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Structure and properties of ultrafine silk fibers produced by *Theriodopteryx ephemeraeformis*

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Abstract *Theriodopteryx ephemeraeformis* commonly known as bag worms produce ultrafine silk fibers that are remarkably different than the common domesticated (*Bombyx mori)* and wild (*Saturniidae)* silk fibers. Bag worms are considered as pests and commonly infect trees and shrubs. Although it has been known that the cocoons (bags) produced by bag worms are composed of silk, the structure and properties of the silk fibers in the bag worm cocoons have not been studied. In this research, the composition, morphology, physical structure, thermal stability, and tensile properties of silk fibers produced by bag worms were studied. Bag worm silk fibers have considerably different amino acid contents from those of the common silks. The physical structure of the bag worm silk fibers is also considerably different compared with *B. mori* and common wild silk fibers. Bag worm's silk fibers have lower tensile strength (3.2 g/denier) and Young's modulus (45 g/denier) but similar breaking elongation (15.3%) compared with *B. mori* silk. However, the tensile strength and Young's modulus of bag worm fibers are similar to those of the common *Saturniidae* wild silk fibers. Bag worm silk fibers could be useful for some of the applications currently using the *B. mori* and wild silk fibers.

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Introduction

Bombyx mori (mulberry) and silk produced by the insects from the *saturniidae* family such as *Antheraea assamensis* (muga)*, Antheraea mylitta* (tasar), and *Phylisomia ricini* (eri) are the most common types of silks in current use [1–3]. Mulberry silks have excellent properties such as good tensile strength, high elongation, and moisture regain and are extensively used for textile, medical, and biotechnology applications [1, 4–7]. Although wild silks generally have inferior properties than *B. mori* silk, wild silks are reared for commercial silk production due to their unique properties and also to provide employment and income to native habitants where these cocoons are found [8, 9]. It has also been recently reported that wild silks have better potential for tissue engineering applications than *B. mori* silk [10].

In addition to the common s*aturniidae* silks, several other uncommon *saturniidae* insects such as *Argema mittrei* that produce cocoons with unique properties are being considered for commercial silk production [9]. It has also been reported that some of the uncommon *saturniidae* insects are easier to rear, produce larger cocoons and have properties similar or better than those of *B. mori* silk [11]. For instance, silk fibers produced by *Coscinocera hercules* moths were found to have fineness and tensile properties very similar to those of *B. mori* silk fibers [11].

Besides the *saturniidae* family, silks produced by insects that belong to different species have been studied for their structure and properties. Silks produced by honey bees are reported to have a coiled-coil structure consisting of four proteins with low molecular weights, unlike most of the common silks that have large repetitive proteins arranged in the form of a β-sheet [12]. Similarly, it was reported that *Hydropsyche siltalai* produces the weakest silk, but it has good elongation and can double its length before breakage [13]. Silk produced by various types of spiders has also been extensively studied for their outstanding properties and applications in various areas [14].

Bag worms named due to the bag-like cocoons they produce belong to the order *Lepidoptera* and family *Pshyhidae* that contains approximately 1,000 species [15]. Bag worms are considered as pests and generally infect ever green plants such as cedar, juniper, spruce, and pine. Although the production of "silken threads" from bag worms has been reported, the structure and properties of the silk produced by bag worms are not known. The cocoons produced by bag worms provide elevated temperature than the outside atmosphere to the insect that accelerates development. The inside of the bag worm cocoon has a temperature of 13 °C compared with the outside temperature of 8 °C [16]. The bag worm cocoons also protect the insects from natural enemies [15].

In this research, we have studied the structure and properties of the bag worm silk fibers in comparison to the properties of *B. mori* and common *saturniidae* silks. Data available from previous studies have been used for *B. mori* and the *saturniidae* silks to include properties reported by various authors.

Materials and methods

Bag worm (*Theriodopteryx ephemeraeformis*) cocoons used for this study were found on Juniper plants in Lincoln, Nebraska during the summer of 2009. Chemicals used for degumming were purchased from VWR International, Bristol, CT.

Degumming

The bagworm cocoons were treated in chloroform to remove any waxes. Treated cocoons were then degummed in water containing 0.5% (w/w) sodium carbonate and 10% ethylene diamine solution at 85 °C for 1 h with a solution to cocoon ratio of 20:1. The degummed cocoons were thoroughly washed in warm water and dried under ambient conditions.

Morphology

Images of the cocoons were obtained using a digital camera. Size of the cocoons was determined using normal rulers. The undegummed cocoons and fibers obtained after degumming were observed using a scanning electron microscope (SEM) (Hitachi S 3000N variable pressure SEM) to determine the longitudinal and cross-sectional features of the cocoons and degummed fibers. Samples were sputter coated with gold palladium and observed in the SEM at a voltage of 20 kV. Twenty fibers from three different SEM pictures were measured to determine the average and ± one standard deviation of the fiber diameter.

Composition

Degummed bag worm silk fibers were analyzed for the proportion of various types of amino acids using a Hitachi L-8800A amino acid analyzer. Fibers were dissolved in 6 N hydrochloric acid under argon atmosphere for 20 h at 110 °C. The samples were then evaporated to dry and then redissolved in 200 μL of 0.02 N HCl. Fifty microliters of the solution was injected automatically into the amino acid analyzer to determine the type and proportion of amino acid. Corrections were made to the amount of internal standard (Norleucine) to minimize dilutional errors.

Thermal behavior

Bagworm silk fibers were observed in a Thermogravimeric Analyzer (TGA) (Sigma Model 701) to understand the thermal behavior of the fibers in comparison to *B. mori* silk fibers. Samples were heated at 10 °C/min up to 600°C in the TGA.

X-ray diffraction

X-ray diffraction studies were conducted to understand the physical structure of the bag worm silk fibers in terms of % crystallinity and positions of the diffraction peaks. Fibers were grounded in a Wiley mill to pass through a 20 μm mesh. Powdered fiber samples were used for X-ray analysis to eliminate the influence of any preferred orientation of the crystals on the X-ray diffraction patterns. Using powdered samples is a common technique used for analyzing X-ray diffraction of fibers. The powdered fibers were compressed to form a pellet. The pellet was mounted on a Rigaku D-max/BΘ/2Θ X-Ray diffractometer (Rigaku Americas, Woodlands, TX) with Bragg–Brentano parafocusing geometry, a diffracted beam monochromator, and a copper target X-ray tube set to 40 kV and 30 mA. Diffraction intensities were determined for 2θ values ranging from 5° to 40°. The % crystallinity of the fibers was determined by integrating the area under the crystalline peaks after subtracting the background and air scatter using the program MICROCAL ORIGIN.

Tensile properties

Fibers were conditioned under standard conditions (21 °C and 65% relative humidity) for at least 24 h before tensile testing. Fineness of the fibers in terms of denier (weight in grams per 9,000 meters) was determined by precisely weighing a known length of fibers determined using a standard ruler. Fiber samples were tested for their tensile properties using an Instron tensile tester (Model 4444). A gauge length of 1 inch and crosshead speed of 18 mm/min were used for testing. About 30 fibers were tested, and the average and standard deviations are reported.

Results and discussion

Bag worm cocoons

Bag worm cocoons are oval shaped, about 3.5 cm in length, dark brown in color and covered with plant material on the outside as seen from Fig. 1. In addition to providing protection from the outside environment, the plant materials on the cocoon also act as a camouflage to predators. Empty cocoons without the plant materials on the surface had an average weight of 85 mg, much lower than the weight of common silk worms. The inside of the cocoon was similar to a woven fabric with some loose white silk fibers (indicated by arrow) as seen from Fig. 2. The silk fibers in the cocoon were compact, and it was very difficult to remove fibers from the cocoon before degumming. SEM image of the cocoon in Fig. 3 shows that the cocoon consists of long and fine fibers. There is no preferred orientation, and the fibers run above and below each other creating a network structure that provides good strength to the cocoons.

Morphology of the bag worm silk fibers

Bag worm silk fibers obtained after degumming had smooth and clean surface as seen from Fig. 4. The fibers in Fig. 4 had an average diameter of 2.9 ± 1 µm. Figure 5 shows that the fibers have a solid cross section, and most of the fibers are circular unlike *B. mori* silk fibers that have a triangular cross section.

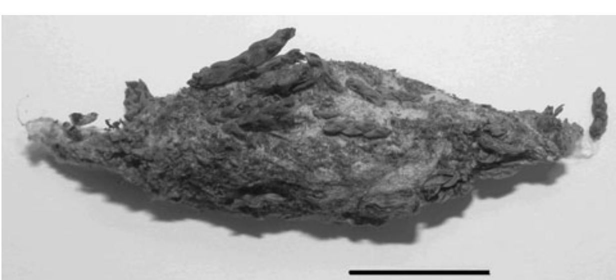

Fig. 1 Digital image of an intact, undegummed bag worm cocoon shows plant materials attached to the cocoon. Scale bar is 1 cm

Composition of bag worm silk fibers

Bag worms have a very unique composition of amino acids than *B. mori* and the common wild silks as seen from Table 1. Alanine and glycine are the two major amino acids in *B. mori* (74%) and wild silks (62–73%), but these two amino acids account for only 6.6% in bag worm silks [2, 3]. The bag worm silks have much higher content of lysine, leucine, phenylalanine, threonine, cysteine, and histidine than *B. mori* and the common wild silks. The amino acids glycine, alanine, serine, and threonine are reported to be in the crystalline region whereas the other amino acids are reported to be in the amorphous region for *B. mori* silk [2, 3, 17, 18]. The ratio of glycine/alanine is reported to determine the crystallographic form of the proteins. The glycine/alanine ratio of bag worm silk fibers is 0.27, but 1.52 for *B. mori* silk fibers indicating that the bag worm silk should have a considerably different crystallographic form [19, 20]. The ratio of amino acids in the crystalline and non-crystalline region is referred to as the disorder ratio and is related to the tensile properties and hydrophilicity of the fibers [18]. Disorder ratio of bag worm silks is 0.18, much lower than the ratio for *B. mori* silk (6.7) suggesting that bag worm silk will have inferior tensile properties compared with *B. mori* silk.

Three groups of silks have been recognized based on the amino acid composition. The first group consists of silks in which the alanine, glycine, and serine content is 60% or higher [21]. This class includes *B. mori* and the other wild silk fibers in Table 1. The second group of silks contains a combination of alanine, glycine, and serine in addition to either proline or glutamine accounting for 60% of the amino acids [21]. The third group consists of silks in which no two amino acids account for 60% or higher amino acid content. Bag worm silk belongs to the third group of insects [21]. The type and amount of amino acids in insects are said to be determined by the diet, cost of producing the silk, and the ecology of the insects [21]. The unique amino acid composition of the bag worms could be due to the lack of proper diet, season in which the cocoons were collected or the inherent biology of the insects.

Table 2 provides a comparison of the various amino acid ratios in the bag worm silks with *B. mori* and the common wild silks. Bag worm silk had relatively more uniform distribution of acidic and basic groups compared with *B. mori* silk which has much higher amounts of acidic amino acids than basic groups. However, the bag worm silk contains substantially higher number of hydrophilic groups than hydrophobic groups compared to the other silks in Table 2. Bag worm silks have much lower ratio of glycine/alanine than the other silks in Table 2. Tables 1 and 2 show that bag worms produce silk containing unique ratio of amino acids than *B. mori* and common wild silks.

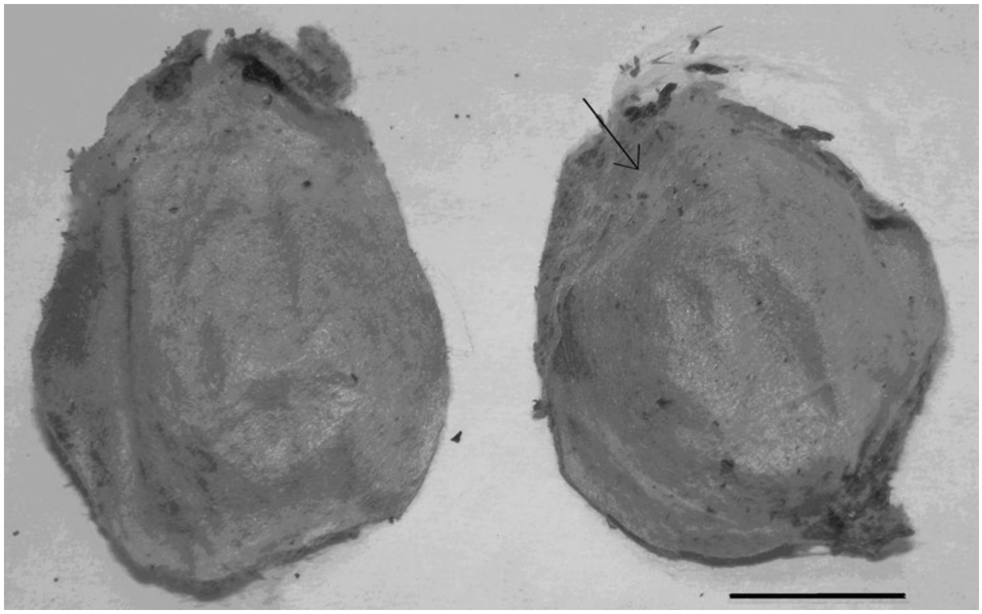

Fig. 2 Image of the inside surface of the bag worm cocoon has a fabric-like appearance. The inside surface had a layer of loosely attached white silk fibers (*arrow*). Scale bar is 1 cm

Fig. 3 SEM image of the surface of the bag worm cocoon shows the random orientation of the silk fibers that helps to create a firm cocoon

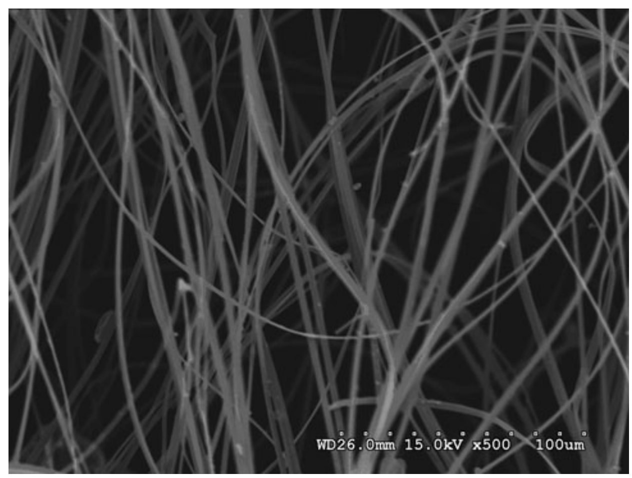

Fig. 4 Degummed bag worm silk fibers have a clean and smooth surface with an average diameter of 2.9 μm

Thermal decomposition

The thermal decomposition of the bag worm silk fibers is compared with that of *B. mori* silk in Fig. 6. Both the bag worm and *B. mori* silk have similar thermal behavior up to about 200 °C and have a weight loss of about 12%. The bag worm silk shows a slightly higher weight loss between 200 and 300 °C. However, the weight loss of the bag worm silk is much lower than that of *B. mori* silk above 300 and up to about 600 °C. At 600 °C, the weight loss of the bagworm silk was about 82% compared with 91% for the *B. mori* silk. The better thermal stability of the bag worm silk to decomposition at high temperatures should mainly be due to the different amino acid compositions of the bag worm silks compared with *B. mori* silk despite the bag worm silks having lower % crystallinity Fig. 7.

Physical structure

X-ray diffractogram of the bag worm silk fibers is compared with *B. mori* silk fibers in Fig. 7. The bag worm silk fibers show similar diffraction peak compared with the *B. mori* silks. Both silks show a single prominent diffraction peak at 2θ angle of 19.8° corresponding to the 201 plane. The 201 plane in *B. mori* is reported to correspond to a d-spacing of 4.43 Å. Five types of fibroin have been

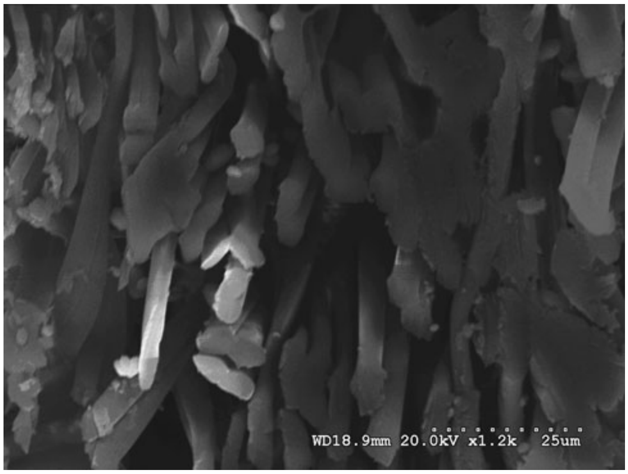

Fig. 5 Bag worm silk fibers have a solid cross section, and most of the fibers are circular

Table 1 Comparison of the amino acid composition of the bag worm silk fibers with *B. mori* and three varieties of common wild silks

Amino acids	% Amino acids				
	Bag worm	*B. mori*	*A. mylitta*	*A. pernyi*	*P. ricini*
Lysine	11.1	0.2	0.2	0.2	0.2
Alanine	5.2	29.4	32.1	34.1	36.3
Glycine	1.4	44.6	40.6	27.7	29.4
Serine	3.6	12.1	4.2	9.9	8.9
Tyrosine	6.8	5.2	4.6	6.8	5.8
Leucine	10.9	0.7	0.8	0.7	0.7
Aspartic acid	7.8	1.3	0.9	6.1	3.9
Arginine	5.5	0.5	2.4	5.0	4.1
Glutamic acid	14.5	1.0	1.3	1.3	1.3
Phenylalanine	7.2	0.1	0.3	0.3	0.2
Threonine	5.1	0.9	0.3	0.2	0.2
Valine	4.8	2.4	1.7	1.5	1.3
Cysteine	4.7	0.1	0.2	0.1	0.1
Histidine	3.7	0.1	0.8	0.7	0.8
Proline	3.9	0.8	2.2	2.2	2.0
Isoleucine	2.2	0.8	0.6	0.5	0.5
Methioine	1.3	0.2	0.3	0.3	0.3

Data for *B. mori* and the wild silks are from [2, 3]

Table 2 Comparison of the amino acid ratios in bag worm silk fibers with the common silks

Ratio	Bag worm	*B. mori*	*A. mylitta*	*P. ricini*
Basic/acidic	0.91	0.65	0.97	1.30
Hydrophilic/hydrophobic	1.9	0.28	0.44	0.35
Glycine/alanine	0.3	1.58	0.81	0.80

Data for *B. mori* and wild silks are from reference [2, 3]

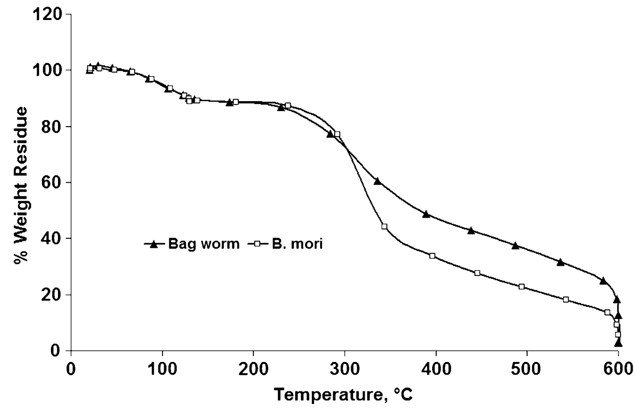

Fig. 6 Thermal decomposition of bag worm silk fibers compared with *B. mori* silk fibers

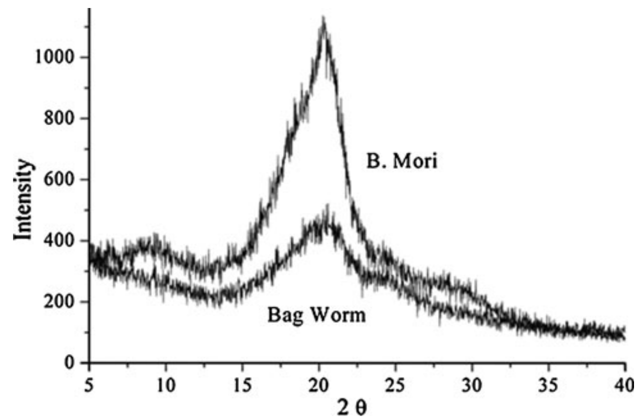

Fig. 7 Diffractogram of bag worm silk fibers compared with *B. mori* silk fibers

classified based on the spacing and intensities of diffraction arcs [22]. Silk produced by the *Psychidae* family has been classified under a different group than *B. mori* and *Saturniidae* silks but with similar fiber repeat distance [22]. More than 80% of the polypeptides in *B. mori* fibroin have been reported to consist of Gly-X (GX) sequence in the crystalline domain whereas the sequence of amino acids in bag worm silks is not known [23, 24].

The % crystallinity of bag worm silk fibers was found to be 33.8% from the diffractogram shown in Fig. 7 compared with 30–40% for *B. mori* and common wild silks [25, 26]. It is reported that the crystalline regions in *B. mori* and common wild silks consists of the amino acids glycine, alanine, serine, and threonine [23–27]. However, the sum of these amino acids in bag worm silks is only 15.2% whereas the % crystallinity was found to be 33.8%. This suggests that the crystalline regions in bag worm silks consist of other amino acids than those found in *B. mori* and common wild silks. The considerably different amino acid compositions of bag worm silks as given in Table 1 and the previous report that classified fibroin from

Table 3 Tensile properties of bag worm silk fibers compared with *B. mori*, *A. mylitta,* and *P. ricini* silks

Fiber	Bag worm	*B. mori*	*A. mylitta*	*P. ricini*
Fineness, denier	0.9 ± 0.1	0.4–1.1	4.7–10.7	2.3–3.6
Breaking tenacity, g/denier	3.2 ± 1.0	4.3–5.2	2.5–4.5	1.9–3.5
Breaking elongation, %	15.3. ± 6.2	10.0–23.4	26–39	24–27
Young's modulus, g/denier	45 ± 12	84–121	66–70	29–31

Data for *B. mori* and wild silks are from references [2, 3]

Psychidae family in a different group than *B. mori,* and wild silks based on the differences in diffraction patterns substantiates our assumption.

Tensile properties

Bag worm silk fibers have fineness similar to those of *B. mori* silk, but are much finer than the wild silks as seen from Table 3. However, the breaking tenacity of the bag worm fibers at 3.2 g/denier is lower than that of *B. mori* and in the range of breaking tenacity for the wild silk fibers. Breaking elongation of the bag worm silk fibers is similar to that of *B. mori* silk but considerably lower than that of the wild silks. The modulus of bag worm fibers is also considerably lower than the modulus of *B. mori* and *A. mylitta* but similar to the modulus of *P. ricini* silk as seen from Table 3. Lower breaking tenacity of the bag worm fibers is most likely due to the low amounts of crystalline amino acids as indicated by the low disorder ratio. Size of the insects, bag worms being considerably smaller than that of *B. mori* or the wild silk caterpillars, probably makes it difficult for the bag worms to secrete the crystalline amino acids. Low modulus of the bag worm fibers indicates that the fibers are soft to touch. Although the breaking tenacity of the bag worm fibers is lower than that of *B. mori* silk, natural cellulose fibers such as cotton have similar (2–3 g/denier) breaking tenacity but much lower (8–9%) breaking elongation. This indicates that bag worm fibers will be suitable for commercial applications.

Conclusions

Bag worms produce very firm oval-shaped cocoons with average weight of about 85 mg. Silk fibers in the cocoons had an average diameter of about 2.9 µm. The fibers are composed of very distinct amino acids than those in the common silk worms. The alanine and glycine content in bag worm silk fibers was 6.6% compared with 74% for *B. mori* silk suggesting that bag worm silks have a considerably different crystallographic forms than those of *B. mori* silk. Bag worm silk fibers also show better stability to thermal degradation than those of *B. mori* silk from 300 to 600 °C. Tensile strength of bag worm silk fibers is considerably lower, but elongation and modulus are similar to that of *B. mori* silk. This article shows that bag worm silk has unique composition and properties than that of the common silk fibers. Other species in the *Pshyhidae* family may have also unique structure and properties.

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