Tensile Properties of Silkworm Silk Obtained by Forced Silking

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ABSTRACT: Bave was acquired by the forced silking of three *Bombyx mori* silkworms, and its tensile properties were characterized. The material collected from any given silkworm yielded reproducible force-displacement plots, which were qualitatively similar to the plots obtained from silk collected from the other silkworms. This uniformity contrasts with the highly variable properties exhibited by silk which had been reeled from degummed cocoons. Scanning electron microscopy images were used to obtain information about the sample cross-sectional area, so that force-displacement plots could be rescaled as stress–strain curves. Surprisingly, the scatter in the tensile properties increases after such rescaling. This finding can be explained in terms of the sericin coating of the bave (which contributes to the cross-sectional area but not significantly to the load-bearing capacity) having a variable thickness. When the sericin coating was eliminated by a degumming treatment, it was found that the fibers showed more consistent cross-sectional areas. Therefore, stress–strain curves of forced *B. mori* silk are reproducible, provided that force-displacement data are rescaled by the correct cross-sectional area. Finally, the Weibull parameters of the forced silk were determined. The Weibull modulus, m, has a value of 13.0 ± 0.3, which is more than double the value obtained previously from silk reeled from a cocoon, demonstrating that the process of degumming cocoons has a detrimental effect on the distribution of defects in the silk microstructure. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 1928–1935, 2001

Key words: silkworm silk; forced silking; tensile test; Weibull modulus

INTRODUCTION

Silkworms (*Bombyx mori*) spin silk in their fifth instar larval stage,[1] when building a cocoon that protects the pupa during its metamorphosis to an adult moth. The as-spun fiber is composed of two cores of fibroin surrounded by a layer of sericin (Fig. 1) in a structure known as a *bave* (each individual core is known as a *brin*).[2] Although both fibroin and sericin consist of protein, their compositions are widely different. Fibroin is a single protein and contains the motif –Gly–Ala–Gly–Ala–Gly–Ser– repeated along its sequence, while sericin is a mixture of proteins and contains a large number of amino acids with hydroxyl groups (serine, threonine, and tyrosine).[3,4] The microstructures and biological functions of the two materials are also different: The –Gly–Ala–Gly–Ala–Gly–Ser– motif in fibroin forms a large number of β-sheet microcrystallites[5] which act as

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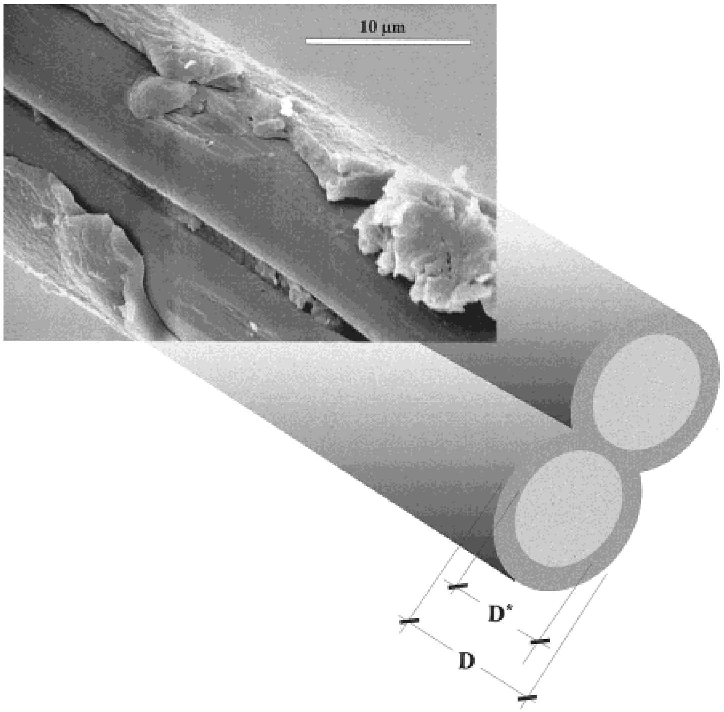

Figure 1 SEM micrograph where the fibroin cores and the sericin coating of a *bave* can be clearly identified. A schematic representation of a bave is included to define the values of the apparent diameter, D, and the diameter of the fibroin core, D^*.

a reinforcement and contribute to the strength and stiffness of the silk. Sericin, on the other hand, is amorphous and acts as an adhesive binder to maintain the structural integrity of the cocoon.

Although cocoons are a convenient source of silk in that they contain a significant length of fiber in a reduced space, bave can only be reeled from them after the sericin coating has been eliminated. Removal of the sericin requires thermochemical treatment of the cocoon in a process conventionally known as *degumming*. A degumming treatment consists of submerging the cocoon in boiling water, usually with salt or detergent to increase the efficiency of the process.[6,7] Since degumming imposes a relatively harsh environment on the silk fibroin, the possibility of changes occurring in the fibroin structure and mechanical properties must be considered.

The possible effect of degumming on silkworm silk has been studied by microstructural characterization techniques that do not require the isolation of individual fibers: X-ray diffraction,[8] differential scanning calorimetry, and birefringence measurements.[9] Although these techniques did not detect any significant microstructural consequences of degumming, it would be instructive to perform tensile tests on isolated bave to check directly whether degumming has an effect on the mechanical properties. Moreover, it is possible that degumming could be a contributing factor to the large variability observed in the tensile properties of silkworm silk when comparing nominally identical samples.[10] Obviously, an answer to the latter question would require that tensile tests be performed on fiber that has not been degummed.

In this context, the testing of fiber obtained by forced silking (i.e., intercepting the fiber before the silkworm can incorporate it into the cocoon) offers information that complements the microstructural characterization techniques referred to above. This collection technique precludes the need for a degumming treatment to obtain isolated bave. Forced silking, also known as reeling, is used routinely by silk researchers to obtain major ampullate (drag line) silk from immobilized spiders.[11, 12] In this procedure, the fiber is collected on a mandrel that combines rotational and translational motions so that the silk is not wrapped onto itself. Long (2–3 m) fibers can be obtained in this way. However, to our knowledge, the adaptation of this technique to obtain silkworm silk has not been described before. The aim of the present work was to characterize the tensile properties of *B. mori* silk that was collected by forced silking, with special attention to the variability of these properties.

EXPERIMENTAL

Three *B. mori* silkworms (to be referred to as Bm1, Bm2, and Bm3) were reared to the fifth larval stage on a diet of mulberry leaves. (Since the worms were of the same genetic strain and were subjected to identical diets and growth environments, the variability in the properties of their silk as described in this article are not intrinsic to the silk protein.) Immediately after they stopped feeding, they were subjected to constant surveillance to detect the onset of cocoon spinning. When silk was first observed, the worm was placed on a black surface and allowed to spin a short length of fiber. The spun fiber was grasped with tweezers and reeled from the worm by hand at a nominal speed of 1 cm/s, equivalent to the rate at which silkworms spin their fiber naturally.[13] Bave of length 25–30 cm was obtained at each attempt. Between 10 and 15 such lengths were collected from each silkworm. A period of approximately 1 h was needed to carry out the complete collection procedure with any one worm.

Samples 5 cm long were cut from the reeled fiber and mounted across the hole in a cardboard frame as described elsewhere,[2, 10] so that the gauge length for the tensile tests was $L_0 = 30$ mm. Tensile tests were performed with an Instron 4411 machine, in order to control the strain rate accurately. All tensile tests were conducted at a strain rate of $0.0002\ \mathrm{s}^{-1}$. A balance (Precisa 6100C, resolution ±10 mg) attached to the lower end of the sample was used instead of a conventional load cell.[10] The displacement of the crosshead was taken as the deformation of the sample, since the compliance of silk is approximately 1000 times greater than that of any other part of the system.[10]

After tensile testing to failure, selected samples were metallized with gold and examined in a scanning electron microscope (JEOL 6300; observation conditions $V = 10$ kV, $I = 0.6$ nA) to characterize their cross-sectional geometry. In occasional cases (when force values at any displacement were approximately half the expected values), it became apparent that the tensile test sample had consisted of a single brin rather than bave. Raw data from such samples were not carried forward into the results described in this article.

RESULTS AND DISCUSSION

Load-displacement Results

Tensile tests were performed on several samples of silk collected from each worm, so that force-displacement (F-d) curves could be established reliably. A selection of F-d plots is shown in Figure 2 to allow the behavior of silk from the three silkworms to be compared. Lines with the same pattern in Figure 2 correspond to silk obtained from the same silkworm, so it is apparent from these data that each silkworm produces silk with consistent tensile properties.

The reproducible F-d response of material obtained by forced silking is a surprising result: *B. mori* silk exhibits highly variable properties when obtained from a degummed cocoon.[10] Spider drag line also exhibits significant variability, even when samples are obtained from the same animal by forced silking.[14, 15] At present, the only procedure for reducing scatter in the measured tensile properties involves testing adjacent samples (i.e., pieces cut successively from the same original fiber). This approach was first established for spider drag line[16] and subsequently confirmed for silkworm silk,[10] and depends on any variations in the microstructure occurring over length scales which are significantly larger than the sample length. It is also possible to reduce scatter by normalizing the F-d data with respect to the initial slope of the plot.[17] At this point, it must be emphasized that the plots shown in Figure 2 were obtained from *non*adjacent samples, although, unsurprisingly, it was found that adjacent samples of bave obtained by forced silking also exhibit reproducible mechanical properties. Moreover, Figure 2 shows that different silkworms (Bm1 and Bm3) can produce silk characterized by comparable F-d curves. Even Bm2 produced silk which is qualitatively similar to the Bm1 and Bm3 silks, the only difference being the scale of the vertical axis of the plots.

Stress–Strain Analysis

Differences between the F-d curves for silk collected from different worms could be the result of different sample cross-sectional areas. Therefore,

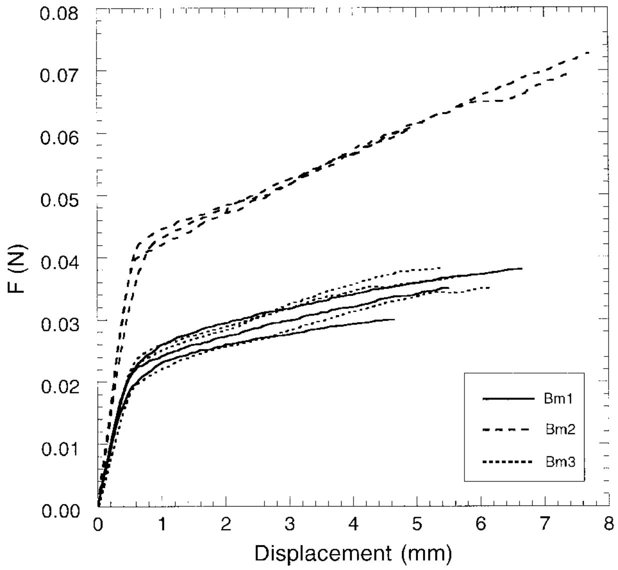

Figure 2 F-d plots obtained from bave that was reeled directly from *B. mori* silkworms Bm1, Bm2, and Bm3. For clarity of presentation, only selected plots are shown.

it is interesting to convert F-d curves into stress–strain curves to determine whether the three silkworms produce material with the same properties.

Characterization of the cross-sectional geometry was performed in a scanning electron microscope (SEM). Fibers were imaged in an SEM to measure their cross section after tensile testing. To allow for variations in the cross-sectional area and shape, the apparent diameter was measured on micrographs recorded at two different positions along the sample, in each case at two different orientations (0°–50°).[2,10] The orientation was changed by rotating the sample about its long axis in the microscope. Ideally, the two orientations would have differed by 90°, but rotation was limited by the geometrical specifications of the microscope.

Results of the characterization of sample cross-sectional geometry (five samples from each of Bm1, Bm2, and Bm3) are summarized in Table I. The average diameter D, maximum diameter $D_{\max}$, minimum diameter $D_{\min}$, and average shape anisotropy are presented for bave collected from each worm. Shape anisotropy is calculated as the ratio of D_0 (or D_{50}, whichever number is greater) to D_{50} (or D_0, whichever number is smaller) at each position where the diameter was measured; subscripts denote the sample orientation. The average diameter of each sample characterized by SEM was used to calculate its area, assuming a circular cross section. (The shape anisotropy of 1.3 equates to a maximum ovality of 1.5,[2] so no significant loss of accuracy results from fitting the cross section to a circle having as its diameter the average of the experimentally measured values.[18])

Since the samples were observed in the SEM *after* tensile testing, we obtained their initial cross-sectional area from their experimentally determined area by using the expression

$$A_0 = A_f l_f / l_0 \tag{1}$$

where A_0 and A_f are the initial and final cross sections, respectively, and l_0 and l_f are the initial

Table I Geometrical Parameters of Bave Obtained from *B. mori* by Forced Silking

Silkworm	D (μm)	$D_{\max}$ (μm)	$D_{\min}$ (μm)	Shape Anisotropy
Bm1	7.3 ± 0.3	9.2	5.1	1.3 ± 0.2
Bm2	9.2 ± 0.2	10.7	5.8	1.3 ± 0.2
Bm3	8.2 ± 0.3	9.9	6.7	1.29 ± 0.09

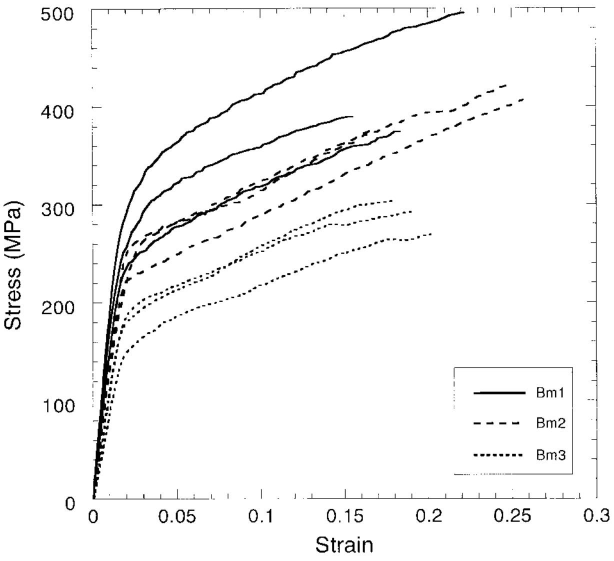

Figure 3 Stress–strain curves obtained when the F-d plots in Figure 2 are normalized by the corresponding sample cross sections (based on measured *D* values).

and final sample lengths, respectively. Equation (1) implies that the volume of the sample remains constant during the tensile test and is used consistently with previous work on *B. mori* silk.[2,10]

When the F-d data presented in Figure 2 are rescaled as stress–strain curves by using the corresponding sample cross sections, the plots shown in Figure 3 are obtained. From the behavior of samples spun by Bm1, it is immediately evident that the scatter in the stress–strain curves of fibers produced by a single worm can be greater than that of the corresponding F-d plots. This effect is unusual, in that normalization of F-d data with respect to sample cross-sectional area is usually seen as a method for reducing scatter. The effect cannot be attributed to experimental artifacts, since the conversion from F-d plots to stress–strain curves does not significantly change the scatter for Bm2 or Bm3 samples. Also, samples from two worms that show dissimilar F-d characteristics may yield similar stress–strain curves (Bm1 and Bm2), while samples from two worms that show similar F-d characteristics may yield dissimilar stress–strain curves (Bm1 and Bm3).

Table II summarizes the mechanical parameters of silk from each of the three sources. Each entry is the mean of five experimental values. All the symbols in Table II have their usual meaning; σ_p and ε_p, respectively, correspond to stress and strain at the proportional limit, which, in turn, is defined as the point where an F-d curve intersects a straight line which passes through the origin and has a slope equal to 95% of the initial slope of the curve. It is instructive to compare the data in Table II with published mechanical properties of silkworm silk obtained from degummed cocoons. In a previous study,[10] *B. mori* silk fibers were classified into three groups according to the value of their elastic modulus. It was suggested that the differences could be attributed to variations in the extent to which the degumming treatment had achieved degradation of the sericin coating. Following this classification, Bm1 and Bm2 belong to the "high elastic modulus" group (*E* lies in the range 17.4 ± 0.4 GPa identified in ref. 10), while Bm3 belongs to the "medium elastic modulus" group (*E* lies in the range 12.8 ± 0.7 GPa identified in ref. 10).

The interpretation of these data requires the effect of the sericin coating to be considered. The load-bearing capacity of sericin is negligible compared to that of fibroin.[2] However, sericin contributes significantly to the cross section of the fibers, accounting for approximately 25% of the weight of a cocoon.[19] Differences among Bm1 samples in Figure 3, and between the stress–strain curves of samples from Bm1 and Bm3, can be explained in terms of two simple suppositions: (a) All the samples have fibroin cores of similar intrinsic mechanical properties, and (b) the thickness of the sericin coating varies among the samples.

An apparent diameter, *D*, is obtained for each fiber from SEM micrographs (see Fig. 1). This

Table II Mechanical Parameters of Bave Obtained from *B. mori* by Forced Silking

Specimen	E (GPa)	σ_p (MPa)	ε_p	σ_u (MPa)	ε_u
Bm1	17.9 ± 0.3	230 ± 20	0.013 ± 0.001	430 ± 40	0.18 ± 0.02
Bm2	16.6 ± 0.6	290 ± 10	0.017 ± 0.001	530 ± 20	0.23 ± 0.01
Bm3	12.4 ± 0.5	200 ± 10	0.016 ± 0.001	360 ± 10	0.21 ± 0.02

E, elastic modulus; σ_p, stress at proportional limit; ε_p, strain at proportional limit; σ_u, tensile strength; ε_u, strain at breaking.

diameter, D, was used to convert F-d into stress–strain curves. However, the apparent diameter, D, contains contributions from both the sericin coating and the fibroin cores (both the fibroin cores and the sericin coating can be easily identified in the micrograph shown in Fig. 1). Since only fibroin can sustain mechanical loads, stresses should be computed only from the cross-sectional area of the fibroin core (whose diameter D^* is shown in Fig. 1). Therefore, differences in the thickness of the sericin coating of different fibers would yield different apparent diameters, D, and, consequently, differences in the stress–strain curves. These differences would arise even if the cross-sectional areas of the fibroin cores were similar, so that they yield similar F-d curves.

To test the validity of the latter supposition, two samples from each silkworm, adjacent to the samples characterized in Figures 2 and 3, were degummed in boiling water for 90 min to eliminate the coating. The duration was chosen on the basis of a previous study which showed that sericin removal does not continue beyond this time[9] (although it must be emphasized that 100% removal is not necessarily achieved). The diameter of the degummed samples (indicated in Fig. 1 as D^*) was measured from SEM micrographs and was found to be almost the same for Bm1 silk ($D^* = 7.1 \pm 0.2$ μm) and Bm3 silk ($D^* = 7.0 \pm 0.4$ μm), in contrast to the diameters measured for the nondegummed bave collected from these silkworms (Table I). Bm2 yielded a value of $D^* = 9.0 \pm 0.1$ μm. F-d curves of fibers adjacent to the degummed ones, and whose F-d plots are shown in Figure 2, were rescaled to stress–strain curves using the diameters of the corresponding degummed fibers (D^*) and the results are collected in Figure 4; they demonstrate that the fibroin cores of all three silkworms exhibit comparable and reproducible tensile properties.

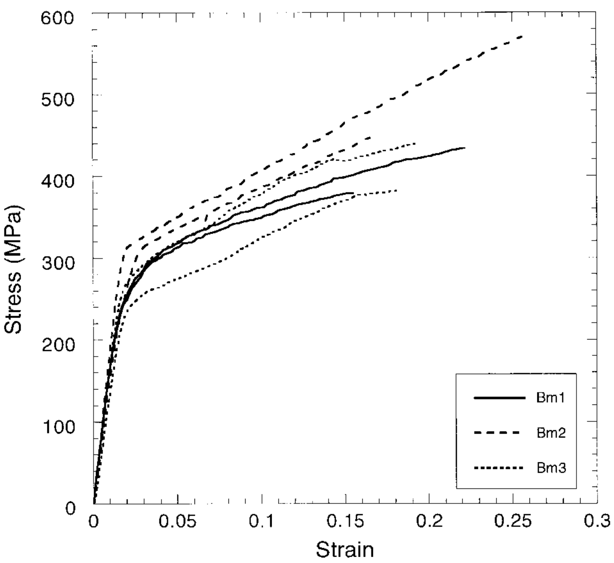

Figure 4 Stress–strain curves obtained when the F-d plots in Figure 2 are normalized by the sample cross sections of adjacent degummed fibers (based on measured D^* values).

Weibull Analysis

Finally, as with any material that may be considered for engineering applications, it is useful to quantify the variability in tensile strength, since this parameter limits the possible use of a material in structural contexts. Tensile strength reproducibility is commonly quantified in terms of the Weibull modulus, m.[20] It was found previously[10] that degummed silkworm silk harvested from cocoons has a low value of $m = 5.8$. The present investigation sought to determine whether this is an intrinsic characteristic of the material or the result of the relatively harsh degumming and harvesting processes.

Twenty-three samples of Bm1 bave were tested. Their tensile strengths were calculated from F-d plots using the average diameter for degummed fiber, under the assumption that all had the same cross-sectional area. (This assumption is justified by recalling that the diameter of this material after degumming is reproducible as noted above, and is further supported by the closely similar F-d plots for Bm1 bave as illustrated in Fig. 1.) The computational details can be found elsewhere,[20] but the Weibull analysis requires construction of an Ln[Ln(1/1 − F)] versus Ln σ_u plot, where F is a measure of the probability of fracture of a sample and σ_u is its tensile strength. When the result of this plot is a straight line, the slope gives the Weibull modulus, m, and the point where an extrapolated Weibull plot intersects the y-axis, $-m$ Ln σ_0, provides a measurement of the average tensile strength of the samples.

The Weibull plot constructed from the data is presented in Figure 5 and yields values of $m = 13.0 \pm 0.3$ and $\sigma_0 = 416 \pm 2$ MPa. The Weibull modulus is more than double the value measured for degummed bave harvested from cocoons,[10] showing that the standard process of degumming does, indeed, decrease the reproducibility of tensile tests, which, in turn, implies that degumming has a significant detrimental effect on the distri-

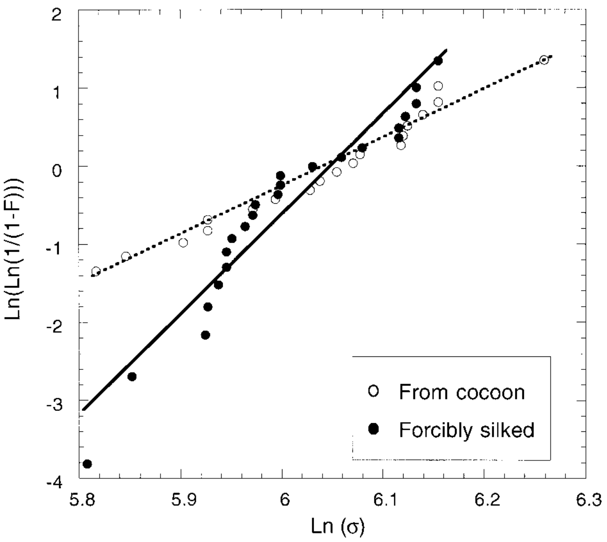

Figure 5 Weibull plot constructed from tensile-strength measurements performed on 23 samples of *B. mori* bave reeled directly from silkworm Bm1 (solid circles). The Weibull plot of fibers obtained from a cocoon[10] is shown to facilitate comparison.

bution of flaw sizes in the silk microstructure. (According to the Weibull model of fracture strength, if all flaws were of the same size, failure would always be triggered by the same applied stress.[20]) The value of σ_0 for bave collected directly from silkworm is similar to the value obtained from degummed fibers (σ_0 = 403 MPa). Therefore, although the *spread* of failure strengths is increased by degumming, there is relatively little change in the *average* failure strength, suggesting that degumming has little effect on the intrinsic molecular order (e.g., the amount and perfection of crystallinity) in the silk.

CONCLUSIONS

1. Bave can be obtained from silkworms by *forced silking*, in amounts that enable detailed characterization of its tensile properties.
2. Samples of bave from the same silkworm exhibit closely similar F-d characteristics.
3. Samples of bave from *different* silkworms exhibit qualitatively (and sometimes also quantitatively) similar F-d characteristics.
4. The cross-sectional geometry of bave samples *from a given silkworm* may exhibit significant variability. Therefore, stress–strain curves are less reproducible than F-d plots are.
5. *Different silkworms* produce bave with different mean diameters, so similarities and differences between F-d plots are not necessarily preserved when data are rescaled as stress–strain curves.
6. The preceding experimental facts can be accounted for by two simple suppositions: (a) *B. mori* produces fibroin cores with consistent mechanical properties, although the cross section may vary depending on the specific silkworm, and (b) the thickness of the sericin coating has a relatively large variability when samples from either a given silkworm or different silkworms are considered.
7. The Weibull parameters for *B. mori* bave obtained by forced silking are m = 13.0 ± 0.3 (more than twice the value for bave harvested from degummed cocoons) and σ_0 = 416 ± 2 MPa, which is similar to the value previously obtained for degummed silkworm silk.

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