SPIDER DRAGLINE SILK: CORRELATED AND MOSAIC EVOLUTION IN HIGH-PERFORMANCE BIOLOGICAL MATERIALS

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Abstract.—The evolution of biological materials is a critical, yet poorly understood, component in the generation of biodiversity. For example, the diversification of spiders is correlated with evolutionary changes in the way they use silk, and the material properties of these fibers, such as strength, toughness, extensibility, and stiffness, have profound effects on ecological function. Here, we examine the evolution of the material properties of dragline silk across a phylogenetically diverse sample of species in the Araneomorphae (true spiders). The silks we studied are generally stronger than other biological materials and tougher than most biological or man-made fibers, but their material properties are highly variable; for example, strength and toughness vary more than fourfold among the 21 species we investigated. Furthermore, associations between different properties are complex. Some traits, such as strength and extensibility, seem to evolve independently and show no evidence of correlation or trade-off across species, even though trade-offs between these properties are observed within species. Material properties retain different levels of phylogenetic signal, suggesting that traits such as extensibility and toughness may be subject to different types or intensities of selection in several spider lineages. The picture that emerges is complex, with a mosaic pattern of trait evolution producing a diverse set of materials across spider species. These results show that the properties of biological materials are the target of selection, and that these changes can produce evolutionarily and ecologically important diversity.

Key words.—Biomaterials, biomechanics, independent contrasts, major ampullate silk, phylogenetic signal, tensile test, web.

Differences among species in traits such as morphology, physiology, and behavior are often thought to be adaptations to species-specific habitats or niches, and extensive research has examined how natural selection might produce these differences (Barlow 1968; Wainwright 1988; Garland and Carter 1994; Lauder and Reilly 1996; Swanson et al. 2003). However, variation among species in the properties of biological materials has received much less study (Opell and Bond 2001; Erickson et al. 2002; Summers and Koob 2002). Biomaterials, whether intrinsic to the organism, as in bone or chitin, or those used to modify the environment, such as silks and glues, respond to applied forces in ways that have performance and fitness consequences for the organisms that produce them (Wainwright et al. 1980; Craig 1987, 1992; Summers and Koob 2002).

Some biomaterials, such as vertebrate bone, exhibit little variation in material properties across individuals or species (Erickson et al. 2002). However, if there is enough variation in biomaterials for selection to occur, then comparative studies of material properties may demonstrate connections among genes, attributes (phenotypes), and ecological uses of biomaterials (Craig 1992; Fedič et al. 2003). Spider silk is an ideal system for examining the evolution of material properties because silk fibers are composed of structural proteins, and the gene sequences that encode these proteins are increasingly accessible to researchers (Xu and Lewis 1990;

Craig 1992; Gosline et al. 1999; Hayashi et al. 1999). An extensive literature on the performance and ecological function of spider webs provides a framework for understanding variation in the material properties of silk (Denny 1976; Craig 1987; Eberhard 1990; Opell and Bond 2001; Blackledge et al. 2003). However, investigations of the phylogenetic patterns of change in silk material properties are needed to make connections between gene evolution, material properties, and ecology (Craig 1987, 1992; Gosline et al. 1999; Hayashi et al. 1999; Opell and Bond 2001).

Spiders are a diverse group of arthropods with over 39,000 described species, and silk use is central to the ecology and life history of nearly every species (Eberhard 1990; Coddington et al. 2004). Spiders spin silk throughout their lives for a variety of functions, including constructing egg sacs, communicating with conspecifics, and ensnaring flying insects with aerial nets (Foelix 1996). These varied uses for silk place diverse pressures on the mechanical and material properties of the fibers (Denny 1976; Stauffer et al. 1994; Gosline et al. 1999). An individual spider may spin as many as seven types of silk that emerge from morphologically distinct spigots on their abdominal spinnerets (Coddington 1989; Platnick et al. 1991; Foelix 1996). Each type of silk is assembled from proteins synthesized in uniquely specialized glands, and different types of glands vary in the suite of silk genes that they express (Gosline et al. 1986; Guerette et al. 1996; Garb and Hayashi 2005). Each type of silk also displays a unique combination of mechanical characteristics within individual species (e.g., Blackledge and Hayashi 2006).

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One of these silk types, the dragline silk of orb-weaving spiders has received the most attention from biomechanists and bioengineers (Denny 1976; Work 1978; Köhler and Vollrath 1995; Gosline et al. 1999; Osaki 1999; Porter et al. 2005). Dragline silk is attached by spiders to substrates for use as trailing safety lines and for the structural frameworks of capture webs. Dragline is considered a ''high-performance'' material because it is tougher than, and almost as strong as, any man-made fiber (Gosline et al. 1999, 2002). Several researchers have suggested that toughness, the energy that can be absorbed by a material prior to failure, has been the target of intense selection in order to maximize the weight of a spider that can be supported by a safety line or to maximize the inertia of prey that can be captured in an aerial web (Köhler and Vollrath 1995; Osaki 1999; Blackledge et al. 2005a; Porter et al. 2005). However, nearly all Araneomorphae (true spiders)—not only the aerial web weavers—spin dragline silk from major ampullate spigots (Platnick et al. 1991; Foelix 1996) and a recent study suggested that the high-performance characteristics of dragline silk predate the evolutionary origin of aerial orb webs (Swanson et al. 2006). Despite its diverse functions among araneomorph spiders, dragline silk is thought to be homologous across spiders, and the genes that encode the silk proteins are all members of a single gene family (Guerette et al. 1996; Gatesy et al. 2001).

The material properties of silk are determined by interactions between the amino acid sequences of the proteins that form the silk fibers (Hayashi et al. 1999), the fiber spinning process (Garrido et al. 2002a; Porter et al. 2005) and, in some cases, absorbed moisture (Vollrath and Edmonds 1989; Blackledge et al. 2005b). Silk fibers are constructed from large (>250 kDa) proteins called fibroins that are synthesized in specialized abdominal glands (Xu and Lewis 1990; Foelix 1996). The amino acid sequences of these proteins can be partially determined by peptide analyses or more fully inferred by translating cDNA sequences for the fibroins (e.g., Xu and Lewis 1990; Hinman and Lewis 1992). Studies of these fibroin sequences have revealed diversity among silk types within a species (Guerette et al. 1996; Dicko et al. 2004) and within homologous silk types across species (Gatesy et al. 2001; Garb and Hayashi 2005). These differences in sequence are hypothesized to result in a variety of material properties through the production of different secondary and tertiary structures (Craig 1992; Hayashi et al. 1999; Gosline et al. 1999; Fedič et al. 2003).

The observed variation in spider silk fibroin sequences and preliminary examinations of dragline silks suggests that there is extensive variation in fiber properties across the phylogeny of spiders (Stauffer et al. 1994; Madsen et al. 1999; Gatesy et al. 2001; Pouchkina-Stancheva and McQueen-Mason 2004; Tian et al. 2004; Swanson et al. 2006). In this study, we examine the evolution of material properties as performance measures (Wainwright 1988; Garland and Carter 1994) across the phylogeny of true spiders. Gathering material property data from many species across this diverse group allows us to test for correlations among properties and for associations between properties and ecological factors (Garland et al. 1992). We ask several questions: First, to what extent do material properties of dragline silks vary among species, and is the variation significant from a statistical or functional standpoint? Second, are different material properties evolutionarily correlated with one another? Third, are there phylogenetic signals in silk material properties? Fourth, are there connections between silk use ecology and material properties?

MATERIALS AND METHODS

Phylogenetic Sampling

Twenty-one species were chosen to span the diversity of true spiders. The basal split in the Araneomorphae is between the Paleocribellatae and Neocribellatae (Coddington et al. 2004). Our exemplars included the Hypochilidae, the only extant family of the Paleocribellatae, and multiple representatives from the Haplogynae (five species) and Entelegynae (15 species), the two large clades in the Neocribellatae (Fig. 1). In sampling both of these clades, we included spiders that exhibited diversity in silk use and ecology.

Silk Collection

Spiders were housed individually in cages at approximately 23°C, fed crickets, and misted with water. Because of differences in silk spinning behaviors among species, it was not feasible to use a single protocol for silk collection (Swanson et al. 2006). Instead, the choice of collection method was dictated by silk spinning behaviors that could be reliably elicited in the lab and generally reflected the way each species uses dragline silk. Silk was collected from *Agelenopsis aperta* (Agelenidae), *Metaltella simoni* (Amphinectidae), *Dysdera crocata* (Dysderidae), *Kukulcania hibernalis* (Filistatidae), *Schizocosa mccooki* (Lycosidae), and *Plectreurys tristis* (Plectreuridae) by allowing individuals to lay silk while walking in clean terrariums. The fibers were collected from the cages on C-shaped cards that were covered with double-sided sticky tape. Silk from *Latrodectus hesperus* (Theridiidae) was collected from webs using techniques described in Blackledge et al. (2005b). Silk from *Araneus gemmoides* (Araneidae), *Argiope argentata* (Araneidae), *Gasteracantha cancriformis* (Araneidae), *Mastophora hutchinsoni* (Araneidae), *Deinopis spinosa* (Deinopidae), *Peucetia viridans* (Oxyopidae), *Scytodes* sp. (Scytodidae), *Nephila clavipes* (Tetragnathidae), and *Uloborus diversus* (Uloboridae) was collected by forcible silking, following techniques outlined in Blackledge et al. (2005a). Silk from *Metepeira grandiosa* (Araneidae), *Hypochilus pococki* (Hypochilidae), *Holocnemus pluchei* (Pholcidae), *Phidippus ardens* (Salticidae), and *Leucauge venusta* (Tetragnathidae) was collected by allowing spiders to lower themselves on a dragline from a raised platform. The silk was then collected on cards covered with double-sided tape. Although different silk collection techniques can affect the material properties of silks (Pérez-Rigueiro et al. 2001; Garrido et al. 2002a; Blackledge et al. 2005b; Porter et al. 2005), our data show no bias associated with silk collection method (i.e., silks collected with similar methods do not show similar properties; see below). Numbers of individuals and samples for each species are summarized in Table 1.

Tensile Testing

For each sample, we measured four properties that describe fiber performance and might have different implications for
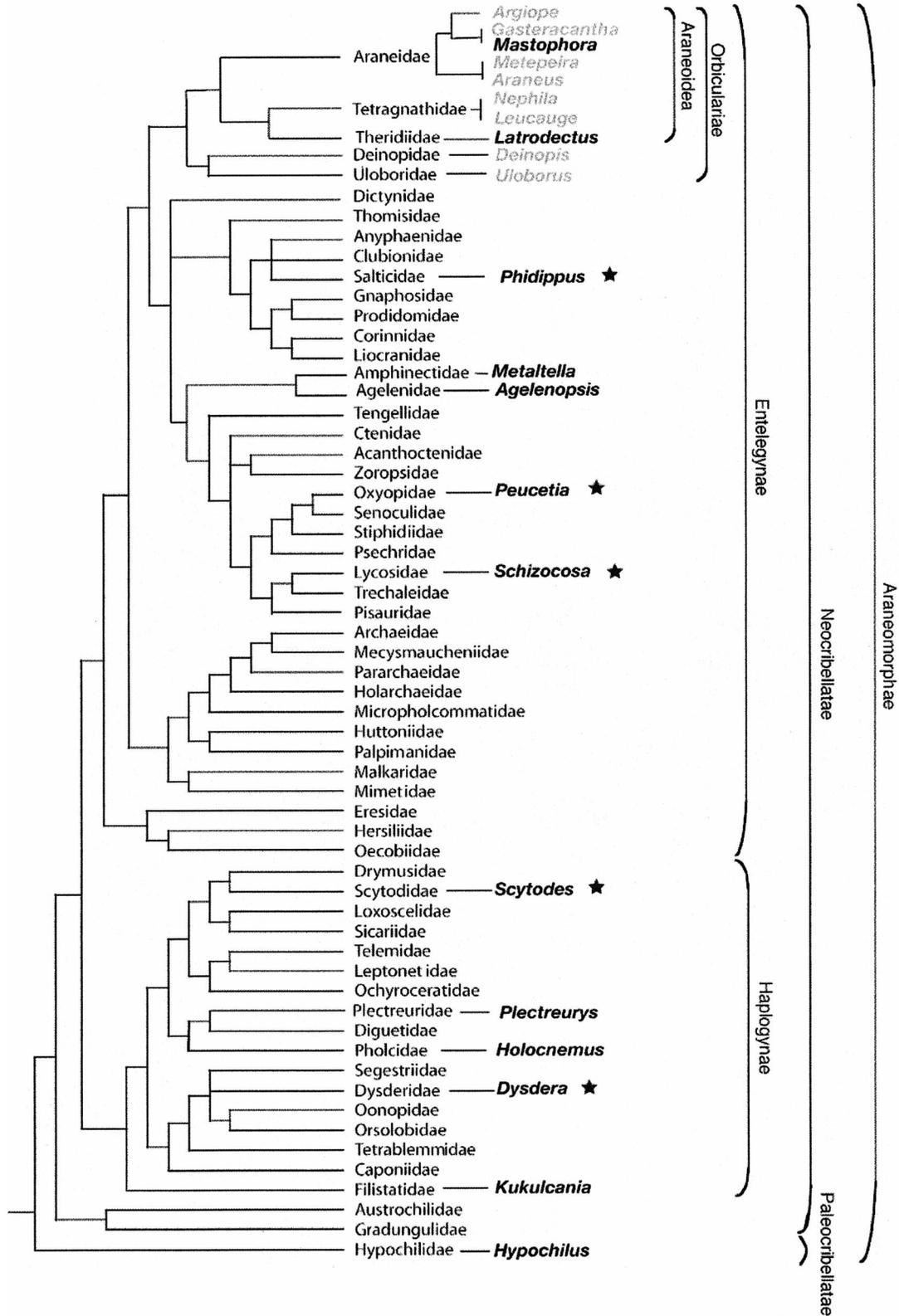
FIG. 1. Phylogeny of true spider families (Araneomorphae) with selected genera used in this study in bold. Spiders used in this study that spin aerial orb webs are indicated by gray type, and spiders that are not known to use silk in prey capture are marked with a star. Phylogeny modified from Scharff and Coddington (1997) and Coddington et al. (2004). Branch lengths are arbitrary.

TABLE 1. Material properties (mean $\pm$ 1 SD) of dragline silk for each species. Deviation calculations based on the number of individual spiders examined per species.

Species	n spiders, silk samples	Stiffness (GPa)	Extensibility (ln(mm/mm))	Strength (MPa)	Toughness (MJ/m^3)
Hypochilus pococki	10, 97	10.947 $\pm$ 3.907	0.170 $\pm$ 0.047	944.87 $\pm$ 377.38	95.59 $\pm$ 49.45
Kukulcania hibernalis	12, 102	22.161 $\pm$ 12.566	0.222 $\pm$ 0.091	1044.33 $\pm$ 384.36	132.18 $\pm$ 72.37
Dysdera crocata	8, 40	8.047 $\pm$ 5.159	0.177 $\pm$ 0.088	544.59 $\pm$ 357.07	47.85 $\pm$ 37.42
Holocnemus pluchei	11, 105	14.267 $\pm$ 4.936	0.153 $\pm$ 0.029	1244.12 $\pm$ 440.03	114.77 $\pm$ 48.60
Plectreurys tristis	11, 108	16.100 $\pm$ 3.737	0.241 $\pm$ 0.073	829.01 $\pm$ 192.82	112.13 $\pm$ 44.27
Scytodes sp.	4, 26	10.693 $\pm$ 3.687	0.357 $\pm$ 0.062	1179.22 $\pm$ 359.74	230.02 $\pm$ 84.53
Schizocosa mccooki	6, 52	4.559 $\pm$ 2.530	0.242 $\pm$ 0.054	553.15 $\pm$ 223.52	59.57 $\pm$ 24.21
Peucetia viridans	3, 22	10.060 $\pm$ 2.083	0.178 $\pm$ 0.023	1088.82 $\pm$ 289.54	107.78 $\pm$ 31.57
Agelenopsis aperta	10, 88	12.093 $\pm$ 4.685	0.183 $\pm$ 0.062	958.45 $\pm$ 349.53	101.36 $\pm$ 47.63
Metaltella simoni	6, 54	8.600 $\pm$ 2.941	0.281 $\pm$ 0.088	764.60 $\pm$ 242.45	113.81 $\pm$ 40.70
Phidippus ardens	10, 95	14.179 $\pm$ 5.839	0.189 $\pm$ 0.058	974.51 $\pm$ 346.13	116.22 $\pm$ 50.28
Uloborus diversus	7, 61	9.085 $\pm$ 2.443	0.234 $\pm$ 0.055	1078.27 $\pm$ 310.10	128.69 $\pm$ 39.20
Deinopis spinosa	3, 24	13.537 $\pm$ 3.292	0.185 $\pm$ 0.027	1328.87 $\pm$ 375.70	135.86 $\pm$ 30.93
Latrodectus hesperus	9, 70	10.167 $\pm$ 2.572	0.303 $\pm$ 0.058	1440.68 $\pm$ 310.18	180.98 $\pm$ 47.67
Leucauge venusta	6, 61	10.596 $\pm$ 2.347	0.233 $\pm$ 0.051	1469.34 $\pm$ 263.15	151.09 $\pm$ 41.50
Nephila clavipes	17, 66	13.803 $\pm$ 3.642	0.172 $\pm$ 0.035	1215.09 $\pm$ 232.91	111.19 $\pm$ 30.54
Araneus gemmoides	3, 23	8.325 $\pm$ 1.038	0.224 $\pm$ 0.032	1375.89 $\pm$ 106.00	141.18 $\pm$ 21.01
Metepeira grandiosa	10, 88	10.628 $\pm$ 4.403	0.235 $\pm$ 0.075	1048.84 $\pm$ 373.32	120.73 $\pm$ 64.51
Mastophora hutchinsoni	3, 21	9.385 $\pm$ 1.295	0.268 $\pm$ 0.050	1137.28 $\pm$ 116.11	140.38 $\pm$ 24.60
Gasteracantha cancriformis	3, 38	7.975 $\pm$ 2.093	0.301 $\pm$ 0.041	1315.22 $\pm$ 337.55	177.57 $\pm$ 44.73
Argiope argentata	8, 59	8.180 $\pm$ 1.898	0.184 $\pm$ 0.020	1463.45 $\pm$ 230.96	116.25 $\pm$ 24.65

organismal fitness (Fig. 2). The first property is the strength, or true breaking stress, of the fiber. This is the amount of force (in newtons; N) required to break a fiber divided by the instantaneous cross-sectional area of the fiber (MPa = MN/m^2). The instantaneous cross-sectional areas of fibers were calculated using an assumption of constant volume during extension (Vollrath et al. 2001). The second property is the extensibility, or true breaking strain, a measure of the stretchiness of a fiber. True breaking strain is the natural log of the length at rupture divided by original length. The standard isovolumetric assumption was the basis for computing "true" from "engineering" stress and strain (Guinea et al. 2006). The third property is stiffness, or Young's modulus, the amount of stress required to strain the sample a given amount. Stiffness (in GPa) is calculated as the slope of the stress-strain curve over the initial elastic region and is an important character for describing structural rigidity. In fact, most construction materials used by humans are selected for their high stiffness (Vogel 1998). The final property, toughness, is the energy required to break a fiber (MJ/m^3), calculated as the area under the stress-strain curve divided by the volume of the sample. Toughness, which takes into ac-
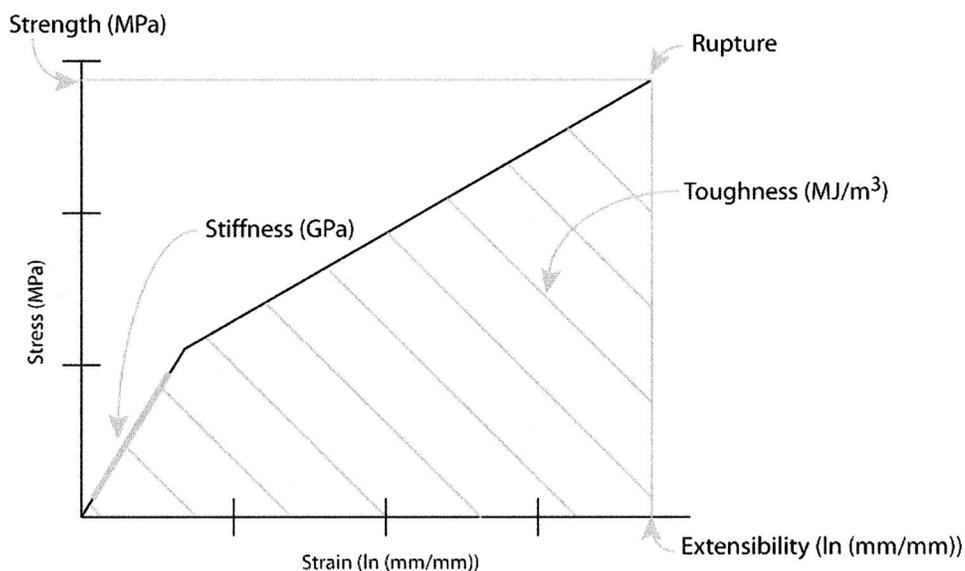
FIG. 2. A schematic of a stress versus strain curve similar to those produced by the Nano Bionix tensile testing machine with material properties measured from the graph. Strength is the stress at rupture. Extensibility is the strain at rupture. Stiffness, or Young's modulus, is the slope of the stress-strain curve over the first linear portion of the curve. Toughness is the area under the stress-strain curve, or the energy required to break the fiber divided by the volume of the fiber.

count both a fiber's strength and its extensibility, measures how good a fiber is at absorbing energy input before rupture (Fig. 2; Denny 1976; Wainwright et al. 1980; Porter et al. 2005).

The material properties of strength, extensibility, stiffness, and toughness were quantified for each sample (Table 1). Tensile testing was conducted using techniques described in Blackledge et al. (2005a,b). Briefly, silk was glued to C-shaped cardboard cards using cyanoacrylate. The diameter of each silk sample was determined by averaging nine measurements taken along the length of the fiber using polarized light microscopy (Blackledge et al. 2005c). Morphological studies demonstrate that spider silk fibers can exhibit mild to moderate shape anisotropy, such that they are elliptical rather than circular in cross-section (Pérez-Rigueiro et al. 2001), and that the diameters of threads can also vary along their length (Madsen and Vollrath 2000). However, we measured the diameter of each fiber at nine different locations to control for this variability, thereby allowing us to estimate the average cross-sectional area of each fiber using a single value (Dunaway et al. 1995; Blackledge et al. 2005c). This imaging also confirmed that each sample consisted of one or two fibers of approximately the same diameter, and did not include smaller fibers that would have originated from other silk glands. Each 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. The sample was extended with a constant cross head speed at a rate of 1% strain/sec to failure. The testing environment ranged from 22.5°C to 24.4°C with 30–55% relative humidity.

Statistics

Conventional statistical analyses were first conducted to describe variation in material properties across species and to test for correlations between these properties without considering phylogenetic relatedness. Correlations among traits were then examined with the effects of phylogenetic relatedness removed by using phylogenetically independent contrasts (reviewed in Garland et al. 2005). Variation in silk properties among spiders that exhibit different silk use ecologies was also tested both conventionally and with the effects of phylogenetic relatedness removed (Grafen 1989; Garland et al. 1993). Finally, individual traits were tested for phylogenetic signal, a tendency for traits in closely related species to be more similar than traits in distantly related species, due to inheritance from more recent common ancestors (Blomberg et al. 2003).

Multivariate analysis of variance (MANOVA) was used to test for differences among species with respect to the material properties described above. ANOVAs were used post hoc to identify which variables differed among species. Pearson's product-moment correlations were calculated using each species as an observation (mean values as reported in Table 1) to examine associations between material properties. Variables were chosen for correlation analysis based on a priori hypotheses about the relationships between properties from materials science (Wainwright et al. 1980). All statistics were conducted in JMP IN version 5.1 (SAS Institute Inc., Cary, NC).

Phylogenetically independent contrasts (Felsenstein 1985) were then calculated using the PDAP:PDTree module of Mesquite to assess evolutionary correlations among the observed material properties (Maddison and Maddison 2004; Midford et al. 2005). The available higher-level spider phylogeny was a supertree without calculated branch lengths (Coddington et al. 2004). Therefore, several sets of arbitrary branch lengths were assigned to the data with the PDAP:PDTree module of Mesquite. These included: all branch lengths = 1, Grafen's (1989) arbitrary, Pagel's (1992) arbitrary, and Nee's arbitrary branch lengths (Purvis 1995). Grafen's (1989) arbitrary branch lengths were used to calculate the independent contrasts because they produced the least correlation between the absolute values of the calculated contrasts and their standard deviations for all traits (Garland et al. 1992; Maddison and Maddison 2004; Midford et al. 2005). These scaled contrasts were then used in correlation analyses through the origin to assess evolutionary associations between material properties. Independent contrasts were also used to estimate the ancestral values and confidence intervals of material properties at the basal node in the tree using the PDAP:PDTree module of Mesquite (Garland et al. 1999; Maddison and Maddison 2004; Midford et al. 2005).

Silk properties of spiders using different prey capture strategies (Fig. 1) were compared using regression with dummy variables. Specifically, spiders that did not use capture webs were compared to web-spinning taxa, and spiders that spin aerial orb webs were compared to those that do not. Then, generalized least-squares (GLS) regressions, using dummy variables and the tree and branch lengths described above, were calculated for these same comparisons using the regression.m program (Grafen 1989; Garland et al. 1993). This analysis allowed testing for differences in material properties among silks spun by spiders with varying foraging ecologies with the effects of phylogenetic relatedness removed (Grafen 1989; Garland et al. 1993).

To test for phylogenetic signal in material properties, a randomization test was performed with the PHYSIG program (Blomberg et al. 2003), which calculated the mean squared error (MSE) of the trait data given the hypothesized phylogeny (topology and branch lengths), then randomly shuffled the positions of the species on the tree and recalculated the MSE for 1000 permutations. The shuffling of species without regard to phylogeny should destroy any phylogenetic signal. A P-value was calculated as the proportion of permutations with a lower MSE than the observed tree. Permutations with these lower MSEs had more similarity between species that were closely related on the randomly assigned tree than between species that were evolutionarily closely related on the actual tree (Blomberg et al. 2003). A P-value less than 0.05 suggested that closely related taxa resembled each other more than expected by chance alone (i.e., due to inheritance from a recent common ancestor). Conversely, P-values greater than 0.05 indicated a failure to discover significant phylogenetic signal due to substantial evolutionary change in traits among relatives. The power of this randomization test decreases in analyses with small numbers of taxa. However, we included 21 taxa in a well-resolved phylogeny for our study, and this

was predicted to yield a statistical power of at least 0.8 (Blomberg et al. 2003).

The descriptive K-statistic was calculated for each of the four material properties to estimate the strength of the phylogenetic signal compared to that expected under a Brownian motion model of character evolution (Blomberg et al. 2003). K-values greater than one indicated phylogenetic signal greater than that predicted by Brownian motion evolution, while K-values less than one indicated that the silk properties of relatives resembled one another less than expected by Brownian motion evolution. This latter case can be interpreted as possible adaptive change in material properties (Blomberg et al. 2003).

RESULTS

We found significant multivariate differences among species in dragline silk material properties (MANOVA, approximate $F_{80/538.9} = 15.129$, $P < 0.05$). In univariate analyses of the data, species varied significantly for each of the four silk material properties measured. Extensibility varied more than twofold from 0.15 to 0.36 ($F_{20} = 15.43$, $P < 0.05$, Fig. 3a). Strength varied almost threefold from 545 to 1469 MPa ($F_{20} = 13.27$, $P < 0.05$, Fig. 3b). Toughness varied more than fourfold from 48 to 230 MJ/m^3 ($F_{20} = 14.83$, $P < 0.05$, Fig. 3c). Stiffness also varied more than fourfold from 4.6 to 22.1 GPa ($F_{20} = 15.8$, $P < 0.05$, Fig. 3d).

Unlike many materials in which stiffness and strength are positively correlated (Wainwright et al. 1980; Hancox 1981), we observed no relationship between these properties in the dragline silk data ($r = 0.11$, $P > 0.05$, Fig. 4a). Strength and extensibility are negatively correlated in single-species studies of dragline silk, suggesting a trade-off between these two properties (Garrido et al. 2002b; Porter et al. 2005). However, again, we found no association between these traits across species ($r = 0.11$, $P > 0.05$, Fig. 4b). As expected, because toughness encompasses both extensibility and strength, our results show that both extensibility ($r = 0.71$, $P < 0.05$, Fig. 4c) and strength ($r = 0.68$, $P < 0.05$, Fig. 4d) were positively correlated with toughness. Additionally, there was neither a significant relationship between stiffness and extensibility ($r = -0.24$, $P > 0.05$) nor between stiffness and toughness ($r = -0.11$, $P > 0.05$). The 95% confidence intervals of the estimated basal node values enclosed most of the data. The estimated ancestral states at the base of the tree were 0.213 $\pm$ 0.083 ln(mm/mm) for extensibility, 1043.4 $\pm$ 338.92 MPa for strength, 119.21 $\pm$ 53.08 MJ/m^3 for toughness, and 11.8 $\pm$ 4.65 GPa for stiffness (Fig. 4).

Phylogenetically independent contrasts demonstrated the same patterns of correlation among material properties as the species data. Again, there was no relationship between either stiffness and strength or extensibility and strength (stiffness/strength, $r = 0.25$, $P > 0.05$, Fig. 5a; extensibility/strength, $r = -0.15$, $P > 0.05$, Fig. 5b). Correlations of independent contrasts still revealed a significant, positive correlation between both extensibility and strength with toughness (extensibility/toughness, $r = 0.64$, $P < 0.05$, Fig. 5c; strength/toughness, $r = 0.61$, $P < 0.05$, Fig 5d).

The only material property that differed across ecological web use types in the conventional regression was strength (other results not shown). Orb-web weavers had significantly stronger silk than non-orb-web weavers ($t_{18} = 3.39$, $P < 0.05$) and spiders that did not build foraging webs had significantly weaker silk than web-spinning spiders ($t_{18} = 2.23$, $P < 0.05$). However, there was no significant difference in strength when using the GLS regression (orb weavers, $t_{18} = 1.70$, $P > 0.05$; non-web weavers, $t_{18} = 0.31$, $P > 0.05$).

The different material properties of dragline silk varied in the amount of phylogenetic signal they retained. However, none of the properties had phylogenetic signal significantly different from a random shuffling of the species, suggesting substantial divergence in the performance of the silk between closely related species. All of the measured properties also had K-values less than one (Fig. 3), indicating less similarity in the material properties of dragline silk among related taxa than predicted by a Brownian motion model of evolution along the specified phylogenetic tree (Blomberg et al. 2003). Extensibility and toughness had K-values of 0.480 and 0.619, respectively, and P-values for the randomization test of 0.688 and 0.193, respectively. Strength and stiffness had K-values of 0.723 and 0.724, respectively. Strength and stiffness had randomization P-values of 0.057 and 0.060, respectively, suggesting that, although neither of these traits had significant phylogenetic signal at $P < 0.05$, at least 94% of the randomized character sets had less phylogenetic signal than the observed tree.

DISCUSSION

Variation and Phylogenetic Signal in Dragline Silk

This study presents the largest comparative dataset on biomaterial properties, with 21 species, 560 individuals, and 1300 individual silk samples (for other significant datasets see Stauffer et al. 1994; Madsen et al. 1999; Opell and Bond 2001; Summers and Koob 2002; Erickson et al. 2002; Fedič et al. 2003). This gives us both confidence in our species values and the power to make phylogenetic conclusions (Garland et al. 1999; Blomberg et al. 2003). We observed large interspecific variation in the strength, extensibility, toughness, and stiffness of dragline silk spun by different taxa of spiders. However, the patterns of evolutionary change in the material properties differ from one another and from our previous understanding of silk evolution. Prior studies suggested that, within a particular species, dragline silk material properties vary because of plasticity in how the liquid dope of silk proteins is polymerized into a solid fiber as it passes through the duct of the silk gland and exits the spigot of the spider (Carmichael et al. 1999; Garrido et al. 2002a; Knight and Vollrath 2002). This model can explain variation in properties of silk based on the amount of order imparted to the alignment of molecules within the fiber during spinning and on how it is drawn from the spinnerets (Porter et al. 2005). These studies have demonstrated variation up to 50% in a particular property depending on either the conditions under which the silk is spun or individual variation (Garrido et al. 2002a; Guinea et al. 2005). However, our study focuses on differences between species of spiders and we find even larger (200–400%) differences in material properties of dragline silk among species in a controlled laboratory environment than the variation exhibited within species. Therefore, the inter-
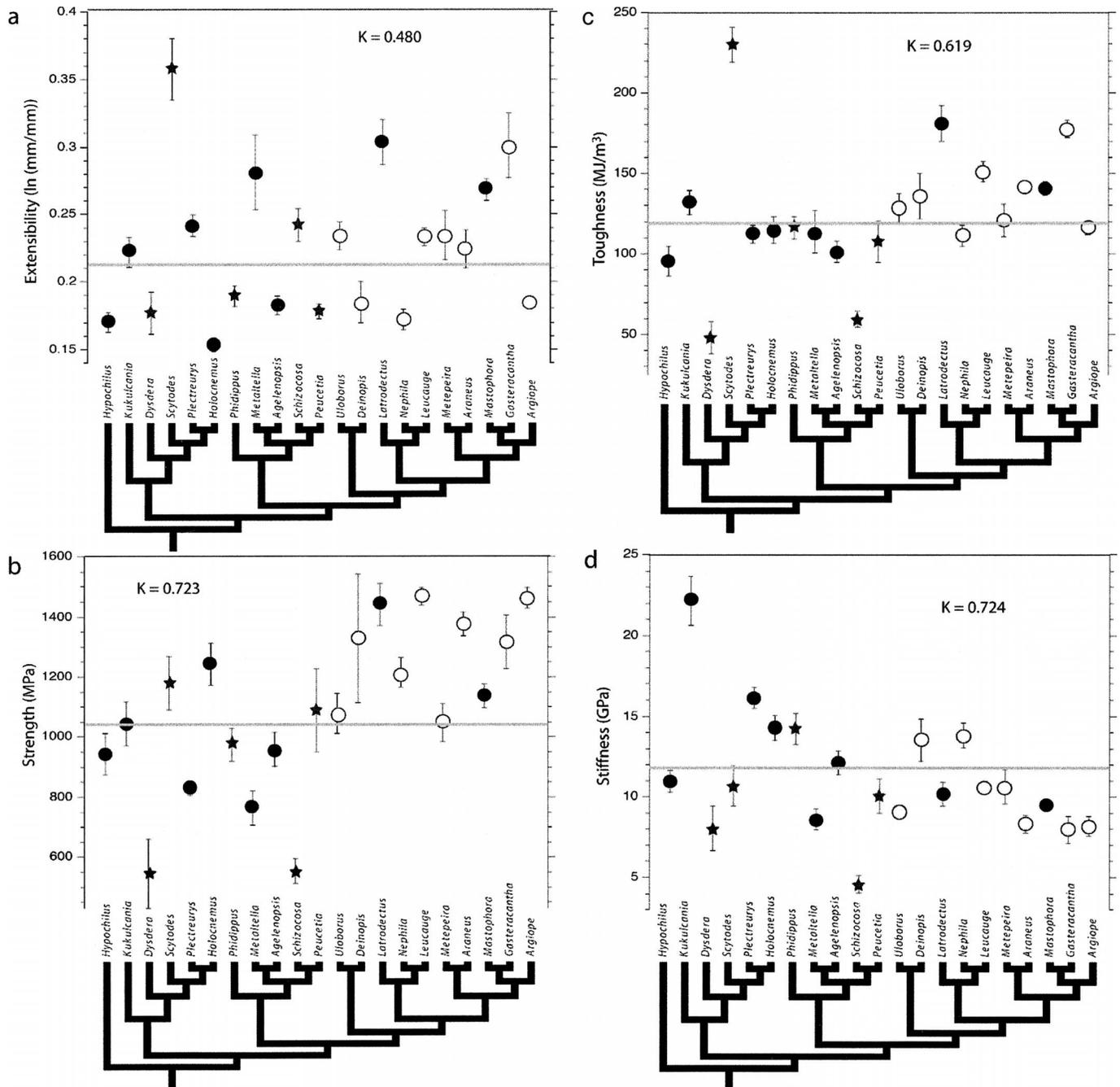
Fig. 3. Mean values ± 1 SEM for each material property across the phylogeny of spiders redrawn to include only the taxa used in this study. Open circles denote spiders that spin aerial orb webs, closed circles denote spiders that use non-orb capture webs, and stars denote spiders that are not known to use webs in prey capture. Horizontal gray lines denote the hypothesized value at the basal node of the tree calculated as the weighted average of the observed (tip) values (Garland et al. 1999). K-statistics for each property (see text; Blomberg et al. 2003) are included in the panels. (a) Extensibility, (b) strength, (c) toughness, (d) stiffness.

specific differences in material properties that we found are likely to represent functionally important, biologically relevant differences among species, and are unlikely to be due simply to plasticity in spinning conditions.

If we examine the pattern of variation in the properties of dragline silk across the spider phylogeny, we find that there is no significant phylogenetic signal for any of the traits measured (MSE tests). However, for strength and stiffness,

more than 94% of the randomized character sets had less signal than the observed data in their correct phylogenetic positions, suggesting that there may be weak conservation in the mechanical performance of dragline silk between related species of spiders, but that we do not have the power to resolve it (see Fig. 2 in Blomberg et al. 2003). The K-values for all of the properties were also less than one, indicating that silk spun by related spiders exhibited less similarity than
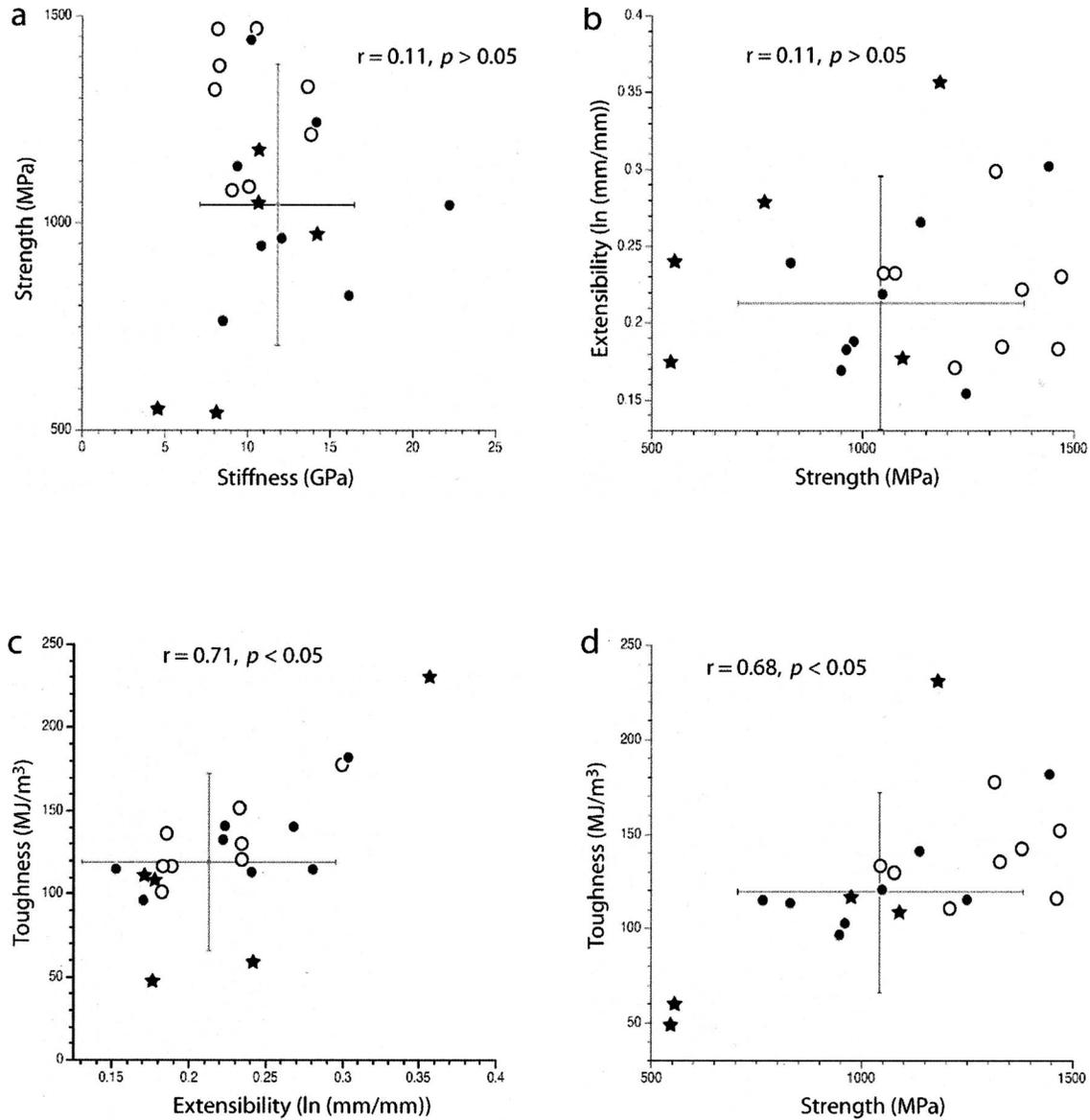
FIG. 4. Bivariate plots of selected material properties. Each marker represents a species mean. Open circles denote spiders that spin aerial orb webs, closed circles denote spiders that use non-orb capture webs, and stars denote spiders that are not known to use webs in prey capture. Coefficients are Pearson's product-moment correlations. Points with whiskers denote the hypothesized ancestral values at the basal node in the tree, calculated as the phylogenetically corrected mean with 95% confidence intervals (Garland et al. 1999). (a) Strength/stiffness, (b) extensibility/strength, (c) toughness/extensibility, (d) toughness/strength.

expected by a Brownian motion model of trait evolution (Blomberg et al. 2003). Toughness and extensibility deviated from the random expectation more than strength and stiffness. These deviations from the Brownian motion model suggest that different parts of the tree are under different selective regimes and that these regimes are sufficient to erase similarities caused by relatedness and random evolutionary change (Blomberg et al. 2003). However, K-values lower than one can also be caused by errors in the measurement of silk properties or by errors in the phylogenetic topology or branch lengths. Strength and stiffness had K-values relatively close to one and P-values approaching significant deviation from random phylogenetic signal, suggesting that they may be constrained, or under less selection than other properties. It is

important to note that most of the traits measured by Blomberg et al. (2003) had K-values less than one and that the descriptive statistics reported here fall well within reported values for morphological and physiological traits. To our knowledge this is the first estimate of phylogenetic signal in biomaterial traits, and it appears from these data that material properties are under similar levels of selection as other phenotypic traits and may be as evolutionarily labile as morphology and physiology.

An alternative argument for the lack of phylogenetic signal in the properties of dragline silk is that the variation among species is due to unmeasured factors that confound our analysis. We can rule out environmental effects on silk performance, such as temperature and humidity, because our tests
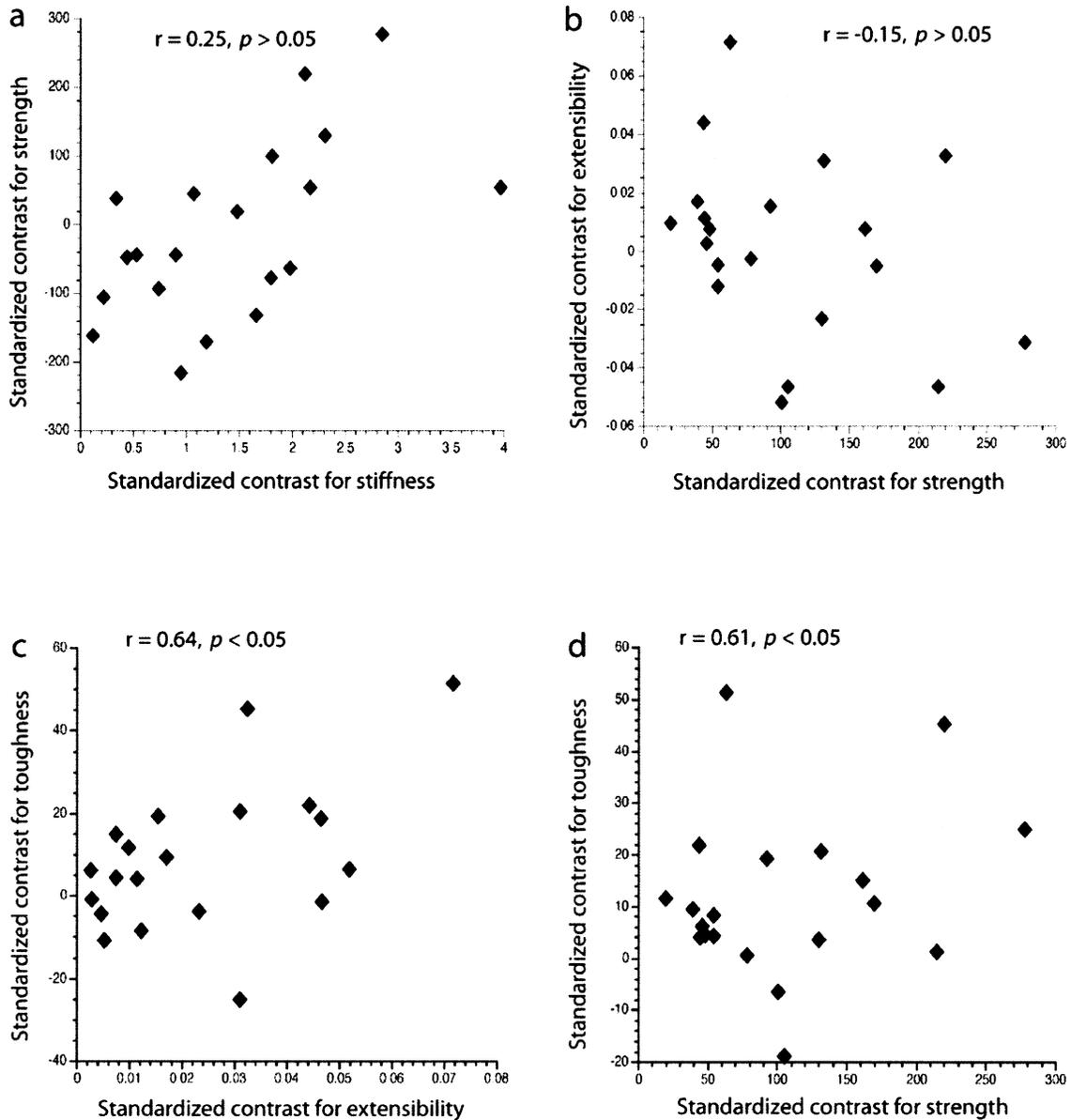
FIG. 5. Bivariate plots of standardized, phylogenetically independent contrasts of selected material properties. Because the signs of the contrasts are arbitrary, values on the x-axis are "positivized" for consistency (see text). Coefficients are Pearson's product-moment correlations constrained to pass through the origin. Each marker represents a node in the tree. (a) Strength/stiffness, (b) extensibility/strength, (c) toughness/extensibility, (d) toughness/strength.

were performed on silk in controlled laboratory conditions. Perhaps spiders are able to exert some control over these properties that we do not yet understand. A likely candidate factor would be spinning effects (e.g., Madsen et al. 1999; Garrido et al. 2002a), such as the rate or amount of tension a spider uses to pull fibers from its spinnerets. Dragline silks from several spider species show different material properties under different spinning conditions. For instance, forcibly silked fibers are less extensible and stiffer than draglines laid down by a walking spider (Garrido et al. 2002a; Guinea et al. 2005; Blackledge et al. 2005a). If the variation in material properties that we measured was mostly due to silk collection method, then we would expect fibers from the forcibly silked species to be less extensible and stiffer than fibers from the

nonforcibly silked species. On the contrary, we find that some of the species with exceptionally extensible fibers were forcibly silked (e.g., *Scytodes* and *Gasteracantha*; Fig. 3a). Furthermore, several of the species with fiber stiffness below the hypothesized ancestral value came from forcibly silked species (e.g., *Araneus*, *Gasteracantha*, and *Argiope* and others; Fig. 3d), and *Kukulcania*, the only species with exceptionally stiff silk, had fibers collected from freely walking individuals. We assume that spinning conditions did affect the results to some extent; however, the directionality of the differences that we found among species suggests that spinning effects did not produce the pattern of variation across species. It is also possible that, in some instances, silk collection method may have reduced apparent interspecific differences. Because

it is not known to what extent most of the species in our study can adjust the material properties of their silks (but see Garrido et al. 2002a; Guinea et al. 2005; Blackledge et al. 2005b), more research will be needed to understand the interaction between intrinsic material differences and spinning processes. Nevertheless, it is clear that we found meaningful variation among species of spiders in the mechanical performance of dragline silk.

Correlated Evolution

Most elastic solids, whether man-made or natural, show a strong positive correlation between stiffness and strength because the chemical bonds responsible for strength and stiffness are the same (Wainwright et al. 1980; Hancox 1981). In spider silk, however, different portions of polymeric proteins are responsible for stiffness (hydrogen bonds) versus strength (cross-linked beta sheets; Termonia 1994; Gosline et al. 1999). Our data show that over a broad range of species there is no obvious relationship between stiffness and strength, supporting this structural hypothesis (Figs. 4a, 5a). The lack of association between stiffness and strength also implies that natural selection can shape these properties independently of one another to produce dragline silk with performance characteristics that cannot be easily mimicked by man-made materials, but that may be well suited to the diverse ecological demands placed upon this type of silk.

For many man-made fibers, strength and extensibility vary inversely, meaning that strong materials are usually brittle whereas highly deformable materials rupture under modest loads (Wainwright et al. 1980). A similar trade-off between stretchy and strong has been suggested in previous studies examining variation in dragline silk performance within single species of spiders (Garrido et al. 2002a,b; Guinea et al. 2005; Porter et al. 2005). This trade-off may represent a constraint at the species level. However, our data do not demonstrate an evolutionary correlation between strength and extensibility across species (Figs. 4b, 5b). This suggests, once again, that these properties are independently shaped by selection.

Toughness can be calculated using the area under the stress-strain curve. Because toughness is a function of strength and extensibility, it should be affected by changes in both of these properties (Fig. 2; Denny 1976; Wainwright et al. 1980). As expected, our data support the evolutionary correlation of toughness with both strength and extensibility (Figs. 4, 5). Fiber toughness has been suggested by several authors to be very important to the function of prey capture webs. Therefore, web performance may provide the selective force explaining the extraordinary toughness of dragline silk (Denny 1976; Gosline et al. 1999; Porter et al. 2005). If toughness is a target of selection, then evolution can result in adjustments to either the strength or the extensibility of the fiber, or both. Accordingly, similar toughness could be produced by fibers with very different tensile behaviors (Denny 1976; Gosline et al. 1999) via alternative evolutionary pathways. For example, Scytodes produces an exceptionally tough silk that is only moderately strong, yet very extensible, whereas Latrodectus produces an exceptionally tough silk, which is very strong but not as extensible.

Patterns of Trait Evolution

We plotted the estimated basal node values (a weighted mean of the observed values) with 95% confidence intervals onto the bivariate plots of the data (Fig. 4), to provide some idea about the direction of material property evolution for dragline silk in different groups of spiders (Garland et al. 1999). As expected, the 95% confidence intervals enclose most of the species, with four notable exceptions. First, three orbicularian species (*Argiope*, *Latrodectus*, and *Leucauge*) produce dragline silk stronger than the 95% confidence interval of the basal node. All three species share an orb-weaving ancestor, although *Latrodectus* now spins a derived three-dimensional cobweb (Agnarsson 2004; Arnedo et al. 2004; Blackledge et al. 2005b). The multiple examples of exceptionally high strength in orbicularian spiders suggest that either high strength has evolved multiple times, or it has evolved once and then was reduced multiple times. A second group of outliers are the extremely tough silks produced by the orbicularian species, *Latrodectus* and *Gasteracantha*, and the distantly related spitting spider (*Scytodes*). Again, these exceptions imply multiple evolutionary acquisitions of high toughness. *Kukulcania*, the southern house spider, produced exceptionally stiff dragline silk, which may be related to the large body masses of these spiders and their long-enduring webs. The fourth group of outliers is perhaps the most revealing because it may illustrate what happens when selection on dragline silk is relaxed. *Dysdera* and *Schizocosa* are species that lay down dragline silks with exceptionally low strength and toughness. These distantly related spiders independently abandoned web spinning to become ground-dwelling predators that no longer use webs for prey capture or to support their body weight (Pollard et al. 1995; Suter and Stratton 2005).

Connections to Ecology

Based on previous studies, the need for orbicularian spiders to capture flying insects in orb webs had been hypothesized to select for high strength and toughness in dragline silk (Foelix 1996; Gosline et al. 1999; Porter et al. 2005). However, we find that high strength and toughness evolved before the Orbiculariae, the clade of orb web weaving spiders (Figs. 1, 3c,b). In fact, dragline silk across almost all spiders is an impressive, high-performance material, when compared to man-made materials and other natural fibers (Gosline et al. 2002). For example, the estimated basal node value for dragline toughness is higher than any other man-made or natural fiber known (Gosline et al. 2002). Even the lower 95% confidence limit of this basal node is higher than toughness values of high-performance fibers such as Kevlar, carbon fiber, and mussel byssus, and is an order of magnitude higher than the toughness values of collagen and high-tensile steel (Gosline et al. 1999, 2002). This finding suggests that the toughness of dragline silk may have evolved early in the evolution of spiders in response to a need to support the weight of the spider or capture ambulatory insects rather than to slow and stop flying prey.

Although the number of species sampled here makes it difficult to directly test correlations between ecological variables and material properties, one of our broad questions is

whether we can identify shifts in material performance that are related to specific selective factors. While all of the spiders in our study use dragline silk to spin trailing lines (Coddington 1989; Platnick et al. 1991), the majority also use this silk in their webs (Fig. 1). The taxa we sampled exhibit two major shifts in the construction of prey capture webs. The first is a shift away from using webs to capture prey, and the second is a shift from webs that capture ambulatory prey to webs that capture aerial prey.

Prey capture webs are plesiomorphic for the Araneomorphae, but five of our taxa have secondarily and independently abandoned capture webs (Eberhard 1990; Coddington and Levi 1991; Pollard et al. 1995; Suter and Stratton 2005). We expect that capture webs require higher performance silk, and in those taxa that do not make webs, we predict that silk is not as strong or as tough. Spiders that do not use webs in prey capture do have significantly lower dragline strength (conventional regression results). However, the GLS regression, which reduces the effects of phylogenetic relatedness, found no difference between these two groups in dragline strength. Hence, we cannot conclude that the observed variation in extant taxa is due to differences in silk use ecology. As mentioned above, two of the taxa that have abandoned capture webs (*Dysdera* and *Schizocosa*) produce the poorest performing silk in terms of both strength and toughness. However, the dragline silk of *Peucetia*, *Phidippus*, and *Scytodes*, the other taxa in our study that forage without webs, have highly variable properties and demonstrate no clear pattern, with *Scytodes* making very tough silk as mentioned above. The spinning of prey capture webs could provide an important selective force shaping the material performance of dragline silk, and relaxed selection in some groups may have resulted in loss of some high-performance characteristics, although more species will need to be sampled to resolve this question.

Our analysis included several members of the Orbiculariae, which use dragline silk as the supporting framework of their aerial orb webs. In these webs, spiders capture flying insects on the wing, subdue the insects, and occasionally leave the prey for consumption at a later time (Foelix 1996). We have already emphasized that the high-performance characteristics of dragline silk predate the evolution of orb webs. However, aerial web weaving should place increased demands on silk, in particular to dampen the kinetic energy of insects impacting webs and to provide stiff supports for webs. Because the orb web appears to have evolved only once (Coddington 1989; Coddington and Levi 1991; Garb et al. 2006), we cannot statistically test for changes in silk performance across this single node. Yet, silks from orb-weaving spiders are significantly stronger than silks spun by the other species (conventional regression results). In addition, two of the three species with exceptionally tough silk (outside the 95% confidence limits of the basal node) and all three species with exceptionally strong silk are found within the Orbiculariae. Both of these results suggest that although the overall high performance of dragline silk evolved prior to its use in orb webs, this foraging strategy may result in additional selective pressures that have shaped material performance.

Conclusions

The role of the material properties of biomaterials in the ecology of organisms is as variable as more traditional aspects of phenotype, such as color, morphology, behavior, and physiology, and may have as important an effect on fitness. In examining a single biomaterial with a clear ecological impact, we have documented that different material properties are under different selective regimes, and that some properties coevolve whereas others are decoupled. Evidence from tendon, bone, and cartilage (Currey 2002; Summers and Koob 2002; Hall 2005) suggests that the variability and evolutionary decoupling of mechanical properties may be generally representative of biomaterials. Although our study is broad in taxonomic scope, there are more than 39,000 other spider species (Coddington et al. 2004) that offer opportunities to correlate material properties of silk with a myriad of feeding, locomotor, and reproductive behaviors. The multiple independent acquisitions of a variety of ecological strategies and the ubiquity of silk make spiders an excellent model system for examining biomaterial evolution. Our study indicates that the conventional view that dragline silk is a single substance with a relatively narrow range of material properties is misleading. Instead, specific biomaterials, such as dragline silk, are best thought of as classes of materials that may have important variation at the species level where different aspects of performance have been independently shaped and tuned by natural selection.

Literature Cited

Agnarsson, I. 2004. Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). Zool. J. Linn. Soc. 141:447–626.

Arnedo, M. A., J. Coddington, I. Agnarsson, and R. G. Gillespie. 2004. From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 31: 225–245.

Barlow, G. W. 1968. Ethological units of behavior. Pp. 217–232 *in* D. Ingle, ed. The central nervous system and fish behavior. Univ. of Chicago Press, Chicago.

Blackledge, T. A., J. A. Coddington, and R. G. Gillespie. 2003. Are three-dimensional spider webs defensive adaptations? Ecol. Lett. 6:13–18.

Blackledge, T. A., J. E. Swindeman, and C. Y. Hayashi. 2005a. Quasistatic and continuous dynamic characterization of the mechanical properties of silk from the cobweb of the black widow spider *Latrodectus hesperus*. J. Exp. Biol. 208:1937–1949.

Blackledge, T. A., A. P. Summers, and C. Y. Hayashi. 2005b. Gum-

footed lines in black widow cobwebs and the mechanical properties of spider capture silk. Zoology 108:41–46.

Blackledge, T. A., R. A. Cardullo, and C. Y. Hayashi. 2005c. Polarized light microscopy, variability in spider silk diameters, and the mechanical characterization of spider silk. Invertebr. Biol. 124:165–173.

Blackledge, T. A., and C. Y. Hayashi. 2006. Silken toolkits: biomechanics of silk fibers spun by the orb web spider *Argiope argentata* (Fabricius 1775). J. Exp. Biol. 209:2452–2461.

Blomberg, S. P., T. Garland, Jr., and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717–745.

Carmichael, S., J. Y. J. Barghout, and C. Viney. 1999. The effect of post-spin drawing on spider silk microstructure: a birefringence model. Int. J. Biol. Macromol. 24:219–226.

Coddington, J. A. 1989. Spinneret silk spigot morphology: evidence for the monophyly of orb-weaving spiders, Cyrtophorinae (Araneidae), and the group Theridiidae-Nesticidae. J. Arachnol. 17(1):71–95.

Coddington, J. A., and H. W. Levi. 1991. Systematics and evolution of spiders (Araneae). Annu. Rev. Ecol. Syst. 22:565–592.

Coddington, J. A., G. Giribet, M. S. Harvey, L. Prendini, and D. E. Walter. 2004. Arachnida. Pp. 296–319 *in* J. Cracraft and M. J. Donoghue, eds. Assembling the tree of life. Oxford University Press, New York.

Craig, C. L. 1987. The ecological and evolutionary interdependence between web architecture and web silk spun by orb web weaving spiders. Biol. J. Linn. Soc. 30:135–162.

———. 1992. Aerial web-weaving spiders: linking molecular and organismal processes in evolution. Trends Ecol. Evol. 7:270–273.

Currey, J. D. 2002. Bones: structure and mechanics. Princeton Univ. Press, Princeton, NJ.

Denny, M. 1976. The physical properties of spider's silk and their role in the design of orb-webs. J. Exp. Biol. 65:483–506.

Dicko, C., D. Knight, J. M. Kenney, and F. Vollrath. 2004. Secondary structures and conformational changes in flagelliform, cylindrical, major and minor ampullate silk proteins: temperature and concentration effects. Biomacromolecules 5:2105–2115.

Dunaway, D. L., B. L. Thiel, S. G. Srinivasan, and C. Viney. 1995. Characterizing the cross-sectional geometry of thin, noncylindrical, twisted fibers (spider silk). J. Mater. Sci. 30:4161–4170.

Eberhard, W. G. 1990. Function and phylogeny of spider webs. Annu. Rev. Ecol. Syst. 21:341–372.

Erickson, G. M., J. I. Catanese, and T. M. Keaveny. 2002. Evolution of the biomechanical material properties of the femur. Anat. Rec. 268:115–124.

Fedič, R., M. Žurovec, and F. Sehnal. 2003. Correlation between fibroin amino acid sequence and physical silk properties. J. Biol. Chem. 278:35255–35264.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Foelix, R. F. 1996. Biology of spiders. Oxford Univ. Press, New York.

Garb, J. E., and C. Y. Hayashi. 2005. Modular evolution of egg case silk genes across orb-weaving spider superfamilies. Proc. Natl. Acad. Sci. USA 102:11379–11384.

Garb, J., T. DiMauro, V. Vo, and C. Hayashi. 2006. Silk genes support the single origin of orb-webs. Science 312:1762.

Garland, T., Jr., and P. A. Carter. 1994. Evolutionary physiology. Annu. Rev. Physiol. 56:579–621.

Garland, T., Jr., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst. Biol. 41:18–32.

Garland, T., Jr., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. Syst. Biol. 42:265–292.

Garland, T., Jr., P. R. Midford, and A. R. Ives. 1999. An introduction to phylogenetically based statistical methods with a new method for confidence intervals on ancestral values. Am. Zool. 39:374–388.

Garland, T., Jr., A. F. Bennett, and E. L. Rezende. 2005. Phylo-

genetic approaches in comparative physiology. J. Exp. Biol. 208:3015–3035.

Garrido, M. A., M. Elices, C. Viney, and J. Pérez-Rigueiro. 2002a. Active control of spider silk strength: comparison of drag line spun on vertical and horizontal surfaces. Polymer 43:1537–1540.

———. 2002b. The variability and interdependence of spider drag line tensile properties. Polymer 43:4495–4502.

Gatesy, J., C. Y. Hayashi, D. Motriuk, J. Woods, and R. V. Lewis. 2001. Extreme diversity, conservation, and convergence of spider silk fibroin sequences. Science 291:2603–2605.

Gosline, J. M., M. E. DeMont, and M. W. Denny. 1986. The structure and properties of spider silk. Endeavour 10:37–43.

Gosline, J. M., P. A. Guerette, C. S. Ortlepp, and K. N. Savage. 1999. The mechanical design of spider silks: from fibroin sequence to mechanical function. J. Exp. Biol. 202:3295–3303.

Gosline, J. M., M. Lillie, E. Carrington, P. Guerette, C. Ortlepp, and K. Savage. 2002. Elastic proteins: biological roles and mechanical properties. Philos. Trans. R. Soc. Lond. B 357:121–132.

Grafen, A. 1989. The phylogenetic regression. Philos. Trans. R. Soc. Lond. B 326:119–157.

Guerette, P. A., D. G. Ginzinger, B. H. Weber, J. M. Gosline. 1996. Silk properties determined by gland-specific expression of a spider fibroin gene family. Science 272:112–115.

Guinea, G. V., M. Elices, J. I. Real, S. Gutiérrez, and J. Pérez-Rigueiro. 2005. Reproducibility of the tensile properties of spider (*Argiope trifasciata*) silk obtained by forced silking. J. Exp. Zool. 303A:37–44.

Guinea, G. V., J. Pérez-Rigueiro, G. R. Plaza, M. Elices. 2006. Volume constancy during stretching of spider silk. Biomacromolecules 7:2173–2177.

Hall, B. K. 2005. Bones and cartilage: developmental skeletal biology. Academic Press, San Diego, CA.

Hancox, N. L. 1981. Fibre composite hybrid materials. Applied Science Publishers Ltd., London.

Hayashi, C. Y., N. H. Shipley, and R. V. Lewis. 1999. Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins. Int. J. Biol. Macromol. 24:271–275.

Hinman, M. B., and R. V. Lewis. 1992. Isolation of a clone encoding a second dragline silk fibroin. J. Biol. Chem. 267:19320–19324.

Knight, D. P. and F. Vollrath. 2002. Biological liquid crystal elastomers. Philos. Trans. R. Soc. Lond. B 357:155–163.

Köhler, T., and F. Vollrath. 1995. Thread biomechanics in the orb-weaving spiders *Araneus diadematus* (Araneae, Araneidae) and *Uloborus walckenaerius* (Araneae, Uloboridae). J. Exp. Zool. 271:1–17.

Lauder, G. V., and S. M. Reilly. 1996. The mechanistic bases of behavioral evolution: a multivariate analysis of musculoskeletal function. Pp. 104–137 *in* E. P. Martins, ed. Phylogenies and the comparative method in animal behavior. Oxford Univ. Press, New York.

Maddison, W. P., and D. R. Maddison. 2004. Mesquite: A modular system for evolutionary analysis. Available via http://mesquiteproject.org/mesquite/.

Madsen, B. and F. Vollrath. 2000. Mechanics and morphology of silk drawn from anesthetized spiders. Naturwissenschaften 87:148–153.

Madsen, B., Z. Z. Shao, and F. Vollrath. 1999. Variability in the mechanical properties of spider silks on three levels: interspecific, intraspecific and intraindividual. Int. J. Biol. Macromol. 24:301–306.

Midford, P. R., T. Garland, Jr., and W. P. Maddison. 2005. PDAP package of Mesquite. Available via http://mesquiteproject.org/pdap_mesquite/.

Opell, B. D., and J. E. Bond. 2001. Changes in the mechanical properties of capture threads and the evolution of modern orb-weaving spiders. Evol. Ecol. Res. 3:567–581.

Osaki, S. 1999. Is the mechanical strength of spider's drag-lines reasonable as lifeline? Int. J. Biol. Macromol. 24:283–287.

Pagel, M. D. 1992. A method for the analysis of comparative data. J. Theor. Biol. 156:431–442.

Pérez-Rigueiro, J., M. Elices, and C. Viney. 2001. Tensile properties of *Argiope trifasciata* drag line silk obtained from the spider's web. J. Applied Poly. Sci. 82:2245–2251.

Platnick, N. I., J. A. Coddington, R. R. Forster, and C. E. Griswold. 1991. Spinneret morphology and the phylogeny of haplogyne spiders (Araneae, Araneomorphae). Am. Mus. Novit. 3016:1–73.

Pollard, S. D., R. R. Jackson, A. Van Olphen, and M. W. Robertson. 1995. Does *Dysdera crocata* (Araneae Dysderidae) prefer wood-lice as prey? Ethol. Ecol. Evol. 7:271–275.

Porter, D., F. Vollrath, and Z. Z. Shao. 2005. Predicting the mechanical properties of spider silk as a model nanostructured polymer. Eur. Phys. J. E16:199–206.

Pouchkina-Stancheva, N. N., and S. J. McQueen-Mason. 2004. Molecular studies of a novel dragline silk from a nursery web spider *Euprosthenops* sp. (Pisauridae). Comp. Biochem. Physiol. B 138: 371–376.

Purvis, A. 1995. A composite estimate of primate phylogeny. Philos. Trans. R. Soc. Lond. B 348:405–421.

Scharff, N., and J. A. Coddington. 1997. A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). Zool. J. Linn. Soc. 120:355–434.

Stauffer, S. L., S. L. Coguill, and R. V. Lewis. 1994. Comparison of physical properties of three silks from *Nephila clavipes* and *Araneus gemmoides*. J. Arachnol. 22:5–11.

Summers, A. P., and T. J. Koob. 2002. The evolution of tendon: morphology and material properties. Comp. Biochem. Physiol. A 133:1159–1170.

Suter, R. B., and G. E. Stratton. 2005. *Scytodes* vs. *Schizocosa*: predatory techniques and their morphological correlates. J. Arachnol. 33:7–15.

Swanson, B. O., A. C. Gibb, J. C. Marks, and D. A. Hendrickson. 2003. Trophic polymorphism and behavioral differences decrease intra-specific competition in a cichlid, *Herichthys minckleyi*. Ecology 84:1441–1446.

Swanson, B. O., T. A. Blackledge, J. Beltrán, and C. Y. Hayashi. 2006. Variation in the material properties of spider dragline silk across species. J. Appl. Phys. A 82:213–218.

Tian, M. Z., C. Z. Liu, and R. V. Lewis. 2004. Analysis of major ampullate silk cDNAs from two non-orb-weaving spiders. Biomacromolecules 5:657–660.

Termonia, Y. 1994. Molecular modeling of spider silk elasticity. Macromolecules 27:7378–7381.

Vogel, S. 1998. Cat's paws and catapults. W. W. Norton and Company, New York.

Vollrath, F., and D. T. Edmonds. 1989. Modulation of the mechanical properties of spider silk by coating with water. Nature 340:305–307.

Vollrath, F., B. Madsen, Z. Z. Shao. 2001. The effect of spinning conditions on the mechanics of a spider's dragline silk. Proc. R. Soc. Lond. B. 268:2339–2346.

Wainwright, P. C. 1988. Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. Ecology 69: 635–645.

Wainwright, S. A., W. D. Biggs, J. D. Currey, J. M. Gosline. 1980. Mechanical design in organisms. Princeton Univ. Press, Princeton, NJ.

Work, R. W. 1978. Mechanisms for the deceleration and support of spiders on draglines. Trans. Am. Microsc. Soc. 97:180–191.

Xu, M., and R. V. Lewis. 1990. Structure of a protein superfiber: Spider dragline silk. Proc. Natl. Acad. Sci. USA 87:7120–7124.

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