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Wild Silkworm Cocoon as a Natural Tough Biological Composite Structure

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Abstract
Silk cocoons are biological composites with intriguing characteristics that have evolved through a long natural selection process. Knowledge of structure-property-function relationship of multi-layered composite silk cocoon shells gives insight into the design of next-generation protection materials. The current investigation studied the composite structure and mechanical performance of a wild silkworm cocoon (Chinese tussah silkworm cocoon, *Antheraea pernyi*) in comparison with the domestic counterpart (Mulberry silkworm cocoon, *Bombyx mori*). 180º peel and tensile tests were performed on the cocoon walls to understand both their interlaminar and in-plane mechanical properties. The fracture surfaces were investigated under SEM. The wild cocoon showed substantially higher toughness over the domestic cocoon, which explains their unique capability to tackle severe environmental adversaries.

Introduction
Many natural materials are hierarchical and multi-functional composites, which embrace outstanding properties and functionalities far superior to that of many equivalent synthetic materials manufactured by current technologies. Evolved over thousands of years' natural selection, very thin and lightweight silkworm cocoons can protect silkworms from physical attacks from predators or environment while supporting their metabolic activity. A cocoon is a multilayer composite material formed by continuous twin silk filaments (fibroin) bonded by silk gum (sericin). Depending upon silkworm species and the rearing environment, cocoons vary in weight, thickness, colour and stiffness.

Despite the rapid growth of research interest in silk fibres, silk protein and the silk extrusion process, limited studies have been conducted to understand the structure and functions of silkworm cocoons. For example, Zhao *et al.* tested the tensile properties of *Bombyx mori (B. mori)* cocoons and found graded mechanical properties from the outer layers to inner layers [1, 2]; Chen *et al.* recently examined a wide range of silkworm cocoons to correlate their tensile, compressive and gas diffusion properties with the cocoon structure [3, 4]. Compared with domestic silkworms such as *B. mori*, wild silkworms are reared in the open environment and conceivably need much greater protection from environmental, biotic and physical hazards. This work has been carried out to explore the unique structure and mechanical property of wild silkworm cocoon (*Antheraea pernyi,* i.e. *A. pernyi)* in comparison with the domestic cocoon (*B. mori*).

Materials and Methods
B. mori silkworm cocoons were purchased from silk rearing houses in North East India and *A. pernyi* cocoons were collected from North East China. They were received as stifled cocoons, as commonly used prior to reeling silk filament for textile applications. The 180 degree peel tests, which are modified from the ASTM standard test D 1876-08 used for peel resistance of adhesives

[5], were performed on a Lloyd LR30K tester with a 100 N load cell. A loading rate of 2 mm/min was applied to delaminate cocoon samples with the dimension of 20 mm × 5 mm. The cocoon samples were peeled artificially for a length of 5 mm before they were pulled apart by the tester. Three samples for each type of cocoons were peeled into multiple layers. The peeled layers were numbered according to the sequence from the outer to inner layers (e.g. layer 1 is the outermost layer). The average peeling load for each loading curve was determined as the average of load readings at a 2 mm increment of crosshead motion and the peel strength was determined by the average peeling load divided by sample width. The work of fracture (WOF) was obtained by the work under the load vs. extension curves divided by the delamination area. The morphologies of the cocoons and the fracture surfaces from failed samples were investigated by a scanning electron microscopy (SEM) (Supra 55VP).

Results and Discussion

Cocoon structure and morphology

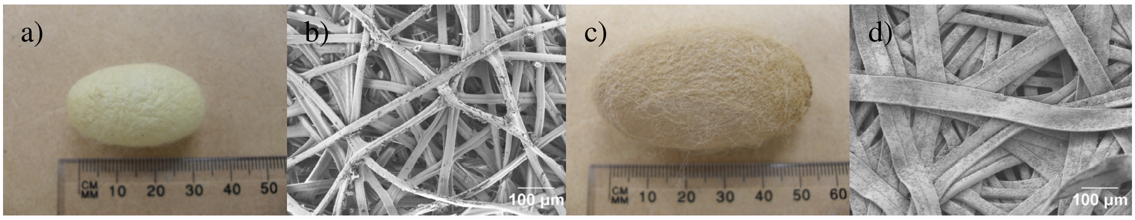

Figure 1 Silk cocoons and their morphology at outer surface. (a), (c) are the *B. mori* and *A. pernyi* cocoons and (b), (d) show their outer surface morphology, respectively.

The cocoon photos and SEM micrographs of the silk cocoon structure for two types of cocoons are shown in Figure 1. The silkworm cocoons show a non-woven composite structure with twin silk fibres coated with sericin. Both types of cocoons show a graded structure, i.e. the fibre bonding length (single fibre width) decreases along the direction from outer layer towards inner layers. For the *B. mori* cocoon, the fibre bonding length is 12.64 µm for the outermost layer and is 8.98 µm for the innermost layer; similarly, the fibre bonding length for the *A. pernyi* outmost layer is 35.68 µm and that for its innermost layer is 29.07 µm. The fibre bonding width for the wild *A. pernyi* is 200 % higher than the domestic *B. mori*. In comparison with the domestic cocoon *B. mori*, the wild cocoon *A. pernyi* has a more compact structure [6]. The nominal density of the cocoon is 377 kg/m^3 for the *B. mori* but 711 kg/m^3 for the *A. pernyi*.

Interlaminar peel resistance of cocoon walls

Figure 2 shows the loading curves for peel tests on different cocoons. For the *B. mori* cocoon, the peeling loads were less than 1 N and the load values were comparable for three peeled layers (Layer 1, 2 and 3). In the case for the *A. pernyi* cocoon, the peeling curves became more fluctuated with much higher peak loads (the maximum load values reached 4 ~ 5 N); the peeling loads were higher for the outer layer (Layer 1') than the inner layer (Layer 2'), which indicates a graded structure with tougher layers on the outside. This is related to the more stringent requirement from the wild environment to protect the silkworm against physical attack from natural predators and harsh surroundings. In addition, the wild cocoon showed higher peak loads and larger load drops during delamination, signifying much greater peel resistance and interlaminar fracture toughness over the domesticated cocoon type. The comparison of peel resistance between two cocoon types was made based on the layers where the highest peeling loads occurred. The maximum peeling load (P_{max}) was 0.74 N and the average peeling load ($P_{average}$) was 0.35 N for the *B. mori* cocoon. By comparison, the *A. pernyi* cocoon had a P_{max} of 4.45 N and a $P_{average}$ of 2.51 N. The maximum work of fracture (WOF) of all tested cocoon layers was 981 J/m^2 from the *A. pernyi* outer layer, suggesting the highest bonding energy. The WOF and peel strength calculated for the *A. pernyi* cocoon were about 8 times of the *B. mori* cocoon.

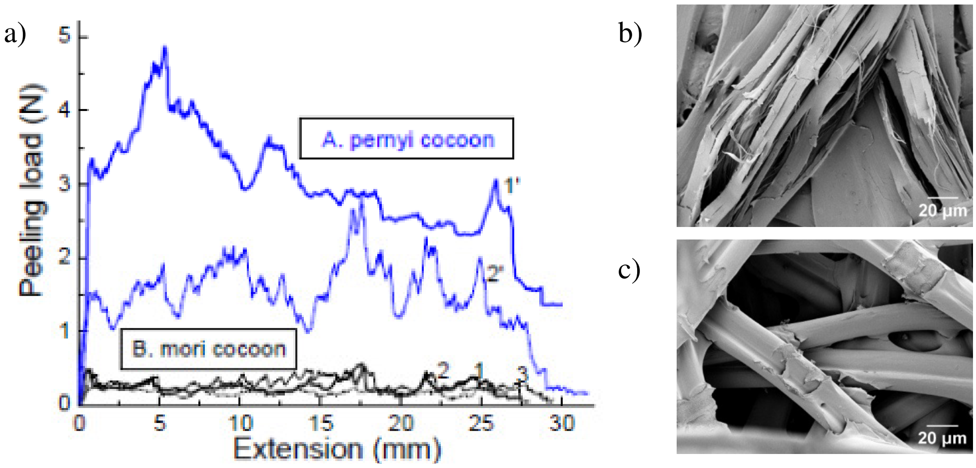

Figure 2 Loading curves for peel tests (a) and peel surface of the *A. pernyi* (b) and the *B. mori* (c) 1, 2, 3 indicate the peeled cocoon layers from the *B. mori* and 1', 2' indicate the peeled cocoon layers from the *A. pernyi*, following the sequence from outside to inside.

The peel fracture surfaces of different cocoon types are presented in the SEM images. As shown in Figure 2c, slight fibre damage can be visualized at the intersections where *B. mori* silk fibres meet. However, fibre splitting is clearly shown on the fracture surface of *A. pernyi* cocoon layers (Figure 2b). The fracture surface of the *A. pernyi* showed more severe fibre damage. The silk fibres of these wild cocoons split into many fibrils indicating the stronger fibre/matrix adhesion and the higher bonding between different layers. The stronger sericin matrices, rougher fibroin surface of wild cocoons and larger bonding area between layers all make important contributions to its substantially higher peel resistance.

Table 1 Peel test results of cocoon walls from *B. mori* and *A. pernyi* silk cocoons

Cocoon type	Wall thickness (um)	Thickness of the peeled layer 1 (um)	Thickness of the peeled layer 2 (um)	Thickness of the peeled layer 3 (um)	Average peeling load (N)	Maximum peeling load (N)	Peel strength (N/m)	Work-of-fracture (J/m^2)
B. mori	393±21	135±21	75±21	80±14	0.35±0.05	0.74±0.17	62±6	119±19
A. pernyi	387±31	190±14	160±57	--	2.51±0.55	4.45±0.63	469±75	981±211

Tensile properties of cocoon walls

The typical tensile stress strain curves for two types of cocoon walls are shown in Figure 3a. The average tensile stress for the *A. pernyi* cocoon is 56 MPa, which is almost twice the value for the *B. mori* cocoon (30 MPa). The maximum load for the *A. pernyi* cocoon is 140 N which is almost triple the value for the *B. mori* cocoon (49 N). The Young's modulus is also significantly higher for the wild cocoon wall than the domestic cocoon wall. The *B. mori* cocoon walls have an average modulus of 365 MPa, in contrast to the value of 872 MPa for the *A. pernyi* cocoon wall. The *A. pernyi* cocoon walls are more ductile and tough, indicated by the bottle neck-shaped tensile failure occurred in the rectangular test piece. The energy absorption capacity of the wild cocoon structure is 700 % higher than the domestic counterpart.

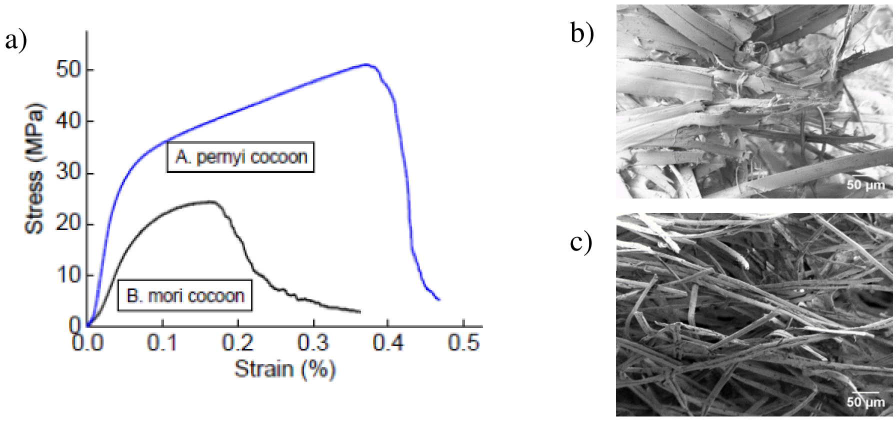

Figure 3 Tensile stress strain curves (a) and the fracture surface of the *A. pernyi* (b) and *B. mori* (c).

The fracture surfaces (shown in Figures 3b and 3c) were investigated under SEM. It is difficult to find a clear boundary between the failed two test pieces of *B. mori* cocoon wall. However, the *A. pernyi* cocoon wall showed obvious breakage between the two failed pieces. The contributing factors to the substantially higher tensile strength and toughness include the higher bonding area (the intersection of silk fibres) between cocoon layers, the rougher interface between fibre and matrix, the stronger sericin and the more compact cocoon structure of the *A. pernyi* cocoon. The fibre bonding length of the *B. mori* is about 13 μm from the outermost layer and 9 μm for the innermost layer, which is in contrast to the 36 μm fibre bonding length for the outermost layer and 29 μm fibre bonding length for the innermost layer of the *A. pernyi* cocoon. It has been noted that the silkworm cocoons have a graded structure with higher mechanical properties at the outer layers [7]. Although both *B. mori* and *A. pernyi* exhibited graded cocoon structures, the interlaminar bonding area for the *A. pernyi* is approximately 3 times of the *B. mori* cocoon outer layers. The flat silk fibre of the *A. pernyi* cocoon also contributes to the more compact structure in comparison with the triangle cross section of the *B. mori* silk fibres. The more porous structure of the *B. mori* cocoon also strongly affects its mechanical performance.

Conclusions

In comparison with the domestic cocoon *B. mori*, wild cocoon (*A. pernyi*) showed significantly higher interlaminar peel resistance, tensile strength and toughness. This helps to protect the wild silkworms from physical attacks and environmental hazards in the wild.

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