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Spider Silk Aging: Initial Improvement in a High Performance Material Followed by Slow Degradation

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ABSTRACT Spider silk possesses a unique combination of high tensile strength and elasticity resulting in extraordinarily tough fibers, compared with the best synthetic materials. However, the potential application of spider silk and biomimetic fibers depends upon retention of their high performance under a variety of conditions. Here, we report on changes in the mechanical properties of dragline and capture silk fibers from several spider species over periods up to 4 years of benign aging. We find an improvement in mechanical performance of silk fibers during the first year of aging. Fibers rapidly decrease in diameter, suggesting an increase in structural alignment and organization of molecules. One-year old silk also is stiffer and has higher stress at yield than fresh silk, whereas breaking force, elasticity, and toughness either improve or are unaffected by early aging. However, 4-year old silk shows signs of degradation as the breaking load, elasticity, and toughness are all lower than in fresh silk. Aging, however, does not reduce the tensile strength of silk. These data suggest initially rapid reorganization and tighter packaging of molecules within the fiber, followed by longer-term decomposition. We hypothesize that possibly the breakdown of amino acids via emission of ammonia gas, as is seen in long-term aging of museum silkworm fabrics, may contribute. Degradation of spider silk under benign conditions may be a concern for efforts to construct and utilize biomimetic silk analogs. However, our findings suggest an initial improvement in mechanical performance and that even old spider silk still retains impressive mechanical performance. *J. Exp. Zool. 309A:494–504, 2008.* © 2008 Wiley-Liss, Inc.

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In the design of synthetic polymers, combining high tensile strength and elasticity to maximize toughness remains a major challenge (Booth and Price, ’89; Guan, 2007). However, spider silk possesses an extraordinary combination of these normally orthogonal material properties (Gosline et al., ’86; Vollrath, 2000; Vollrath and Knight, 2001; Vollrath and Porter, 2006). Spider silk is also an attractive model for biomimetic fibers because it is immunologically compatible with living tissue and the silk is spun under environmentally benign conditions (i.e. inside a living organism) (Vadlamudi, ’95; Vollrath and Knight, 2001).

Spiders are capable of producing up to seven different kinds of silk, each of which is spun from discrete glands (Blackledge and Hayashi, 2006). Two of these silks play a dominant role in the spinning of orb webs and are the main focus of biomimetic research (Fig. 1). Spiders use dragline silk, spun from major ampullate glands, to build the dry frame threads of their webs and also as lifelines when dropping from high places. Dragline silk can match steel in strength but is also highly elastic and thus outperforms the best synthetic fibers in terms of toughness (Gosline et al., ’86). These properties result from stiffening by β-sheet crystals that cross-link the proteins. The crystals

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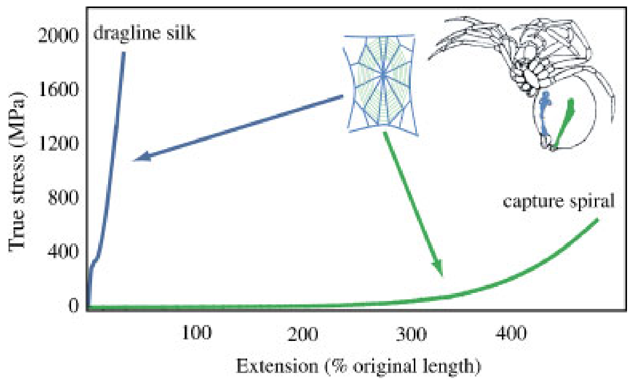

Fig. 1. Spider orb webs are composed of two different fibrous silks that are highly divergent in material properties and each of which function as a model for the development of biomimetic fibers. Stiff and strong dragline silk (blue) forms the supporting frame of webs and is also spun as spiders walk through the environment. Highly elastic capture silk (green) forms the core of the sticky spiral of orb webs. Spider redrawn from Vollrath ('92).

are embedded within a network of less organized, ''amorphous'' regions of the proteins that provide mobility (Gosline et al., '99). Capture silk, spun from flagelliform glands, forms the fibrous core of the sticky spiral of orb webs. Capture silk is weaker than dragline silk, but far more elastic and almost equally tough (Denny, '76; Blackledge and Hayashi, 2006). Capture silk proteins are characterized by large numbers of β-spiral ''molecular nanosprings'' that result in an extremely compliant and elastic fiber (Hayashi and Lewis, 2001; Becker et al., 2003). In contrast to dragline silk, capture silk functions in a rubber-like state because it is surrounded by an aqueous glue (''silk'' from aggregate glands) that plasticizes the silk proteins (Gosline et al., '84; Vollrath and Edmonds, '89). Thus, the two types of silk fibers provide very different functional models for the development of synthetic analogs.

Orb weaving spiders typically renew their webs on a daily basis, first removing and consuming the old silk and then spinning a new orb. As a result, neither of these silks is normally required to function for periods of time longer than $\sim$ 1 day in nature. Other spiders, such as cobweb weavers, spin webs with backbones of dragline that may persist longer, up to several days or weeks. In contrast, most applications for biomimetic silk analogs, such as high performance cables or clothing, depend upon long-term durability of mechanical performance. Thus, it is essential to understand how the mechanical performance of spider silk is affected by the environment (Altman et al., 2003). For instance, very short exposure to UV radiation, or brief aging, can increase tensile strength of spider dragline silk (Osaki et al., 2004; Pérez-Rigueiro et al., 2007). Aging also increases stiffness in the egg sac silk produced from the cylindrical gland, making those fibers more brittle (Van Nimmen et al., 2003; Gellynck, 2006). At least some of these changes result from cleavage of protein bonds or from the formation of new cross-linking between proteins within fibers. However, longer exposure to UV radiation results in degradation of performance, likely owing to decay of amino acids via emission of ammonia gas from the fiber and to oxidation, as happens with many polymers (Clough et al., '96). Aging within the laboratory decreased the toughness and diameter of dragline silk (Van Nimmen et al., 2003). Even under museum archival conditions, amino acids appear to be lost via various processes from ancient silk fabrics, resulting in weight loss of naturally aged silkworm (*Bombyx mori*) fabrics (Becker et al., '95;Yanagi et al., 2000; Hermes et al., 2006). Finally, dragline spider silk degrades very rapidly when implanted onto pig skin, (Vollrath et al., 2002) with important implications for its use in biomedical applications.

Here, we report on changes in the mechanical properties of dragline and capture silk fibers from several spider species after aging for short (up to 1 year) or long periods (up to 4 years) under very benign conditions. We compare the properties of the aged fibers with fibers taken from the same individual spiders and tested fresh up to 4 years earlier and then we compare our results with studies looking at short-term (less than 1 month) effects of aging (Griffiths and Salanitri, '80; Elices etal., 2005).

MATERIALS AND METHODS

Major ampullate dragline silk

To test the effect of aging dragline silk for 4 years, we chose nine species from among the ''true spiders'' (Araneomorphae) to represent variation in silk use and ecology (Table 1). For each species, we used 1–4 spiders and measured 3–11 silk samples from each individual. Exemplars include eight orb weaving spiders, one species with a modified orb web (*Deinopis*) and another that has secondarily lost the behavior (*Mastophora*). Some of these species have been intensively studied as models for biomimetic silk. We also included the non-orb weaving green lynx spider *Peucetia* that produces dragline silk but does not spin prey

TABLE 1. Number of individuals used per species (N), and the total number of samples (individual fibers) tested, both fresh and after aging

Spider	No. fresh	No. aged	Silk use
Dragline—4-year aging			
Araneus gemmoides ($N=2$)	15	10	Orb webs, dragline
Argiope argentata ($N=4$)	24	17	Orb webs, dragline
Argiope trifasciata ($N=1$)	4	5	Orb webs, dragline
Deinopis spinosa ($N=2$)	19	10	Modified orb webs, dragline
Gasteracantha cancriformis ($N=2$)	11	6	Orb webs, dragline
Latrodectus geometricus ($N=1$)	6	5	Cobwebs, dragline
Latrodectus hesperus ($N=2$)	6	5	Cobwebs, dragline
Mastophora hutchinsoni ($N=3$)	19	19	Dragline (orbweb lost)
Mastophora phrynosoma ($N=1$)	7	5	Dragline (orbweb lost)
Peucetia viridans ($N=3$)	22	15	Dragline
Dragline—1-year aging			
Achaearanea tepidariorum ($N=7$)	21	21	Cobwebs, dragline
Dragline—continuous aging			
Latrodectus hesperus ($N=1$)	5	20	Cobwebs, dragline
Capture—continuous aging			
Argiope argentata ($N=5$)	59	59	Sticky spiral of orb webs

Four- and 1-year aging refer to samples that were tested fresh and only at one later age. Continuous aging refers to samples that were tested at several time intervals. A total of 418 silk samples were tested.

capture webs. In addition, we measured three samples of silk from each of seven individuals of the cobweb spinning common house spider (*Achaearanea tepidariorum*) to examine changes in silk over a shorter period of time (up to 1 year) and to focus on changes in variability of performance. Finally, we sampled silk from a single cobweb spinning black widow, *Latrodectus hesperus*, to examine changes over even shorter periods (7–260 days), testing five silk samples each at day 1 (fresh), and aged 7, 26, 50, and 260 days.

Spiders were housed individually in cages at room temperature (about 23°C), fed crickets two to three times per week, and misted periodically with water. We collected fibers by forcible silking onto paper slides as described in Blackledge et al. (2005c). Silk collected in this manner is mounted under minor tension, analogous to native tension of silk threads in webs, draglines etc., thereby minimizing the potential influence of creep on future performance. For each spider, we initially tested some of the fibers fresh, the same day they were spun, while storing the remainder on the paper slides in slide boxes. The boxes were sheltered from UV radiation, but subject to normal changes in temperature and humidity as ambient conditions varied in the storage room. This ''unassisted'' aging contrasts with most previous work on polymers, where aging effects are accelerated with the aid of temperature, UV radiation, or chemicals (Clough et al., '96; El Shafee, 2003; Vlasveld et al., 2005; Boukezzi et al., 2006). However, natural aging may be a useful framework for understanding the durability of fibers and causes of degradation.

For each sample, we measured force–extension curves and calculated four mechanical properties. (1) Ultimate strength, or true breaking stress, was calculated as the amount of force required to break a fiber relative to the instantaneous cross-sectional area of the fiber. The instantaneous cross-sectional area of a fiber was calculated using an assumption of constant volume during extension (Vollrath et al., 2001). (2) Extensibility or true breaking strain. True breaking strain was calculated as the natural log of the breaking length divided by original length. The standard isovolumetric assumption was applied (Guinea et al., 2006). (3) Initial stiffness or Young's modulus was calculated as the slope of the stress–strain curve over the initial elastic region. (4) Toughness or the energy absorbed by a fiber prior to rupture was calculated as the area under the stress–strain curve divided by sample volume (calculated from the initial cross-sectional area and length of the fiber). For each spider, we used the average performance of all silk samples tested to estimate the percent change in mechanical performance induced by aging as

$$(\text{aged silk} - \text{fresh silk})/\text{fresh silk} * 100$$

Tensile testing followed earlier work (Blackledge et al., 2005b,c). We glued silk onto cardboard holders across 20.7 mm gaps using Superglue® (Hollys, NY) (cyanoacrylate). We determined the diameter of each silk sample earlier to testing by averaging six measurements taken along the length of the fiber using polarized light microscopy, which has been shown to be as accurate as measurements from electron microscopy (Blackledge et al., 2005a). We also examined each sample to confirm that it consisted solely of one or two equi-dimensional fibers, thereby excluding any samples that included other types of silk besides major ampullate fibers or exhibited obvious flaws. We attached silk samples to the grips of a Nano Bionix tensile tester (MTS, Oakridge, TN) and extended them at a rate of 1% strain/sec to failure. The testing environment ranged from 21 to 28°C with a range of 16–54% relative humidity during initial testing, and 16–45% humidity during testing of 4–year-aged silk. Despite the overall broad range in humidity across the study, most aged silk samples were tested at within ~10% of the humidity at which their fresh counterparts were tested.

We originally tested silk fibers the same day as they were collected and subsequently about 4 years later. We used paired *t*-tests to compare the diameters, and performance, of freshly collected major ampullate silk fibers to aged fibers, averaging within each spider. For diameter comparisons we used uncorrected α value (0.05). For mechanical performance we compared several parameters, therefore, in addition to comparing results with $\alpha = 0.05$ we also more conservatively compared test values with sequential Bonferonni correction values (a global $\alpha = 0.05$, and sequential single test critical $\alpha = 0.0500$, 0.0250, 0.0167, 0.0125, 0.0100, and 0.0083).

We tested dragline fibers from seven individuals of *A. tepidariorum*, at an intermediate age of approximately 1 year. For the intermediate-aged silk, we also examined the variance in mechanical properties of aged and fresh silk. We compared two potential sources of variation—within individual spiders and between spiders. To compare variability within individual spiders we calculated the average coefficient of variation (CV) for each of the seven spiders' silk. We then used a paired *t*-test to compare fresh vs. aged silk. To compare variability across spiders we calculated the CV of each individual sample of silk, from the global mean by pooling all spiders. We then compared the average CV of fresh vs. aged silk using a χ^2 test. This allowed us to determine whether changes in variability of silk properties during aging resulted from different spiders' silk becoming more similar to one another or from simple reduction in the variability of each spider's silk.

Flagelliform capture silk

The protocol differed slightly for testing capture silk in the following ways. First, we mounted samples using Elmer's® glue (Columbus, OH) because cyanocrylate dehydrates the surrounding coating of aqueous glue (Blackledge and Hayashi, 2006). Second, we analyzed a total of 65 directly paired samples of silk collected from five spiders. For each sample, we collected three adjacent segments of silk from a single continuous capture thread, mounting the outer two onto cardboard across gaps as described above. We adhered the middle sample directly to a glass slide to visualize the diameters of the core capture silk fibers, which are normally obscured by the aqueous glue that surrounds them. We then immediately mechanically tested the first sample, as described above, testing the second sample after a period of aging in a covered glass petri dish. Thus, we were unable to compare potential changes in diameters of capture silk through time but could make tightly controlled comparisons in mechanical performance.

We mechanically tested the capture silk in the same manner as the dragline silk with two important differences. First, the surrounding glue plays a vital role in modulating the properties of the capture silk but is known to qualitatively degrade rapidly (Gosline et al., '84; Vollrath and Edmonds, '89). We therefore performed most comparative testing of fresh and aged silk over a scale of 1–20 days and second set of samples aged to ~120 days. Second, the surrounding glue droplets act as windlasses that reel in loose silk thereby visually obscuring when tension is removed from slacked capture threads (Vollrath and Edmonds, '89) and capture threads are already significantly strained within webs. Therefore, to determine the actual gauge length of specimens, we overslacked specimens and then used the point of the force displacement curve at which detectable load was generated to determine the gauge length for each sample (Blackledge and Hayashi, 2006; Swanson et al., 2007).

Linear regression compared variation in age of silk (from 1–20 days) with the percent change in silk performance calculated as above. Multivariate analysis of variance (MANOVA) tested for differences in the performance of briefly aged (1–20 day old) vs. older (~120 days) capture threads.

RESULTS

In the 4-year-aged dragline silk, the diameters of threads were reduced by 14% and the breaking load by 11% (Table 2, Figs. 2 and 3). Young's modulus (stiffness) was 54% higher, whereas extensibility and toughness were reduced (35 and 18%, respectively) (Fig. 4). Furthermore, the yield stress increased by 217% (Table 2, Fig. 4). In contrast, ultimate strength (true stress) did not differ from fresh silk (Table 2, Fig. 4). The effects of aging may show some species-specific patterns as individuals of each species tended to cluster (Figs. 2–4), but our sample sizes were too small to address this quantitatively.

After 1 year of aging (intermediate-aged silk), the diameter of the common house spiders', *A. tepidariorum*, dragline silk was reduced by 23%, but breaking load was maintained (Table 3). Young's modulus was 34% higher in 1-year-old silk compared with fresh silk, and yield stress was 42% higher than in fresh silk. Extensibility, toughness, and ultimate strength did not differ statistically from fresh silk (Table 3), although both toughness and strength showed a trend toward improvement after 1 year aging. The intermediate-aged silk performed in a much more uniform and predictable manner than did the fresh silk. The CV in performance of fresh silk was greater than that of aged silk for all parameters except true strain (Table 3). This seemed due to silk from different individual spiders becoming more similar through time because variation within individual spiders did not differ between fresh and old silk. We had less power to test statistically for changes in variability for 4-year-aged silk because fewer individual spiders per species were examined. However, both across all species and for individual spiders within the most densely sampled species (*A. argentata*), the performance of 4-year-aged silk was as variable as fresh silk (data not shown).

Within the first 50 days, the silk of one individual black widow (*L. hesperus*) decreased in diameter and increased in yield stress, modulus, true stress, and toughness. Those changes were retained until we last tested silk at 230 days (Table 4, trends were statistically insignificant for diameter and yield stress, owing to few data).

The overall performance of capture spiral silk declined within the first 20 days after it was spun ($F_{5,32} = 0.40$, $P = 0.007$) (Fig. 5). The fibers became stiffer and lost extensibility, strength, and toughness (Table 2, Fig. 5). The initial gauge length of aged fibers also increased ($F_{1,36} = 10.9$, $R^2 = 23.3$, $P = 0.002$). We used MANOVA to compare the change in performance of ''young'' fibers (aged <20 days) to ''old'' fibers (aged 120–180 days) and found a continued decline in performance ($F_{5,52} = 11.95$, $P < 0.000$). This was

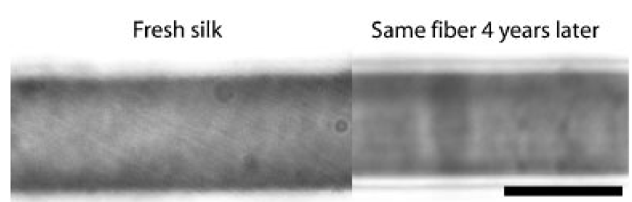

Fig. 2. Change in the diameter of a single silk thread photographed immediately after spinning and 4 years later. Scale = 5 μm.

TABLE 2. Change in diameter and mechanical properties of aged vs. fresh dragline and capture silk

	Dragline 4yo, df = 20	Dragline 1yo, df = 7	Capture silk 20do
Diameter	−14% ($t = -3.8$, $P = 0.001$)	−23% ($t = -2.8$, $P = 0.032$)	N/A
Breaking load	−11% ($t = -3.4$, $P = 0.003$)	($t = -0.66$, $P = 0.53$)	−72% ($F_{1,\ 36} = 12.1$, $R^2 = 25.1$, $P = 0.001$)
Young's modulus	54% ($t = 3.5$, $P = 0.002$)	34% ($t = 2.5$, $P = 0.046$*)	7% ($F_{1,\ 36} = 4.2$, $R^2 = 10.4$, $P = 0.048$)
Yield stress	217% ($t = 3.3$, $P = 0.003$)	42% ($t = 3.2$, $P = 0.018$*)	N/A
Extendibility	−35% ($t = -4.6$, $P < 0.001$)	($t = -0.28$, $P = 0.79$)	−21% ($F_{1,\ 36} = 8.3$, $R^2 = 18.8$, $P = 0.007$)
Toughness	−18% ($t = -3.5$, $P = 0.002$)	($t = 1.43$, $P = 0.20$)	−76% ($F_{1,\ 36} = 6.2$, $R^2 = 14.6$, $P = 0.018$)
Strength (true stress)	($t = 0.6$, $P = 0.53$)	($t = 1.1$, $P = 0.31$)	−80% ($F_{1,\ 36} = 7.6$, $R^2 = 17.3$, $P = 0.009$)

Percent change from fresh to aged silk is shown for significant values ($\alpha = 0.05$). Values that are nonsignificant after sequential Bonferroni corrections are marked with an asterix. Four-year-old (4yo) and 1-year-old (1yo) dragline silk are compared with fresh silk using *t*-tests. Aging in capture silk varied daily. Linear regression was used to test for a significant change in properties across days 1–21, whereas the percent change is reported only for 17–21-day-old (20do) samples.

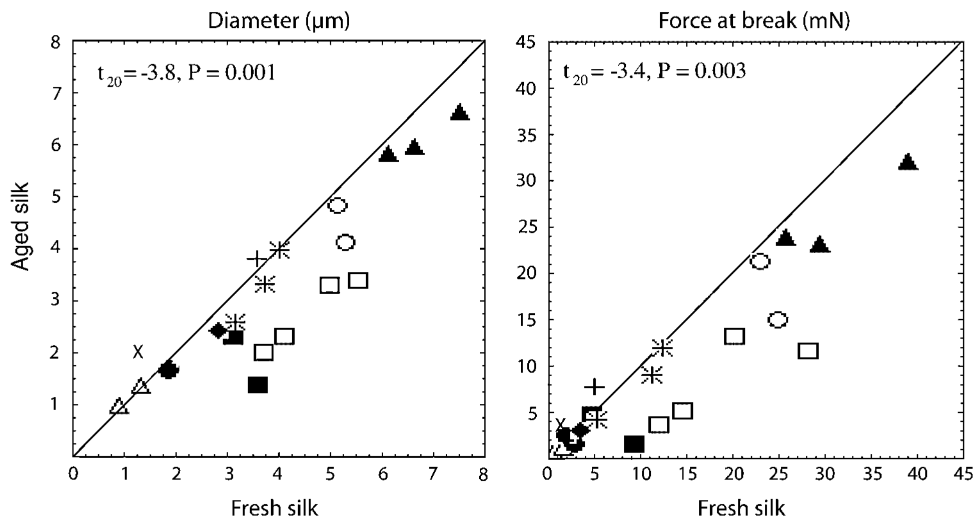

Fig. 3. Comparison of the structural properties of fresh and 4-year-old dragline silk spun by nine species of spiders. The line represents no change in mechanical performance. Symbols: ◦ *Araneus gemmoides*; □ *Argiope argentata*; x *Argiope trifasciata*; Δ *Deinopis spinosa*; ● *Gasteracantha cancriformis*; ♦ *Latrodectus geometricus*; ■ *Latrodectus hesperus*; ▲ *Mastophora hutchinsoni*; + *Mastophora phrynosoma*; ⋇ *Peucetia viridens*.

primarily driven by loss in extensibility ($F_{1,56} = 10.7$, $P = 0.002$), strength ($F_{1,56} = 18.3$, $P < 0.000$) and toughness ($F_{1,56} = 11.8$, $P = 0.001$).

DISCUSSION

Spider dragline silk clearly changes both in structural and material properties with aging. These changes occurred even though the silk was stored under benign environmental conditions where fibers were protected from UV light and maintained at ambient temperature and humidity. Many synthetic polymer fibers exhibit an initial improvement in performance after they are drawn as molecules continue to reorient along the fiber axis and bond more tightly (Davis, '96). Similarly, we found that many of the desirable properties of spider dragline silk are retained, or improved, after aging up to 1 year. Some mechanical properties, such as stiffness and yield stress improved over 10 days of aging (Elices et al., 2005) and continue to improve for up to 4 years year of aging (Tables 2 and 4). However, perhaps the most commonly sought-after quality—tensile strength—was never impacted, even after 4 years. The performance of spider dragline silk also becomes much more uniform and predictable than that of fresh silk (Table 3), as reported for other biological fibers such as wool (Rigby et al., '74).

Capture silk degrades more rapidly and uniformly than dragline silk. All measures of performance reduced continuously across the first 20 days. This rapid and dramatic ''degradation'' likely results primarily from the drying out of the aqueous glue coating because it is essential for maintaining the elastic mobility of capture silk proteins (Vollrath and Edmonds, '89). Like dragline silk, aging of capture silk continues as a gradual process over extended periods of time, with dramatic changes in performance occurring between days 20 and 180 (350% increase in stiffness, and reductions of 23% in strain, 83% in stress, and 71% in toughness).

The thinning of silk with age has been reported before for silkworm fibers (Becker et al., '95) and dragline silk from *Araneus* (Van Nimmen et al., 2003), but the causes are poorly understood (Elices et al., 2005; Gellynck, 2006). Initially, thinning is likely owing to reorganization of the silk fiber that results in a denser packing of molecules (El Shafee, 2003). This explains the overall trend toward improvement in performance. However, the later decline in performance of silk fibers suggests a long-term deterioration of the intermolecular bonding between silk proteins or even degradation of individual peptides. This may result from the emission of ammonia gas from the fibers as amino acids degrade, such as happens under UV radiation (Yanagi et al., 2000), or oxidation disrupting chemical bonds within the fiber (Robinson and Rigby, '81; Clough et al., '96; Gumargalieva et al., '96). The combination of changes in mechanical properties of silk with aging that we found may provide clues. Despite physical changes, the tensile strength of the dragline silk remains constant as silk ages.

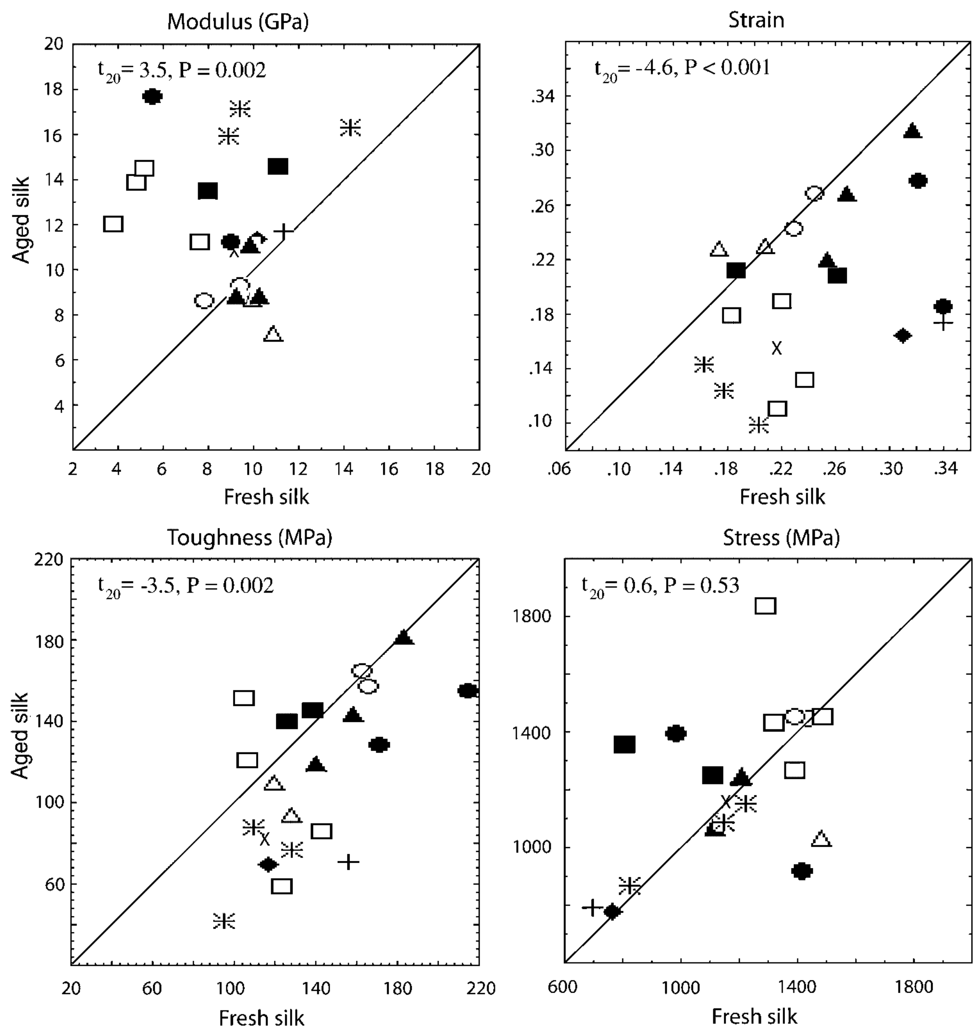

Fig. 4. Comparison of material properties of fresh and 4-year-old dragline silk from nine species of spiders. The line represents no change in mechanical performance. Symbols: ◦ *Araneus gemmoides*; □ *Argiope argentata*; x *Argiope trifasciata*; Δ *Deinopis spinosa*; ● *Gasteracantha cancriformis*; ♦ *Latrodectus geometricus*; ■ *Latrodectus hesperus*; ▲ *Mastophora hutchinsoni*;+*Mastophora phrynosoma*; ⁎ *Peucetia viridens*.

TABLE 3. Comparison of the coefficient of variation (CV) in mechanical performance of fresh vs. 1-year-aged A. tepidariorum dragline silk

	CV fresh	CV aged	χ^2	*P*	df
Diameter	10.965	6.306	1.257	n/s	1
Modulus	20.884	2.766	13.88	<0.001	1
Yield stress	20.688	2.718	13.797	<0.001	1
True stress	24.032	5.641	11.399	<0.001	1
True strain	4.955	8.394	0.886	n/s	1
Toughness	21.594	6.404	8.241	0.004	1
Breaking force	37.461	16.424	8.213	0.004	1

Average CVs are calculated for each sample of silk from the global mean. The χ^2 tests compare the average CV of all fresh vs. aged samples of silk. The average CV of silk within individual spiders did not differ between fresh silk and aged silk.

However, the silk becomes stiffer and less elastic, both commonly observed aging effects in polymers that, at least in synthetic polymers, can be attributed to oxidation (Gillen et al., '96). Furthermore, Yanagi et al. (2000) found that under UV radiation silk decomposes first in the amorphous region via emission of ammonia gas and changes in the amino acid composition. The crystalline region, however, decomposes much more slowly. Hence, the degree of crystallinity of the fiber as a whole would increase through time, resulting in increased stiffness and brittleness (Davis, '96). This may also explain why tensile strength is maintained because the crystalline region is thought to be the major contributor to silk's strength.

TABLE 4. Changes in properties of silk from the black widow L. hesperus *over a period of 230 days*

Age	Diameter	Modulus	Yield stress	True stress	True strain	Toughness
1	2.07	7.84	216.80	1455.40	0.32	214.31
7	1.60	8.91	238.02	1200.98	0.26	146.38
26	1.93	6.95	181.37	1328.14	0.33	192.80
50	1.30	12.53	610.88	1839.97	0.26	258.34
230	1.40	19.09	501.46	1926.89	0.27	268.70
	n/s	$F_{(1,24)} = 39.00$ $P < 0.0001$	n/s	$F_{(1,24)} = 8.2$ $P = 0.009$	n/s	$F_{(1,24)} = 6.8$ $P = 0.016$

Data are averages of five samples tested at each time period. Statistics show results of multiple regression analyses using age to predict all parameters simultaneously (yield stress marginally insignificant, $P = 0.06$).

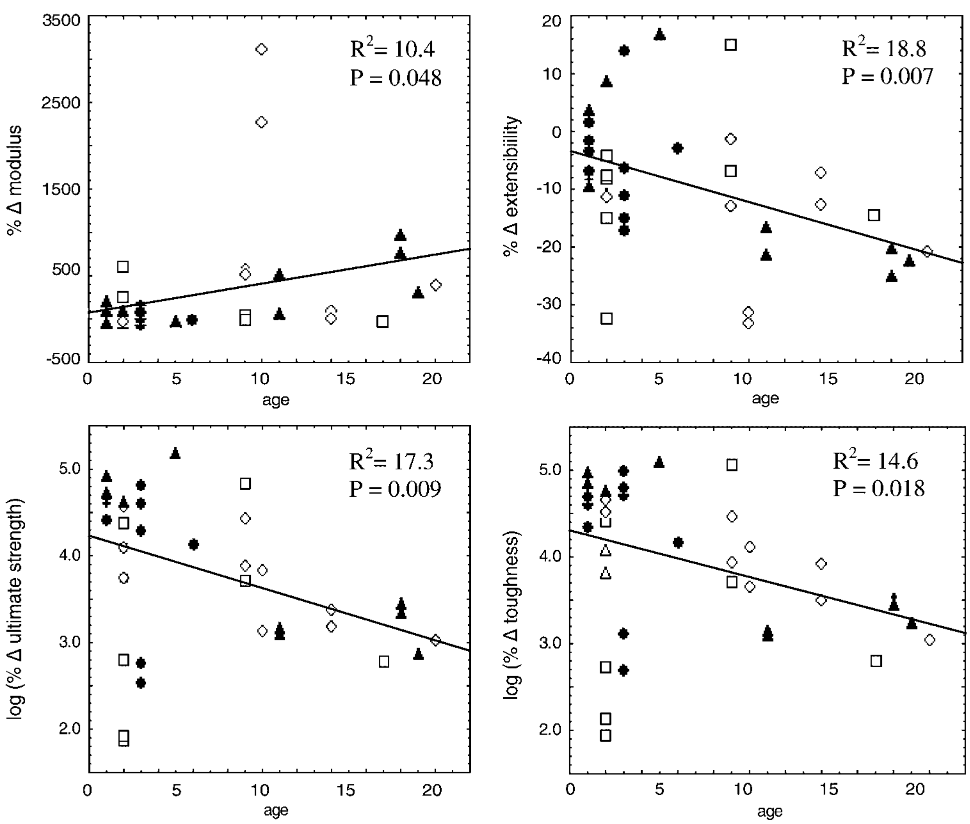

Fig. 5. Short-term degradation of performance for glue-coated capture silk. Symbols indicate silk spun by five different individuals of the orb weaving spider *Argiope argentata*.

Sticky capture silk differs fundamentally from dragline silk in being coated by aqueous aggregate silk glue. This coating dramatically affects the performance of the core fibers of the capture silk by hydrating them (Gosline et al., '84; Vollrath and Edmonds, '89). Thus, at least some of the changes we found with aging, early on, are likely owing to evaporation of water from the glue coating. It should be noted, however, that capture threads contain hydrophilic compounds that can attract atmospheric moisture (Vollrath et al., '90; Townley et al., '91), and at humidity of about 50% and above desiccated capture threads can regain full glue volume (Opell and Schwend, 2008). However, in our case, the average humidity during testing of fresh samples (35±5%) was similar to that of aged silk (31±6%). Moreover, the testing humidity of the oldest capture thread samples (42±2%) was actually higher than at initial testing, yet they still exhibited changes in performance. It is possible that there is a threshold humidity at which atmospheric moisture can be attracted, and that our testing conditions were below it. In addition, processes other than mere drying are likely involved in capture thread degradation. We hypothesize that orientation and cross-linking of proteins might increase over the long term, as seems to be the case with dragline silk. In particular, the incredible elasticity of capture silk is owing to, in part, highly mobile and extensible ''nanosprings'' that are formed by stereotyped amino acid motifs within the protein chains (Hayashi and Lewis, '98; Becker et al., 2003). Even a small increase in the cross-linking of these protein motifs, as described in the aging of other types of spider and silkworm silk, would dramatically stiffen capture silk.

Spider silk's unique high performance properties make it a desirable model for artificial fibers. However, relatively rapid degradation in the performance of spider silk under benign conditions has important implications for biomimicry. For example, spider silk-like material might be ideal for semielastic climbing ropes, but such ropes would gradually become stiffer. Similarly, spider silk used as material in bullet-resistance armor (Lazaris et al., 2002) might reduce in toughness over time. Our findings suggest that spider silk experiences degradation over the long term similar to many synthetic polymers that reduce in mass, increase in stiffness and yield stress, and lose strength, elasticity, and toughness through time (Clough et al., '96; Davis, '96; Gumargalieva et al., '96; El Shafee, 2003). However, even after 4 years the performance of spider dragline silk, in particular its toughness, is still extraordinary. Spider silk may also differ from typical synthetic polymers because the initial improvement exhibited during early aging is retained for 1 year or more. Spider dragline silk is also exceptional for its long-term retention of tensile strength. Understanding the molecular basis for this pattern of aging in mechanical performance will ultimately help in the design of high-quality and long-lasting fibers of many different polymers.

Both dragline and capture spider silk undergo relatively rapid physical and mechanical change with aging under benign conditions. Our findings, combined with earlier studies, suggest that aging may be parsed into short- and long-term changes in molecular structure. First, there is a relatively rapid reorganization, tighter packaging, and cross-linking of molecules within the fiber that generally improves performance as the molecules reach a new equilibrium. This results in the material properties of moderately aged spider silk becoming more homogenous than those of fresh silk. This early process is then followed by a much slower and continuous degradation of performance that we hypothesize may involve the breakdown of amino acids and the seeping of ammonia gas (and, perhaps, water) from the fibers, as seen in ancient silk (Yanagi et al., 2000; Hermes et al., 2006), and increase in the crystallinity of the fibers through changes in the cross-linking of proteins, as seen in other biological fibers such as keratin (Robinson and Rigby, '81). Such degradation may be a concern for biomimetic applications of spider silk, as it is for synthetic polymers in general (Clough et al., '96). The hypotheses we propose here to explain changes in mechanical properties of spider silk during aging can be tested using a variety of techniques. Fiber X-ray diffraction and solid-state nuclear magnetic resonance could reveal details of the molecular orientation of proteins in aging silk, although such methods would typically require aging larger bundles of silk, rather than single fibers (but see Riekel et al., '99; Hermes et al., 2006). Future studies involving larger amounts of silk may also allow estimations of change in mass and chemical composition of silk as explicit tests of these hypotheses.

Perhaps our most exciting finding is the initial ''improvement phase'' of spider silk aging owing to its implications for the synthesis of biomimetic fibers. However, we caution that these data focus on a single species (*Achaearanea)*. Although our data from the broader set of experiments all consistently point in the same direction—initial improvement followed by slow degradation—future work could fruitfully focus on verifying the initial ''improvement phase'' of spider silk across multiple individuals and species.

Despite a decrease in performance in some properties over time, it is important to note that spider silk is unusual among polymers (Clough et al., '96) in that it improves mechanically after a year of aging and retains impressive mechanical performance even after 4 years of aging. Silk from different species of spiders may also age differently—silks of some species did not show evidence of degradation after 4 years (Figs. 3 and 4). Generally, aged spider silk retains full strength, increases in stiffness, and although toughness is reduced, 4-yearold spider silk is still more than twice as tough as Kevlar® (Richmond, VA), one of the toughest synthetic fibers. Hence, spider silk remains an attractive model for high-performance synthetic polymers.

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