

Structure and properties of cocoons and silk fibers produced by *Hyalophora cecropia*

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Abstract This paper shows that silk fibers produced by *cecropia* (*Hyalophora cecropia*) have similar tensile properties but different amino acid composition than that of mulberry (*Bombyx mori*) silk. The *cecropia* fibers are also much finer and have better strength and modulus than tasar silk, the most common non-mulberry silk. *Cecropia* is one of the largest silk producing moths and has similar lifecycle to that of mulberry silk but is easier to grow and produces larger cocoons than mulberry silk. In this study, we have characterized the composition, morphology, physical and tensile properties, and thermal behavior of the *cecropia* silk. *Cecropia* cocoons have a three tier structure and are larger (750 mg) than the cocoons produced by *B. mori* (650 mg). Fibers in the three layers in *cecropia* cocoons have tensile properties similar to that of *B. mori* silk but are finer (1.7–2 denier) and have higher strength (3.8–4.3 g/denier) and modulus (68–92 g/denier) than tasar silk.

Introduction

For centuries, mulberry (*Bombyx mori*) silk has been the most common type of silk. However, wild varieties of silk such as tasar silk, produced by the insect *Antheraea mylitta* commonly found in India and *Antheraea pernyi* found in China; muga silk, produced by *Antheraea assamensis*; and eri silk, produced by *Phylisomia ricini* have been partially domesticated and used to rear silk for commercial applications [1–3]. Silks are known for their high strength, durability, luster, drapeability, and other unique features compared to the common cellulose and synthetic fibers in current use. However, the amount of silk produced worldwide is only about 1.6 million tons compared to about 65 million tons of fibers produced worldwide every year. Therefore, efforts have been made since 1930s to produce regenerated protein fibers using various protein sources. Soyproteins, wheat gluten and gliadin, zein, keratin in feathers are some of the sources used to develop regenerated protein fibers [4–6]. Our group has demonstrated that byproducts such as soyprotein, zein, and wheat gluten obtained during processing of food grains for food and biofuels can be used to develop high quality regenerated protein fibers [4–6]. The regenerated protein fibers developed from wheat gluten and soyproteins were crosslinked with carboxylic acids and reported to have properties similar to wool and suitable for textile and medical applications [7, 8]. Plant proteins have also been chemically modified to develop biodegradable protein materials [9].

The four common wild silks currently in use belong to the *Saturniidae* family that consists of several cocoon producing species. In addition to the common wild silks, other *Saturniidae* insects such as *Coscinocera hercules*, *Antheraea oculea*, *Eupackardia callata*, and *Rothschildia lebeau* are reported to produce silk fibers with unique

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properties compared to *B. mori* and the common wild silks [10], *Hyalophora cecropia* moth also belongs to the *Saturniidae* family and is commonly referred to as the giant silk worm moth and is one the largest moths found in North America with a wing span of 10–15 cm and the *cecropia* caterpillar reaches a length of up to 10 cm [11]. Although *cecropia* moths are commonly found in vegetation in the mid-west and east coast of the United States, they are generally considered as pests and have not been domesticated and reared for their silk. *H. cecropia* caterpillars feed on leaves from several trees (crab, elder, sugar maple, wild cherries, birch, and dogwood) and are therefore easier to rear compared to *B. mori* silk, which only feeds on mulberry leaves [11, 12]. *H. cecropia* has similar length of life-cycle but produces larger cocoons to that of *B. mori* silk. It has also been reported that the proteins generated by *cecropia* have antimicrobial and anticancer activity [13–15].

Understanding the structure and properties of the silks produced by *cecropia* moths could be useful to identify the unique aspects and understand the possibility of commercial rearing of *cecropia* for silk. It may also be possible to develop recombinant and/or regenerated protein fibers from *cecropia* silk for various applications. For instance, considerable efforts are being made to understand the properties of silks produced by various types of spiders. It has been shown that spider silk has strength comparable to that of steel. Studies have also been conducted to develop regenerated and recombinant films and fibers from spider silk for various applications [16, 17].

The primary functions of cocoons constructed by moths are to resist mechanical damage, protect the insects from harsh environmental conditions, support the development and pupation of the larvae, and also be immune to microbial degradation [18]. Different insects construct their cocoons in various shapes and sizes depending on their habitat and their life-cycle. *Cecropia* moths construct two types of cocoons, the most common “baggy” cocoons and the less familiar “compact” cocoons [19–21]. It has also been reported that *cecropia* moths can spin flat sheets of silk in a two-dimensional environment [12, 22]. *Cecropia* moths turn various angles as they construct the three layers in their cocoons and also repair damaged cocoons and continue their construction [12, 22].

The shape, size, and composition of the cocoons, the environment in which the insect grows, and the function of the cocoons influence the properties of the cocoons and the fibers in the cocoons. In this research, we have studied the structure and properties of the cocoons and silk produced by *cecropia* in comparison to *B. mori* and the common wild silks *A. mylitta* and *P. ricini* in an effort to identify the unique properties and to understand the possibility of using the *cecropia* silks for various applications.

Materials and methods

Materials

Cecropia cocoons were supplied by the Entomology Department at the University of Nebraska-Lincoln and by Reiman Gardens in Ames, Iowa. Chemicals used for the study were purchased from VWR international (Bristol, CT).

Methods

Degumming

Cecropia cocoons were treated with chloroform at room temperature to remove any waxes on the surface. The cocoons were later washed using 1% sodium dodecyl sulfate to remove impurities. The *cecropia* silk fibers were degummed by treating the silk in 10% ethylenediamine and 0.5% sodium carbonate solution at 80 °C for 50 min with the cocoon to solution ratio of 1:20. The degummed silk was thoroughly washed in warm water and dried.

Morphology

The construction of the cocoons was analyzed visually and images were collected using a digital camera. The structure of the cocoons and silk was analyzed using scanning electron microscope (SEM). Both the longitudinal and cross-sectional features of the three layers of the cocoons were observed under the SEM at a voltage of 20 kV. Samples were sputter coated with gold palladium before observing in the SEM.

Composition

An aminoacid analysis was performed on a Hitachi L-8800A to determine the composition of the *cecropia* silk. In the amino acid analysis, norleucine was used as the internal standard. Norleucine was added to the samples and the standards. The samples were then evaporated to dryness in a speedvac. The samples were later hydrolyzed in liquid 6 N HCl under argon atmosphere. After 20 h of hydrolysis at 110 °C, the samples were evaporated to dryness, then redissolved in 200 µL of 0.02 N HCl. 50 µL was injected automatically onto the Hitachi Amino Acid Analyzer to determine the amino acid type and content. In data analysis, correction is made to the amount of the internal standard, to minimize dilutional errors. Three samples were tested for the amino acid composition and the average and ±1 standard deviations are reported.

Physical structure

X-ray diffraction was used to determine the physical structure of the *cecropia* fibers in terms of % crystallinity and peak positions. Fibers were powdered in a Wiley mill and the powder was pressed to form a pellet. The pellet was used for X-ray analysis on a Rigaku D-max/B Θ /2 Θ 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. The % 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.

Tensile properties and moisture regain

All samples were conditioned for at least 24 h under standard testing conditions of 21 °C and 65% relative humidity. The three layers of the cocoons were carefully separated to determine their tensile properties. Loose fibers between the outer and intermediate layers were discarded. Samples measuring 20 mm in length \times 10 mm in width were cut from each layer and tested on an Instron tensile testing machine with a gauge length of 10 mm and cross-head speed of 25 mm/min. About 25 samples from each layer from eight different cocoons were tested. The degummed fibers from the three layers were tested using a gauge length of 1 in. and cross head speed of 18 mm/min. At least 50 fibers from eight different cocoons were tested for their tensile properties and the average and standard

deviations were obtained. The moisture regain of the fibers was determined according to ASTM standard method 2654 using standard conditions of 21 °C and 65% relative humidity.

Thermal analysis

The thermal behavior of the *cecropia* silk was determined using a Sigma (Model 701) thermogravimetric analyzer at a heating rate of 20 °C/min.

Results and discussion

Properties of the *cecropia* cocoons

Cecropia cocoons have a three-tier structure consisting of an outer, intermediate, and inner layer as shown in Fig. 1a–c. Figure 1a shows an intact cocoon attached to a twig. *Cecropia* cocoons have a brownish color and the cocoons have several shiny fibers. The average weight of the cocoons used in this study was about 750 mg and *cecropia* cocoons with average weights of 800 mg–1 g were reported earlier [12]. Average cocoon weights of about 640 mg for mulberry silk, 3.4 g for *A. mylitta*, and 840 mg for *P. ricini* silks have been reported [2, 3]. In another report, cocoon weights ranging from 7 to 14 g have been reported for *A. mylitta* cocoons [23]. The outer most layer of the *cecropia* cocoons accounts for approximately 38% of the total weight of the cocoon, has average thickness of 210 µm and is crisp and paper-like and probably acts as a camouflage to deceive

Fig. 1 Structure of the *cecropia* cocoons. **a** Shows an intact cocoon attached to a twig on which the cocoon is built. **b** Reveals the fiber (intermediate) layer after the outer most layer has been cut open. **c** Shows the three layers with the inner most layer attached to the fiber layer

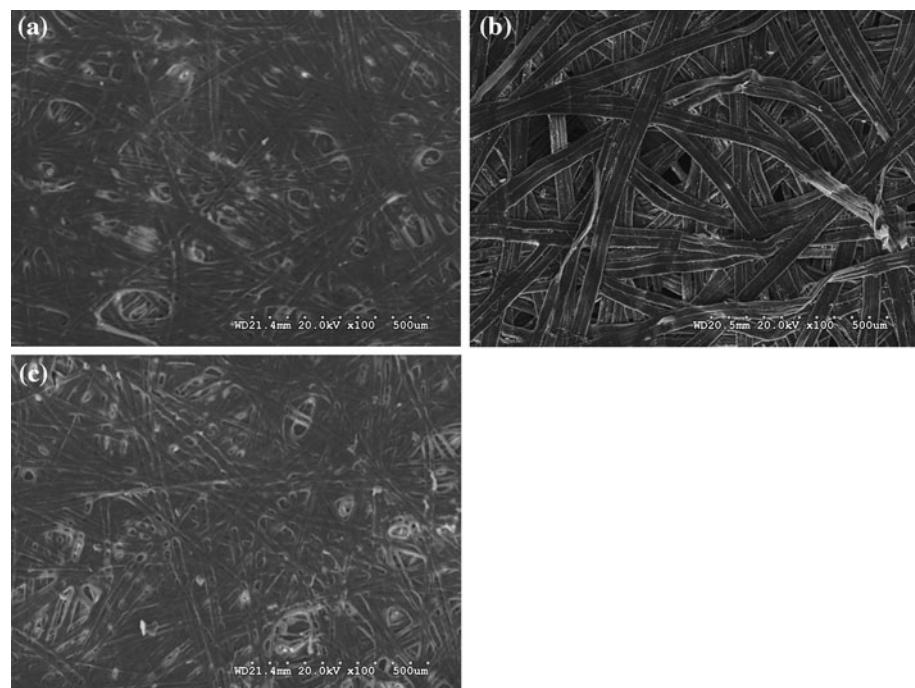


predators. Figure 1b reveals the outer and intermediate layer which has most of the fibers and Fig. 1c reveals the tightly woven inner layer. The intermediate or middle layer containing the fibers forms the bulk of the cocoon and weighs about 45% of the total weight of the cocoon. The intermediate fiber layer is loosely connected to the outer layer but very tightly connected to the inner layer which weighs about 17% of the cocoons and has a very compact construction and probably provides most of the protection to the insect from the environment. However, cocoons with outer, intermediate, and inner layers weighing 50–54%, 5–10%, and 40–42%, respectively, were reported earlier [12]. It has been suggested that the amount of inner layer produced remains fairly constant but that of the outer and intermediate layers keeps changing among cocoons. It has also been reported that the weight of the cocoons is dependent on the sex of the insect and that female insects tend to produce heavier cocoons [12].

Table 1 Properties of the three layers in *cecropia* cocoons

	Outer layer	Intermediate layer	Inner layer
Thickness (μm)	210 ± 10	330 ± 13	180 ± 14
Fiber diameter (μm)	25.5 ± 1.6	35.0 ± 7.5	30 ± 4.3
Strength (MPa)	16.2 ± 6.9	47.0 ± 11.7	20.3 ± 11.5
Elongation (%)	26.2 ± 8.1	10.4 ± 6.6	22 ± 9.1
Modulus (MPa)	242 ± 129	2558 ± 2204	283 ± 150

Fig. 2 SEM images of the outer (a), intermediate (b), and inner layer (c) of *cecropia* cocoons



Morphology of the cocoons

Hyalophora cecropia cocoons have considerable variation in thickness of the three layers and the diameters of the fibers in the three layers as seen from Table 1. The intermediate layer of *cecropia* cocoons have the highest thickness (330 μm) followed by the outer and inner layers. The intermediate layer also has fibers that are relatively coarser compared to fibers in the outer and inner layers, before degumming. The outer layer contains the finest fibers with an average diameter of 25 μm. Cocoons produced from *B. mori* have a thickness of 0.5–0.5 mm and are composed of up to 10 layers but the thickness of each layer has not been reported [24].

Figure 2a–c shows the surface morphology of the fibers in the three layers of *cecropia* cocoons. From Fig. 2b, it can be seen that the intermediate layer that forms the bulk of the cocoons consists of fibers that have a relatively loose structure compared to the outer (Fig. 2a) and inner (Fig. 2c) layers. It can also be seen that the *cecropia* caterpillar spins two fibers simultaneously indicating the presence of two spinnerets in the insect, not uncommon to insect silks. However, the two adjacent fibers vary in diameter and there is no particular pattern or direction in which the fibers are spun. Places where the fibers crossover show the presence of considerable amounts of glue (indicated by arrow in Fig. 3) that holds the fibers together and provides good strength to the layers.

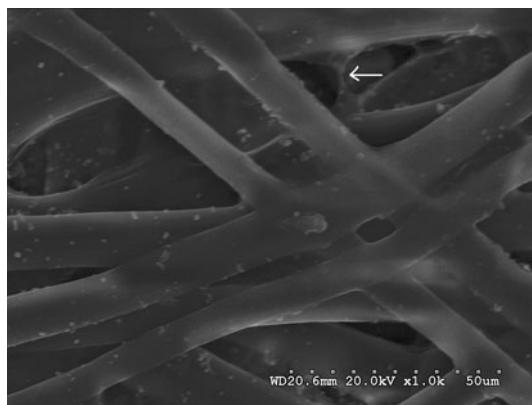


Fig. 3 SEM picture of a layer of *cecropia* cocoon showing the binding of the fibers by glue indicated by the arrow

Tensile properties

Table 1 provides a comparison of the tensile properties of the three layers of *cecropia* cocoons. The intermediate layer which contains most of the fibers has the highest thickness, 50% thicker than both the outer and inner layer. The intermediate layer also has high strength and modulus compared to the outer and inner layers. The strength and modulus of the intermediate layer are higher by nearly 3 and 10 times, respectively, compared to the strength of the outer layer and 2.3 and 9 times, respectively, compared to the inner layer. However, the elongation of the intermediate layer is about 60 and 53% lower than that of the outer and inner layers, respectively. Up to 10 layers could be peeled from *B. mori* cocoons and the tensile strength and modulus of the layers varied from 3.1 to 35 MPa and 170 to 449 MPa, respectively [24]. The tensile properties of the outer and inner layer of *cecropia* cocoons are similar to the layers in *B. mori* cocoons but the intermediate layer of *cecropia* cocoons has much higher strength and modulus compared to any layer of the *B. mori* cocoons. However, it should be noted that the thickness of the various layers of *B. mori* cocoons varied from 0.3 to 0.5 mm, similar to that of the intermediate layer of *cecropia* cocoons. The much better tensile properties of the intermediate layer of *cecropia* cocoons despite having similar thickness to the *B. mori* cocoons should mainly be due to the arrangement of the fibers and the adhesive forces between the fibers. *H. cecropia* cocoons need much harsher degumming than *B. mori* cocoons indicating that fibers in the *cecropia* cocoons are tightly held together that would enable them to provide better mechanical properties.

Effect of degumming

Before degumming, it was not possible to obtain fibers from the outer and inner layers but the loose structure of

the intermediate layer allowed long lengths of fibers to be drawn from the intermediate layer. Degumming results in a weight loss of 17, 13.5, and 13.5%, for the outer, intermediate, and inner layers, respectively. The removal of gums during degumming releases the fibers from all the three layers and loose fibers were formed after degumming. Degumming also results in considerable decrease in the fineness of the fibers from the intermediate layer. The fibers from the intermediate layer had an average fineness of 4 denier before degumming and 2 denier after degumming. However, degumming did not have a major effect on the tensile strength and modulus but the breaking elongation of the degummed fibers was 70% higher compared to the undegummed fibers from the intermediate layer. Before degumming, fibers from the intermediate layer had average breaking tenacity, breaking elongation, and Young's modulus of 3.6 g/denier, 8.7%, and 71 g/denier, respectively, and after degumming the respective values were 3.8 g/denier, 14.8%, 68 g/denier. Degumming is reported to weaken the non-covalent interactions in silks and reduce the tensile strength [25]. The removal of gums allows the polymers in the fibers to move easily and hence the fibers have higher elongation after degumming.

Properties of *cecropia* silk fibers

Composition

Silks produced by *cecropia* have considerably different amino acid composition than the mulberry and wild silks reported in Table 2. The *cecropia*, mulberry, and wild silks have similar alanine contents but the composition of the other amino acids is appreciably different in *cecropia* than the other silks in Table 2. *H. cecropia* silk has much higher amounts of tyrosine but lower amounts of glycine than the other silks in Table 2. Tyrosine with bulky side groups is reported to be in the amorphous region and the simple

Table 2 Comparison of the amino acid composition of *cecropia* silk with *B. mori* and three varieties of common wild silks

Amino acids	% Amino acids				
	<i>H. cecropia</i>	<i>B. mori</i>	<i>A. mylitta</i>	<i>A. pernyi</i>	<i>P. ricini</i>
Alanine	32.5 ± 0.92	29.4	34.1	34.7	36.3
Tyrosine	18.2 ± 0.09	5.2	6.8	5.1	5.8
Glycine	17.6 ± 0.60	44.6	27.7	28.4	29.4
Serine	10.0 ± 0.56	12.1	9.9	9.1	8.9
Aspartic acid	4.8 ± 0.23	1.3	6.1	5.0	3.9
Arginine	4.3 ± 0.15	0.5	2.4	5.0	4.1
Glutamic acid	3.6 ± 0.18	1.3	1.3	1.4	1.3
Histidine	1.9 ± 0.02	0.1	0.8	0.7	0.8

Data for *B. mori* and wild silks are from references [1–3]

amino acids such as alanine, glycine, and serine are in the crystalline regions [26]. The serine, aspartic acid, and arginine content in *cecropia* silk are similar to that in *A. pernyi* and *P. ricini*. The ratio of glycine/alanine for *cecropia* silk is 0.54, much lower than that of 1.5 for mulberry and 0.8 for the common wild silks. The amount of glycine and alanine determines the crystallographic form of the proteins [2, 3]. The lower ratio glycine/alanine in *cecropia* suggests that *cecropia* silk has a considerably different crystallographic structure compared to mulberry and wild silks. *H. cecropia* silk also has higher amounts of hydrophobic amino acids than hydrophilic amino acids. A tyrosine content of about 12% was previously reported for *cecropia* moth silk [27]. It has been reported that cecropins are made from amino acids that are 37-residues long and exist as a random coil.

Morphology

The undegummed *cecropia* fibers have considerable amounts of foreign substances and a thick layer of gum (indicated by arrow) on the surface as seen from Fig. 4. The degummed fibers have a relatively smooth and clean surface as seen from Fig. 5 but all the gums are not removed from the fibers. The striations on the fibers (indicated by arrows) in Fig. 5 show that some gums are still present on the fibers [25]. Cross-section of the fibers in Fig. 6 reveals that the fibers are ribbon-like with a solid rectangular cross-section. The cross-section of the *cecropia* silk fibers are different than mulberry silk fibers which have a triangular shape but similar to the tasar (*A. mylitta*) and muga (*A. assama*) silk fibers [1–3].

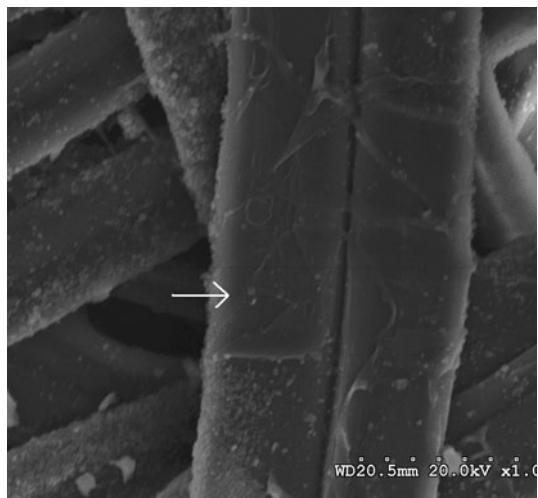


Fig. 4 SEM picture of undegummed *cecropia* fibers showing considerable amounts of glue (arrow) and impurities on their surface

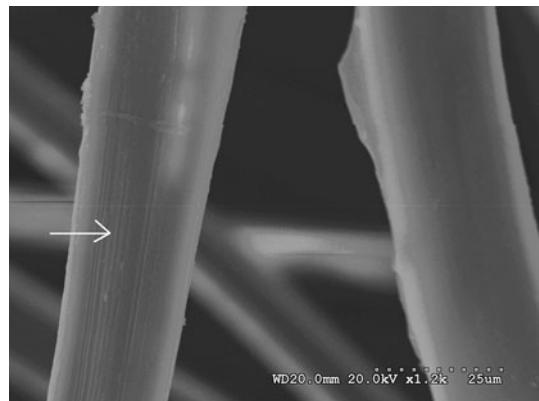


Fig. 5 SEM image of degummed *cecropia* fibers showing a clean and smooth surface. Arrow on the fibers on the left side of the image shows striations on the fibers indicating incomplete degumming

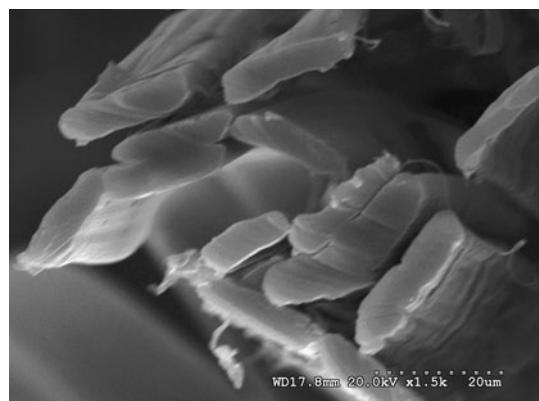


Fig. 6 SEM image of *cecropia* fibers shows that the fibers have rectangular solid cross-section

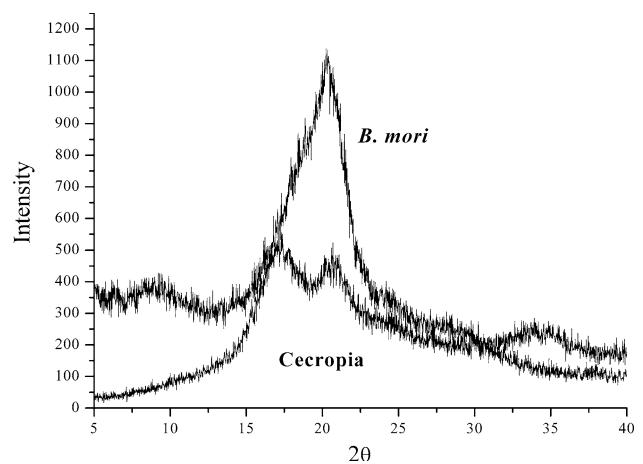


Fig. 7 X-ray diffractogram of *cecropia* fibers in comparison to *B. mori* silk fibers

Physical structure

Figure 7 shows the X-ray diffractogram of *cecropia* and *B. mori* silk fibers. *H. cecropia* fibers show two distinct

diffracting peaks at about 17° and 20° whereas *B. mori* silk has a single peak at about 20.6°. The diffraction peaks seen in *cecropia* are similar to those found in wild silks such as *A. mylitta* [28]. The differences in diffraction peaks between *B. mori* and wild silks were attributed to the differences in the unit cell dimensions [29]. The % crystallinity of *cecropia* was found to 29, lower than that of *B. mori* silk. However, *B. mori* silks with % crystallinities from 20 to as high as 41% have been previously reported [28]. The presence of high amounts of amino acids such as tyrosine that are non-crystalline reduces the % crystallinity of the *cecropia* fibers compared to *B. mori* fibers [26]. Differences in the varieties of silks studied, rearing conditions, and method used to calculate % crystallinity are some of the other major factors that are responsible for the large variation in % crystallinity between silk fibers.

Tensile properties

The *cecropia* silk fibers are much finer than *A. mylitta* and *P. ricini* silk but coarser than the silk produced from *B. mori*. Fibers produced by *cecropia* moths have tensile properties similar to that of *B. mori* silk and better than that of *P. ricini* silk as seen from Table 3. However, *A. mylitta* and *P. ricini* silks have more than twice the elongation of *cecropia* fibers but *P. ricini* silk has much lower modulus than any other silk fiber in Table 3. Fibers from the three layers of *cecropia* cocoons have very similar strength and elongation but the fibers from the intermediate layers have slightly lower modulus than the fibers from the other two layers. Although we did not observe large variations in the properties of the fibers from the three layers of *cecropia* cocoons, it has been reported that the properties of fibers from different layers of mulberry and non-mulberry silks have considerable variations [2, 3]. It was reported that the tenacity of the fibers increase substantially within a cocoon and inner layers have fibers with high tenacity than fibers from the outer layer [2, 3]. The large variations between the various silks should be due to the biology and health of the insect and the environment in which the insects grow. The tensile properties of *cecropia* silk shows that the fibers

from all three layers in *cecropia* cocoons could be used for various applications, similar to *B. mori* silk. The moisture regain of *cecropia* silk fibers is higher than that of *B. mori* silk but similar to that of the wild silks.

The variations in the tensile properties of the *cecropia* fibers compared to *B. mori* and the common wild silks should mainly be due to the differences in the composition and sequence of amino acids and physical structure (% crystallinity and crystal orientation) in the fibers. As seen from Table 2, the *cecropia* fibers contain much higher amounts of tyrosine than *B. mori* or the common wild silks. The benzene rings and hydroxyl groups in tyrosine can form strong interactions such as hydrophobic interactions, hydrogen bondings, and pi-hydrogen bondings with the benzene rings in other tyrosines and the hydroxyl and amino groups in the various amino acids in *cecropia* silk leading to higher strength than the wild silks. However, the strength of the *cecropia* fibers is not higher than that of the *B. mori* silk despite the *cecropia* fibers having higher tyrosine content. This is most likely due to the lower % crystallinity of the *cecropia* fibers compared to the *B. mori* fibers as shown in Fig. 7. The higher content of non-crystalline tyrosine in *cecropia* fibers could also contribute to the lower crystallinity of the fibers [26]. Fibers with low % crystallinity will have lower strength compared to a fiber with higher % crystallinity if all other parameters are the same.

Thermal behavior

Hyalophora cecropia silks have similar thermal behavior compared to the common *B. mori* silk up to a temperature of 300 °C as seen from Fig. 8. Both fibers lose about 25% of their weight at 300 °C and the weight loss increases sharply between 300 and 400 °C and gradually above 400 °C. The *cecropia* fibers show considerably lower weight loss compared to the *B. mori* silk fibers above 300 °C. *B. mori* silk fibers have been reported to have a thermal degradation temperature around 324 °C [29]. This difference in the thermal behavior of *cecropia* silk compared to *B. mori* silk should mainly be due to the

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

Fiber	<i>Cecropia</i>			<i>B. mori</i>	<i>A. mylitta</i>	<i>P. ricini</i>
	Outer	Intermediate	Inner			
Fineness (denier)	1.7	2.0	1.7	0.4–1.1	4.7–10.7	2.3–3.6
Strength (g/denier)	4.3 ± 0.7	3.8 ± 0.6	4.3 ± 1.1	4.3–5.2	2.5–4.5	1.9–3.5
Elongation (%)	12.6 ± 6.5	14.8 ± 6.8	12.6 ± 5.9	10.0–23.4	26–39	24–27
Modulus (g/denier)	92 ± 15	68 ± 9.9	82 ± 19	84–121	66–70	29–31
Moisture regain (%)	13.4	12.6	10.5	8.5	10.5	10.0

Data for *B. mori* and wild silks are from references [1–3]

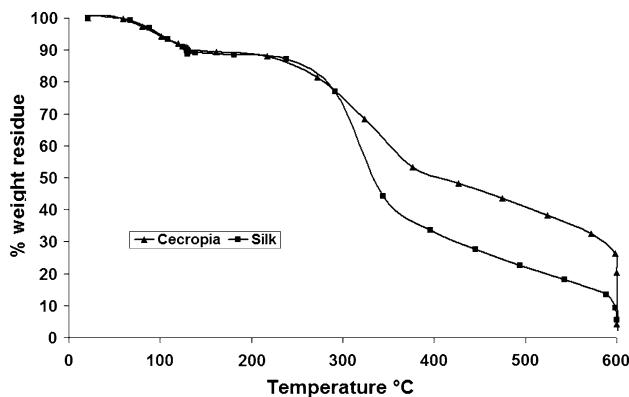


Fig. 8 TGA curves of *cecropia* and *B. mori* silk fibers

differences in the composition of the two fibers. Considerable changes in the color of the cocoons from yellow to black to white are reported to occur when *B. mori* cocoons were heated from 190 to 550 °C for various periods of time [30].

Conclusions

Hyalophora cecropia caterpillars are easy to rear than *B. mori* and offer good potential for commercial production of silk. *H. cecropia* caterpillars also produce much finer fibers and have better properties than common wild silks. The *cecropia* cocoons are made up of three distinct layers and degumming is necessary to extract fibers from the layers. The outer and inner layer of *cecropia* cocoons have similar tensile properties whereas the intermediate layer has much better strength and modulus even with the similar thickness compared to the *B. mori* silk cocoons. The amino acid composition of *cecropia* silks is different than that of *B. mori* and common wild silks, mainly due to the much higher content of tyrosine and much lower content of glycine. The tensile properties of fibers from all the three layers of *cecropia* cocoons are similar to that of *B. mori* silk and better than that of the wild silks. The physical structure of *cecropia* silks is different than that of *B. mori* silk but similar to that of the wild silks. Overall, fibers produced by *cecropia* have structure and properties that make them suitable for various applications that currently use *B. mori* silk.

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