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Variation in the material properties of spider dragline silk across species

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ABSTRACT Spiders produce high performance fibers that compare favorably with the best manmade fibers in strength and toughness. The amino acid sequences of silk proteins have been determined for a number of silk types and species, revealing extensive variation. This variation in sequence is hypothesized to confer different material properties. However, the material properties of silk have been characterized from only a few ecologically similar species, even though spiders are extremely diverse. Using a Nano Bionix® tensile tester, we measured mechanical properties of one type of silk, the dragline, from a broad sample of spider species. These taxa included orb-weavers and representatives of other lineages of true spiders that do not spin aerial capture webs. We found that all of the species sampled produce high-performance dragline fibers, suggesting that the remarkable properties of dragline silk predate the origin of the aerial orb-web. However, we report significant variation in all of the material properties measured. Furthermore, material properties tend not to be correlated, implying that different properties may have been selected upon in different spider lineages. We suggest that the spectrum of dragline silk sequences and material properties that have been produced over evolutionary time provides a rich resource for the design of biomimetic silk fibers.

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1 Introduction

Dragline silk is a proteinaceous fiber used by spiders as a safety line and to construct webs [1]. These silks are synthesized in large, often ampulla-shaped, abdominal glands that are connected via thin ducts to spigots on the anterior lateral spinnerets [2, 3]. The dragline silks spun by orb-weaving spiders have been extensively researched because these silks exhibit both high strength and extensibility. Such combinations of properties result in fibers with toughness values greater than most other natural or even synthetic fibers [2, 4]. Because of the many potential military and industrial applications for strong, fracture-resistant fibers, it is important to

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have a detailed understanding of how spider silks are produced and the diversity of their mechanical behaviors [1, 2].

Spider silks are constructed from large proteins (fibroins) and the sequence of many of these proteins, from a variety of species, have been characterized from cDNA translations and a few peptide analyses [3, 5, 6]. These studies, along with the results from various imaging techniques, indicate that fibroins have repeated amino acid sequence motifs that form stiff crystalline structures embedded in an elastic matrix. Alanine and glycine rich motifs, common in most silks, fold into beta-sheets that are hypothesized to confer tensile strength. Other repeated motifs are predicted to form amorphous or helical, spring-like linking regions that allow extensibility [2, 7, 8]. An individual spider can produce a variety of silks and all spider silks examined thus far contain some of these types of motifs. However, different types of silk can exhibit extensive sequence variation, leading to differences in fiber properties [2, 9]. Even within a single type of silk, such as dragline silk, there is substantial sequence variation across species [3]. Lineages of spiders also vary greatly in their ecological uses of dragline silk and spinning behaviors. However, the extent to which there is significant variation in material performance of dragline silk across the Araneomorphae (true spiders) is largely untested.

Studies of silk material properties and molecular modeling have focused on a few, closely related and ecologically similar species. The broadest sampling is for dragline silk proteins, but even this has been largely restricted to the orb-weaving spiders from the Araneidae and the Tetragnathidae, with additional data from the closely related cobweb spinning black widow spiders in the Theridiidae [1, 2, 4, 9–12]. Yet, spiders are a diverse group with more than 37 000 species in over a 100 families, all of which use silk [13, 14]. Dragline silk is spun by all araneomorph spiders, most of which do not construct aerial orb-webs. Thus, it is possible that diverse ecological functions have led to selection for silks with different material properties [9, 14–17]. However, there have been few attempts to quantify differences in dragline silk material properties among species [16, 17].

Here we examine tensile material properties of dragline silk spun by seven species of phylogenetically and ecologically diverse spiders, and compare these properties to previous studies. The new data significantly add to the variety of species characterized and lay the foundation for examining

the interconnections between fibroin sequence, phylogeny, ecology and mechanical properties.

2 Experimental

2.1 Silk collection

Dragline silk was collected in several ways depending on the spider species. Our initial intention was to collect silk from all taxa using a single method. However, we found it prohibitively difficult to use one method because of species-specific differences in spinning behaviors. Thus, silk from *Nephila clavipes* (Tetragnathidae), *Argiope argentata* (Araneidae) and *Araneus gemmoides* (Araneidae) was collected by forcible silking, following techniques outlined in Blackledge et al. [18]. Silk from *Latrodectus hesperus* (Theridiidae) was collected from webs using techniques described in Blackledge et al. [18]. Silk from *Leucauge venusta* (Tetragnathidae) was collected by allowing spiders to lower themselves on a dragline from a raised platform; the dragline was then affixed to *C*-shaped cards covered with double-sided tape. *Kukulcania hibernalis* (Filistatidae) and *Plectreurys tristis* (Plectreuridae) did not spin webs suitable for in situ silk collection nor could they be forcibly silked or suspended from platforms. Instead, silk was collected from these species by allowing individuals to lay silk while walking in clean terrariums. Silk was then harvested from the terrarium floors onto *C*-shaped cards with double-sided tape. It has been noted that different silk collection techniques produce silks with different material properties [12, 18]. However, our data show no clear bias associated with these different silk collection methods (i.e., silks collected with similar methods do not show similar properties; see results).

2.2 Tensile testing

Testing was conducted using techniques described in detail by Blackledge et al. [18]. Briefly, silk was glued to the *C*-shaped cards with cyanoacrylate. The diameter of each silk sample was measured using polarized light microscopy [19]. Each silk card was then attached to the grips of a Nano Bionix® tensile tester (MTS, Oakridge, TN). The card was cut away so that the tester pulled only on the silk sample between the grips. The sample was extended with a constant cross head speed at a rate of 1% strain/s to failure. For each sample, ultimate stress was measured as both engineering and true stress at break (MPa), ultimate strain was measured as both engineering and true strain at break (mm $*$ mm^{-1} and ln mm $*$ mm^{-1}, respectively), toughness was calculated as the area under the stress-strain curve (MJm^{-3}), and Young's modulus was measured as the initial slope of the stress-strain curve (GPa). Throughout the test, the sample was also oscillated at 20 Hz for continuous dynamic analysis. Continuous dynamic analysis assesses the instantaneous storage modulus, loss modulus and tan ∂ throughout the test by measuring the response of the sample to a sinusoidal oscillation as a function of strain [18]. This allowed calculation of a maximum tan ∂ value as an estimate of how well the fiber dissipates energy, and the amount of strain hardening of the fibers, which was calculated as the increase in storage modulus with increasing strain. Multivariate analysis of variance (MANOVA) was used to test for multivariate differences among species using the variables described above. ANOVAs and student's *t*-tests were used post hoc to identify which variables differed and to make pair-wise comparisons among species. We qualitatively compared our data to published dragline silk values for *Nephila clavipes*, *Nephila edulis*, *Araneus diadematus*, *Latrodectus hesperus* and *Argiope trifasciata* [2, 9–12].

3 Results

The qualitative patterns of the tensile behaviors were the same for all species (Fig. 1). A stiff, initial, elastic region was followed by a yield region (decrease in the slope of the stress–strain curve); then further stiffening until failure. Previous continuous dynamic analysis of the dragline silk of black widows demonstrated that both storage modulus and tan ∂ varied continuously as fibers were strained [18]. The general pattern of dynamic behavior was also similar across species. Storage modulus remained constant, at approximately the calculated Young's modulus, through the elastic region, then increased until the fiber failed. Tan ∂ increased rapidly to a maximum at the yield point, then slowly decreased through the rest of the test.

Despite these qualitative similarities, the details of the tensile behaviors varied across species (Fig. 1) and there were significant differences among species in material properties (MANOVA, Wilks' Lambda$_{(24/196)} = 0.0283$, $p < 0.05$). Post hoc ANOVA tests indicated that all of the material properties measured varied significantly among species (ultimate stress, $F_{(6)} = 17.93$, $p < 0.05$, Fig. 2a; ultimate strain, $F_{(6)} = 18.47$, $p < 0.05$, Fig. 2b; Young's modulus, $F_{(6)} = 24.25$, $p < 0.05$, Fig. 2c; toughness, $F_{(6)} = 10.99$, $p < 0.05$, Fig. 2d; maximum tan ∂, $F_{(6)} = 5.74$, $p < 0.05$, Fig. 2e; strain hardening, $F_{(6)} = 9.94$, $p < 0.05$, Fig. 2f). Pair-wise comparisons of species for each variable indicated complex and shifting groupings,
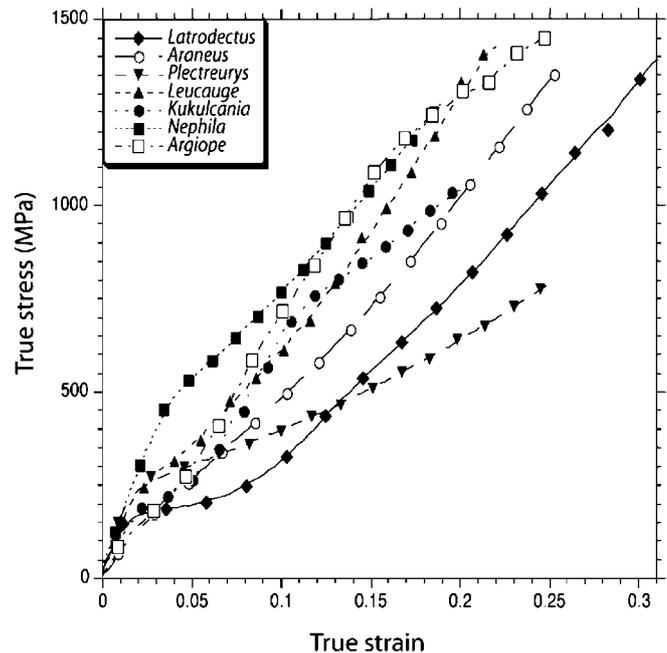
FIGURE 1   Representative stress–strain curves that approximate the mean behavior of all samples for each species
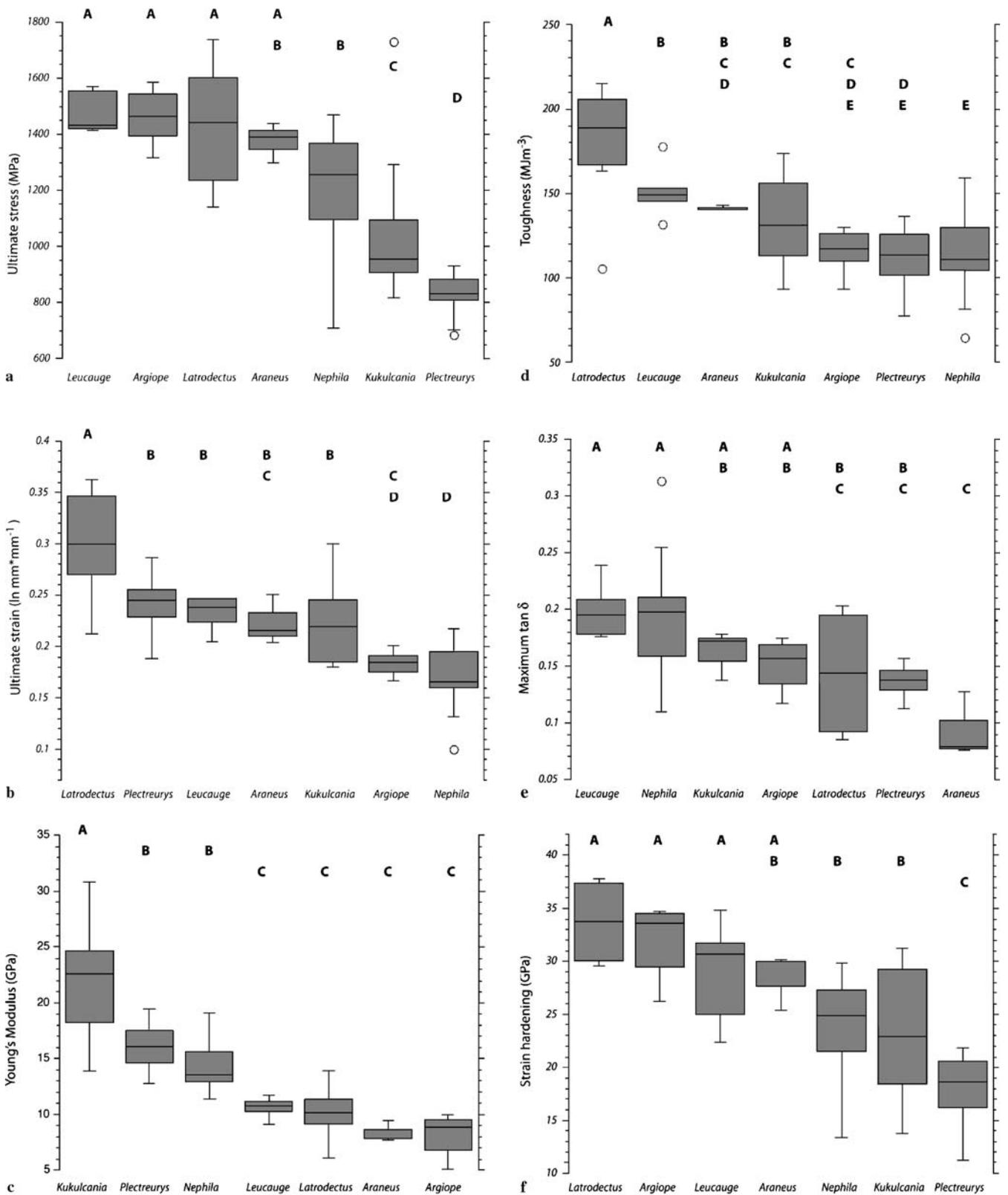
FIGURE 2 Box plots comparing tensile behavior across species. *Lines* denote the median, *boxes* enclose the upper and lower quartiles, and *whiskers* enclose the range. *Circles* are outliers (a value greater than 1.5 times the interquartile distance from the upper or lower quartile) that are included in the calculations. Species not connected by the same letter are significantly different at $p < 0.05$. Species in each graph are in descending order of mean values. (**a**) Ultimate true stress (MPa), (**b**) ultimate strain (ln mm mm^{-1}), (**c**) Young's modulus (GPa), (**d**) toughness (MJm^{-3}), (**e**) maximum tan ∂, (**f**) strain hardening (GPa)
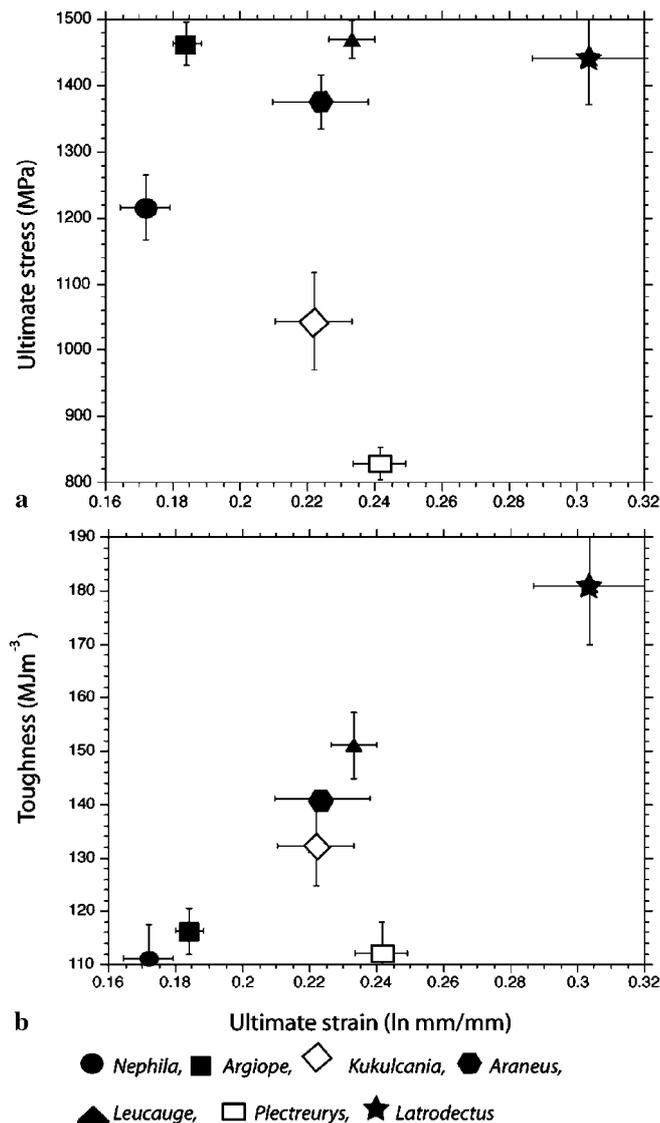
FIGURE 3 (**a**) Scatter plot of ultimate stress and ultimate strain (Mean ±1 S.E.M.) for each species and (**b**) scatter plot of ultimate strain and toughness (Mean ±1 S.E.M.) for each species. Species in the Haplogynae lineage are represented by *open shapes* and spiders from the Araneoidea lineage are represented by *solid shapes*

depending on the variable in question (Fig. 2). *Kukulcania* silk had the highest initial stiffness, more than double that of *Araneus* (Fig. 2c). Toughness was highest for *Latrodectus* silk (Fig. 2d). *Nephila* and *Leucauge* silks had the highest tan ∂ values (Fig. 2e). The directionality of the observed variation suggests that silk collection method could not have been largely responsible for the differences in properties. Forcible silking tends to increase stiffness and strength but decrease toughness and extensibility [18, 20]. Yet in our results, some forcibly silked orb-weaver (*Araneus gemmoides* and *Argiope argentata*) silks exhibited lower stiffness than silks of other taxa. *Araneus gemmoides* silk was also intermediate in extensibility and toughness compared to the other silks. While the silk collection method probably did affect the values of the material properties observed, the predicted influence should have minimized differences among species.

Most material properties were not correlated. For example, there was no relationship between ultimate stress and ultimate strain (Fig. 3a). An exception was toughness and ultimate strain, which demonstrated a strong positive correlation (Fig. 3b).

The visco-elastic nature of spider silk dictates that testing protocol impacts results. Therefore, although it is interesting to compare across studies, caution should be exercised when making quantitative comparisons across studies that utilized different testing methods. However, our values generally fell within the range of reported properties for *Nephila*, *Araneus*, *Argiope* and *Latrodectus* silks reported in other studies (Table 1) [2, 9–12].

4   Discussion

4.1   *Variability in material properties of dragline silk*

All of the species studied thus far have dragline silk properties that fall within the same order of magnitude and are within the range of values reported in other studies (Table 1). The strength and toughness of the silk spun by these species makes dragline silk among the most impressive natural fibers [2]. Yet, we find that dragline material differs

	Young's modulus (GPa)		Ultimate engineering stress (GPa)		Ultimate engineering strain (mm/mm)		Toughness (MJ/m³)	
	This study	Pub.	This study	Pub.	This study	Pub.	This study	Pub.
Araneus N = 3, n = 23	8.3 ± 0.54	4–10	1.06 ± 0.005	0.86–1.4	0.29 ± 0.024	0.27–0.39	141.2 ± 0.77	131–160
Argiope N = 8, n = 62	8.2 ± 0.63	6.9–11	1.2 ± 0.003	0.6 –1.2	0.23 ± 0.006	0.18–0.3	116.3 ± 4.39	90
Nephila N = 17, n = 66	13.8 ± 0.76	7.38–22	1 ± 0.004	1–1.3	0.2 ± 0.011	0.12–0.37	111.2 ± 6.41	80
Latrodectus N = 9, n = 70	10.2 ± 0.75	6	1 ± 0.005	1.1	0.45 ± 0.034	0.22	180.9 ± 11.19	
Leucauge N = 6, n = 61	10.6 ± 0.35		1.2 ± 0.002		0.27 ± 0.013		151 ± 6.18	
Plectreurys N = 11, n = 108	16.1 ± 0.64		0.64 ± 0.002		0.29 ± 0.009		112.1 ± 5.77	
Kukulcania N = 12, n = 102	22.2 ± 1.52		0.83 ± 0.006		0.26 ± 0.014		132.2 ± 7.53	

TABLE 1 A comparison of values from this study (Means ±1 S.E.M.) with published (Pub.) values for the range of material properties of spider dragline silk. (*N*) denotes the number of individuals and (*n*) is the total number of samples used in this study. Published comparative data are mean values from studies on *Araneus diadematus* [1, 2, 10], *Argiope trifasciata* [7, 12], *Nephila clavipes* [9], *Nephila edulis* [16], and *Latrodectus hesperus* [11]

significantly and substantially across spider species in several important properties, including stiffness, ultimate stress, ultimate strain, and strain hardening (Fig. 2).

In previous studies, substantial intra- and inter-individual variability, along with different methodologies, have been obstacles to comparing silks across species [7]. In this study, by using a single testing protocol, we were able to detect a signal through the inherent variation and make comparisons among fiber attributes across species. To explore the effect of sample size on our conclusions, we sub-sampled the data and re-ran the analyses with two individuals per species (not shown). The sub-sample had the same general patterns as the full data set in the MANOVA and ANOVA results, but many of the relatively small differences between species were not detected in the post hoc tests. Thus, although small samples are sufficient to find differences among species and elucidate the general pattern, larger sample sizes are required to resolve the details of variation across species.

4.2 Sampling of diverse spiders silks

Kukulcania and *Plectreurys*, from the Haplogynae lineage of spiders, have dragline silks with higher initial stiffness and lower strength than dragline silks spun by the distantly related orb and cobweb weavers (members of the Araneoidea lineage). This contrast could be due to selective pressures resulting from differences in silk use and spider ecology [15]. While *Kukulcania* and *Plectreurys* use silk for draglines, tangled sheet webs and protective retreats, they do not spin aerial nets like orb weavers. Haplogyne and araneoid spiders also appear to have different fibroin sequences. *Kukulcania* and *Plectreurys* lack the glycine-proline-glycine repeats that are prevalent in the orb weaver dragline silk protein, MaSp2 [3, 5, 6, 21]. Hayashi et al. [22] suggested that the frequency of different sequence motifs might affect the material properties of the fiber because of secondary and tertiary structures formed by the motifs. Data presented here suggest that although dragline silk properties are consistent within an order of magnitude across species, the significant differences in fiber properties may be associated with variation in protein sequences. Although this sampling of spider species is not large enough to allow correlations between specific amino acid motifs and fiber performance, we concur with other authors that variation in sequences and mechanical performance are probably linked [2, 16, 22].

Although we observe significant variation in dragline material properties among species, it must be emphasized that all of the sampled spiders produce mechanically impressive fibers. This means that molecular models in addition to those developed for orb weaver dragline silk may be needed to explain the tensile behavior of silks spun by phylogenetically divergent species. It has been suggested that lepidopterans construct fibers with similar properties from heavy-chain (H) fibroins that have dissimilar sequences by using alternative molecular architectures [23]. For example, the H-fibroin of *Bombyx mori*, the domesticated silkworm, has short repeated amino acid sequence motifs with frequent runs of poly-alanine (A_n) that aggregate to form beta-sheet crystalline structures. Other moths, such as

Galleria mellonella, have longer, highly conserved amino acid repeats, which align to form larger crystals. Although the sequences of these fibroin types are very different, the silk fibers spun by these moths are both very strong and tough [23]. Similarly, we find that spiders seem to produce strong and tough dragline fibers using a variety of amino acid sequences.

Material properties of dragline silk are not only variable, but tend not to be correlated. For instance, species with high breaking strengths do not necessarily have high breaking strains (Fig. 3a). This suggests that some fiber properties may be associated with portions of the protein that have evolved independently in different spider lineages [24]. An exception to this observation is the correlation between ultimate strain and toughness (Fig. 3b). Increased strain greatly increases the total energy required to break the fiber. Hence, changes in ultimate strain are likely to have the most consistent effect on toughness.

Sampling of distantly related species with different silk uses also lets us address questions about the evolution of high performance fibers in spiders. It has been suggested that high performance fibers are an adaptation for building the familiar, aerial orb-web of araneoid spiders [15, 16, 25]. These aerial traps must withstand impacts and dissipate the kinetic energy of large, high-velocity insects. However, we find that non-araneoid spiders also have high performance dragline fibers, implying that selection for strong, tough fibers predates the evolution of the orb-web. Instead, fibers with high strength and toughness may have evolved for use in other web types, or to act as a safety line [4, 18, 26, 27].

4.3 Applications of variation in silk material properties

The potential applications of spider silks and silk inspired man-made fibers are diverse [2]. The toughness and strength of these fibers make them especially promising as kinetic energy absorbers in anti-ballistic applications (e.g., bullet proof vests). The variability in material properties observed among different dragline silks, and the independence of many of these characteristics, suggests that it is beneficial to survey the variation in silk properties on both large and small scales. With this information, it will be possible to select a particular silk spun by a particular spider to serve as the template for a biomimetic fiber optimized for a precise application. For instance, if stiffness were important, a silk similar to that produced by *Kukulcania* would be the best. However, if toughness is the most important characteristic, a silk produced by *Latrodectus* should be mimicked. It should also be possible to tailor dynamic properties. For instance, the silk from the tetragnathid spiders (*Nephila* and *Leucauge*) dissipate a larger portion of the input energy through viscous flow (higher maximum tan ∂) than other species. Variation in strain hardening suggests that it is possible to tailor both pre- and post-yield behavior. Most spider silk research has focused on fibers from two species of spider, *Araneus diadematus* and *Nephila clavipes*, because they are large, abundant, and produce thick, mechanically strong fibers [1, 5, 9]. However, other species may spin silks with more attractive material properties for biomimetic applications.

5   Conclusions

All of the spiders sampled produce high-performance dragline fibers. However, the material properties of dragline silk are not homogeneous across spiders, but vary in a complex manner. Therefore, studies of silk from only a few ecologically and evolutionarily similar species cannot adequately characterize the range of attributes of spider silk. The variability observed in both sequence and material properties will provide an opportunity to apply these differences to the design of high-technology fibers and biomimetic silks. Finally, we must emphasize the need for continued comparative research to elucidate the interactions and relative effects of different fibroin sequences, and different spinning characteristics and behaviors in producing these impressive fibers.

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