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# Investigation of the Structure and Properties of Silk Fibers Produced by *Actias lunas*

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# Investigation of the Structure and Properties of Silk Fibers Produced by *Actias lunas*

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**Abstract**

This paper reports the structure and properties of silk fibers produced by *Actias lunas* in comparison to *Bombyx mori* and the common wild silks. Considerable efforts are being made to find new sources for natural silk and also to develop regenerated protein fibers to supplement the limited amounts of *B. mori* and wild silks available in the market. In addition, it has been found that non-traditional silks have unique properties and utilizing uncommon wild silks can provide income and employment to indigenous people where the wild silks are found. *Actias lunas* belongs to the Saturniidae family of silk producing insects. However, the structure and properties of silk produced by *A. lunas* have not been studied. This research showed that the silk fibers produced by the luna moth had morphological and physical structure similar to that of the common wild silks but tensile properties similar to that of *B. mori* silk. *A. lunas* silk fibers are composed of higher amounts of hydrophobic amino acids and had much less glycine than *B. mori* and common wild silks. With a fineness of 2 denier, breaking tenacity of 4.3 g/den and breaking elongation of 10.9 %, the tensile properties of *A. lunas* silk fibers were similar to that of *B. mori* and much better than that of the common wild silks that are coarser and have lower breaking tenacity. *A. lunas* fibers show good potential to be useful for applications currently using *B. mori* silk.

**Keywords:** *Actias lunas*, silk fibers, wild silks, properties

## Introduction

*Bombyx mori* and the wild silks *Antheraea mylitta*, *Antheraea pernyi,* and *Phylisomia ricini* belonging to the Saturniidae family are the most common types of silks that have been known for centuries. Although silks are preferred fibers for various applications, there is relatively limited quantity of silk available compared to other fibers. The total annual world production of silk is approximately 1.3 million tons compared to 63 million tons of fibers consumed in 2009 [1]. Therefore, attempts have been made to find alternative sources for silk fibers. Such attempts have also led to the discovery of new sources for natural silk fibers and the development of regenerated protein fibers. Recently, we have demonstrated that high quality regenerated protein fibers with properties suitable for textile and medical applications can be produced from wheat gluten and soy-proteins [2–4].

Although *B. mori* and the common wild silks are predominantly used for commercial scale silk production, many other insects have been known to produce silk and silk containing cocoons [5–8]. Efforts are being made to harvest the silks produced by the uncommon wild insects, study their properties and understand the potential of using the silks for various applications. Interests in non-traditional silks are mainly due to the po-

tential for high income generation for indigenous people located in habitats where the wild silks are found [9, 10]. In addition, wild silks may have unique properties not found in the common silks. For instance, it has been reported that weaver ants produce silk in the form of nanofibers unlike any other known silk [11]. Similarly, silks produced by bees have a unique coiled–coiled structure compared to the normal silks. Recent studies have also shown that wild silks have better potential for cell growth than *B. mori* silk [12].

The Saturniidae family consists of some of the largest silk producing insects [5]. However, only a limited number of Saturniidae insects primarily *A. mylitta*, *A. pernyi* and *P. ricini* are currently used for commercial scale silk production. We have recently reported that several uncommon Saturniidae insects produce silk with properties similar or better than that of the common *B. mori* silks [13, 14]. It was found that silk fibers produced by *Coscinocera hercules* had properties very similar to that of *B. mori* silk whereas *Copaxa multifenestrata* produced coarse silk fibers that had considerably lower tensile properties than *B. mori* and the common wild silks [13].

*Actias lunas* also belongs to the Saturniidae family but the properties of silk produced by the luna moths have not been studied. Luna moths are generally found in forested areas but are nocturnal and have a wing span ranging from 70 to 105 mm. Depending on their location, Luna moths are reported to have one to three generations (univoltine or multivoltine). Reared Luna cocoons are reported to have a different color than those found in nature [15].

In this research, the cocoons produced by Luna moths were used to extract silk fibers and the structure and properties of the fibers have been studied in an effort to understand the unique properties and identify potential applications. The properties of the *A. lunas* silk have been compared to the properties of *B. mori* and the common wild silks reported in literature.

## Experimental

### Materials

*A. lunas* cocoons were obtained from Reiman Gardens in Ames, Iowa. Ethylenediamine and sodium carbonate used for degumming were analytical grade chemicals purchased from VWR international (Bristol, CT).

### Degumming

The *A. lunas* cocoons were degummed by treating in a solution containing 0.5 % (w/w) sodium carbonate and 10 % (w/w) ethylenediamine solution at 95 °C for 30 min with a cocoon to solution ratio of 1:20. The fibers obtained after treatment were washed several times in warm water and dried under ambient conditions.

### Composition

The composition of the *A. lunas* fibers was determined in terms of the amino acid content. Silk fibers were hydrolyzed using 6 N HCl at 110 °C for 20 h under argon atmosphere. After the hydrolysis, the samples were evaporated to dryness and then redissolved in 200 µl of 0.02 N HCl. Fifty microliters of the solution was injected onto the Hitachi (L-8800A) amino acid analyzer to determine the amino acid type and content. Norleucine was used as the internal standard and corrections were made to account for the dilutional errors.

### Physical Structure

X-ray diffraction (Rigaku D-max/BΘ/2Θ X-Ray diffractometer, Rigaku Americas, Woodlands, TX) was used to study the physical structure of the silk fibers in terms of the degree of crystallinity and shape and intensity of the diffraction peaks. X-ray diffraction measurements were taken using a Bragg–Brentano parafocusing geometry, a diffracted beam monochromator, and a copper target X-ray tube set to 40 kV and 30 mA. The silk fibers were powdered in a Wiley mill and the powder was pressed to form a pellet. The pellet was fixed to the sample holder and diffraction intensities were recorded from 5° to 40° (2θ). The degree of crystallinity (%) of the fiber was obtained by integrating the area under the crystalline peaks after subtracting the background and air scatter using the program MICROCAL ORIGIN.

### Morphology

Morphology of the undegummed *A. lunas* cocoons and fibers obtained after degumming were observed in a Hitachi S-3000N variable pressure scanning electron microscope (SEM). Longitudinal and cross-sectional images of the fibers were taken on samples mounted on conductive adhesive tapes and sputter coated with gold palladium.

### Tensile Properties and Moisture Regain

Silk fibers obtained after degumming were tested for their tensile properties in terms of breaking strength, breaking elongation, and Young's modulus. Fibers were conditioned under the standard testing conditions of 21 °C and 65 % relative humidity for at least 24 h before conducting the tensile tests. Fineness of the fibers was measured in terms of denier (weight in grams per 9,000

meters) by weighing a known length of fibers. Tensile tests were performed on an Instron (Model 4444) tensile testing machine using a gauge length of 1 inch and cross head speed of 18 mm/min. At least 50 fibers were tested to determine the tensile properties and the average and ± one standard deviations are reported. The moisture regain of the fibers was determined according to ASTM standard method 2654 using standard conditions of 21 °C and 65 % relative humidity.

## Results and Discussion

### Properties of *A. lunas* Cocoons

Cocoons produced by *A. lunas* were white to slightly yellow in color and were extensively covered with leafy materials as seen from Fig. 1. The cocoons had a considerably uniform length of about 4 cm whereas the width of the cocoons ranged from 2 to 2.6 cm. The cocoons used in this study had an average weight of 200 mg and were considerably smaller compared to *B. mori* cocoons that had an average weight of approximately 640 mg [14, 16]. The wild silks *A. mylitta* and *P. ricini* cocoons are reported to produce cocoons with average weight of 3.4 g and 840 mg, respectively [17, 18]. However, cocoons as heavy at 14 grams have also been reported for *A. mylitta* cocoons [16]. The average thickness of the *A. lunas* cocoons was about 420 µm whereas *B. mori* cocoons are reported to have thickness ranging from 500 µm to 5 mm. Fibers in the cocoons were relatively loosely arranged and could be pulled out easily whereas fibers in cocoons produced by some Saturniidae insects such as cecropia are tightly held by the cocoons and cannot be pulled out without degumming [14]. The *A. lunas* cocoons consisted of a single layer of fibers whereas most wild silk cocoons belonging to the saturniidae family are reported to have three or more distinct layers [5, 14].

Figure 2 shows the SEM picture of the undegummed Luna cocoon revealing the morphological features. Luna moth spins two fibers simultaneously, commonly observed in most silk producing insects. The fibers were long and run in random directions and form a network structure providing strength to the cocoon. Considerable amounts of white particles were seen on the fibers reported to be calcium oxalate crystals excreted by the silk worms [19, 20].

### Composition of Fibers

Table 1 provides the amino acid composition of the *A. lunas* silk fibers in comparison to *B. mori* and wild silks. As seen from the table, the amino acids alanine, glycine, serine and tyrosine account for 66 % of the amino acids in *A. lunas* silk fibers whereas these four amino acids account for 90 % in *B. mori* and approximately 78 % in the wild silks [17, 18]. The amino acids alanine, glycine, serine and threonine that are reported to be found in the crystalline region account for 53 % of the amino acids in *A. lunas* silk whereas the amount of crystalline amino acids in *B. mori* silk is about 86 % and about 72 % in the wild silks. The ratio of amino acids in the crystalline and amorphous regions is called the disorder ratio and is reported to affect the structure and properties of the fibers. *A. lunas* silk had lower amounts of crystalline amino acids suggesting that the fibers may have poor mechanical properties compared to *B. mori* silks assuming that all other factors are the same. *A. lunas* silk had much higher amounts of aspartic acid, arginine, glutamic acid, leucine and threonine but very low levels of glycine compared to the common silks.

Table 2 shows the ratio of amino acids in *A. lunas* and the common silks. *A. lunas* silk had similar ratio of basic

**Fig. 1.** Digital image of the *A. lunas* cocoons. Scale bar is 1 cm.

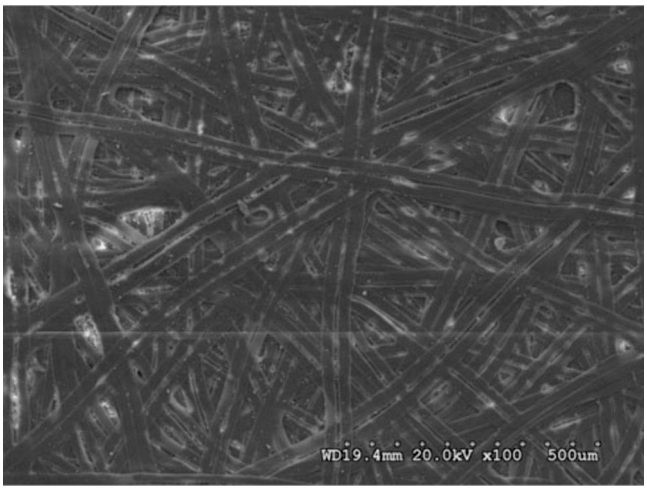


**Fig. 2.** SEM image of the surface of an undegummed *A. lunas* cocoon.

**Table 1.** Amino acid composition of *A. lunas* silk compared to *B. mori* and three varieties of common wild silks

| Amino acids | % Amino acids | | | | |
|---|---|---|---|---|---|
| | *A. lunas* | *B. mori* | *A. mylitta* | *A. pernyi* | *P. ricini* |
| Alanine | 22.3 | 28.4 | 34.1 | 34.7 | 36.3 |
| Glycine | 11.9 | 44.6 | 27.6 | 28.4 | 29.4 |
| Serine | 16.6 | 12.1 | 9.9 | 9.1 | 8.9 |
| Tyrosine | 15.3 | 5.2 | 6.8 | 5.1 | 5.8 |
| Aspartic acid | 6.6 | 1.3 | 6.1 | 5.0 | 3.9 |
| Arginine | 6.6 | 0.5 | 5.0 | 4.7 | 4.1 |
| Glutamic acid | 4.3 | 1.8 | 1.4 | 1.4 | 1.3 |
| Leucine | 7.5 | 0.7 | 0.7 | 0.7 | 0.7 |
| Threonine | 2.8 | 0.9 | 0.3 | 0.2 | 0.2 |
| Histidine | 0.7 | 0.1 | 0.8 | 0.7 | 0.8 |
| Lysine | 1.37 | 0.2 | 0.2 | 0.2 | 0.2 |
| Valine | 2.2 | 2.4 | 1.7 | 1.5 | 1.3 |
| Isoleucine | 0.9 | 0.8 | 0.6 | 0.5 | 0.4 |

Data for *B. mori* and wild silks are from references [17, 18, 21, 22].

**Table 2.** Comparison of the amino acid ratios in *A. lunas* fibers to the common silks

| Ratio | *A. lunas* | *B. mori* | *A. mylitta* | *P. ricini* |
|---|---|---|---|---|
| Basic/acidic | 0.70 | 0.65 | 0.97 | 1.30 |
| Hydrophilic/hydrophobic | 0.91 | 0.28 | 0.44 | 0.35 |
| Glycine/alanine | 0.55 | 1.58 | 0.81 | 0.80 |

Data for *B. mori* and wild silks are from reference [17].

to acidic amino acids compared to *B. mori* but a much higher ratio of hydrophilic to hydrophobic amino acids suggesting a more uniform distribution of hydrophilic to hydrophobic amino acids in *A. lunas* silk. Based on the amino acid composition, *A. lunas* silk could have lower moisture sorption than the common silks.

## Morphology

Silk fibers produced by *A. lunas* are flat and ribbon-like but had twists as seen from Fig. 3. Considerable amounts of white particles were seen on the fibers even after degumming. The white particles are reported to be calcium oxalate crystals secreted by the moths and are also found in other Saturniidae silks. Except for the presence of the crystals, the fibers had a smooth appearance as seen from Fig. 4. The fibers had an oval to rectangular cross-section as seen from Fig. 5 similar to that of the common wild silks but unlike *B. mori* silks that have a triangular cross-section that provides excellent luster to the fibers [21]. Visually, the *A. lunas* fibers were dull compared to *B. mori* silk.

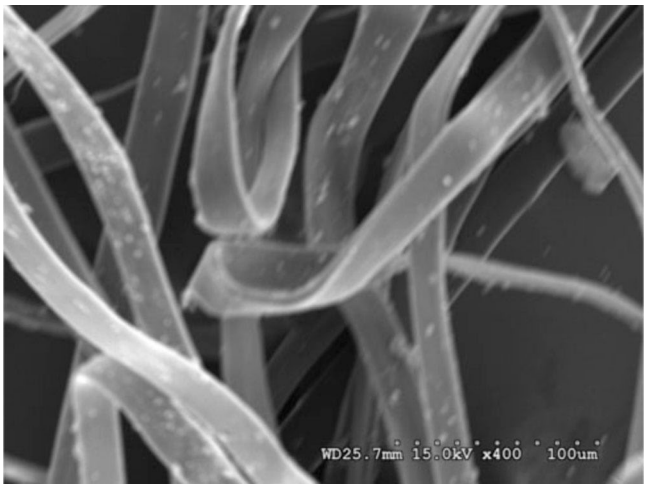


**Fig. 3.** SEM image revealing the flat and ribbon-like *A. lunas* silk fibers.

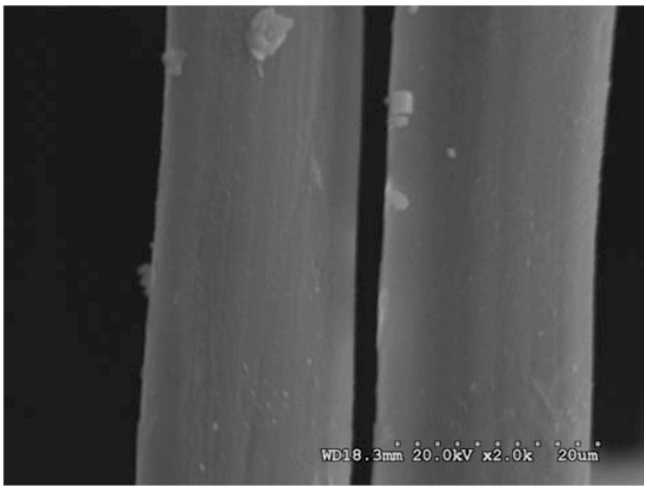


**Fig. 4.** SEM image of the longitudinal surface of degummed *A. lunas* silk fibers shows a clean and smooth surface.

## Physical Structure

The *A. lunas* silk fibers had a considerably different physical structure than the *B. mori* silk fibers as seen from Fig. 6. However, the diffractogram of the *A. lunas* fibers was similar to that of the wild silks. The *A. lunas* silk had two diffracting peaks at about 16.8° and 21.1° corresponding to a d-spacing of 5.27 and 4.41 Å, respectively as seen in A. assama silk [20]. The *B. mori* silk had a single prominent peak at about 20.6°. In addition to the two prominent peaks, the *A. lunas* fibers had a small sharp peak at about 29° which was due to the presence of the calcium oxalate crystals [20]. The % crystallinity of the *A. lunas* fibers was 30.6 % was in the range of % crystallinity reported for *B. mori* (20–41 %) silk. Overall, the physical structure of *A. lunas* silk was found to be more close to that of the wild silk than *B. mori* silk.

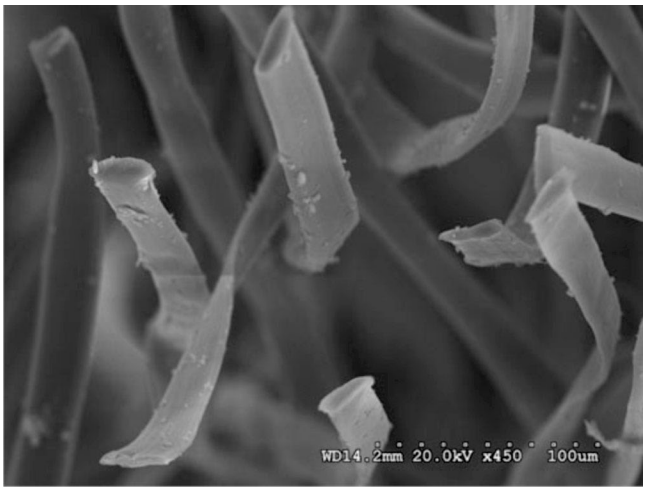


**Fig. 5.** SEM image of the cross-section *A. lunas* silk fibers shows that the fibers are solid and have oval to rectangular cross-sections.

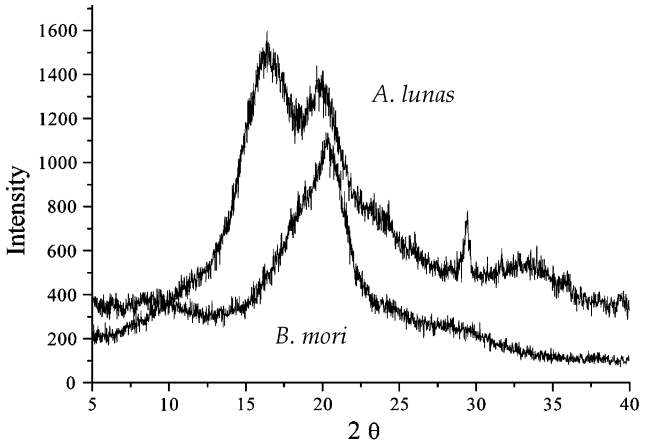


**Fig. 6.** X-ray diffractogram of *A. lunas* silk fibers compared to *B. mori* silk fibers.

## Tensile Properties

Table 3 provides a comparison of the fineness, tensile properties and moisture regain of the *A. lunas* fibers with *B. mori* and two common wild silks. As seen from the table, the *A. lunas* fibers were coarser than B.mori but much finer than the wild silks. It is reported that the coarseness of the wild silks is a major reason for the limited applications of the wild silks compared to mulberry silk [17, 18, 21]. The much finer fibers produced by *A. lunas* compared to the wild silks suggests that the fibers could be more suitable for applications currently using *B. mori* silk. The breaking tenacity of the *A. lunas* fibers was higher than that of the wild silks but similar to that of the *B. mori* silk. However, the breaking elongation of the *A. lunas* fibers was much lower than that of the wild silks. The modulus of the fibers was lower than *B. mori*, similar to that of *A. mylitta* but higher than that of *P. ricini* silk suggesting that the *A. lunas* fibers may not be as smooth as *B. mori* but are not as harsh to handle as the wild silks. The relatively high breaking tenacity of the *A. lunas* fibers despite having low % crystallinity should be due to the presence of amino acids such as trysoine that provide strong interactions. Lower elongation of *A. lunas* fibers compared to the wild silks should be due to the finer fibers and probably better arrangement of the protein crystals along the fiber axis. The moisture regain of the *A. lunas* fibers was higher than that of the other fibers in Table 3 despite the fibers having higher amounts of hydrophobic amino acids than the common silks. This should be due to the differences in the arrangement of the amino acids in the fibers and the % crystallinity.

**Table 3.** Tensile properties of *A. lunas* silk fibers compared to *Bombyx mori*, *A. mylitta* and *P. ricini* silks

| Fiber Property | *A. lunas* | *B. mori* | *A. mylitta* | *P. ricini* |
|---|---|---|---|---|
| Fineness, denier | 2.0 ± 0.2 | 0.4–1.1 | 4.7–10.7 | 2.3–3.6 |
| Breaking tenacity, g/den | 4.3 ± 0.7 | 4.3–5.2 | 2.5–4.5 | 1.9–3.5 |
| Breaking elongation, % | 10.9 ± 4.5 | 10.0–23.4 | 26–39 | 24–27 |
| Young's modulus, g/den | 76 ± 11 | 84–121 | 66–70 | 29–31 |
| Moisture regain, % | 12.1 | 8.5–9.9 | 10.5 | 10.0 |

Data for *B. mori* and wild silks are from references [17, 18, 22].

## Conclusions

*Actias lunas* produced cocoons that were considerably smaller and lighter that the commonly known silks. Fibers obtained after degumming the cocoons had much lower amount of glycine but higher amounts of tyrosine, aspartic acid, arginine and leucine compared to *B. mori* and the common wild silks. The *A. lunas* fibers had lower ratio of crystalline to amorphous amino acids but had higher amounts of hydrophobic amino acids. Physical structure of the *A. lunas* fibers was more similar to that of the wild silks than *B. mori* silk. Morphologically, the fibers were flat and ribbon-like with a oval to rectangular cross-section that makes the fibers less lustrous compared to *B. mori* silk fibers. The *A. lunas* fibers are coarser than the *B. mori* silk but much finer than the wild silks. However, the breaking tenacity of the luna fibers was similar to that of *B. mori* fibers but higher than that of the wild silks. The breaking elongation of the luna fibers was similar to that of *B. mori* silk but lower than that of the wild silks. Overall, the *A. lunas* silk fibers show potential to overcome the limitations of wild silks and be useful for applications that currently use mulberry silks.

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*Actias luna* female taken by Shawn Hanrahan and reared on American sweetgum;
http://en.wikipedia.org/wiki/File:Actias_luna_female_sjh.JPG

*Actias luna* 4th instar larva taken by Shawn Hanrahan. Reared on American sweetgum;
http://en.wikipedia.org/wiki/File:Actias_luna_4th_instar_sjh.JPG