coordinates on the y-axis, \( \text{top} \) and \( \text{bot} \) represent respectively the top and bottom of the block, \( t \) is a given frame and \( t-1 \) is the frame just before \( t \).

Then, speed itself was calculated as:

\[ v = 500 \sqrt{\delta_x^2 + \delta_y^2} \]

The prey speed was graphed against time, and the average speed just before and just after impact was visually estimated (Fig. 7.1A).

From the block's speed, its average kinetic energy before and after impact was calculated as:

Figure 7.1: (A) Block velocity through time in the case where the weight is caught by the web. The quick decrease in speed, marked by a red arrow, corresponds to the impact with the web. Velocity before web impact (\( v_b \)) and after web impact (\( v_a \)) are indicated on the y-axis. After a sharp decrease upon impact, kinetic energy and weight speed increase again as the weight oscillates along with the web and behaves like a pendulum. (B) Proportion of initial kinetic energy absorbed by the web during impact for supercontracted (in grey) and dry (in white) webs of Argiope and Nephila spiders. Error bars represent SE and asterisk means a difference at \( \alpha = 5\% \).
\[ KE = \frac{1}{2}mv^2 \]

where \( m \) is the block's mass, and \( v \) is its speed.

The proportion of the block's initial kinetic energy absorbed by the web during the first web oscillation was calculated as:

\[ \%KE_{abs} = \frac{KE_b - KE_a}{KE_b} \]

where \( KE_a \) is the block's kinetic energy after impact and \( KE_b \) is the block's kinetic energy before impact.

Even when blocks were stopped, this proportion was not 100%, as it takes the web several oscillations to dissipate all of the block's kinetic energy.

Stills of the video were taken just before impact (one frame before impact) and at the web's maximum deflection, using VirtualDub 1.9.8 (Lee, A., http://www.virtualdub.org/, 1998-2009). The two stills were superimposed and the distance by which the web extended was measured using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2007) (Fig. 7.2A).

The behavior of the block in the web (bounce, caught or through) was compared between supercontracted and dry webs using a chi-square test. As few blocks bounced off the web (4 out of 91 trials), we only used trials where the blocks was caught or went through the web in our subsequent analysis.

The proportion of kinetic energy absorbed was compared between supercontracted and dry webs using a Mann-Whitney U test (the data were not normally distributed) independently for \textit{Nephila} and \textit{Argiope}. Although initial kinetic energy did not differ
between supercontracted and dry webs (t-test, P = 0.1640), the effect of initial kinetic energy on kinetic energy absorption was still assessed using an ANCOVA with amount of kinetic energy absorbed as dependent variable and supercontraction state (supercontracted or dry), genus and initial kinetic energy as co-factors.

Similarly, web deflection was compared between supercontracted and dry webs using an ANCOVA with web deflection as dependent variable and supercontraction state, genus and initial kinetic energy as co-factors. Web deflection is expected to increase with initial kinetic energy (the faster and heavier the “prey”, the more the web will extend), therefore initial kinetic energy was used as a co-factor in the ANCOVA.

Figure 7.2: (A) Superimposed stills of a web before impact and at its maximum deflection. The block is circled in red on both stills and the web deflection is represented by a blue line. (B) Web deflection during impact for supercontracted (in grey) and dry (in white) webs of *Argiope* and *Nephila* spiders. Error bars represent SE.
Results

Samples of *Argiope* silk exposed to high humidity had lower supercontraction stress than samples exposed to low humidity (Mann-Whitney U test, $P = 0.0169$, mean ± SE (MPa), $9.3 ± 2.2$ for high humidity vs. $18.2 ± 3.0$ for low humidity) (Fig. 7.3). This confirms that the silk from webs kept at high humidity really supercontracted. However, even webs exposed to high humidity presented a small supercontraction stress. It is possible that the threads collected were stretched, either during prey impact or during collection. This small stretching may increase molecular orientation inside the thread, thereby allowing the thread to supercontract to a certain extent. In addition, small relaxation prior to the supercontraction test (for instance, while mounting samples) will result in increased supercontraction stress, yielding again a false positive (unpublished data). Thus, relaxation and stretching during sample manipulation may explain the existence of a supercontraction stress in samples from webs exposed to high humidity.

![Figure 7.3: Supercontraction stress (mean ± SE) of silk threads from *Argiope* webs exposed to high (> 70%) or low (< 35%) relative humidity. Asterisk mean a difference at $\alpha = 5\%$](image-url)
Proportion of weights caught was higher for supercontracted webs compared to dry webs, with supercontracted webs catching 84% of the weights while dry webs caught 48% of the weights (chi-square, \( P = 0.0004 \)) (Table 7.1).

The proportion of the initial kinetic energy absorbed by the web was higher for supercontracted webs of *Argiope* (Mann-Whitney U test, \( P = 0.0005 \), means ± SE (%), 93 ± 1 for supercontracted webs vs. 84 ± 2 for dry webs) but not for *Nephila* (Mann-Whitney U test, \( P = 0.9723 \), means ± SE (%), 93 ± 1 for supercontracted webs vs. 89 ± 2 for dry webs) (Fig. 7.1B). Similarly, the amount of kinetic energy absorbed was higher for supercontracted webs (ANCOVA, effect of humidity, \( P < 0.0001 \), mean ± SE (mJ), 0.97 ± 0.06 for supercontracted webs vs. 0.81 ± 0.06 for dry webs). How much of the initial kinetic energy was absorbed increased with initial kinetic energy (ANCOVA, effect of initial kinetic energy, \( P < 0.0001 \)). Furthermore, the relation between kinetic energy absorbed and initial kinetic energy differed for supercontracted and dry webs (ANCOVA, interaction of initial kinetic energy and humidity, \( P = 0.0297 \)), with supercontracted webs showing slightly more increase in kinetic energy absorbed with initial kinetic energy (Fig. 7.4). Surprisingly, these relations did not differ between *Argiope* and *Nephila* webs (ANCOVA, effect of genus, \( P = 0.3581 \), effect of the interaction of genus and humidity, \( P = 0.0659 \)).

### Table 7.1: Behavior of the weights in the web, for supercontracted and dry webs.

<table>
<thead>
<tr>
<th>Number of weights:</th>
<th>Supercontracted webs</th>
<th>Dry webs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caught</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>Breaking through</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Bouncing off</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
Web deflection was higher for supercontracted webs (ANCOVA, effect of humidity, $P = 0.0005$, mean ± SE (mm), 93 ± 4 for supercontracted webs vs. 74 ± 3 for dry webs) (Fig. 7.2B). Web deflection also increased with initial kinetic energy (ANCOVA, effect of initial kinetic energy, $P < 0.0001$). The relation between web deflection and initial kinetic energy was similar for supercontracted and dry webs (ANCOVA, interaction of initial kinetic energy and humidity, $P = 0.5709$). Once again, there was no difference in web deflection between *Argiope* and *Nephila* webs (ANCOVA, effect of genus, $P = 0.8368$, effect of the interaction of genus and humidity, $P = 0.1603$), even though the raw data would suggest the opposite (as evidenced in Fig. 7.2B).
Discussion

Supercontraction clearly does not impede the performance of orb-webs. Instead, it enhances the ability of orb-webs to stop prey, leading to 75% greater success. As predicted, supercontracted orb-webs extended more when hit, absorbed more of the projectile's energy and caught projectiles more often than dry webs. This can be explained through differences in the performance of supercontracted and dry spider silk. At low strains, supercontracted silk is more compliant and extensible than dry silk, which means it should deform more easily under the effect of a load. Therefore, when a projectile hits the orb-web, imposing a load on the silk threads, supercontracted webs should extend more. As orb-webs absorb prey energy through viscous dissipation (Sensenig et al., in preparation; Alam et al., 2007; Ko & Jovicic, 2004) webs that extend more (i.e. supercontracted webs) can absorb more energy and stop most projectiles.

This relation appears more pronounced for orb-webs of Argiope sp. than orb-webs of Nephila sp. This finding is consistent with differences in the supercontraction behavior of Argiope and Nephila silk, with Nephila exhibiting less contraction (Boutry & Blackledge, 2010; Work, 1981; Liu et al., 2008b). Differences in web architecture between these genera must also be considered. Nephila webs have more radii than Argiope's, and retain their auxiliary spiral even after the web is completed. They may thus be stiffer structures, which could limit their ability to deform during prey impact.

As initial kinetic energy increases, the amount of this energy that is absorbed by the web also increases. Even though some of the blocks impacted orb-webs with fairly high kinetic energy (more than that of a bee, for instance), the amount of energy absorbed by
webs did not seem to reach a plateau in our experiment, and some webs could absorb nearly 2 mJ of energy without breaking.

This increase in kinetic energy absorbed with initial kinetic energy is very lightly more pronounced for supercontracted webs. Looking at the data, this difference seems mainly driven by the smaller variability of the proportion of kinetic energy absorbed by supercontracted webs compared to dry webs (see Results and Fig. 7.4). This may be due to the more variable properties of dry silk compared to supercontracted silk (Perez-Rigueiro *et al.*, 2003) or more differences in stickiness among threads in dry webs. If dry silk threads differ in their properties more than supercontracted silk threads, then some may be able to absorb more energy than others during impact, explaining the high variation in the kinetic energy absorbed by dry webs. In terms of web function, if supercontracted webs can absorb more kinetic energy, they may be able to stop the large, fast prey that are more profitable to the spiders than smaller prey (Venner & Casas, 2005).

However, our reasoning is based on the properties of supercontracted silk when it is completely relaxed. Within orb-webs exposed to high humidity, silk is under tension when it supercontracts. In effect, when threads that are free at one end supercontract, they shrink, while threads held at both ends (such as threads in orb-webs) instead increase in tension. Silk properties, such as compliance, depend upon thread tension. Silk threads are usually more compliant when under no tension, and become stiffer as they are tensed. Therefore, supercontracted threads within orb-webs may be less compliant than we would expect based on tensile tests on individual threads cut from the web and unrestrained (Fig. 7.5) (Work, 1977).
We estimated the actual properties of supercontracted silk threads within the orb-web of *Argiope* (n=7). Assuming that web architecture and individual thread length do not change during supercontraction (which was confirmed by comparing pictures of dry webs with pictures of the same web after it was misted with water), we calculated that the tension within supercontracted threads would be about 70 MPa. This figure is closer to the tension in supercontracted orb-webs reported by Savage *et al.* (2004) than the values reported by Bell *et al.* (2002). Table 7.2 shows the properties of a supercontracted thread with 70 MPa of tension (a thread within the web), a supercontracted thread with no

Table 7.2: Material properties of three types of *Argiope* silk threads: supercontracted threads that are totally relaxed, supercontracted threads within the orb-web (n=7) and dry threads (mean ± SD). Dry threads data come from Sensenig *et al.*, 2010.

<table>
<thead>
<tr>
<th>Thread type</th>
<th>Compliance (Young's modulus, GPa)</th>
<th>Strength (true breaking stress, MPa)</th>
<th>Extensibility (true breaking strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supercontracted</td>
<td>0.2 ± 0.0</td>
<td>1545 ± 237</td>
<td>53% ± 4%</td>
</tr>
<tr>
<td>(relaxed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supercontracted</td>
<td>1.8 ± 0.3</td>
<td>1020 ± 261</td>
<td>26% ± 1%</td>
</tr>
<tr>
<td>(in web)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>5.2 ± 2.3</td>
<td>1585 ± 288</td>
<td>29% ± 6%</td>
</tr>
</tbody>
</table>
tension (a relaxed thread) and a dry thread. Supercontracted threads within the webs are only ~3 times as compliant as, ~30% weaker than and nearly as extensible as dry threads. In contrast, relaxed supercontracted threads are ~25 times as compliant, nearly as strong and nearly twice as extensible as dry threads. Therefore, the silk of supercontracted webs is not as different as dry silk as originally expected. This may explain that the differences observed in terms of web behavior were not as strong as expected.

This study focused on the mechanical response of the web, but other aspects of prey capture may be affected by humidity. In particular, spiral stickiness depends on humidity (Edmonds & Vollrath, 1992; Townley et al., 1991). Therefore, webs at high humidity may be better at retaining prey than webs at low humidity. Differences in stickiness could explain that supercontracted webs catch more projectiles than dry webs. However, in that case, we could expect weights to bounce off dry webs more than they bounce off supercontracted webs, but this was not the case (1 out of 45 weights bounced off dry webs, vs. 3 out of 46 for supercontracted webs). Furthermore, we ensured that the weights that flew through dry webs actually broke the spiral and / or radii, instead of tumbling through the web. If no damage to the web was observed, the trial was discarded. If differences in stickiness explained that supercontracted webs caught more projectiles than dry webs, then we would expect to see most projectiles tumble through or briefly stick before falling off the dry webs.

Prey detection may also be affected by humidity. Supercontracted silk is more compliant than dry silk, even within webs, and behavioral observations showed that spiders detect prey faster in stiffer webs (Watanabe, 2000). Vibration transmission is also known to increase with thread tension (Walcott, 1963). Thus, spiders may be better at
detecting prey at high humidity. Finally, supercontracted silk functions as a rubber (Gosline et al., 1984), therefore, supercontracted webs may recover better after prey impact and maintain their functional integrity better than dry webs.

There have been some debate on supercontraction function (Boutry & Blackledge, 2010). Supercontraction has been hypothesized to allow webs to remain tight under humid conditions (Work, 1977) and to be used for tailoring of silk properties during spinning (Guinea et al., 2005a). This study may suggest a third possible function of supercontraction: to allow webs to better absorb prey kinetic energy. In general, as supercontracted webs are overall better at stopping prey than dry webs, web performance may have been a selective pressure for silk supercontraction. Our study has only focused on orb-webs and how other web architectures (e.g. cobwebs) would perform when supercontracted compared to non-supercontracted remains unknown. However, we previously suggested that higher compliance and extensibility would allow the capture threads (i.e. gumfooted lines) of cobwebs to better contain prey (Boutry & Blackledge, 2009). In this case, we could expect supercontracted cobwebs to perform better than dry cobwebs. Although this remains to be tested, an improved performance of supercontracted cobwebs would agree with our observation that supercontraction was selected for in cobweb-weavers (Boutry & Blackledge, 2010).

To summarize, supercontraction appears to improve orb-webs' ability to stop prey. However, other aspects of web performance, such as retaining prey or allowing prey detection remain uninvestigated. These findings may point to web performance as a potential selective pressure for spider silk supercontraction.
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CHAPTER VIII

CONCLUSION

Our study showed that variability in major ampullate silk material properties is present in various clades of Entelegyne spiders. Orb-weaving and cobweb-weaving species display a certain control of silk properties, probably through anatomical mechanisms. In contrast, the properties of RTA clade spiders silk seems to mainly depend upon external conditions (Chapter V). The material properties of silk spun by some cobweb-weaving spiders vary in ways that enhance performance, for instance web function (Chapters II and III). However, such pattern has not been found in other species (Chapter IV). Silk properties could also depend on spiders' internal state, such as mass or condition (Chapter II).

Our work also demonstrated that supercontraction of major ampullate silk exists in most spider species, except for tarantulas. Supercontraction is likely due to the silk protein MaSp2. Its function are still debatable, but supercontraction probably help increase variability of silk material properties (Chapter VI). In terms of web performance, supercontraction increases the energy absorbed by webs during impact, thereby probably enhancing prey capture (Chapter VII).

By investigating the mechanisms of silk properties, this study shed light on the relative importance of silk biochemical composition vs. silk dope processing (“spinning effects”) for silk properties (e.g. see Hinman et al., 2000; Vollrath & Porter, 2009). In
particular, “spinning effects” appear important for silk material properties, such as stiffness, strength and extensibility. These findings have implications for biomimicry, as they suggest that processing of silks can influence silk properties. Our investigation into the consequences of silk properties is also relevant to biomimicry. For instance, if silk-like materials are used in membrane structures (Alam & Jenkins, 2005), then how supercontraction, for instance, influence orb-web performance is directly applicable to these membrane structures.

However, the consequences of silk properties for web performance are also important to understand how silk and web architecture co-evolved, a topic that has seldom been investigated (but see Sensenig et al., 2010). Currently, our understanding of how silk properties and web architecture interact during web function is still limited, despite a few recent models (Alam & Jenkins, 2005; Alam et al., 2007; Aoyanagi & Okumura; Ko & Jovicic, 2004). Our findings on evolution of supercontraction relate silk properties in part to web architecture. Furthermore, direct studies on how silk properties affect web performance, such as how well supercontracted and dry webs absorb energy, also point to potential evolutionary pressures on silk properties in relation to certain types of web architecture.

While silk properties and their potential mechanisms have been extensively investigated in the last decades, the relation between these properties and how structures made of silk (such as webs) function has not received much attention. Thus, our study belongs in part to a relatively new area in silk research, one that relates silk to webs and other structures.
LITERATURE CITED


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