Tensile Mechanical Property Evaluation of Natural and Epoxide-Treated Silk Fibers

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SYNOPSIS

Silkworm cocoon silk and spider major ampullate (drag line) silk exhibit macroscopic tensile properties that, while impressive in the context of polymer fibers, are highly variable. The variability is linked to the cross-sectional geometry being nonuniform: silk fiber cross section changes significantly over distances that are small compared to the scale on which diameters are averaged by typical characterization techniques. This characteristic must be taken into account when evaluating chemical treatments (in the present case infiltrating with crosslinkable epoxide) that are aimed at improving strength or stiffness. The magnitude of any change in mechanical property must be considered in relation to the *spread* in values recorded prior to treatment. An apparent improvement in the mean value of a tensile property may turn out to be statistically insignificant when compared to the standard deviations associated with those data. Previous authors do not address this simple assessment of significance. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Spider major ampullate silk (MAS), which forms two of the component fibers in the drag line, exhibits several impressive tensile properties. These include high values of initial stiffness, breaking strength, elongation to failure, and toughness.[1-4] Spiders evolved the ability to spin silks at least 380 million years ago,[5] and have optimized the properties of silk to fit their particular needs. However, one cannot presume that any one of the mechanical properties of silk has been *maximized* by this evolutionary process. If one could control and refine individual properties such as stiffness or strength, the attractiveness of MAS as an engineering material would be enhanced. For example, given that spider MAS has a composite microstructure consisting of microcrystalline regions dispersed in a water-absorbing disordered matrix,[2,6,7] one can envisage increasing water resistance and stiffness by infiltrating a cross-linking moiety into the disordered regions.

Textile industry researchers have tested a variety of treatment procedures aimed at improving the performance of silkworm silk fibers and fabrics.[8-18] The goal of such research is not necessarily limited to improving tensile properties, but also includes attempts to increase the rate of dye uptake, improve crease recovery, decrease moisture regain, and decrease photoyellowing. Previous researchers have argued that treatment of *Bombyx mori* silkworm silk fibers with resorcinol diglycidyl ether (resorcinol DGE) in tetrachloroethylene increases both the breaking strength (by up to 29%) and the elongation to failure (also by as much as 29%).[15] In that study, as is usual for investigations of textile mechanical properties, the fiber cross-sectional area was taken to be a constant, average value determined from a measurement of fiber denier. Thus, the tensile strength is quoted in terms of grams per denier, rather than as a force divided by the initial cross-sectional area at the point of failure in the specific piece of fiber tested. In the case of synthetic textile fibers, this distinction is moot, because the diameter is sufficiently uniform along the 9000 m length of

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artificially spun fiber that is weighed to obtain a standardized measurement of denier.

However, the cross-sectional geometry of natural silk fibers is *not* uniform (Fig. 1), and a rigorous measurement of denier cannot be made on a single fiber because continuous 9000 m lengths are not available. For MAS obtained from *Nephila clavipes* (golden orb weaver) spiders, the thickness as measured by scanning electron microscopy (SEM) over a millimeter length of fiber can vary by as much as ±20% relative to the mean.[19,20] *B. mori* silk exhibits a similar variability at this scale,[19] and the mean thickness itself varies (by up to a factor of two) according to whether the fiber from the inside surface, middle, or outside surface of the cocoon is characterized.[21] Thus, if a long piece of silk is assigned a cross-sectional area based on denier measurement, significant variability can occur in the measured tensile strength and stiffness of individual subsamples, simply because the initial cross-sectional area at the point of failure is not the same in each subsample. Indeed, this source of variability will arise if an average cross-sectional parameter is measured by *any* technique. An apparent difference in the strength or stiffness of native fiber and chemically treated fiber may then reflect just the variability in actual sample cross section. One must therefore be concerned about the statistical significance of any apparent increase in strength or stiffness.

In the present study, we evaluate an epoxide infiltration and crosslinking procedure in terms of its effect on tensile properties of both *N. clavipes* MAS and *B. mori* silk that has undergone extensive characterization of cross-sectional geometry. The chemical treatment is adapted from a published method[15] that was devised to modify the tensile properties of silkworm fiber, and is novel in the context of spider MAS. The two silks have different native microstructures.[7]

EXPERIMENTAL

Fiber Collection and Sample Group Assignment

MAS fiber was collected from the spider, *N. clavipes*, using an adaptation[22] of a procedure initially developed by Work and Emerson.[23] The procedure enables silking to be performed at a controlled nominal rate (10.3 cm s^{-1} in the present case), and allows the two strands of MAS in the dragline to be separated easily. Single strand fiber was divided into short lengths. These were glued with small beads of RTV 108 silicone adhesive (GE, Silicone Product Division, Waterford, NY) across 0.75 × 0.5-in. notches cut in 3 × 1-in. glass microscope slides (Fig. 2). The adhesive was chosen for its resistance to degradation by the chemical environment in which fibers were subsequently treated. Several consider-

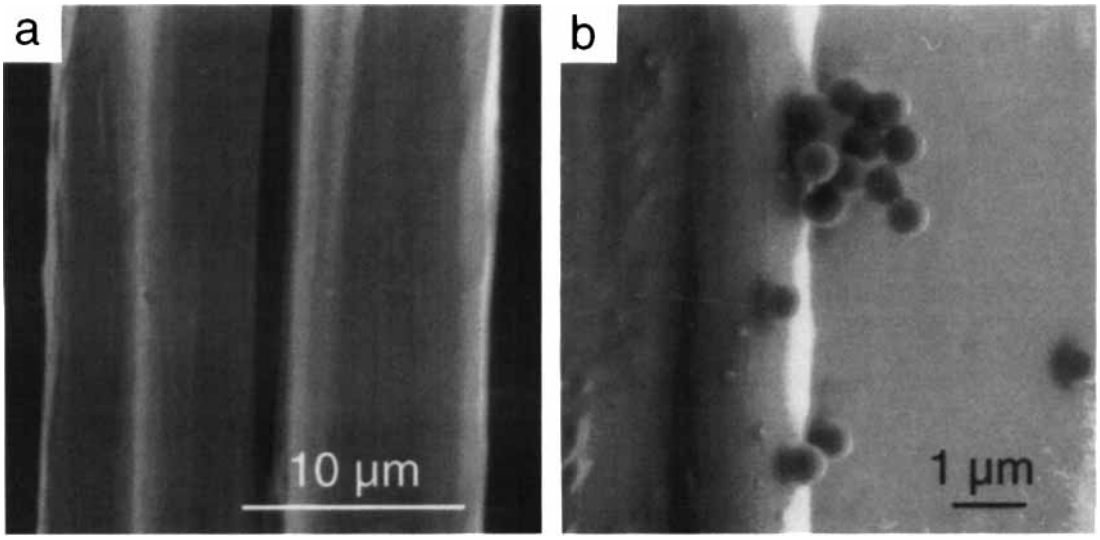

Figure 1 Scanning electron micrographs of (a) *B. mori* cocoon silk, which consists of two filaments (brins), and (b) *N. clavipes* major ampullate silk, which consists of a single filament. Scale bars denote nominal magnifications as recorded by the microscope; view (b) contains some of the 0.73-μm polystyrene spheres used as a calibration standard for accurate diameter measurement. Fiber cross sections are irregular and nonuniform.

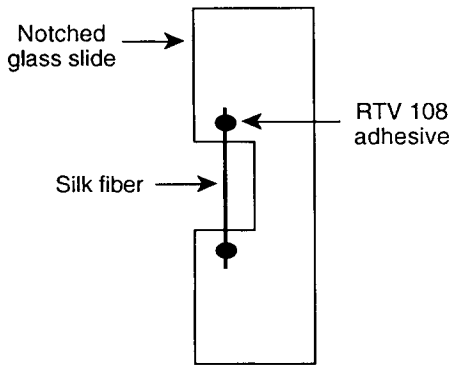

Figure 2 Schematic representation of notched glass microscope slide used to support silk fibers during both chemical treatment and diameter measurement by laser diffraction. For tensile testing, specimens could easily be transferred to jigs of the type shown in Figure 3.

ations led us to mount fibers in this way: (1) the diameter of single strand fiber could easily be characterized nondestructively by laser diffraction (see below); (2) it facilitated immersion in the chemicals used for infiltrating the cross-linking agent; (3) the specimen could easily be remounted later for tensile property measurement; and (4) fibers that are axially (or radially) restrained are not prone to undergo supercontraction[7,24-28] when immersed in aqueous media, so microstructural changes will be localized to the disordered matrix and changes to crystallinity and molecular orientation will be minimized—in other words, the process of chemical treatment will not affect complex changes in the silk microstructure. A group of three specimens cut successively from the same fiber constituted a set of "adjacent subsamples" (following terminology used previously in the literature of spider MAS[29]). The three successive members of such a set were denoted MAS_T (subsequently treated with a tetrachloroethylene-based solution of the active crosslinking agent resorcinol diglycidyl ether), $MAS_{T\text{-}R}$ (control subjected to a similar treatment, without any active crosslinking agent present), and MAS_C (untreated control). Mounted samples were stored in a desiccator at room temperature prior to chemical treatment.

Three white *B. mori* cocoons (obtained from the U.S. Army Research, Development and Engineering Laboratories, Natick, MA) were boiled in distilled water for 4, 5, and 6 h, respectively. This treatment is sufficient to degum the material, i.e., to remove the sericin coating.[30,31] Degumming ensures that subsequent processing and characterization pertain only to the fibroin component of the silk. Loosened fiber (bave), consisting of separate [Fig. 1(a)] but irregularly entwined filaments (brins) was reeled off by hand from the outer region of each cocoon. To avoid damaging the silk (for example, by stretching it and thus affecting its mechanical properties and susceptibility to chemical treatment), we did not attempt to separate the entwined brins in the bave recovered from *B. mori* cocoons. This precluded nondestructive diameter characterization by laser diffraction. Therefore, diameter characterization was performed by SEM on a subsample adjacent to each of the specimens used for mechanical property measurement. Sets of three adjacent subsamples were designated: SW_T (treated with resorcinol diglycidyl ether), SW_{DM} (diameter measurement), and SW_C (untreated). The *B. mori* silk studies were performed after the work on *N. clavipes* MAS, which showed no significant difference in the mechanical properties of samples MAS_T and $MAS_{T\text{-}R}$. For that reason, subsamples $SW_{T\text{-}R}$ (i.e., analogous to $MAS_{T\text{-}R}$) were not used. SW_T specimens were mounted on notched glass slides identical to those used for *N. clavipes* silk, again to facilitate immersion in the media used for chemical treatment. The mounted samples were stored in a desiccator at room temperature prior to infiltration with epoxide.

Chemical Treatment

MAS_T, $MAS_{T\text{-}R}$, and SW_T samples were swelled by soaking in a flask of 8.1 g/L NaSCN at 23.5°C for 5 min, and then removed. MAS_T and SW_T samples were each placed in a flask with 10 g of resorcinol diglycidyl ether (resorcinol DGE) in 200 mL tetrachloroethylene (i.e., a 5% solution). The $MAS_{T\text{-}R}$ samples were instead placed in a flask containing 200 mL tetrachloroethylene only. Flasks were covered with aluminum foil and placed for 3 h in a preheated water bath maintained at 68–70°C on a Corning PC 351 hot plate. After this treatment, all samples were washed with acetone in a Soxhlet extractor for one hour (to remove non-crosslinked oligomers in the case of MAS_T and SW_T samples). Then, samples were washed at room temperature ($\sim$ 22°C) for 2 min in each of methanol, tap water, and distilled water. All treated samples were then placed in an oven (VWR Scientific Model 1500E) at 37°C for either 4 days (MAS, thinner) or 7 days (SW, thicker), and were subsequently stored at room temperature in a desiccator prior to characterization.

Diameter Measurement

Average diameters of MAS samples (attached to their notched glass slides) were characterized non-

destructively by laser diffraction.[19,20] Thus, the diameter measurements were made on the actual specimens used later in tensile tests. Our method of diameter measurement allows for the fact that MAS has an elliptical or oval cross section. Diameters of MAS_T and $MAS_{T\text{-}R}$ samples were not affected measurably by chemical treatment.

Laser diffraction was not used to characterize the cross-sectional geometry of SW samples; accuracy is severely limited because the natural fibers consist of two irregularly intertwined brins. Diameters of SW samples were instead measured by SEM. The SW_{DM} samples were mounted on an aluminum specimen stub with an adhesive graphite tab (Ted Pella, Redding, CA). As a calibration standard, polystyrene spheres (Ted Pella; mean diameter 0.730 μm, standard deviation 0.001 μm) were sprinkled across the stub. Samples were sputtered with Au-Pd, and observed in a JEOL JSM 6300F field emission SEM, at a working distance of 15 mm and with an accelerating voltage of 3 kV. The specimen preparation method will preclude significant supercontraction of the fiber under the desiccating conditions in the SEM: fibers that are restrained in either the axial or radial dimension are not prone to supercontraction (vide supra), and the deposited metal presents a barrier to water diffusion. Photomicrographs were recorded of regions where the two brins were clearly separated. Strand diameters measured from the photomicrographs were used to determine the cross-sectional area of the adjacent subsamples SW_T and SW_C, with the significant assumption that the strands are cylindrical.[20] In fact, the cross section of each brin is better described by a triangle with convex (bulging) sides[32]; however, the area that we determine will differ by a constant factor from the value that would be obtained by consistently assuming any other cross section geometry. Our assumption of a cylindrical fiber therefore cannot be a source of the mechanical property variability that is the focus of this paper.

Tensile Testing

After diameter measurement and/or chemical treatment, samples were remounted across half-inch holes punched in cardboard jigs (Fig. 3). The jigs were used to facilitate alignment and clamping of specimens for tensile tests; they provided enough support to hold the fiber straight as it was clamped, yet they could easily be burned through so that silk and cardboard were not loaded in parallel during the actual test.

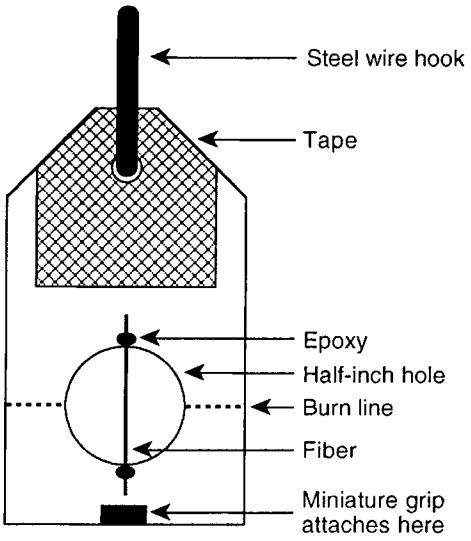

Figure 3 Schematic representation of cardboard jig used to support silk fibers during tensile testing.

Specimens were transferred from their notched glass slides to the cardboard jigs as follows: The glass slide was placed fiber side down on the jig, and the specimen was aligned across the vertical diameter of the half-inch hole. Devcon 5 min® epoxy (Devcon Corporation, Danvers, MA) was used to secure the fiber to the jig at the edge of the hole. After the glue dried, a scalpel was used to cut the specimen between the beads of silicone and epoxy adhesive at each end, freeing the specimen from the glass slide.

Tensile tests were performed on an Instron model 4500 with a 4505 load frame, controlled by an IBM PS-2 model 50 Z running Series IX Automated Testing System Software—Version 5.20. A 500 g load cell was used at a full scale deflection equal to 3.91 g, i.e., 1/128 of this maximum load. Over this load range, the load cell was accurate to ± 0.01 g.

A steel wire hook passing through the tape-reinforced small hole at the top of the jig was attached to the load cell, while the bottom of the jig was attached to the crosshead with a miniature fiber grip. This arrangement facilitated vertical alignment of the silk specimens. The sides of the card were burned through with a hot wire, and a cardboard shield was placed around the specimen to prevent air convection from causing fluctuations in the measured load.

An extension rate of 100%/min was used on MAS samples, consistent with established precedent in the spider silk literature.[1,33] SW samples were tested at 30%/min, consistent with the rate used by previous researchers.[15] Ambient temperature and humidity were measured to be 21–25°C and 46–52%, respectively, using a VWR digital

thermometer/analog hygrometer (VWR Scientific, Philadelphia, PA).

The silk specimens have cross sections that are small compared to those of the card and hook, which are loaded in series with the specimens. Therefore, most of the deformation during tensile testing is confined to the silk. Calibration determined that in the initial high-modulus regime of specimen deformation, card/hook extension accounted for less than 0.17% of the total measured extension when MAS samples were tested, and approximately 3% of the total measured extension in the case of SW samples.

RESULTS AND DISCUSSION

Spider MAS

Principal features of our MAS stress–strain curves are illustrated schematically by the solid portion of curve *A* in Figure 4:

- an initial elastic regime (modulus E_{el}),
- a yield point (at a strain and stress denoted by ε_{y1} and σ_{y1}, respectively), and
- a second, extended, linear region (modulus E_2).

Similar curves have been reported in the spider silk literature: stress–strain curves of silk from *N. clavipes*[34] and *Argiope aurantia*,[1] and representative elongation curves of silk from unidentified spiders.[33] In addition, as illustrated by the dashed continuation of curve *A* in Figure 4, some of our specimens exhibited

- a second yield point (at strain ε_{y2} and stress σ_{y2}), and
- a third linear region (modulus E_3)

before breaking at strain ε_{br} and stress σ_{br}.

In a few instances, a stress–strain curve for MAS exhibited irregular sawtooth features, presumed to be the result of stick–slip motion at the fiber/epoxy interface or at the fiber grip. Data from such specimens were used only up to the point at which the irregularity set in. Samples that were significantly thicker (typically by a factor of about 2), and that simultaneously exhibited greatly diminished mechanical properties (typically by about 50%) relative to other samples from the same strand, were excluded from the data analysis. Previous studies[35] have suggested that such enlarged and weakened fibers are formed when the spinneret fails during forced silking.

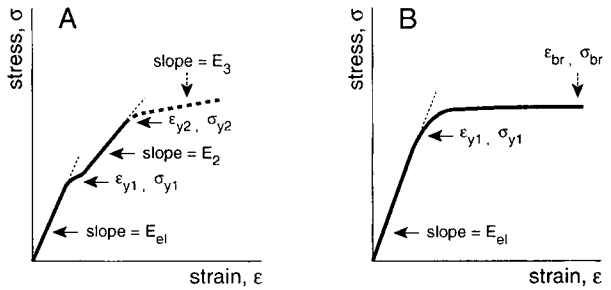

Figure 4 Characteristic features of silk fiber stress–strain curves. Curve *A* pertains to *N. clavipes* major ampullate silk, which exhibits either a single yield (solid portion of curve) or a double yield (entire curve). *B. mori* cocoon silk exhibits either the double yield behavior shown in curve *A*, or the single yield response shown in curve *B*.

The principal features consistently exhibited by stress–strain curves were used to compare the mechanical properties of MAS_T, $MAS_{T\text{-}R}$, and MAS_C samples. Mean values and standard deviations are plotted in Figure 5. MAS_T and $MAS_{T\text{-}R}$ fibers gave rise to stress-strain curves that were slightly offset toward lower strains, compared to the curves obtained from MAS_C samples. Thus, ε_{y1}, ε_{y2}, and ε_{br} of MAS_T and $MAS_{T\text{-}R}$ were smaller than the corresponding values for MAS_C. While this shift has poor statistical significance, as indicated by the error bars, its presence both in MAS_T and in $MAS_{T\text{-}R}$ suggests that the resorcinol DGE (crosslinking agent) is no more effective at changing mechanical properties than the other chemicals used in the treatment procedure. The data also suggest that treatment with resorcinol DGE may actually decrease the load-bearing ability of MAS samples, compared to untreated fiber. This is in contrast to reports that similar treatments enhanced the mechanical properties of silkworm silk,[15] which is why fibers of that material were included in our study.

Silkworm Fiber

Silkworm silk exhibited two types of stress strain curve: (1) double-yield curves similar to those obtained from MAS samples, illustrated schematically by curve *A* in Figure 4, and (2) single-yield curves characterized by an initial regime of high stiffness, followed by a yield point, followed in turn by a regime in which the slope decreased continuously until the specimen broke, as illustrated by curve *B* in Figure 4. The latter specimens typically exhibited elastic deformation to larger strains, as well as a greater overall strain to failure. Both types of behavior were recorded in silk from each of the three

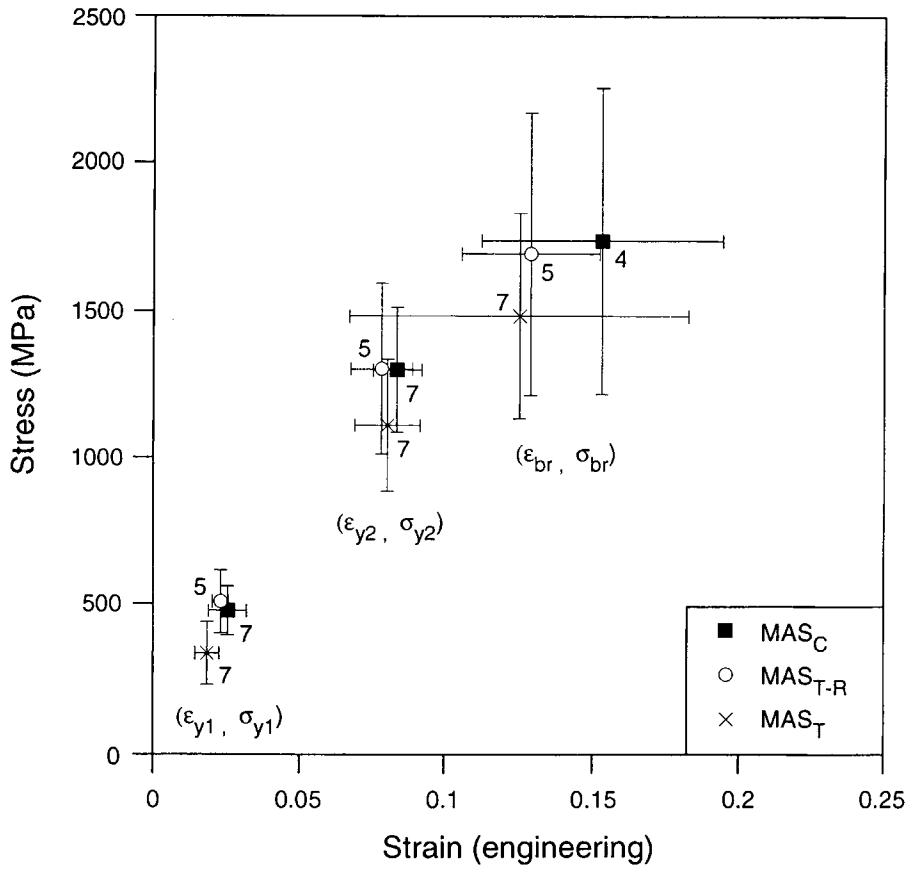

Figure 5 Mechanical properties of *N. clavipes* major ampullate silk specimens. Values of stress and strain for the first yield point, second yield point, and breaking are shown. Error bars denote standard deviations. Numbers adjacent to data points denote how many samples were used to obtain the mean and standard deviation in each case.

cocoons that were characterized. Results similar to our curve *B* in Figure 4 were reported by Nadiger and Halliyal.[36]

The mechanical properties of samples were compared on the basis of those features consistently exhibited by stress–strain curves. Thus, averages and standard deviations were obtained for E_{el}, ε_{y1}, σ_{y1}, ε_{br}, and σ_{br}. Figure 6 separates the results for SW_T and SW_C samples, in each case combining data from single-yield and double-yield curves. In contrast, Figure 7 displays separately the results from single-yield and double-yield curves, combining data from SW_T and SW_C material in each case. From Figure 6, it is apparent that the mechanical properties after treatment do not show a statistically significant difference relative to the properties of the control group. Indeed, the changes are even less statistically significant than we observed for MAS (Fig. 5), primarily due to the intrinsic variability (i.e., single-yield vs. double-yield behavior) of silkworm silk. Comparison of Figures 6 and 7 shows that the changes caused by treatment are substantially smaller than the intrinsic differences between material exhibiting single- and double-yield tensile curves.

Tsukada et al.,[15] who subjected *B. mori* silk to an epoxide infiltration treatment that is similar (but not identical) to the one described here, have reported 29% increases in both elongation to failure and breaking strength after treatment for 3 h. While these improvements are certainly within the *range* of our data, they are quoted without reference to standard deviations or any other measure of statistical significance. Recalling from above that *B. mori* silk fibers do not have a circular cross section, and that the local diameter of a silk fiber can be as much as 20% larger or smaller than the average measured over as little as a millimeter length, we emphasize that a substantial *apparent* difference in strength (of even 40%) may simply reflect our ignorance of the initial geometry at the point of failure.

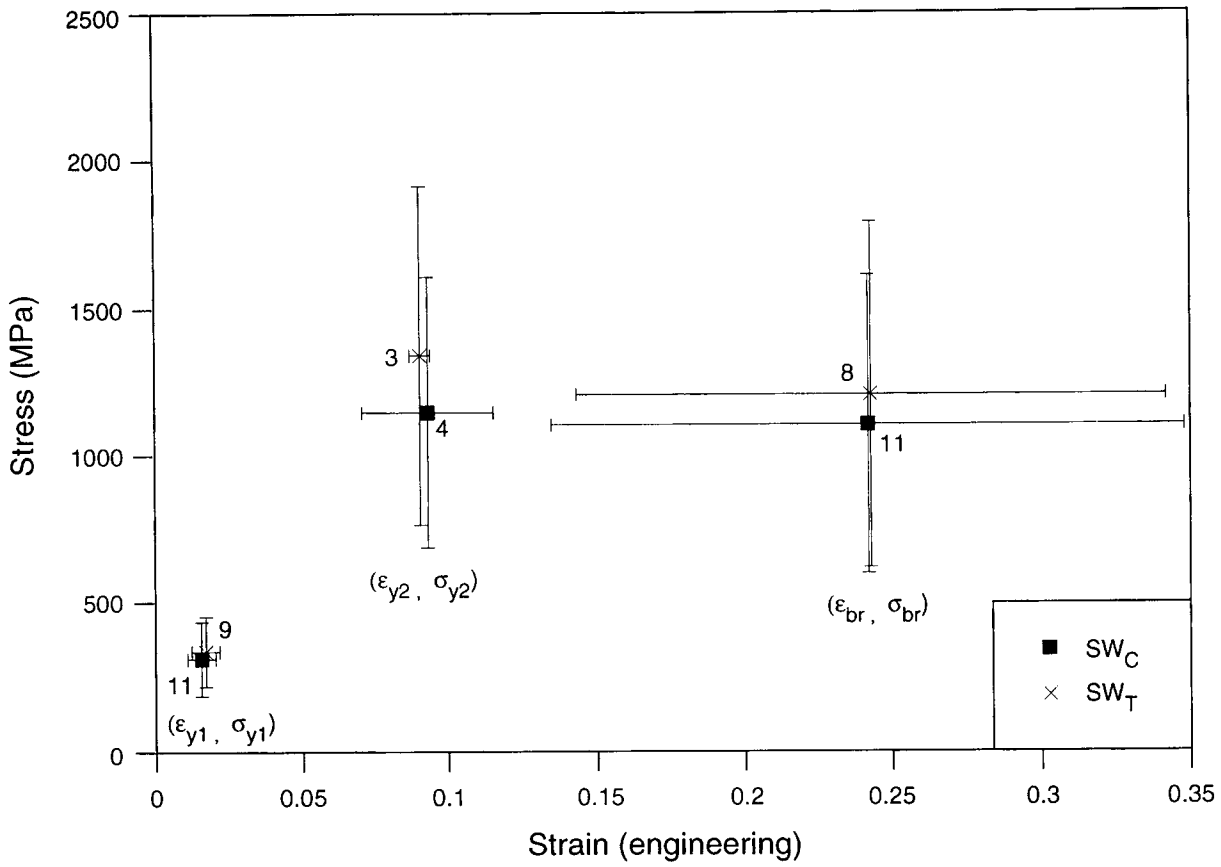

Figure 6 Mechanical properties of *B. mori* cocoon silk specimens. Values of stress and strain for the first yield point, second yield point, and breaking are shown. Data from specimens exhibiting single-yield and double-yield behavior were combined in the data points for the first yield point and for breaking. Error bars denote standard deviations. Numbers adjacent to data points denote how many samples were used to obtain the mean and standard deviation in each case.

To reliably engineer materials that exploit the highest potential strength and stiffness of silk fibers, it would be expedient to work with fibers that have a more uniform diameter. Spinning reconstituted fibers from solution, or spinning recombinant silk analogs, may provide routes to fibers with reproducible macroscopic properties. In addition, such processing routes would facilitate the introduction of crosslinking, property-modifying agents.

CONCLUSIONS

1. The mechanical properties of single-filament spider major ampullate silk and silkworm silk are highly variable. The variability is evident in the overall profile of tensile stress–strain curves, as well as in the magnitude of specific tensile properties, e.g., initial elastic modulus, yield strength, breaking strength, and elongation to failure.
2. The magnitude of the mechanical property variability is consistent with the ±20% variability in cross-sectional geometry, which is not taken into account by techniques that simply measure an average diameter. In particular, it is easy to lose sight of this variability when diameter is described in terms of denier measured from a single, short piece of fiber.
3. It is essential that, when a chemical treatment is devised to modify the mechanical properties of silk, the magnitude of any apparent modification is compared to the intrinsic variability in the data.

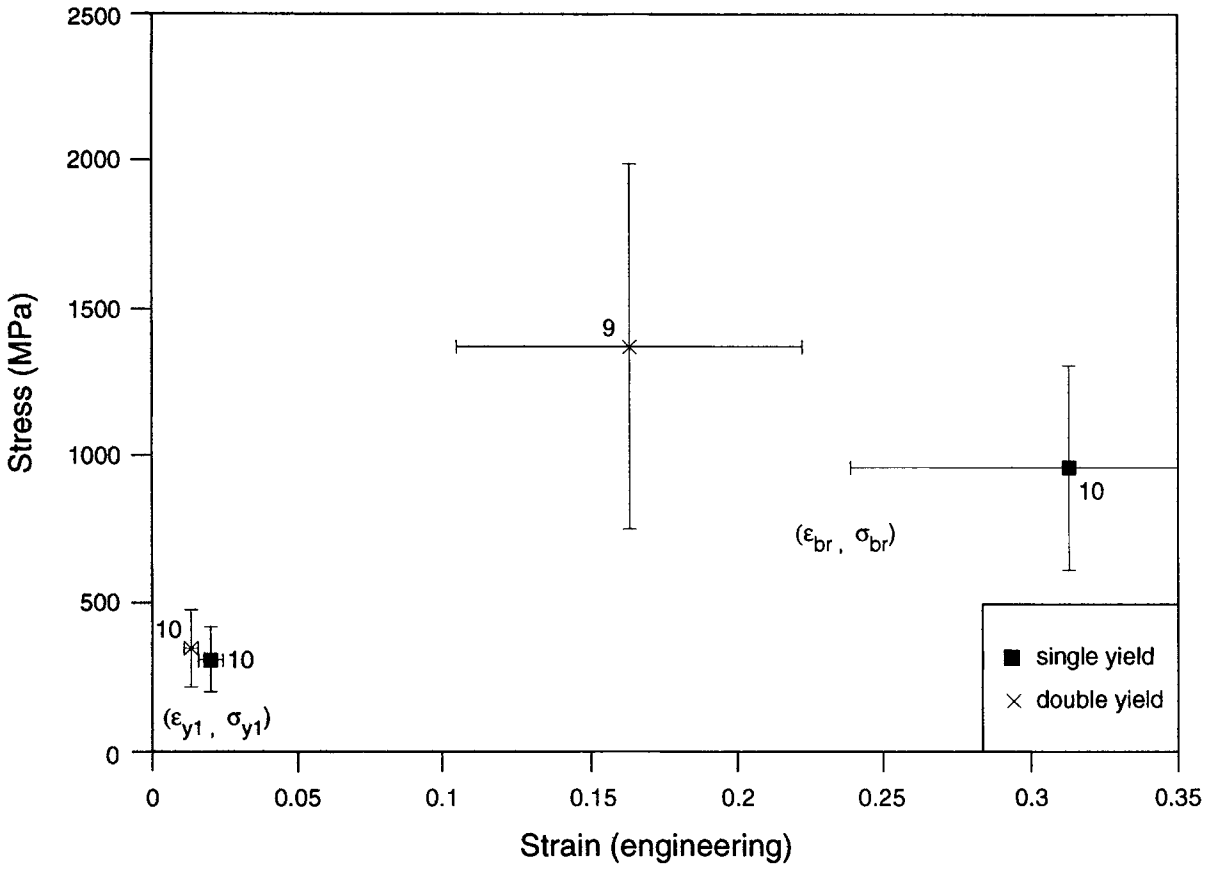

Figure 7 Mechanical properties of *B. mori* cocoon silk specimens. Values of stress and strain for the first yield point and for breaking are shown. Data from treated and control specimens were combined in each data point. Error bars denote standard deviations. Numbers adjacent to data points denote how many samples were used to obtain the mean and standard deviation in each case.

4. Changes in strength, elastic modulus, and elongation arising from our epoxide treatment of axially restrained spider MAS and silkworm fibers are small compared to the intrinsic variability in these properties, and are not statistically significant.
5. Changes in strength, elastic modulus, and elongation that arise from treatment of axially restrained spider MAS in epoxide solution are not significantly different from the changes effected by immersing the silk in pure solvent.

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