

## Polarized light microscopy, variability in spider silk diameters, and the mechanical characterization of spider silk

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**Abstract.** Spider silks possess a remarkable combination of high tensile strength and extensibility that makes them among the toughest materials known. Despite the potential exploitation of these properties in biotechnology, very few silks have ever been characterized mechanically. This is due in part to the difficulty of measuring the thin diameters of silk fibers. The largest silk fibers are only 5–10  $\mu\text{m}$  in diameter and some can be as fine as 50 nm in diameter. Such narrow diameters, coupled with the refraction of light due to the anisotropic nature of crystalline regions within silk fibers, make it difficult to determine the size of silk fibers. Here, we report upon a technique that uses polarized light microscopy (PLM) to accurately and precisely characterize the diameters of spider silk fibers. We found that polarized light microscopy is as precise as scanning electron microscopy (SEM) across repeated measurements of individual samples of silk and resulted in mean diameters that were  $\sim 0.10 \mu\text{m}$  larger than those from SEM. Furthermore, we demonstrate that thread diameters within webs of individual spiders can vary by as much as 600%. Therefore, the ability of PLM to non-invasively characterize the diameters of each individual silk fiber used in mechanical tests can provide a crucial control for natural variation in silk diameters, both within webs and among spiders.

*Additional key words:* major ampullate, flagelliform, fibers, tensile test, orb web

Spider silks are among the strongest and toughest fibers known to science. Using a diverse array of proteins, spiders are able to construct silk fibers that vary tremendously in their mechanical properties, from major ampullate silk with a tensile strength rivaling that of steel to flagelliform silk with a stretchiness approaching that of rubber (Gosline et al. 1986). Despite this immense variation in physical properties and the potential exploitation of spider silks by industry, the material properties of most spider silks have never been investigated. This is due in part to the difficulty of working with silk fibers that are only a few  $\mu\text{m}$  in diameter at their largest, with some fibers as thin as 50–100 nm (Foelix 1996).

Characterization of the mechanical properties of spider silks typically begins with measurement of the stress generated as fibers are extended until breaking (Denny 1976). Because stress is a measurement of

force/cross-sectional area of a fiber, accurate and precise assessment of the diameters of fine silk threads is necessary. One of two different strategies is usually employed. The first approach is to use scanning electron microscopy (SEM) of a subsample of “focal” fibers that are assumed to be identical to the fibers that are to be tested mechanically. This method is expected to yield highly precise measurements of the focal fibers, but then assumes that the diameters of those focal fibers provide an accurate estimation of the diameters of the fibers that are actually tested. This could be particularly problematic when working with silks that have highly irregular diameters, such as silk collected from native structures like webs or egg sacs. The alternative strategy is to measure the diameters of each thread using a non-destructive method, such as compound light microscopy, and then to apply those diameters to each thread as they are tested mechanically. While the technical difficulties of using this procedure on fibers whose diameters approach longer wavelengths of light may decrease precision, this is the only method that can account for variation in fiber diameter within the population of samples to be tested, potentially

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increasing overall accuracy of the characterization of individual fibers.

Accurate characterization of fiber diameter is also important for understanding the biology of spiders, because spiders can actively control the diameters of silk threads spun under different environmental conditions (Vollrath & Köhler 1996; Garrido et al. 2002). For instance, the major ampullate fibers that constitute draglines are sometimes used as lifelines by falling spiders (Osaki 1996). When spinning draglines under conditions where they are more likely to fall, such as climbing a vertical surface versus moving horizontally, spiders will increase fiber diameter, thereby increasing the load-bearing capacity of their draglines (Garrido et al. 2002). Furthermore, spiders will increase the diameters of threads in their orb webs in proportion to increases in their body weights, which increases the ability of webs to support the spiders (Vollrath & Köhler 1996). Thus, control of fiber diameter is an important way in which spiders can alter the physical performance of draglines, webs, and other silk structures while using the same material to spin different silk fibers.

Here we report on a technique for the measurement of the diameters of fine silk fibers that utilizes polarized light microscopy (PLM). We first provide a quantitative comparison of the precision and accuracy of PLM versus SEM. We then provide a quantitative assessment of variability in the diameters of threads within two common types of spider webs. Finally, we discuss the relative merits of PLM and SEM approaches to measuring spider silk diameters.

## Methods

### Collection of silk samples

We collected a total of 21 silk samples from a variety of spiders in an effort to sample a wide range of variation in silk types and thread diameters (Table 1). All fibers were initially fixed onto “c”-shaped mounts cut from thin poster board, secured with a fast-drying cyanoacrylate glue (Superglue™). Major ampullate fibers were collected from a phylogenetically diverse group of spiders, by forcible silking of spiders that were anesthetized with CO<sub>2</sub>. The species sampled were *Araneus gemmoides* CHAMBERLIN & IVIE, *Gasteracantha cancriformis* LINNAEUS, *Latrodectus hesperus* CHAMBERLIN & IVIE, and *Peucetia viridans* HENTZ. One sample included a double-stranded minor ampullate thread and a single-stranded major ampullate fiber from *G. cancriformis*, and another included a double-stranded major ampullate thread from *L. hesperus*, to assess how multiple strands may affect determination of the total cross-sectional area of multistrand samples. A single fiber was collected from an egg sac of *Argiope argentata* FABRICIUS. A single fiber from the paired axial core fibers of a capture thread was collected from the web of *Uloborus diversus* MARX. Three samples of wrapping silks, composed of single aciniform fibers, were collected from *Argiope trifasciata* (FORSKÅL), as the spiders attacked and swathed prey. Finally, three samples of paired flagelliform capture fibers were collected from the webs of *A. argentata*.

**Table 1.** Silks measured using both polarized light microscopy and scanning light microscopy. Numbers in parentheses refer to the number of individual fibers that constituted a single sample of silk. Silks were collected during prey attack (PA), directly from webs (W), through forcible silking of restrained spiders (FS), and from egg sacs (E).

Silk (#fibers)	Species	Family	Ecological function	Source
Aciniform (1)	<i>Argiope trifasciata</i>	Araneidae	Prey wrapping	PA
Flagelliform (2)	<i>Argiope argentata</i>	Araneidae	Capture spiral of ecribellate orb webs	W
Major ampullate (1)	<i>Araneus gemmoides</i>	Araneidae	Dragline, frame, and radii of orb web	FS
Major ampullate (1)	<i>Gasteracantha cancriformis</i>	Araneidae	Dragline, frame, and radii of orb web	FS
Major ampullate (1 and 2)	<i>Latrodectus hesperus</i>	Theridiidae	Dragline and tangle of cobweb	FS
Major ampullate (1)	<i>Peucetia viridans</i>	Oxyopidae	Dragline	FS
Major and minor ampullate (3)	<i>G. cancriformis</i>	Araneidae	Dragline, frame, and radii of orb web	FS
Minor ampullate (2)	<i>A. trifasciata</i>	Araneidae	Temporary spiral of orb web	FS
Pseudoflagelliform (1)	<i>Uloborus diversus</i>	Uloboridae	Capture spiral of cribellate orb webs	W
Tubuliform (1)	<i>A. argentata</i>	Araneidae	Interior of egg sacs	E

### Measurements of focal samples: PLM vs. SEM

To compare variability within and between methods, we first measured the diameters of each thread using a novel method described below that is based upon polarized light microscopy (PLM). This entire process was repeated a total of five times for each sample of silk. These same silk samples were then measured using a Philips XL30 FEG scanning electron microscope (SEM). Silk threads were mounted onto carbon tape stubs and sputter coated for 1 min with a gold–palladium mixture at each of three angles, to a thickness of  $\sim 15\text{--}20$  nm. We then used SEM to measure the diameter of each sample five times. For the gluey capture threads of *Argiope argentata*, it was necessary to adhere the threads to glass slides to visualize them under the light microscope (Opell & Bond 2001). This prevented us from measuring the exact same sample with both PLM and SEM. Therefore, for each sample of flagelliform silk that was measured using PLM, we collected a second sample of silk from the sticky spiral immediately adjacent to the first sample, and measured that second sample using SEM. Regardless, capture threads consisted of a pair of flagelliform fibers that loosely twisted around one another. We were therefore careful to measure fiber diameter only along sections of the thread where the two fibers appeared parallel to one another or where there were small gaps between the two fibers. We used Wilcoxon matched-pairs tests to compare the coefficients of variation (cv) of the five repeated measurements for each silk sample, between the PLM and SEM measurements. We also used Wilcoxon matched-pairs tests to compare the mean diameters of each sample, calculated from the five repeated measurements, for both PLM and SEM.

### Polarized light microscope

The polarized light microscope used in these studies was based on a Leica DMLB platform with a rotatable stage. The microscope was fitted with a rotatable polarizer between the condenser and the specimen, and a fixed analyzer above the specimen. When illuminating the sample, the condenser was adjusted so that the specimen was evenly illuminated to maximize contrast. For maximum magnification, a “dry”  $100\times$ , 0.9 NA infinity-corrected objective was used. The use of the “dry” objective allowed high-resolution measurements to be made without contaminating the fibers with either oil or water, which could alter the material properties of silks. The image was sent either to the ocular or to a Canon S40 Powershot

camera through a 0/100 splitter. The sample stage was rotated so that the fiber was oriented horizontally. In this orientation, the polarizer and the analyzer were typically crossed (perpendicular to one another) and, in this configuration, higher order interference fringes that gave rise to apparently thicker images were abolished. Polarized light images were sent to the camera and analyzed using NIH Image 1.63 (US National Institutes of Health) to determine their width. Measurements were calibrated using a standard hemacytometer grid on the stage.

### Variation in silk fiber diameter within webs

We also examined the extent to which silk threads vary in diameter within individual webs as well as between webs constructed by different spiders. Using the PLM measuring technique described above, we measured the diameters of multiple samples of threads from individual webs to determine the extent to which fiber diameter varies within single webs. We sampled scaffolding threads from the cobwebs of nine western black widows (*L. hesperus*). All spiders were penultimate to adult females and weighed 0.09–0.82 g. From each spider’s web, we collected six to nine threads from the scaffold region (i.e., tangle) of the cobweb. We also examined capture threads from the orb webs of eight adult female silver garden spiders (*A. argentata*). For each spider, we measured the diameters of the core flagelliform fibers from the capture spirals of three webs. From each of these three webs, four samples were taken from the outermost rows of the sticky spiral and four samples were taken from the innermost rows of sticky spiral. The three webs were constructed under very different physiological conditions: the first after about five days of starvation, the second after about five days of feeding to satiation, and the third after about five additional days of starvation during which time all spiders also produced egg sacs. The weight of spiders changed by as much as 100% across each of these transitions. This allowed us to determine whether there were any consistent differences in the diameters of threads between the inner and outer spiral zones of orb webs that were maintained across a variety of physiological conditions.

### Mechanical characterization of silk

To test the effect of variation in fiber diameters on the repeatability of the mechanical characterization of spider silk, we performed tensile tests on six samples of scaffolding silk collected from a single *L. hesperus* cobweb. Force–extension data were generated

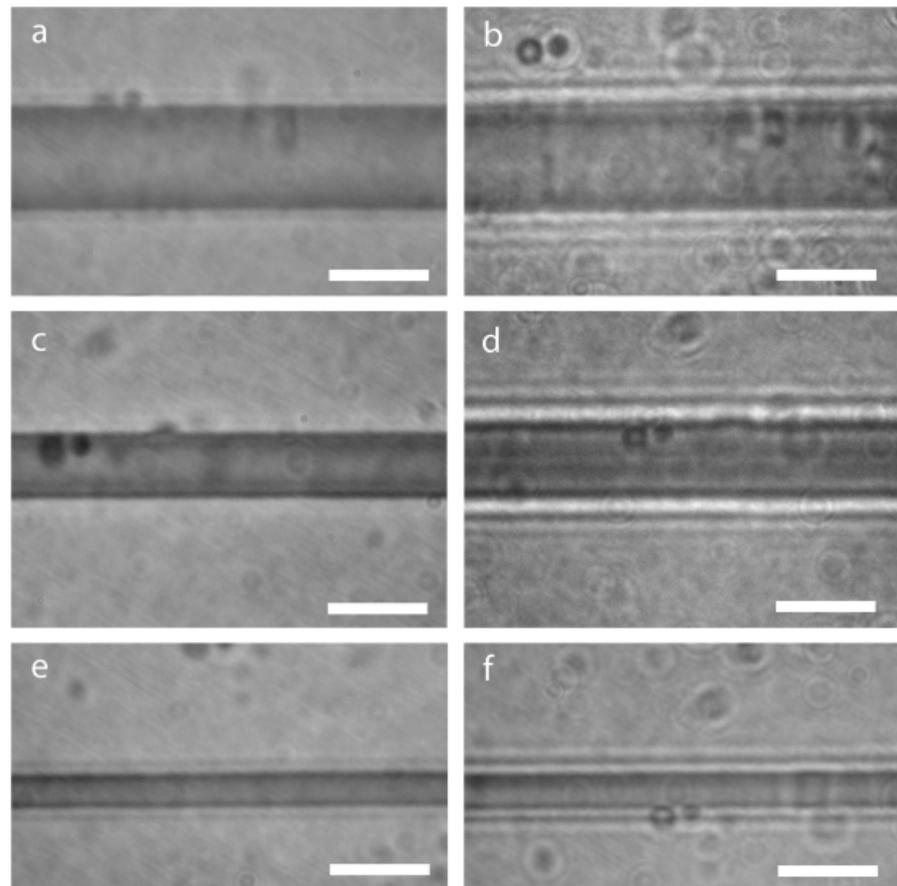
for each sample using a Nano Bionix tensile tester (MTS Corp.). Fibers were extended at a constant rate of 1% strain/s, relative to original length, until the samples failed. We then used Testworks 4.0 software (MTS Corp.) to calculate engineering stress (force/initial cross-sectional area of fibers) and engineering strain (extension/original length of sample) from the force and extension data in two ways. Initially we calculated stress using an identical cross-sectional area for all six fibers, calculated as the mean of diameters measured for each of the fibers with PLM. This was analogous to using SEM to measure the diameters of one set of fibers while mechanically testing a second set of fibers. Then we recalculated stress for each sample using the PLM diameter measurements for each individual fiber.

## Results

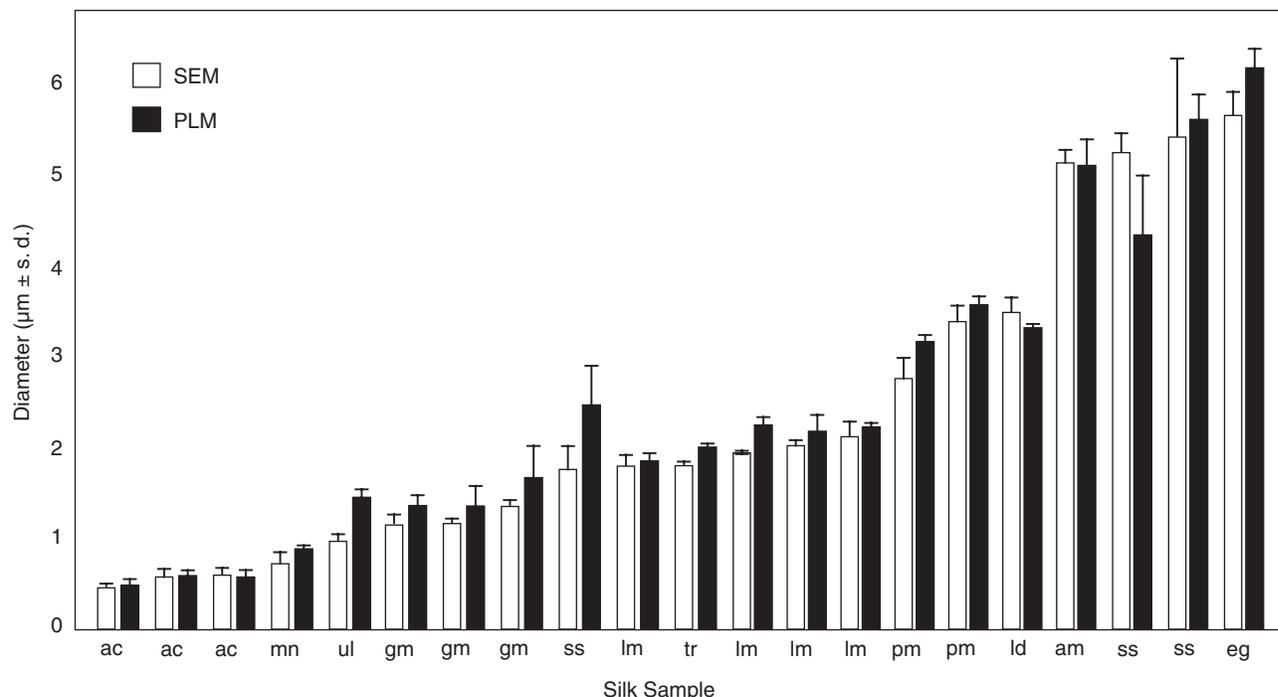
### Measurements of focal samples: PLM vs. SEM

Figure 1 illustrates the dramatic reduction in distortion that can be achieved using polarized light

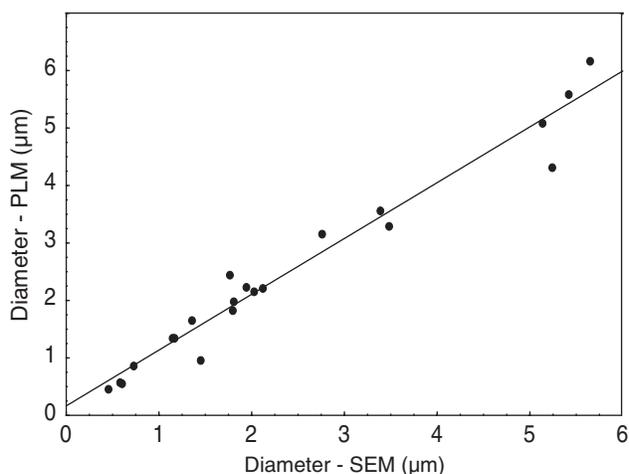
microscopy. There was no difference in the coefficients of variation across the five repeated measurements made for each silk sample between techniques (mean  $\pm$   $s\bar{x}$  =  $8.6 \pm 1.4\%$  and  $7.7 \pm 1.1\%$  for PLM and SEM respectively; Wilcoxon matched-pairs test,  $n = 21$ ,  $t = 102$ ,  $p = \text{n.s.}$ ). PLM tended to result in larger measurements of fiber diameters than SEM (mean difference  $\pm$   $s\bar{x}$  =  $0.10 \pm 0.07 \mu\text{m}$ ; Wilcoxon matched-pairs test,  $n = 21$ ,  $t = 55$ ,  $p < .05$ ; Fig. 2). The absolute difference in the mean diameter of each sample as measured by PLM and SEM was  $12.2 \pm 2.3\%$  (mean  $\pm$   $s\bar{x}$ ) and ranged from 0.5% to 39%. Absolute differences between the two methods were greatest for the sticky silk samples and excluding those three samples yielded a mean ( $\pm$   $s\bar{x}$ ) of  $9.2 \pm 1.5\%$  and a range from 0.5% to 22%. Regression of the mean PLM fiber diameters as a function of SEM diameters resulted in a linear relationship ( $F_{1,19} = 469$ ,  $p < .00001$ ,  $R^2 = 0.98$ ) with  $\beta = 0.98$  (Fig. 3). This relationship had a non-zero intercept of  $0.16 \pm 0.13 \mu\text{m}$  (mean  $\pm$   $s\bar{x}$ ), although it was not significantly different from zero ( $p < .25$ ).



**Fig. 1.** Comparison of photographs of major ampullate spider silk taken using polarized light microscopy (left) and standard light microscopy (right). **a,b.** *Peucetia viridans*. **c,d.** *Latrodectus geometricus*. **e,f.** *Gasteracantha cancriformis*. Scale bar, 2  $\mu\text{m}$ .



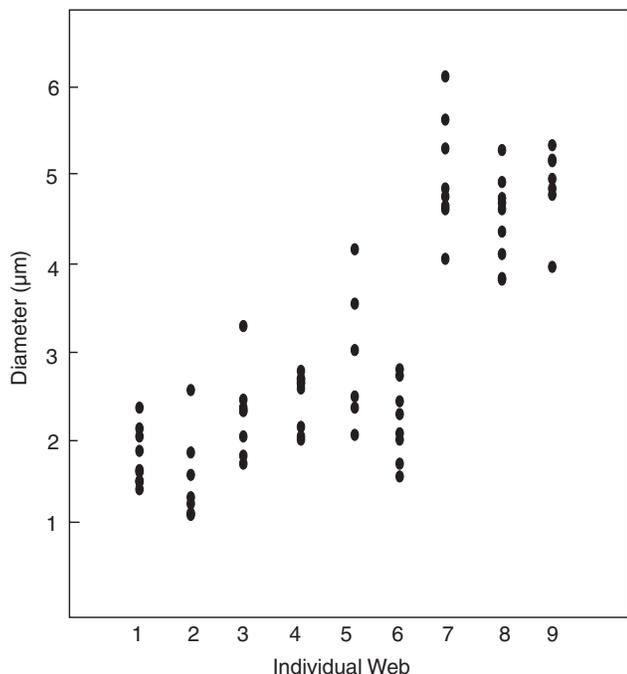
**Fig. 2.** Comparison of the mean  $\pm$  SD diameters of 11 silk samples, each of which was measured five times, using scanning electron microscopy (SEM) and polarized light microscopy (PLM). (ac) aciniform wrapping silk from *Argiope trifasciata*; (mn) double-stranded minor ampullate silk from *A. trifasciata*; (ul) single-stranded axial fiber from *Uloborus diversus* capture thread; (gm) single-strand major ampullate fiber from *Gasteracantha cancriformis*; (ss) double-strand flagelliform fiber from *Argiope argentata* capture thread; (lm) single-strand major ampullate from *Latrodectus hesperus*; (tr) double-strand minor ampullate and single-strand major ampullate from *G. cancriformis*; (pm) single-strand major ampullate from *Peucetia viridans*; (ld) double-strand major ampullate from *L. hesperus*; (am) single-strand major ampullate from *Araneus gemmoides*; (eg) single-strand from egg sac of *A. argentata*. For all double- and triple-stranded threads, the reported diameters are of hypothetical single-stranded fibers with cross-sectional areas equivalent to those of the total cross-sectional areas of the actual multi-stranded structures.



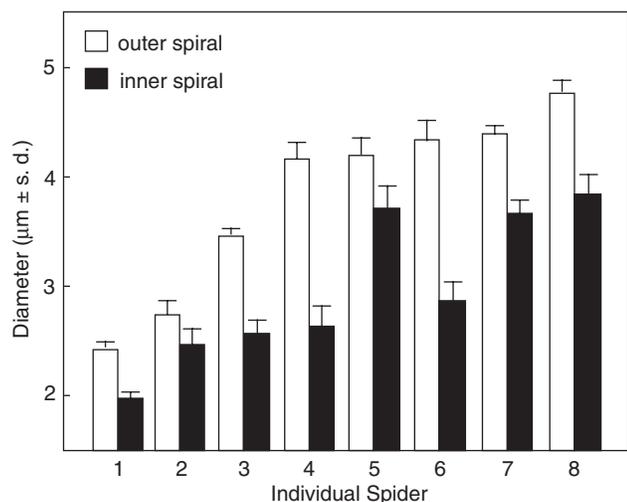
**Fig. 3.** Relationship between mean fiber diameters measured using scanning electron microscopy (SEM) and polarized light microscopy (PLM). Note that PLM tended to give slightly larger measurements than SEM.

#### Variation in fiber diameter within individual webs

We found that the diameters of different scaffolding threads in webs of *L. hesperus* can vary by as much as 100% within a single web (Fig. 4). Furthermore, the mean diameters of the scaffolding threads varied by  $\leq 600\%$  among the webs of the nine individuals of *L. hesperus* we examined. The nine spiders in Fig. 4 are arranged by weight, from lightest to heaviest, and silk diameter generally increases with spider weight. For all eight individuals of *A. argentata*, the diameters of flagelliform fibers from the outermost sticky spirals of webs were larger than the diameters of samples from the innermost sticky spirals (mean  $\pm$   $s\bar{x}$  =  $3.9 \pm 0.9 \mu\text{m}$  vs.  $3.0 \pm 0.9 \mu\text{m}$ , respectively; Fig. 5). There was also substantial variation between individuals. Even after averaging across all samples for each individual, the mean diameters of flagelliform threads differed by  $\leq 200\%$  across the eight spiders.



**Fig. 4.** Variation in diameter of silk threads from the scaffold region of cobwebs for nine western black widows (*Latrodectus hesperus*). Six to eight threads were sampled from each web. Individual spiders are arranged from lightest to greatest body weight, left to right. Spiders 7–9 were gravid females.



**Fig. 5.** Comparison of the diameter of the flagelliform core fibers from the outermost and innermost rows of the sticky spiral of orb webs for eight silver garden spiders (*Argiope argentata*). Data are averaged across three webs for each spider. From each web, four samples were measured from the outermost rows of capture silk and four additional samples were measured from the innermost rows of those same webs. Individuals are arranged from smallest to largest threads.

## Discussion

The difference between measurements of thread diameters made using PLM and SEM was small, even for relatively fine fibers such as aciniform wrapping silks (Fig. 2). Overall, diameters measured using PLM were  $0.10 \pm 0.07 \mu\text{m}$  (mean  $\pm$   $s\bar{x}$ ) larger than measurements made using SEM, but were similarly precise across repeated measurements of the same sample. Regression of PLM measurements upon SEM measurements (Fig. 3) suggested that PLM consistently measured fiber diameter  $0.16 \pm 0.13 \mu\text{m}$  (mean  $\pm$   $s\bar{x}$ ) larger than SEM, regardless of thread diameter. Together, these results suggest that PLM provides a valid alternative to SEM for the characterization of spider silk diameters.

Our data are remarkably similar to those obtained by Knight & Parsons (1985) in their comparison of the use of PLM and SEM to measure the diameters of fine glass fibers (0.1–2.0  $\mu\text{m}$  in diameter). Knight and Parsons found a highly correlated positive relationship ( $r^2 = 0.997$ ) that suggested that PLM gave slightly higher measurements than SEM (mean  $\pm$   $s\bar{x}$  of the  $y$  intercept was  $0.17 \pm 0.02 \mu\text{m}$  with a slope of mean  $\pm$   $s\bar{x} = 0.97 \pm 0.03$ , compared to a  $y$  intercept of  $0.16 \pm 0.13 \mu\text{m}$  and a slope of  $0.98 \pm 0.04$  for our study). This led Knight and Parsons to conclude that measurement differences between methods were largely independent of diameter and that they could use their regression relationship to correct PLM values to give SEM values. Following their logic, we have used our data to generate the following correction equation to produce equivalent SEM diameters from PLM measurements using our technique:

$$\begin{aligned} \text{estimated SEM diameter} \\ = \frac{\text{PLM diameter} - 0.16[\pm 0.13]}{0.98[\pm 0.04]} \mu\text{m} \end{aligned}$$

Interestingly, Knight and Parsons suggested that the linear relationship between PLM and SEM measurements was not only independent of diameter, but could be extended down to measurements of 0.1  $\mu\text{m}$ . This suggests that our technique may prove a viable way to measure the diameters of very fine silk fibers below the lower limit of 0.5  $\mu\text{m}$  that we studied here (e.g., Hayashi et al. 2004).

The question of how well PLM performs relative to SEM carries with it the implicit assumption that SEM measurements are correct. However, one clear methodological advantage of PLM is that it allows measurement of silks in their natural states, while SEM requires that fibers first be sputter coated and then measured in a vacuum. Most silks readily

absorb atmospheric water and are partially hydrated in their native state, which can affect their volume (Gosline et al. 1986; Vollrath 1999). This means that dehydration of silk fibers during preparation for SEM may shrink the fibers. This could explain why SEM measurements of silk fiber diameter are consistently smaller than PLM measurements.

We also found that SEM performed poorly when trying to measure sticky silk. Sticky silk consists of two core fibers of flagelliform silk coated with aqueous glue, and it was difficult to discern the flagelliform fibers within this sheath of glue when using SEM. In contrast, PLM clearly distinguished the flagelliform fibers from the aggregate glue once the sticky silk was adhered to a glass slide.

An important assumption for both SEM and PLM measurement of silk diameter is that the fibers are circular in cross section. While this is a standard assumption made in most studies on spider silk mechanics, at least some spiders with highly specialized silks spin fibers that have strongly elliptical cross sections (Eberhard 1980; Coddington et al. 2002). Furthermore, major ampullate fibers can be slightly elliptical ( $\sim 5\%$  anisotropy; Pérez-Rigueiro et al. 2001). This would increase the variability of diameter measurements as fibers are sometimes measured across the thinner and at other times across the thicker axis. However, such minor anisotropy likely has little effect on the estimation of cross-sectional areas for most spider silks. When the anisotropy is  $< 20\%$ , and several measurements of diameter made from different axes of rotation are averaged, then the cross-sectional areas of elliptical fibers can be closely approximated by using the average of those measurements as though it was the diameter of a circle (Dunaway et al. 1995).

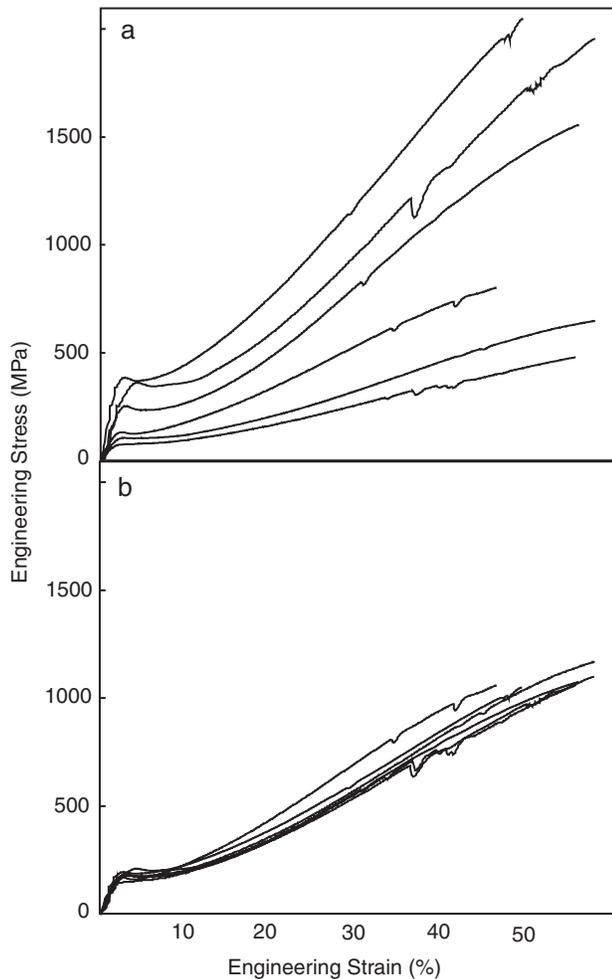
Because of its molecular nature, spider silk is an anisotropic crystal that has crystallographically distinct axes that interact with light in a manner dependent upon the orientation of the crystalline lattice with respect to incident light (Carmichael & Viney 1999; Carmichael et al. 1999). As a result, light is refracted into two rays, each of which polarized with the vibration directions oriented at right angles to one another. This phenomenon is known as birefringence, and without the proper use of polarized light the measurement of birefringent objects will be highly variable. Although the diameters of many silk fibers are well within the resolution limit of the light microscope ( $\sim 0.2 \mu\text{m}$  for visible light), their birefringent nature makes quantitative measurements of those diameters nearly impossible. Use of standard light microscopy results in higher-order interference fringes that make the fiber appear to have a larger

diameter than it actually has (Fig. 1). However, when using polarized light, it is possible to take advantage of the fiber's birefringence by aligning the electric field vector component along the axis of the fiber, thereby resulting in a reliable, repeatable measurement. Indeed, we have found that the use of PLM yields silk diameters that are quite similar to those obtained using SEM.

Techniques such as forcible silking of spiders under controlled conditions in the laboratory can yield multiple samples of threads that have fairly regular diameters. In these situations, SEM measurements of a few exemplar fibers could be used to infer an average diameter for the fibers that are mechanically tested, avoiding the need to independently measure each fiber tested. However, diameters of fibers can vary greatly even under these highly controlled conditions as a function of the speed at which fibers are drawn (Madsen et al. 1999), of anesthetization with  $\text{CO}_2$  (Madsen & Vollrath 2000), and of spider size or weight (Vollrath & Köhler 1996). In addition, the spigots of at least the major ampullate silk glands contain internal friction breaks that allow spiders to control the tension applied to silk fibers as they are extruded (Ortlepp & Gosline 2004). This means that spiders can also actively manipulate the mechanical properties and the diameters of fibers as silk is spun (Garrido et al. 2002).

Given this capacity for spiders to exercise active control over the diameters of silk fibers, PLM offers the advantage of measuring the fibers that are actually being mechanically tested. In contrast, preparation of samples for SEM renders silk unusable in mechanical testing such that researchers must measure the diameters of one set of fibers while physically testing a different set. This distinction is particularly important when working with silk fibers whose diameters vary dramatically from sample to sample, or even within the same web. For instance, we found that the diameters of fibers from cobwebs of *Latrodectus hesperus* can vary by as much as 100% within webs (Fig. 4), and that the outermost end of the sticky spirals in orb webs of *Argiope argentata* are consistently 30% thicker than the innermost ends, even when those differences are averaged across webs built by spiders under very different physiological conditions (Fig. 5).

Figure 6 illustrates how accounting for such variation in diameters among threads can greatly enhance the repeatability of individual mechanical tests. Figure 6a shows the results of stress-strain curves for six samples of scaffolding silk from a single cobweb of *L. hesperus* (individual 5 in Fig. 4) under the assumption that all samples have the same



**Fig. 6.** Controlling for sample-to-sample variation in the diameters of fibers tested can improve precision of mechanical testing. **a.** Stress–strain curves for six samples of scaffolding silk from a single cobweb of *Latrodectus hesperus* under the assumption that they all had similar diameters (the mean of the diameters for all six fibers measured using PLM). **b.** Stress–strain curves for those same six samples when stress values were scaled by the diameters measured for each fiber using PLM.

diameter as computed from the mean of the PLM measurements of all six threads. This approach is analogous to using SEM to measure a sample of focal fibers and then applying that diameter to the set of fibers being mechanically tested. The stress–strain curves do not resemble one another and range in their breaking stresses from values that approach the weakest spider silks ever measured ( $< 500$  MPa) to values that are 50% higher than the typical values for major ampullate silks. However, when stress is scaled relative to the individually measured diameters of each fiber (Fig. 6b), the curves become very consistent with one another, and breaking stress varies by only a

few MPa. This increase in precision allows new avenues of research, such as studying how individual spiders may manipulate the mechanical properties of silks, as well as increasing our ability to make fine-scale distinctions in the performance of different types of silks or between silks from different spiders.

The ability of spiders to manipulate the diameters of silk fibers and thereby alter mechanical performance of silk structures is clear (Vollrath & Köhler 1996; Garrido et al. 2002). Yet, such manipulation is rarely considered, although it could have an important influence on our interpretation of spider behavior and evolution. For instance, the increased diameter of draglines spun by spiders on vertical surfaces (Garrido et al. 2002) adds to the energetic costs of climbing, and energetic costs of movement along vertical surfaces have recently been suggested as an important factor in the evolution of sexual dimorphism in spiders (Moya-Laraño et al. 2002). Spiders increase the size of orb webs and capture spirals when starved, which has been interpreted as an increase in foraging effort (Sherman 1994). However, such starved spiders may be conserving energetic resources while still building larger webs if they used thinner fibers of silk, a hypothesis that has not previously been considered. Finally, active control of fiber diameter could affect how selection acts upon silk genes by allowing a change in the physical performance of silks without necessitating a change in the protein sequences used to construct those silk fibers.

Most types of spider silk cannot be readily obtained through forcible silking, but are still of interest to materials scientists. In these instances, it is necessary to sample flagelliform, tubuliform, and acini-form fibers from natural sources such as webs, prey wrapping attacks, and egg sacs. This may increase the likelihood that individual samples will vary in their diameters even when taken from the same web or structure. Yet, these silks may have novel mechanical characteristics (e.g., Hayashi et al. 2004). PLM can clearly provide an important means by which to reduce variability in the measurement of the mechanical properties of silk fibers by better controlling for variation in diameters. Furthermore, variation in fiber diameter is as much a trait of ecological interest to biologists as it is a technical challenge for mechanical analysis.

Many other experimental parameters can influence the precision and accuracy of the mechanical characterization of spider silk, including length of fibers tested, rate at which fibers are strained, temperature, and humidity (e.g., Denny 1976; Gosline et al. 1986; Madsen et al. 1999). However, experimental error in the measurement of fiber diameter can be particularly

influential because of its non-linear effect upon measurements of stress, which depends upon cross-sectional area. We suggest that the use of PLM can provide an important tool for the mechanical characterization of spider silks. By precisely controlling for variation in the diameters of different silk samples, PLM can enhance the repeatability of mechanical tests on fibers. This should increase our ability to make comparative distinctions between individual spiders as well as between different species, thereby facilitating exploration of the remarkable mechanical properties of a wide range of spider silks.

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