

Spider Capture Silk: Performance Implications of Variation in an Exceptional Biomaterial

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ABSTRACT Spiders and their silk are an excellent system for connecting the properties of biological materials to organismal ecology. Orb-weaving spiders spin sticky capture threads that are moderately strong but exceptionally extensible, resulting in fibers that can absorb remarkable amounts of energy. These tough fibers are thought to be adapted for arresting flying insects. Using tensile testing, we ask whether patterns can be discerned in the evolution of silk material properties and the ecological uses of spider capture fibers. Here, we present a large comparative data set that allows examination of capture silk properties across orb-weaving spider species. We find that material properties vary greatly across species. Notably, extensibility, strength, and toughness all vary approximately sixfold across species. These material differences, along with variation in fiber size, dictate that the mechanical performance of capture threads, the energy and force required to break fibers, varies by more than an order of magnitude across species. Furthermore, some material and mechanical properties are evolutionarily correlated. For example, species that spin small diameter fibers tend to have tougher silk, suggesting compensation to maintain breaking energy. There is also a negative correlation between strength and extensibility across species, indicating a potential evolutionary trade-off. The different properties of these capture silks should lead to differences in the performance of orb webs during prey capture and help to define feeding niches in spiders. *J. Exp. Zool.* 307A:654–666, 2007. © 2007 Wiley-Liss, Inc.

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Organismal morphology can determine feeding performance and therefore ecological niche. For instance, jaw structure determines available food resources in fish (Wainwright, '88) and tooth morphology correlates with feeding niche in mammals (Gunnell et al., '95). However, less is known about how the properties of biological materials affect the ecology and evolution of species (Summers and Koob, 2002; Opell and Bond, 2001; Erickson et al., 2002; Podos and Hendry, 2006). Some biological materials, such as vertebrate bone, have remarkably conserved material properties even across taxonomic classes (Erickson et al., 2002), whereas others, such as vertebrate tendon, show properties that vary greatly across those same groups (Summers and Koob, 2002). The properties of biological materials used to build structures can be as important to performance, and therefore ecology, as the morphology of those same structures. Spiders

(Araneae) use silk throughout their lives for a variety of essential functions, including prey capture, reproduction and predator avoidance (Stauffer et al., '94; Foelix, '96; Gosline et al., '99). This tight association between silk and key tasks that affect spider fitness dictates that variation in silk performance has both evolutionary and ecological impacts (Craig, '87, '92; Madsen et al., '99; Opell and Bond, 2000, 2001; Swanson et al., 2006a,b).

An individual spider can produce a variety of different silk fibers that are used for diverse

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functions. Each silk type is composed of its own distinctive suite of proteins and has unique material properties (Stauffer et al., '94; Guerette et al., '96; Gosline et al., '99; Blackledge and Hayashi, 2006a). Much of the research on spider silk has focused on only one type of silk, the dragline, which is stiff, strong, and tougher than almost any synthetic or natural fiber (Gosline et al., '86; Pérez-Rigueiro et al., 2001). However, dragline silk is not the only high-performance fiber produced by spiders (Denny, '76; Stauffer et al., '94; Blackledge and Hayashi, 2006a).

The Orbiculariae, encompassing the Araneoidea and Deinopoidea superfamilies, is a large clade of ecologically important orb-web weaving spider species. Multiple lines of evidence (morphological, behavioral, genetic, and fossil) support the hypothesis that the orb-web architecture evolved once in this clade in an ancient orbicularian ancestor (Griswold et al., '99; Gatesy et al., 2001; Garb et al., 2006; Peñalver et al., 2006). The subsequent radiation of orb-weaving spiders is associated with their target prey, flying insects, the most diverse animals on the planet (Dudley, 2000; Opell and Bond, 2001). Orb webs (Fig. 1) function as aerial snares that intercept flying prey, adhere to the insects, and dissipate their kinetic energy (Denny, '76; Eberhard, '90). Prey capture by orb webs depends on a complex suite of variable characteristics, including web architecture, web location, web stickiness, attack behaviors of spiders, and possibly material properties of the component silks (Craig, '87; Miyashita, '97; Opell and Bond, 2001).

A key component of orb webs is the sticky, extensible, capture spiral that is constructed from a pair of core silk fibers (from the flagelliform or pseudoflagelliform glands) that is coated with either aqueous glue from the aggregate glands in the Araneoidea, or a sticky matrix of dry cribellar microfibrils in the Deinopoidea (Coddington, '89; Hayashi and Lewis, '98; Blackledge and Hayashi, 2006b). Capture threads act like rubber, being orders of magnitude more compliant than other types of spider silk, and are able to extend more than 500% of their resting length before breaking (Denny, '76; Vollrath and Edmonds, '89; Gosline et al., '99). In fact, with their combination of extreme extensibility and moderate strength, capture silks have toughness (energy required to break the material) similar to that of the renowned dragline silks, which in contrast have moderate extensibility and extreme strength. Thus, capture silks, like dragline silks, are tougher than nearly all



Fig. 1. Image of an orb web from *Cyclosa simplicicauda*. The radial threads and frame material are constructed from dragline silk. The spiral is constructed from capture threads from the flagelliform glands. Inset is a $1,000\times$ polarized light micrograph. The central flagelliform silk is coated with droplets of viscid, aggregate gland secretions.

natural and man-made materials (Gosline et al., '99, 2002).

The impressive mechanical properties of capture threads (i.e. extensibility and toughness) have been attributed to the complex secondary and tertiary structures of their constituent silk proteins (fibroins). Flagelliform proteins include numerous tandem repeats of small, relatively simple amino acid sequence motifs (e.g. GPGG(X)_n, where X indicates one of a small subset of possible amino acid residues). Protein structural modeling suggests that these amino acid sequences produce series of consecutive β -turns that form winding, helical structures ("nanosprings"; Becker et al., 2003). These nanosprings possess sacrificial hydrogen bonds that rupture as the fibroins are stretched. Thus, the sacrificial bonds absorb energy while the

strong polypeptide backbones are preserved. This nanospring structure is thought to impart the exceptional extensibility, strength, and toughness seen in capture fibers (Hayashi and Lewis, '98; Hayashi et al., '99; Becker et al., 2003). Recent analyses have found similar structural elements in the homologous silk proteins comprising pseudoflagelliform silk in the Deinopoidea (Garb et al., 2006).

All of the capture silk proteins sequenced thus far contain similar types of amino acid motifs that are organized in tandem arrays (Guerette et al., '96; Hayashi and Lewis, 2000; Gatesy et al., 2001; Garb et al., 2006). However, the details of the sequence and the number of repeated units vary across species (Hayashi and Lewis, 2000). Additionally, several studies suggest that there is variation in the performance properties of capture spiral silk across species (Craig, '87; Bond and Opell, '98; Opell and Bond, 2000, 2001). Finally, according to extensive research on spider foraging ecology, the webs of different spider species capture different sets of insect prey (Robinson and Robinson, '70; Howell and Ellender, '84; Craig, '87; Uetz and Hartsock, '87; Bishop and Connolly, '92; Miyashita, '97; McReynolds, 2000). It is largely unknown how fibroin sequence variation might affect the material properties of capture silks and how silk properties might be adapted for capturing different types of insects.

Here, we sample silks from species across the orbicularian clade to address several questions regarding capture spiral fibers. First, we ask whether material and mechanical properties vary significantly across species. Second, we ask whether these properties are evolutionarily correlated. Third, we ask whether observed variation in material or mechanical properties could contribute to differences in prey capture performance and therefore the foraging ecology of spiders.

METHODS

Phylogenetic sampling

Thirteen spider species were chosen to span the phylogeny of orb-weaving spiders in the Orbicularia (Scharff and Coddington, '97; Coddington et al., 2004). The species sampled were *Argiope argentata* (number of individuals (N) = 6, San Diego Co., CA), *Argiope aurantia* (N = 4, Riverside Co., CA), *Araneus diadematus* (N = 4, San Diego Co., CA), *Cyclosa turbinata* (N = 7, Riverside Co., CA), *Deinopis spinosa* (N = 5, Alachua Co., FL), *Hyptiotes cavatus* (N = 3, Alachua Co., FL), *Hyptiotes gertschi* (N = 2, Riverside Co., CA), *Leucauge*

venusta (N = 8, Broward Co., FL), *Metepeira grandiosa* (N = 7, Riverside Co., CA), *Micrathena sagittata* (N = 5, Alachua Co., FL), *Neoscona oaxacensis* (N = 5, Riverside Co., CA), *Nephila clavipes* (N = 8, Alachua Co., FL), and *Uloborus diversus* (N = 15, Riverside Co., CA). Numbers of individuals and silk samples for each species are summarized in Table 1.

Tensile testing

Spiders were housed in the lab at approximately 23°C in individual, large enclosures that allowed the construction of orb webs. Spiders were misted with water and fed crickets. Tensile testing was conducted using techniques based on those described in detail in Blackledge et al. (2005c). Briefly, silk was collected from freshly spun orb webs onto c-shaped cards and glued to those cards using Elmer's™ white glue (Elmer's Products, Inc., Columbus, OH). This differs from many studies on other spider silks, which typically use cyanoacrylate adhesives, because nonwater based glues can dehydrate capture silk. For each individual web, four to 12 silk samples were collected at native tension from different parts of the web. Because capture silk fibers are coated with either a glue-like secretion (araneoid aggregate silk) or puffy fibrils (deinopoid cribellate silk), it was difficult to measure the diameter of the actual silk samples that were used in tensile testing. Instead, for each silk sample, its diameter was estimated by mounting an adjacent silk sample onto a microscope slide and measuring the fibers on the slide using polarized light microscopy (Blackledge et al., 2005a). Flagelliform fibers can be elliptical in cross-section (Pérez-Rigueiro et al., 2001), however our technique of taking multiple measurements from multiple photographs allowed us to control for this effect by averaging diameters across nine separate measurements (Blackledge et al., 2005b).

Each silk card was then attached to the grips of a Nano Bionix tensile tester (MTS, Oakridge, TN). Most of the card was cut away so that the tester pulled only on the silk sample between the grips. Capture threads are under tension in the web (Blackledge et al., 2005b), therefore gage length would be overestimated if we used the in web length of the fibers. Some researchers have used this "in web" length as the gage length for their studies because it reflects the conditions under which the silk functions in webs (Opell and Bond, 2000). However, we wanted to maximize the comparability of our data with past and future

TABLE 1. Material properties of capture spiral fibers from orb-weaving spiders

Species	<i>N, n</i>	Strain at break (ln(mm/mm))	SD strain	Stress at break (MPa)	SD stress	Toughness (MJ/m ³)	SD toughness
<i>Argiope argentata</i>	6, 50	1.63	0.13	362.74	120.73	50	16.00
<i>Argiope aurantia</i>	4, 41	1.58	0.53	389.21	134.97	65	23.26
<i>Araneus diadematus</i>	4, 33	1.53	0.47	501.48	227.12	80	30.11
<i>Cyclosa turbinata</i>	7, 44	1.68	0.36	157.59	68.99	26	14.88
<i>Deinopis spinosa</i>	5, 27	0.72	0.33	403.62	144.98	92	43.08
<i>Hyptiotes cavatus</i>	3, 22	0.43	0.06	1439.97	25.38	206	23.21
<i>Hyptiotes gertschi</i>	2, 24	0.40	0.00	1171.50	320.46	121	24.32
<i>Leucauge venusta</i>	8, 66	0.62	0.21	511.03	246.15	85	44.54
<i>Metepeira grandiosa</i>	7, 50	1.32	0.15	365.28	119.97	50	20.41
<i>Micrathena sagittata</i>	5, 37	1.97	0.28	510.92	211.23	111	37.63
<i>Neoscona oaxacensis</i>	5, 49	1.39	0.19	510.83	91.04	92	17.14
<i>Nephila clavipes</i>	8, 45	1.82	0.49	142.28	56.81	27	11.10
<i>Uloborus diversus</i>	15, 103	0.42	0.14	1174.55	477.77	103	41.49

N, number of individuals used from each species; *n*, total number of silk samples tested from each species. Standard deviations (SD) are calculated based on the means of individual spiders.

research on spider silk mechanics and attempted to measure the actual gage length of the fibers from slack. It was also impossible to simply measure the lengths of fibers once they were shortened enough to become slacked because the sticky droplets of glue around the flagelliform fiber can act as windlasses that “reel in” slack (Vollrath and Edmonds, '89). Thus, flagelliform fibers would seem to still be under tension long after they were slacked, thereby underestimating gage length. We therefore calculated the gage length of flagelliform samples post-test using the force-extension data. First, we determined the extension at which stress was first detected within the fiber (i.e. the length of the thread when it was first tensed). Then we calculated the difference between that extension and the extension at the thread's original length in the web. Finally, we subtracted the difference in lengths (difference in extensions) from the in web length of the fiber to calculate the “true” gage length. Therefore, our gage length was the fiber length at which we first observed measurable stress.

The samples were extended at a constant rate of 1% strain/sec to failure. For all calculations, each gage length was adjusted (decreased) so that zero strain corresponded to the point where stress began to increase as explained above. Diameter was adjusted (increased) based on this new gage length, assuming constant volume (Guinea et al., 2006). For each sample, true stress at break (strength in MPa), true strain at break (extensibility in ln(mm × mm⁻¹)), energy to break divided by volume (toughness in MJm⁻³), and Young's modulus (stiffness in GPa) were calculated. The

unscaled force needed to break the fiber (not standardized by cross-sectional area), and the unscaled energy required to break the fiber for a standardized 10 mm length (not standardized by volume) were also recorded. Although the evolution of silk proteins is best characterized through material properties such as stress and toughness, it is the mechanical performance of threads that ultimately determines how silk functions in webs. In other words, spiders can improve the performance of webs either by spinning threads from fibroins with stronger material properties or by increasing the diameters of threads. Thus, both types of measurements are important to assess. The testing environment ranged from 22.5 to 24.4°C with 30–55% relative humidity.

The capture threads spun by spiders in the Deinopoidea function as composite structures. Initially, tensile performance is dominated by the extension of the pseudoflagelliform core fibers, but much of the work carried out by the capture threads occurs after the rupture of the core fibers, as the surrounding sticky cribellar fibrils continue to stretch (Blackledge and Hayashi, 2006b). Because the pseudoflagelliform core fibers, not the cribellar fibrils, are the homologues of the flagelliform fibers of araneids, we only present data from the initial extension of the fibers until the core fibers ruptured for *Deinopis*, *Hyptiotes*, and *Uloborus*.

Statistics

Multivariate analysis of variance was carried out to test for multivariate differences among species

using the variables described above. Analysis of variance was used post hoc to identify which variables differed among species. All statistics were conducted in JMP IN v. 5.1 (SAS Institute Inc., Cary, NC). Phylogenetically independent contrasts were calculated using the PDAP:PDTree module in Mesquite to assess correlations between variables (Maddison and Maddison, 2004; Midford et al., 2005). Several sets of arbitrary branch lengths (branch length transformations) were assigned to the data with the PDAP:PDTree module of Mesquite. These included all branch lengths = 1, Grafen's arbitrary (Grafen, '89), Pagel's arbitrary (Pagel, '92), and Nee's arbitrary (Purvis, '95). The assignment of different arbitrary branch lengths is a standard procedure in calculating independent contrasts. This allows an empirical determination of the statistical adequacy of a given set of branch lengths in removing the effect of phylogenetic signal and is analogous to transforming data to avoid violating the assumptions of statistical tests (see Garland et al., '92 for a detailed discussion of this procedure). Nee's arbitrary branch lengths were used in further analyses for all traits because this minimized the relationship between the absolute values of the calculated contrasts and their standard deviations, removing the effect of phylogenetic relatedness in the data (Garland et al., '92). Pearson's product moment correlation (constrained to pass through the origin) was used to identify associations among the calculated scaled contrasts (Felsenstein, '85).

RESULTS

We found that the tensile properties of spider capture silk can vary over sixfold across species (Tables 1 and 2; Fig. 2). The multivariate analysis of variance revealed multivariate differences among silks from different species (Wilk's $\lambda_{(81/267)} = 0.0002313$, $P < 0.05$). In the post hoc analyses of variance, all of the silk variables, except stiffness (data not shown), differed significantly among species [true stress: $F_{(12)} = 6.14$, $P < 0.05$ (Fig. 3A); true strain: $F_{(12)} = 12.2$, $P < 0.05$ (Fig. 3B); stiffness: $F_{(12)} = 0.994$, $P = 0.46$; toughness: $F_{(12)} = 7.09$, $P < 0.05$ (Fig. 3C); diameter: $F_{(12)} = 34.22$, $P < 0.05$ (Fig. 4A); breaking force: $F_{(12)} = 7.00$, $P < 0.05$ (Fig. 4B); breaking energy: $F_{(12)} = 6.25$, $P < 0.05$ (Fig. 4C)]. *H. cavatus* silk had the highest ultimate stresses, whereas *Nephila* and *Cyclosa* silks had the lowest values. Silks produced by congeneric *H. cavatus* and *H. gertschi*

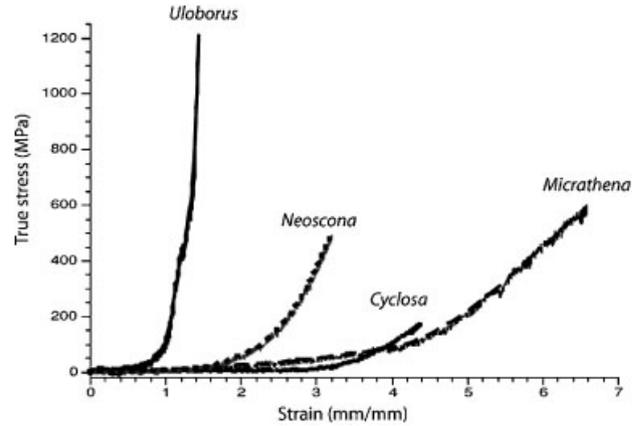


Fig. 2. Example stress strain curves for capture silk from different species. Species were chosen to illustrate extreme differences in tensile behavior. Each trace represents a single tensile test that approximates the mean silk behavior for the species.

along with *Micrathena* had the highest toughness, whereas silks produced by *Nephila* and *Cyclosa* once again had the lowest values (Table 1; Fig. 3). The mechanical properties measuring the performance of whole fibers (i.e. not normalized by fiber size) were even more variable, with energy and force required to break a fiber varying by more than an order of magnitude across species. In this case, the largest diameter fibers, produced by *A. argentata* and *Nephila*, required the most energy and force to break them (Table 2; Fig. 4).

Correlations between standardized independent contrasts revealed significant associations between many of the measured variables (Table 3). For instance, contrasts in diameter were positively correlated with strain at break, whole fiber breaking force, and whole fiber breaking energy, whereas diameter was negatively correlated with both toughness and true stress at break (Fig. 5; Table 3). There were also significant correlations between some of the material properties (Table 3). For instance, contrasts in true breaking stress were positively correlated with toughness and contrasts in breaking strain and breaking stress were inversely correlated (Fig. 6; Table 3).

DISCUSSION

Variability in properties

Although this study presents the largest comparative data set to date on capture silks (with 79 individuals and 591 silk samples tested), it includes only 13 of the over 3,000 orb-weaving species in the Orbiculariae (Coddington et al.,

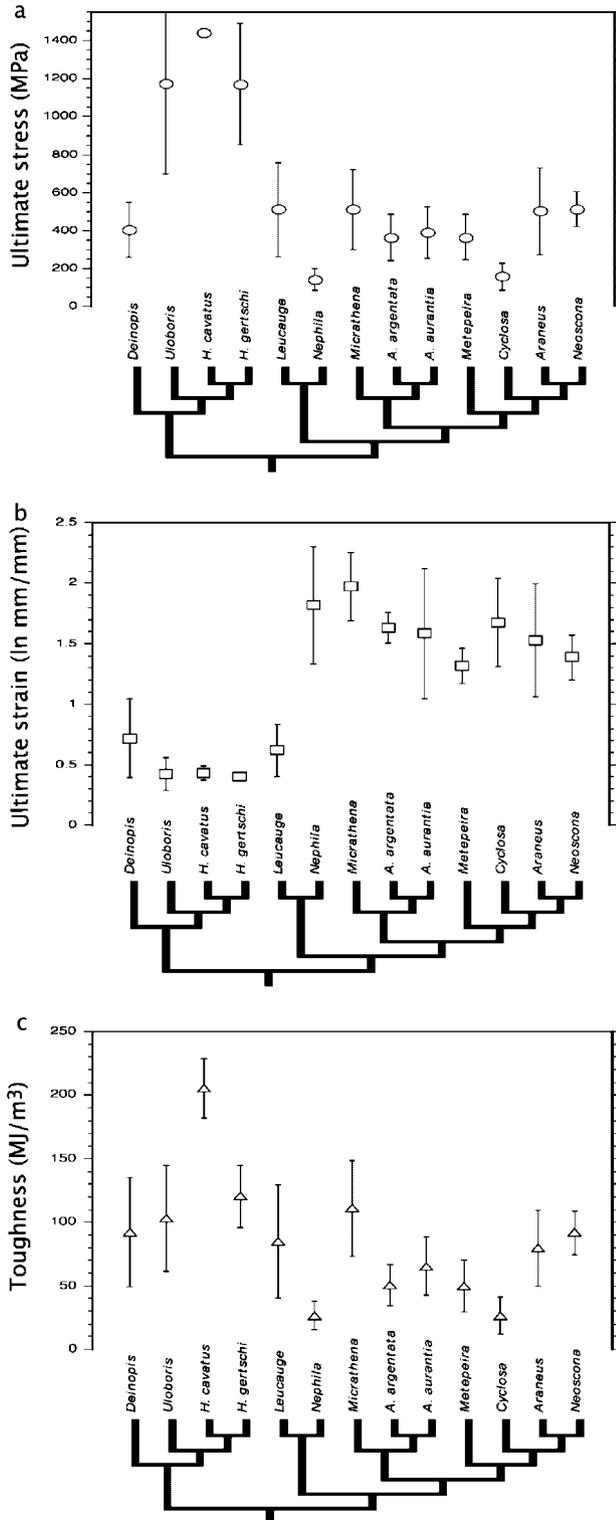


Fig. 3. Material properties of capture silk for each species (mean \pm 1SD). Deviations are small enough to be hidden by the symbols if not shown. Tree topology is drawn from Scharff and Coddington ('97) and branch lengths are arbitrary. (a) Ultimate stress, (b) ultimate strain, and (c) toughness.

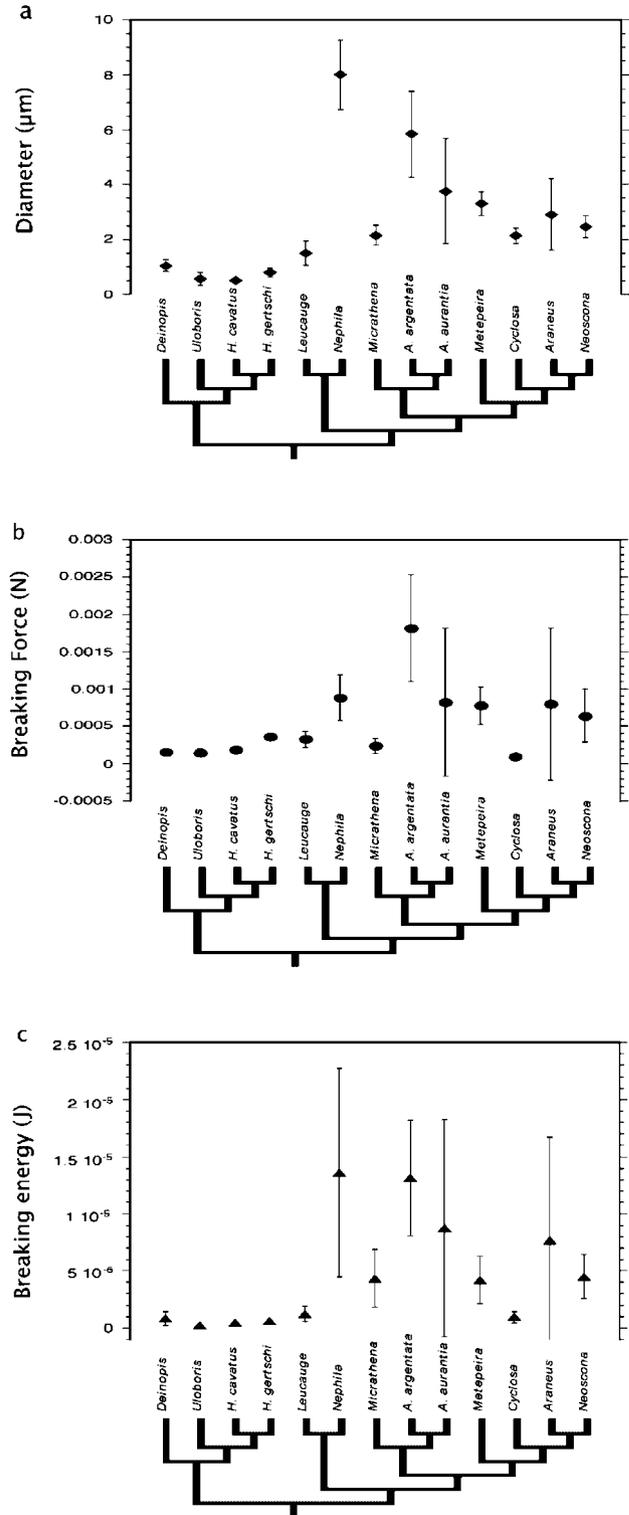


Fig. 4. Mechanical properties of capture silk for each species (Mean \pm 1SD). Deviations are small enough to be hidden by the symbols if not shown. Tree topology is drawn from Scharff and Coddington ('97) and branch lengths are arbitrary. (a) Diameter of silk fibers, (b) force required to break the whole fiber, and (c) energy required to break the whole fiber.

TABLE 2. Structural and mechanical properties of capture spiral fibers from orb-weaving spiders

Species	<i>N, n</i>	Diameter (μm)	SD diameter	Energy to break (J/10 mm section)	SD energy	Force to break (N)	SD force
<i>Argiope argentata</i>	6, 50	5.84	1.57	1.31E-05	5.04E-06	1.82E-03	7.20E-04
<i>Argiope aurantia</i>	4, 41	3.76	1.92	8.77E-06	9.51E-06	8.19E-04	9.92E-04
<i>Araneus diadematus</i>	4, 33	2.91	1.30	7.64E-06	9.09E-06	7.96E-04	1.01E-03
<i>Cyclosa turbinata</i>	7, 44	2.13	0.28	9.69E-07	5.09E-07	9.58E-05	2.40E-05
<i>Deinopis spinosa</i>	5, 27	1.03	0.22	8.63E-07	6.05E-07	1.58E-04	5.43E-05
<i>Hyptiotes cavatus</i>	3, 22	0.51	0.02	4.15E-07	5.28E-08	1.87E-04	1.33E-05
<i>Hyptiotes gertschi</i>	2, 24	0.79	0.16	5.71E-07	1.21E-07	3.63E-04	3.17E-05
<i>Leucauge venusta</i>	8, 66	1.50	0.44	1.24E-06	6.43E-07	3.27E-04	1.08E-04
<i>Metepeira grandiosa</i>	7, 50	3.30	0.44	4.19E-06	2.09E-06	7.79E-04	2.51E-04
<i>Micrathena sagittata</i>	5, 37	2.15	0.37	4.37E-06	2.54E-06	2.40E-04	9.95E-05
<i>Neoscona oaxacensis</i>	5, 49	2.47	0.39	4.51E-06	1.92E-06	6.39E-04	3.54E-04
<i>Nephila clavipes</i>	8, 45	8.00	1.28	1.36E-05	9.12E-06	8.82E-04	3.11E-04
<i>Uloborus diversus</i>	15, 103	0.56	0.23	2.15E-07	9.80E-08	1.49E-04	4.35E-05

N, number of individual spiders used from each species; *n*, total number of silk samples tested from each species. Standard deviations (SD) are calculated based on the means of individuals.

TABLE 3. Pearson's product moment correlation coefficients for standardized independent contrasts of material and mechanical properties for capture spiral silk from orb-weaving spiders

	Diameter	Breaking strain	Breaking stress	Toughness	Energy	Force
Diameter	1	0.684	-0.472	-0.535	0.95	0.714
Breaking strain		1	-0.53	-0.35	0.63	0.173
Breaking stress			1	0.747	-0.337	-0.150
Toughness				1	-0.397	-0.331
Energy					1	0.790
Force						1

Coefficients in bold are significant at $P < 0.05$.

2004). Even with this relatively small subset of species, we find extreme variation in the material and mechanical properties of capture fibers across the orbicularian clade (Figs. 2–4; Tables 1 and 2). This variation, which can range over sixfold for material properties and 10-fold for mechanical properties, should have profound impacts on the foraging performance and ecology of these species. Spiders construct orb webs of diverse designs and these webs capture different sets of insect species (Craig, '87; Eberhard, '90; Foelix, '96; Ibarra-Nunez et al., 2001). The ability of a web to effectively retain prey for capture by spiders is related to a variety of factors including web architecture, orientation, adhesiveness of the silk, and the material and mechanical properties of the fibers themselves (Shear, '86; Craig, '87; Eberhard, '90; Bishop and Connolly, '92; Opell and Bond, 2000). The genes that encode these capture spiral proteins can also vary greatly among species (Hayashi and Lewis, 2000; Garb et al., 2006). The

observed differences in material properties reported here provide a mechanism by which the different amino acid sequences might have a direct impact on the performance and ecology of foraging spiders.

On the basis of the hypothesized molecular structure of capture silk (Hayashi and Lewis, '98; Gosline et al., '99; Becker et al., 2003) and the known inter-specific fibroin sequence variation, we suggest that differences in material properties are driven by sequence-induced protein structural changes (Hayashi et al., '99). Fibroin sequences are not known for most of the species studied here, making direct sequence to property connections difficult. However, on the basis of the subset of spiders where both material properties and sequence data are available, we can make hypotheses about how protein structure might affect material performance. All of the araneoid flagelliform fibroin sequences have serially repeated GPGG(X)_n amino acid motifs that are thought to

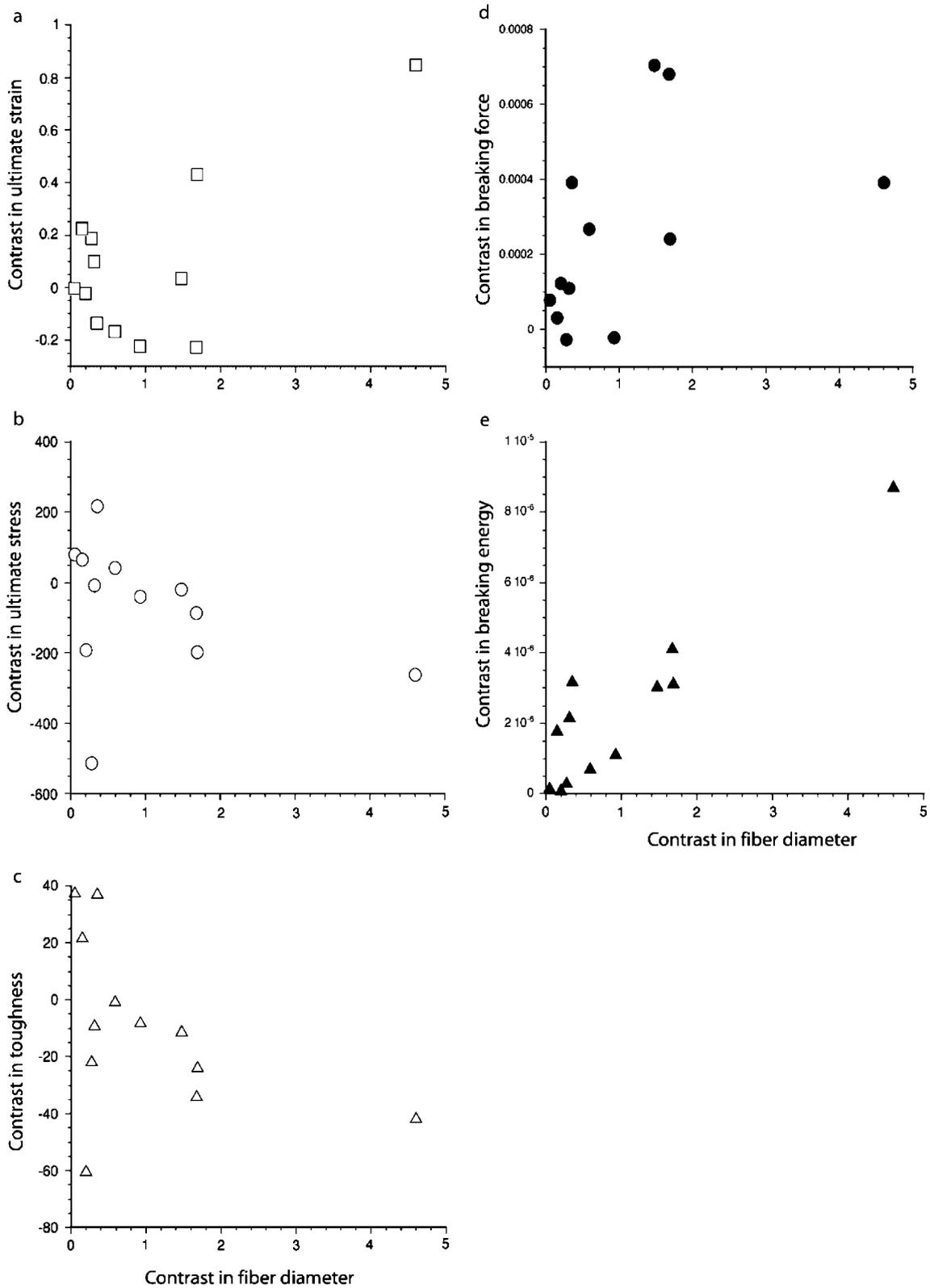


Fig. 5. Bivariate plots of standardized independent contrasts in material and mechanical properties plotted against standardized contrasts in fiber diameter. These correlations are all significant at $P < 0.05$, see Table 3. (a) Ultimate strain, (b) ultimate stress, (c) toughness, (d) whole fiber breaking force, and (e) whole fiber breaking energy.

induce the β spiral structure of these proteins. Having more repeated GPGG(X)_n motifs in the fibroin sequence may lead to a more extensible fiber by producing greater numbers of nanospring β spirals (Hayashi et al., '99). Flagelliform fibroin cDNA sequences are published for *Araneus*, *Nephila*, *Argiope trifasciata*, and *Deinopis* (Guerette et al., '96; Hayashi et al., '99; Gatesy et al., 2001; Garb et al., 2006). Of these species, *Nephila* has the highest number of serially repeated GPGG(X)_n amino acid motifs and its capture silk is also the most extensible of this group, supporting this hypothesis (it is the second-most extensible in our sample of species; Fig. 3). We also found a consistent difference in the performance of araneoid versus deinopoid silks. Deinopoid silks tended to show higher strengths and lower extensibilities than araneoid silks. The addition of aqueous glue (aggregate gland secretions) to the capture spiral fibers by araneoid species should affect properties by increasing plasticization, and this could help to explain the relatively stronger and less extensible silk from deinopoid species that do not use aqueous glue (Gosline et al., '86; Vollrath and Edmonds, '89). However, experimental addition of sticky aggregate and other liquids to dry fibers has not produced the degree of property variation observed here (Vollrath and Edmonds, '89; Blackledge et al., 2005b). Instead of the GPGG(X)_n amino acid motifs, the capture silk fibroin sequence from *Deinopis* has a serially repeated motif of GPQG(X)_n (Garb et al., 2006). It is possible that this variation in motif sequences could confer higher strength and lower extensibilities to deinopoid capture threads by either strengthening hydrogen bonds between sequential β spirals, or decreasing the total extensibility of the "nanospring" structures (Blackledge and Hayashi, 2006b; Garb et al., 2006).

Several other hypotheses have also been advanced to explain intra- and inter-specific differences in silk materials. Porter et al. (2005) suggest that the amount of nanoscale order determines the relative strength and extensibility of fibers. This model predicts a trade-off between strength and extensibility, with relatively constant toughness. Although we do not find constant toughness across species, our data do show a negative correlation between strength and extensibility (Fig. 6). This is in contrast to recently published data for dragline silk where there is no evolutionary relationship between strength and extensibility (Swanson et al., 2006b). We propose that the most likely proximal cause of the observed

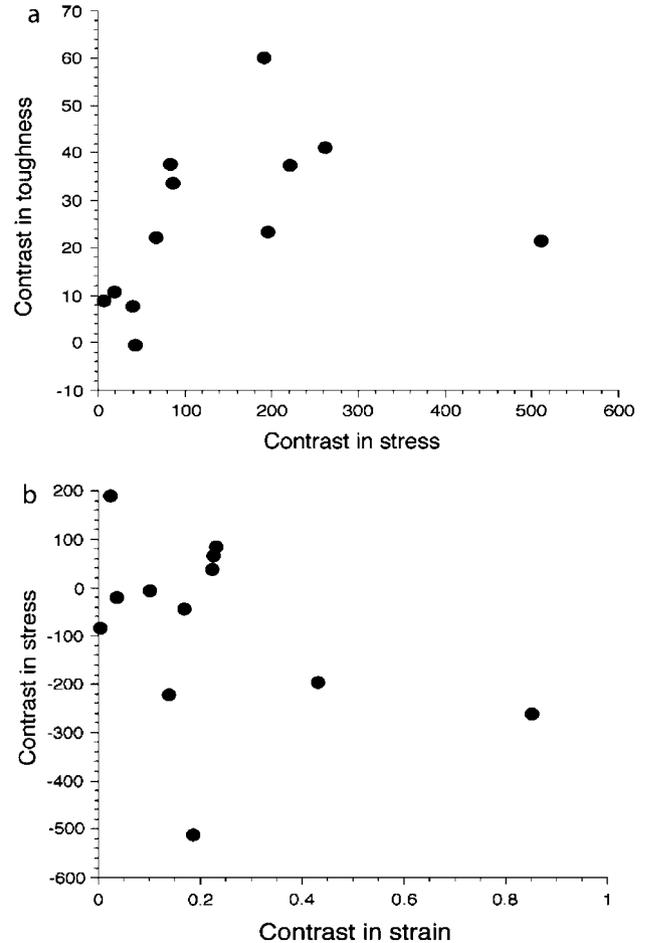


Fig. 6. Bivariate plots of selected standardized independent contrasts for material properties. Both of these correlations are significant at $P < 0.05$, see Table 3. (a) Toughness/true stress and (b) true stress/true strain.

material variation is in fact fibroin sequence. Several authors have further suggested that because of its ecological importance, silk performance should be under selection (Craig, '92; Opell and Bond, 2001; Swanson et al., 2006a,b) and we agree with these authors that prey capture ecology is likely the ultimate cause of the observed property variation in capture silks.

Correlated properties

In a visual examination of the property values across the phylogeny of spiders in our study, little obvious phylogenetic pattern is observed. Closely related species can produce silks with substantially different properties, or the properties can be very similar (Figs. 3 and 4). This lack of consistent pattern indicates that these properties may be quite labile or under intense divergent selection in

different lineages. Yet, there appear to be a few potential phylogenetic patterns, such as capture silks spun by the clade of cribellate orb weavers (Deinopoidea, represented in this study by *Deinopis*, *Uloborus*, *H. cavatus*, and *H. gertschi*) showing higher ultimate stresses and lower ultimate strains than capture silks produced by species in the Araneioidea (see also Blackledge and Hayashi, 2006b). As more silks from more species are characterized, it will be possible to test statistically for phylogenetic signal (Blomberg et al., 2003) in these properties.

Correlations between phylogenetically independent contrasts allow us to assess associations between variables and to make conclusions about correlated evolution of traits (Felsenstein, '85; Garland and Carter, '94; Garland et al., '99). Most obviously, fiber diameter is correlated with several material and mechanical properties. First, diameter is a good predictor of the force and energy required to break a fiber. Because thicker fibers have more cross-sectional area and volume than thinner fibers, we expect that thicker fibers should be mechanically stronger and require more energy to rupture. Second, diameter is also correlated with ultimate strain. This could be due to differences in fibroin sequence resulting from genetic variation, or it could be a direct size effect, where thicker fibers require more extension before they reach some minimum diameter and rupture. Third, diameter is negatively correlated with both ultimate stress and toughness. Spiders that spin thin fibers may be under more intense selection to produce materials with high strength and toughness to compensate for small fiber size and capture relatively larger prey. Some of these diameter correlations may be driven by a diameter contrast outlier (the contrast between *Nephila* and *Leucauge*). However, the trends exist even without this data point and more data will be required to test the robustness of these results.

We also find a correlation between ultimate stress and toughness, but not between ultimate strain and toughness. Both of these properties contribute to toughness, and a significant correlation with only one (ultimate stress) suggests that evolutionary increases in capture silk toughness are driven by changes in fiber strength more than by fiber extensibility. This observation is understandable considering the J-shaped stress/strain curve of capture fibers (Fig. 2). Most of the extensibility of these fibers occurs at very low forces, and changes in the amount of low force extension across species should not substantially

affect the overall area under the stress/strain curve (i.e. toughness). Finally, there is an inverse relationship between ultimate stress and ultimate strain, suggesting an evolutionary trade-off between having capture spiral materials that are either strong or extensible. This trade-off between strength and extensibility is exactly what we would expect based on most biological and man-made materials (Wainwright et al., '82). Yet, this inverse relationship is not seen in dragline silk, which shows no strength/extensibility correlation or trade-off across species (Swanson et al., 2006b). We do not know the reason for this difference in property correlation between these two types of silks, but it is possible that the difference in phylogenetic scope (much broader sampling in the dragline study) contributes to the different pattern.

Performance and ecology

To assess the performance of capture fibers as they function in prey capture, both material properties and size must be taken into account. A mechanically strong fiber, that can withstand high forces, can be produced by either a fiber with a high breaking stress or a large cross-section. It is in fact the variation in mechanical properties, rather than material properties per se, that should lead to differential performance in capturing flying insects. The data presented here suggest that although material properties contribute to variation in fiber mechanical performance, the size of the fiber (diameter) is a much better predictor of critical properties such as breaking force (Fig. 5, $r = 0.71$) and breaking energy (Fig. 5, $r = 0.95$).

Here, we attempt to connect fiber mechanical performance with the range of flying insects that may be retained by capture fibers. This is a complex question and we recognize that capture spiral performance depends on a suite of additional characteristics, including stickiness of the fiber, capture spiral spacing in the web, and distance between web radials. However, the energy that can be absorbed by a length of capture fiber should be an important determinant of silk performance, because successful foraging requires that prey be arrested (kinetic energy dissipated) and retained in the web. The target prey of almost all orb-weaving spiders is flying insects. Examinations of flight speeds and body masses in insects reveal that kinetic energy can vary by several orders of magnitude across species (Dudley and Srygley, '94). However, Dudley (2000) suggests that most small insects (where most

insect abundance and diversity is found) are not able to fly more than 1 m/sec.

If we assume a 1 m/sec flight speed, then the thinnest fibers studied here could dissipate the kinetic energy of a 1 mg insect without rupture (approximately the size of a mosquito) and the thickest fibers could stop a 20 mg insect (approximately the size of a large housefly). We can compare this range of performance to the range of insects that are actually captured in orb webs. Although insect prey items are often small and relatively slow, they can also be much more massive than 20 mg and much faster than 1 m/sec (Craig, '87; Blackledge and Zevenbergen, 2006). These larger and faster species can have at least 2.7×10^{-2} J of kinetic energy (Dudley and Srygley, '94), which is more than two orders of magnitude more energy than can be absorbed by a single strand of the largest and toughest capture silk tested here (Table 2; Fig. 4).

An extensive body of field research examining the prey that are observed to be caught in orb webs indicates that spider species vary greatly in their diets and exploit different subsets of the available flying insect fauna in a habitat (Robinson and Robinson, '70; Howell and Ellender, '84; Craig, '87; Uetz and Hartsock, '87; McReynolds, 2000). For instance, *Leucauge* and *Cyclosa* species may specialize in very small dipteran and hemipteran prey (Bishop and Connolly, '92; Miyashita, '97). In this case, our data suggest that individual capture spiral fibers spun by these spider species should be sufficient to arrest and retain such prey items. In contrast, large insects are not retained in the webs of some small spider species such as *Cyclosa*, *Uloborus*, and *Leucauge* (Craig, '87; Uetz and Hartsock, '87; Miyashita, '97). The mechanical data indicate that the fibers in these webs are simply not able to absorb the kinetic energy of larger insects. Denny ('76) suggested that spiders may select for prey insects that are small enough for them to subdue without injury by using silk fibers that are too weak to arrest larger insects. However, some of the species studied here (e.g. *Nephila*, *Argiope*) are known to capture large lepidopterans, hymenopterans, orthopterans, and other high kinetic-energy insects (Robinson and Robinson, '70; Howell and Ellender, '84; Foelix, '96; McReynolds, 2000; Blackledge and Zevenbergen, 2006). Capture of these large, energetically rewarding, but also rare, insects is in fact crucial for the effective reproduction of orb-weaving spiders (Venner and Casas, 2005). Our data suggest that single capture fibers of any of the

spider species studied here are not capable of arresting and capturing these large living missiles. Many of the larger diameter silks function at in web lengths greater than 10 mm and we are therefore underestimating the energy that it should take to break these fibers. It is also known that insects often strike multiple spiral strands. For instance, if two 10 cm long capture spiral strands of a *Nephila* web (the thickest silk tested) were struck, together they could arrest a 500 mg insect assuming a 1 m/sec flight speed (approximately a large bumble bee flying well under maximum velocity). However, if we assume that some large insects can have 1×10^{-2} J of kinetic energy, it would require almost 10 m of capture spiral from the largest species studied here to arrest them. Clearly, orb-weaving spiders must depend on the entire web acting as a composite, energy absorbing structure to capture insects that could not be arrested by individual fibers (Denny, '76; Lin et al., '95). These webs must use energy absorption by the capture spiral, the frame and radial fibers (constructed of much stiffer dragline materials), and air resistance from the displacing web (Denny, '76; Lin et al., '95). Only with this whole set of energy-absorbing strategies and the web fibers operating at the limit of their mechanical performance are spiders able to capture the largest, fastest insects (Denny, '76). This may constrain the evolution of web architectures in these spiders in ways that differ from species that capture small prey (Blackledge and Eliason, 2007). For instance, the architectures of the webs spun by spiders specializing in prey small enough to be retained effectively by single capture threads may be free to maximize the number of prey intercepted. In contrast, the web architectures of spiders capturing larger insects may evolve under stronger selection to maximize the retention of insects by the variety of energy-absorbing strategies described above (Blackledge and Zevenbergen, 2006; Blackledge and Eliason, 2007).

The material and mechanical properties of capture silks help determine the feeding niche of orbicularian spiders, a species-rich group of ecologically important terrestrial predators. Webs can have different orientations, architectures, or can be constructed and tended at different times of the day (Craig, '87; Eberhard, '90; Miyashita, '97). In our study, we document that the extreme variation in material properties observed for capture spiral silk adds to the complexity and variation known for spider orb webs. This complexity allows evolution in web

building along several axes, from architecture to material and mechanical properties of constituent fibers. Thus, natural, inter-specific differences in the properties of biological materials have the potential to directly affect feeding performance and therefore will have significant impacts on species ecology and evolution.

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