A study of the extraordinarily strong and tough silk produced by bagworms

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Global ecological damage has heightened the demand for silk as ‘a structural material made from sustainable resources’. Scientists have earnestly searched for stronger and tougher silks. Bagworm silk might be a promising candidate considering its superior capacity to dangle a heavy weight, summed up by the weights of the larva and its house. However, detailed mechanical and structural studies on bagworm silks have been lacking. Herein, we show the superior potential of the silk produced by Japan’s largest bagworm, *Eumeta variegata*. This bagworm silk is extraordinarily strong and tough, and its tensile deformation behaviour is quite elastic. The outstanding mechanical property is the result of a highly ordered hierarchical structure, which remains unchanged until fracture. Our findings demonstrate how the hierarchical structure of silk proteins plays an important role in the mechanical property of silk fibres.
Severe global concerns about growing ecological damage and depletion of non-renewable resources have heightened the demand for silk as a structural material made from renewable resources\textsuperscript{1–3}. More than 200,000 different silks are known to exist in nature\textsuperscript{4}. When we focus on toughness, i.e., a balance of strength and extensibility, we find the dragline silks of some kinds of spiders, such as those of the \textit{Araearius nephila}, and \textit{Laridectus} genera, to be among the most attractive\textsuperscript{5–7}. Particularly, the \textit{Caerostris darwini} (\textit{C. darwini}) (aka Darwin's bark spider) dragline silk had been considered the toughest silk in nature, showing five times higher toughness than that of the \textit{Bombyx mori} (\textit{B. mori}) silkworm silk\textsuperscript{8} (see Table 1). However, many spider dragline silks, especially that of \textit{C. darwini}, show typical plastic deformation in their tensile behaviours, showing a distinct yield point after the initial elastic region, followed by a subsequent levelling-off or plateau region before strain-hardening\textsuperscript{9}. To realise their practical application as structural materials, stronger and tougher silks with more elastic deformation behaviours are required. Towards this goal, many trials are being conducted worldwide to produce ideal silk products, for instance, by controlling the hierarchical structure of regenerated silk proteins\textsuperscript{10,11}, producing transgenic (or genome-edited) artificial silk proteins\textsuperscript{12–14}, and searching for superior un-explored silks in nature\textsuperscript{8,15,16}. When we search for superior un-explored silks, spiders provide a major hint that the silks used for dangling should be strong and tough\textsuperscript{17–19}. Bagworm silk, produced by larvae of bagworm moths (\textit{Lepidoptera} order and \textit{Psychidae} family)\textsuperscript{20} especially of the largest and heaviest Japanese bagworm \textit{Eumeta variegata} (\textit{E. variegata}) (a synonym for \textit{Eumeta japonica} and \textit{Clania variegata})\textsuperscript{21,22}, might be a promising candidate, considering its superior capacity to dangle a heavy weight, summed up by the weights of the larva and its house (generally called larval case, bag, or nest). However, little is known about the mechanical and physical properties of bagworm silks\textsuperscript{23–25}.

In this report, based on the detailed analyses of the fibre morphology and mechanical properties, we reveal that the \textit{E. variegata} bagworm silk is extraordinarily strong and tough compared to other known silks, and that its tensile deformation behaviour is quite elastic. To investigate the relationship between the structure and the outstanding mechanical properties of this bagworm silk, a comprehensive analysis of the hierarchical structure, made up of crystalline and amorphous phases, is conducted by in situ time-resolved simultaneous measurements of synchrotron wide-angle X-ray diffraction (WAXD) and small-angle X-ray scattering (SAXS) during tensile deformation.

**Results**

**Morphology of bagworm silk.** While the exterior of the nest of \textit{E. variegata} bagworms is covered with dead branches and leaves (Fig. 1a), the interior is made of fine, densely stacked nest silk fibres, like a nonwoven fabric (Fig. 1b, c). It is well-recognised that bagworms use their silk also as lifeline for dangling. Furthermore, we found that they use it as a foothold as well, by spinning it in a zigzag manner with slightly greater widths than that of the space between their legs, in which folded points are attached with adhesive glue. While spiders have plural pairs of silk glands and spinnerets for different purposes, the multi-task thread is produced by a pair of silk glands via a single spinneret in the case of bagworms. The individual silk threads are composed of a pair of thin filaments, as in the cases of silkworm silks and spider dragline silks, and their cross-sections were revealed to be rather elliptical, and not circular (Fig. 1d). The fact that \textit{E. variegata} bagworms spin silk over several tenths of a metre to several hundreds of metres is worthy of mention; therefore, one can wound it on a bobbin (Fig. 1e) or twist it into multiple threads (Fig. 1f).

**Mechanical properties.** In the estimation of the tensile properties, the cross-sectional area of the sample filament should be evaluated as accurately as possible. Our detailed morphological observation revealed that the cross-sections were well-approximated by an ellipse, with a pair of major and minor axes \(L_a\) and \(L_b\), satisfying an axial ratio of \(L_a/L_b\) of 1.7 (Fig. 1g–j; Table 2). This axial ratio was used to estimate the cross-sectional area of the single filaments, needed for converting the tensile force to stress values in the tensile test (further details in the Methods section).

A typical stress–strain curve of \textit{E. variegata} bagworm silk is shown in Fig. 1k, and the average tensile properties of modulus, strength, extensibility, and toughness are summarised in Table 3. The values of modulus, strength, and toughness are extraordiarily high compared to other known silks (note that the toughness is almost comparable with that of \textit{C. darwini} silk\textsuperscript{8}). In addition, the \textit{E. variegata} bagworm silk exhibits an ideal stress–strain behaviour, without a levelled-off stress after the yield point, but followed by a linear and steep strain-hardening just after elastic deformation.

**Amino acid sequence.** The structural and mechanical properties of the silk produced by \textit{Lepidoptera} are predominantly attributable to the contribution of heavy-chain silk fibroin (H-Fib). An analysis of the amino acid composition, carried out for \textit{E. variegata} bagworm silk, revealed that the molar ratios of Gly and Ala are approximately equal, and their sum accounts for about 80% (Supplementary Fig. 1), which is consistent with the results in earlier reports\textsuperscript{26,27}. In accordance with this, we found, via silk gland transcriptomic analysis, that the bagworm \textit{h-fib} gene hypothetically encodes the Gly and Ala–rich protein. The molecular structure of the H-Fib was investigated by a long-read transcriptomic analysis. At least 5 tandem repeat motifs were identified, each of which is composed of polyalanine block (PAB) and non-polylalanine block (NPAB) sequences (Fig. 2). This kind of combination motif of PAB and NPAB sequences is widely observed in the \textit{Saturniidae} silkworm and spider dragline silks\textsuperscript{28–30}. However, one can find several decisive distinctions from their motifs. Firstly, the length of each sequence motif,

| Table 1 Tensile properties of various silks |
|------------------------------------------|
| **Young's modulus (GPa)** | **Fracture strength (GPa)** | **Extensibility** | **Toughness (MJ m\(^{-2}\))** | **Ref.** |
|------------------------------------------|
| \textit{Araearius diadematus} Spider silk (MA) | 10 | 1.1 | 0.27 | 160 | 54 |
| \textit{Nephila clavipes} Spider silk (MA) | 13.8 | 1 | 0.20 | 111.2 | 61 |
| \textit{Laridectus hesperus} Spider silk (MA) | 10.2 | 1 | 0.45 | 180.9 | 61 |
| \textit{Caerostris darwini} Spider silk (MA) | 11.5 | 1.7 (1.0\textsuperscript{a}) | 0.52 (0.69\textsuperscript{b}) | 354 | 8 |
| \textit{Bombyx mori} Silkworm silk | 7 | 0.6 | 0.18 | 70 | 54 |
| \textit{Eumeta minuscula} Bagworm silk | 25 | - | - | - | 24 |

\(^{a}\)The true stress and \(^{b}\)true strain values from the original paper were converted into engineering stress and strain and are given in parentheses.
residues in the PAB is also very unique, compared to that of the *Saturniidae* silkworm (*~3–15* repeats) and spider dragline silks (*~5–8* repeats)*28–30*. It should be noticed that a short non-repeated sequence of *~5–8* residues, made up of the relatively bulky residues of mainly Ser, Val, and Tyr, is characteristically inserted in each NPAB sequence (coloured blue).

**Crystal modification and hierarchical structure.** To understand the structural origin of the outstanding mechanical properties of the bagworm silk, the crystal modification and hierarchical structure, which is a combination of crystal and amorphous phases, were investigated. The two-dimensional (2D) WAXD fibre diagram shows a typical β-sheet pattern*12* (Fig. 3a) widely observed in the silkworm and spider dragline silks. Although the β-sheet crystals formed in a variety of silks are commonly indexed with the orthogonal unit cell, the detailed unit cell parameters differ slightly among the silks depending on their amino acid composition*33,34*. The β-sheet unit cell parameters of the bagworm silk were evaluated, using the 200, 210, and 002 reflections, under the assumption of a rectangular unit cell, to be *a*= 9.39 Å, *b*= 9.50 Å, and *c* (fibre axis) = 6.98 ± 0.05 Å, which are quite similar to those of the *B. mori* silk (*a*= 9.38 Å, *b*= 9.49 Å, and *c*= 6.98 Å)*32*. The crystallinity was estimated to be about 44% by peak fitting the crystalline and amorphous diffraction peaks (Fig. 3b).

The meridional 1D-SAXS Kratyk profile (*q*I(*q*) vs. *q* plot)*35* (Fig. 3d) (the original *I*(*q*) vs. *q* plot is shown in Supplementary Fig. 2) obtained from synchrotron SAXS measurement (Fig. 3c) clearly revealed at least five scattering peaks, the peak positions of which approximately satisfy an integer ratio of 1:2:3:4:5. This relation revealed the existence of a well-ordered repeating structure, composed of alternating crystal and amorphous phases. The first-order long period, that is, the real period (*L*(1st)), was estimated to be about 38.5 nm. Analysis of the correlation function *K*(*)36,37* (Fig. 3e) for the electron density distribution *η* (2) of the repeating system made up of phases I and II (Fig. 3f) revealed that the 38.5 nm periodic structure consists of phase I with thickness of 15.7 nm and phase II with a thickness of 22.8 nm. The meridional periodic scatterings in SAXS are observed not only in this bagworm silk, but also in many kinds of spider dragline and wild silkworm silks. However, to the best of our knowledge, such a clear periodicity for fifth-order scattering has never been reported in any kind of β-sheet silk. In addition, the long period is roughly five times longer than those (~6–8 nm) reported for spider dragline silks and wild silkworm silks*38–43* (see Supplementary Fig. 3). This highly ordered and long-range hierarchical structure is considered to contribute significantly to the outstanding mechanical properties, and might be directly related to the amino acid sequence.

The SAXS data also revealed two-point scatterings on the equatorial line, indicating the presence of a nanofibril bundle with a neighbouring fibril distance of 4.7 nm (Fig. 3g). This periodicity is approximately the same as the periodicities observed for *B. mori* silk, which is the most studied among silk proteins. The similarity in the periodicity may suggest that the spider dragline silk and wild silkworm silks have a similar structural arrangement. The SAXS data also revealed that the nanofibril bundle thickness of ∼150 nm, which is considerably thicker than those of other silks (Supplementary Fig. 3c).

Fig. 1 Morphological and mechanical characteristics of the bagworm silk. Photographs of a exterior and b interior of larval house (or nest) of a last-instar female larva of *E. variegata*. c SEM image of inner surface of the larval house (from rectangular part in b). d Magnified SEM image of single fibres with their cross-sections. e Continuous bagworm silk wound on a bobbin, and f its twisted yarn. g Optical micrograph of cross-sections of bagworm silks. Typical cross-sections of a single fibre composed of h, a pair of straight conjugated filaments, and i a pair of filaments conjugated with a tilting angle θ, are shown together with schematic illustrations. In each filament in h, i, the cross-section is traced by an elliptical shape with a broken line. j Plot of axial ratios of lengths of major and minor axes in elliptical cross-sections of bagworm silk. The straight line with a slope of 1.70 was obtained by linear regression for the plots. k Typical stress-strain curve of single bagworm silks. For comparison, a typical stress-strain curve of *N. clavipes* dragline silk, measured in this study using the same procedure and instruments used for bagworm silks, is included. The average tensile properties of *N. clavipes* (*n*= 5) were as follows: modulus = 8.13 GPa, strength = 0.9 GPa, extensibility = 0.26, and toughness = 139.7 MJ m–3. Scale bars: 10 mm (a), 10 μm (d), and 10 μm (g).
Structural changes during tensile deformation. WAXD and SAXS analyses of the as-spun bagworm silk revealed a distinctive structural nature, that is, a highly ordered hierarchical structure made up of about 45% $\beta$-sheet crystalline phase and 55% amorphous phases. To clarify how the ordered structure contributes to the strength and toughness, we investigated the deformation behaviour of this highly ordered structure using in situ time-resolved simultaneous measurements of synchrotron WAXD, SAXS, and tensile deformation behaviour (the experimental setup is shown in Supplementary Fig. 4). The stress–strain plot is shown at the top of Fig. 4d, and the changes in the 2D-WAXD and SAXS patterns during tensile deformation are shown in Fig. 4a. Compared with the case of single fibres (Fig. 1k; Table 3), the fibre bundle (top of Fig. 4d) showed a non-negligibly lower fracture stress, and the stress decreased gradually in a broader strain region (stage III) due to the imperfection of parallelism of the bundle fibres. However, except for this difference in the stress–strain curve, the mechanical behaviour can be assumed to be essentially the same as that of a single fibre, allowing us to investigate the deformation process of the silk fibre via the in situ measurements performed for the bundle of fibres.

It was found that the lattice spacing of the meridional 002 crystal reflection ($d_{002}$) changed against the bulk strain in an approximately similar manner as that of the stress–strain curve behaviour (Fig. 4b, d). The once-deformed $d_{002}$ value recovered almost perfectly after fracturing (in Stage III). While the bulk strain shows an inflection point at a tensile stress of 0.4 GPa, the crystal strain increased linearly against the tensile stress, until the fracture point (~1.4 GPa) (Fig. 5a). This is one of the characteristic differences from other kinds of silk, as will be discussed later in Fig. 5b, c, and is considered to be of crucial importance for understanding the structural origin of the strength and toughness of the bagworm silk, as discussed below.

The changes in the long periods $L_{p2nd}$ and $L_{p3rd}$ (Fig. 4c) are plotted against the bulk strain, in Fig. 4d, and the changes in the true long period ($L_{p1st}$) were estimated from the changes in the $L_{p3rd}$ to be from 37.5 to 44.7 ($\epsilon_L = 19.2\%$) (Table 4). The ratio of the long periods ($L_{p2nd}/L_{p3rd}$) remained at a constant value of 1.5 throughout the tensile deformation (Fig. 4d). This demonstrated that the highly ordered repeating structure of crystal and amorphous phases was maintained throughout the stretching deformation. While the c-axis lattice spacing $d_{002}$ was elongated more pronouncedly in the initial elastic region (stage I) compared to the subsequent stage II after the yield point, the changes in the long period showed an opposite tendency. The elongated long period was not recovered, but remained constant after the tensile force was removed upon fracture. Similarly, the degree of crystal orientation was not recovered (Fig. 4d). These irreversible behaviours are essentially different from those observed for the dimensional change in the crystal unit cell.

**Discussion**

We have revealed that the *E. variegata* bagworm silk is a promising candidate for sustainable structural materials, owing to its
Fig. 3 Structural information of the bagworm silk. a WAXD fibre diagram obtained from the bagworm silk and b its 2θ profile scanned in the 2θ range from 10° to 37°, and for the azimuthal angle range from 0° to −180°, which is enclosed by the broken blue line in a. The crystallinity was evaluated from the 2θ profile by peak fitting. Each peak number corresponds to the following: 1: (200) crystalline reflection, 2: (210), 3: (211), 4: (002), 5: (102)* + (300)**, 6: (202), and 7: amorphous peak. The peak fitting was performed using the spectroscopy software Grams Suite 9.3 (Thermo Fisher Scientific Inc., USA) by fixing the peak positions for the crystalline reflections at −1°. c Synchrotron SAXS pattern and d its meridional q-profile (q^2*I(q) vs. q Kratky plot). To avoid beam damage of the detector due to the strong equatorial-streak scattering, a narrow metal plate was attached on the detector surface along the equatorial line, and it appears as the white gap running along the equatorial line in the SAXS pattern. e The electron density correlation function K(z) and f the corresponding electron density distribution η(z), for the repeating phases. Q, Lp, dp, and (dp) denote the invariant, long period (phase I + II), core thickness of phase I, and mean thickness of phase I, respectively. The Lp, dp, and (dp) were estimated to be 38.2, 12.5, and 15.7 nm, respectively. g Equatorial SAXS q-profile (q^2*I(q) vs. q Kratky plot) scanned from the 2D pattern shown in Supplementary Fig. 3a-(1)
Fig. 4 Time-resolved tracing of structural changes during tensile deformation. **a** Changes in time-resolved synchrotron WAXD and SAXS patterns during the tensile deformation process. The corresponding stress–strain curve is shown at the top of **d**. **b** The selective meridional WAXD 2θ profiles and **c** the meridional SAXS q²-l(q) vs. q profiles, both of which were obtained from the strains at 0, 1.3, 5.7, 10.0, 13.3, 18.0, and 22.0% in the stress–strain curve shown at the top of **d**. **d** Summary of the time-resolved simultaneous measurement. From top to bottom, the changes in the tensile stress, crystal unit cell dimension along the c-axis direction (d₀₀₂), crystalline orientation index, and meridional long periods (L_p(2nd) and L_p(3rd)), estimated from the 2nd and 3rd periodic scatterings, are plotted against the bulk strain. In the bottom plots of the long periods, the ratio of L_p(2nd)/L_p(3rd) is also plotted.

Fig. 5 Relationships between crystal strain and tensile stress in different kinds of silk. The changes in bulk strain (ε) and of crystal strain (ε_c) against the tensile stress in the time-resolved simultaneous synchrotron X-ray analysis for **a** E. variegata bagworm silk, **b** B. mori (Bombycidae) silkworm silk, and **c** A. assama (Saturniidae) silkworm silk. The changes in tensile stress and crystal strain against the bulk strain, using the same experimental data, are shown in Supplementary Fig. 5.

Table 4 Summary of the dimensional changes observed in the bulk sample, crystal unit cell, and hierarchical structure

|                     | Stage I (Initial elastic region) | Stage II (Strain hardening) | Total (I : II) | Stage III (After fracturing) |
|---------------------|----------------------------------|-----------------------------|---------------|-----------------------------|
| Sample length (mm)  | 15.0 → 15.5                      | 15.5 → 18.3                 | 15.0 → 18.3   | not evaluated               |
| Bulk strain (ε) (%) | 3.3                              | 18.7                        | 22.0          | not evaluated               |
| d₀₀₂ (Å)            | 3.52 → 3.54                      | 3.54 → 3.57                 | 3.52 → 3.57   | 3.57 → 3.52                 |
| Crystal strain (ε_c) (%) | 0.57                           | 0.85                        | 1.41          | 19.2                        |
| Long period (L_p(1st)) (nm) | 37.5 → 38.1                  | 38.1 → 44.7                 | 37.5 → 44.7   | 44.7 → 44.0                 |
| Strain of long period (ε_L) (%) | 1.6                            | 17.6                        | 19.2          | −1.6                        |
superior mechanical properties and their quite elastic tensile-deformation behaviour. While the β-sheet crystal modification revealed by WAXD analysis is common in the other kinds of silkworm and spider silks, the hierarchical structure was found to be quite unique. The SAXS analysis revealed a highly ordered and long-range repeating structure. Additionally, while the B. mori silk does not show the meridional long period, many Saturniidae silkworm and spider dragline silks show it. In other words, such ordered structure is considered to be constructed only in the silks having a PAB sequence, and the PAB plays an important role in the formation of the periodic structure. The PAB of the Saturniidae silkworm or spider dragline silks is considered to form a helical conformation in their silk glands. It is reasonably speculated that the polyalanine helices in the neighbouring H-Fib proteins (or spidroins) are gathered around each other, probably forming a hexagonal packing via hydrophobic–hydrophobic self-assembly interactions. The long PAB sequence of 22 residues (corresponding to 6 helical pitches) in the bagworm silk (Fig. 6a, b) may gather together more effectively compared to the shorter alanine repeats of the Saturniidae and spider dragline silks. This self-assembly of the helical polyalanine sequences occurs at approximately constant intervals determined by the amino acid residues of the tandem repeat motifs (~160 residues in the case of the E. variegata bagworm silk) (Fig. 6c), and these intervals are speculated to correspond to the long period detected by SAXS measurement (Fig. 6d). Here, the question is: how many amino acid sequence units contribute to the β-sheet crystal formation from the end of the PAB sequence? We paid attention to the presence of the non-repeated short sequences inserted in each NPAB sequence region. It was found that around 45 residues exist between the PAB and non-repeated short unit. The 67 residues (22 in PAB and 45 in NPAB) were calculated to measure 23.5 nm (~0.35 nm/residue in the β-sheet crystal), which is consistent with the length of phase II (22.8 nm) experimentally estimated by SAXS analysis (Fig. 6d). We made an assumption, which is widely believed, that the polyalanine helical conformation changes to β-sheet crystals due to the stress-induced helix-sheet transition. We can assign the lengths of phases I and II to the amorphous and crystalline phases, respectively (Supplementary Note 1). The remaining NPAB sequence of around 90 residues should behave

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**Fig. 6** Relationship between primary structure and hierarchical structure. **a** A representative sequence motif, corresponding to the top of Fig. 2, identified in the H-Fib protein of the E. variegata bagworm silk, and **b** the repeating model of the motifs. **c** Assumed aggregation model of the motifs in the silk gland, and **d** after spinning. In the silk gland, the PAB has a helical conformation, and the neighbouring helical PABs gather together to form the aggregation bundle (or nanofibril) with 4.7 nm thickness and hexagonal packing, as shown in c. During the spinning process, the neighbouring NPAB sequences between the PAB and blue-coloured non-repeated sequences crystallise with β-sheet modification as shown in d. Simultaneously, the helical PAB bundle exhibited a stress-induced structural transition from helix to β-sheet crystals (as shown in d). SAXS analysis revealed a well-developed nanofibril bundle with a thickness of around 150 nm for a single filament, as shown in e. A stretching deformation model of the nanofibrils, during which the ordered repeating structure is maintained, is proposed in f. In situ X-ray analysis revealed that the deformations of the crystal and amorphous phases are reversible and irreversible modes, respectively.
as amorphous, by shrinking about 50% from the β-sheet con-
formation ((90 × 0.35–15.7)/(90 × 0.35) × 100 (%)) (picture on the
left in Fig. 6d).

The tensile deformation behaviours of the different silks differ
significantly among the species. The in situ time-resolved syn-
chrotron X-ray measurement during the tensile deformation of
the bagworm silk successfully revealed a remarkable structural
feature, wherein the crystal strain increased linearly against the
tensile stress throughout the stretching, up to the fracture point
(Fig. 5a). For comparison, we conducted the same measurements
for the B. mori and A. assama silks, which yielded different types
of stress–strain curves. All the three kinds of silk initially show a
linear increase in the crystal strain for a low tensile stress, as
reported by Seydel et al. for the B. mori silk18, but saturated at a
later stage in the B. mori and A. assama silks (Fig. 5b, c, and
Supplementary Fig. 5). This indicates that the stress distribution
to the crystal phases in the bagworm silk is more homogeneous
throughout the tensile process than that in the other two silks.
This superior stress distribution mode should be realised owing to
the continuous and highly ordered periodic structure revealed by
SAXS analysis. By combining all the experimental results
obtained in the present study, we have proposed a hierarchical
structure model for bagworm silks (Fig. 6e). The ellipsoid single
filament is made up of the 150 nm-thick bundles of nanofibrils,
each of which has a diameter of about 4.7 nm. These nanofibrils
are considered to be assembled together and form the repeatedly-
arranged structure with the amorphous phases. It is reasonably
speculated that the amorphous chain segments gather together
randomly and the entire system of nanofibrils appears like a
network of nanofibrils (image on the left in Fig. 6e). The tension-
induced structural deformation of the nanofibril bundle is sche-
matically illustrated in Fig. 6f, where the ordered repeating
structure is maintained in this process. Our findings obtained by
X-ray scattering techniques are consistent with the intrinsic
nanofibrillar nature of silkworm and spider silks revealed by
nanoscale imaging techniques49,50. Verification of the proposed
structural model and the deformation process of bagworm silks
using nanoscale imaging and spectroscopy techniques remains an
avenue for further study.

Our experimental results presented an important fact: the
mechanical property of the crystal phase of silk β-sheets is con-
siderably higher than what we had considered so far. The slope of
the stress vs. crystal strain plot of the bagworm silk gave a crystal
modulus of 106 GPa (Fig. 5a), which is four times higher than the
values for B. mori silkworm silks estimated by the X-ray dif-
fraction method51,52. The methodology for the measurement of
the crystal modulus is established, but it is made under an
assumption of homogeneous stress distribution (Supplementary
Note 2). The high modulus of the bagworm silk proposed a
possibility that the true crystal modulus of the β-sheet crystal is
much higher than what we had considered53, and that the stress
distribution to the crystal phases is far from an ideal homo-
geneous stress distribution, but quite heterogeneous, in natural
silks (Supplementary Note 3). In contrast, the highly ordered
network structure of bagworm silk results in a highly homo-
geneous stress distribution mode and gives the crystal modulus
approximately close to the true ultimate modulus.

In summary, we found that the bagworm silk is extraordinarily
strong and tough compared to other known natural silks, and its
tensile deformation behaviour is quite elastic. Although a variety
of silks with different mechanical properties should be candidates
for various practical applications, bagworm silk seems especially
appropriate for use as a structural material. The detailed struc-
tural analyses, from amino acid sequence to hierarchical structure
level, clarified that the outstanding mechanical properties origi-
nated from a highly ordered hierarchical network structure,
axial ratio ($L_1/L_2$) of 1.7, as described in the main text. By this approximation, one can calculate the cross-sectional area by measuring the length of the major axis of the filament using a well-calibrated RZ-X700 instrument. At this stage, $\theta$ should be taken into account, because the length of the major axis measured using the optical microscope ($L_0$) is $L_0 \times \cos \theta$, as illustrated schematically in Fig. 1. Therefore, the actual cross-sectional area of a single filament is $$\frac{\pi L_1^2 - \pi L_2^2}{4 \tan \theta} \times \cos \theta.$$

Tensile test. The tensile properties of the individual fibres of *E. variegata* bagworm silk were measured at 22–25 °C and 40–60% relative humidity, using a mechanical tensile stage (EZ Test/CE, Shimadzu Co., Japan) equipped with a 5 N load cell. All the measurements were carried out at a cross-head speed of 10 mm min$^{-1}$. Single fibre made up of a pair of thin filaments with an elliptical cross section was attached to a hand-made paper flame using epoxy glue, with a window distance (i.e., sample distance) of 15 mm. After positioning the paper flame holding a single fibre in the tensile stage, both sides of the flame were cut gently, and then the measurement was started. The cross-sectional area of each fibre was estimated for every sample, based on optical microscope observations. The procedure for estimating the elliptical cross-sectional area of bagworm silks was described in the previous section. In all, 19 measurements (4 or 5 measurements each for 4 different bagworms) were carried out and averaged (Supplementary Fig. 6).

Analysis of amino acid composition. The amino acid composition of the bagworm silk fibres was analysed with a Shimadzu amino acid analysis system using a high-performance liquid chromatography CBM-20A controller (Shimadzu Co., Japan), equipped with the columns Shim-pack Amino-Na (100 mm (L) × 6.0 mm (φ) and ISC-30 (Na) (50 mm (L) × 4.0 mm (φ)) (Shimadzu Co., Japan). The degummed fibres were dissolved in 6 N hydrochloric acid under vacuum at 110 °C for 22 h. The sample was dried under nitrogen gas purging, re-dissolved in pH 2.2 sodium citrate buffer, and examined, for the amino acid analysis.

RNA sequencing and bioinformatics analysis. The larvae of the bagworm moth were collected in Abiko City, Chiba (Japan) in September 2015. The silk gland was dissected from one larva and the total RNA was extracted from the posterior region of the silk gland using the ISOGEN (NIPPON GENE, Tokyo, Japan) and SV Total RNA isolation kit (Promega, Madison, WI). The total long-read sequencing analysis (Isoform Sequencing) was carried out using a PacBio RS II sequencer. The library was prepared using the PacBio SMARTer RNA Sample Prep Kit v3 (Takara, Otsu, Japan) and the −5 to 10 kb libraries were selected using the BluePippin Size-Selection System (NIPPON Genetics, Tokyo, Japan). After purification and end-repair, the blunt-end SMARTbell adaptors were ligated. The libraries were quantified using a QuantiT Horiba Green kit (Thermo Fisher Scientific, Waltham, MA), and qualified using the Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Subsequently, the libraries were sequenced using the PacBio P6/C4 chemistry in an 8-well SMART Cell v3 with a PacBio RS II system. For sequence correction, the Illumina short-read sequence was used. The total RNA extracted from the posterior region of the silk gland was applied for the RNA library construction using a TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA), and the 100 bp paired-end reads were obtained using an Illumina HiSeq 2500 sequencer. Adapter sequences and low-quality bases in the Illumina short reads were trimmed using the Trimmomatic version 0.32.36, and the cleaned short reads were used for the long-read correction with ColloMap25. The raw RNA sequencing data have been deposited in the DNA Data Bank of Japan (DDBJ) Centre (https://www.ddbj.nig.ac.jp/index-e.html) and can be accessed via accession numbers DRA007344 (short-reads) and DRA007345 (long-reads).

Static structural analysis by WAXD and SAXS. A parallel-aligned fibre bundle with a thickness of about 0.1 mm was prepared, and the surface adhesive was washed with a 0.05 M sodium carbonate buffered aqueous solution for 3 min. The fibre bundle was then thoroughly rinsed and dried, and transferred as samples for the subsequent X-ray analysis.

For references, the de-gummed *B. mori* and Indian *Antheraea assama* (A. assama) (aka Maga silk) (*Saturniidae*) silks were also gently collected in a parallel-aligned fibre bundle with a bundle thickness of about 0.5 mm, and used for the subsequent X-ray analyses during the tensile deformation test. The cross-sectional area for each fibre bundle, needed for the data conversion from the stress-to-the-strain curves, was estimated from the length and weight of the initial fibre bundle and the density for each silk. The values of density, 1.35 and 1.31 g cm$^{-3}$, reported by Gupta et al. for *B. mori* and *A. assama* silks, respectively, were used.32

The crystalline modification of the bagworm silk was investigated by WAXD analysis, using an R-Axis Rapid II X-ray diffractometer (Mo-Kα (Rigaku Co., Japan) equipped with the highly sensitive single-photodiode counting pixel detector HyPix-6000 (Rigaku Co., Japan). Cerium oxide and silver behenate were used to calibrate the camera distances for the WAXD and SAXS measurements, respectively. All X-ray measurements were performed at 22–25 °C and 40–60% relative humidity.

Time-resolved synchrotron X-ray scattering investigation. The structural changes in the bagworm silk, occurring in the stretching processes, were investigated by time-resolved simultaneous measurements of the force-strain curve and synchrotron WAXD and SAXS patterns. The fibre bundle with a bundle thickness of about 0.1 mm was set on a stretching device, the Micro-stretcher (Linkam Microscopy Ltd., UK), which was set in a SPring-8 4802 X-ray beamline (Hyogo, Japan). The setup geometry is shown in Supplementary Fig. 4. The WAXD and SAXS data were measured every 9 s (8 s exposure time + 1 s interval) during the continuous stretching process, with a stretching rate of 10 μm s$^{-1}$. The wavelength of the incident X-ray beam was 0.0709 nm, and the sample-to-camera distances for the WAXD and SAXS measurements were 95 and 763 mm, respectively. The beam size at the sample position was around 0.8 mm$^2$. Tuning of the best beam intensity, which yields the best quality of scattering without serious sample damage during the repetitive measurements, was carefully performed using the attenuator system. A flat panel detector with a pixel size of 50 × 50 μm$^2$ (Hamamatsu Photonics K. K., Japan) and CMOS detector with a pixel size of 43.1 × 43.1 μm$^2$ (Hamamatsu Photonics K. K., Japan) were used for the WAXD and SAXS measurements, respectively. For the accurate calibration of the WAXD camera distance, silicon powder was dusted onto the fibre bundle. The force-strain data obtained from the tensile test was approximately converted to a stress-strain curve on the basis of the stress-strain curves obtained from the single fibre. The sample strain (%) was estimated as $\epsilon = \frac{L_f}{L_0} \times 100$ (%), where $L_f$ is the initial sample length of 15 mm between the tensile clamps, and $L_0$ is the stretched length.

WAXD data analysis. For all the cases of WAXD analysis, air scattering was subtracted from the original 2D-WAXD pattern. The crystallinity was estimated from the WAXD 2θ profile. The 2θ profile was scanned from the 2D-WAXD fibre image, and ranged from 10 to 37°. For the 2θ profile obtained, peak fitting analysis was performed to separate the contributions from the crystalline reflections and amorphous halo-scattering. For the peak fitting, the contributions from the 6 crystalline lattice planes, (200), (210), (211), (002), (102) + (001) + (002), and (202), and that from the single amorphous scattering, were considered. Each 2θ position of the crystalline reflections was calculated on the basis of the unit cell parameters of the β-sheet crystal determined for the bagworm silk in this study. The peaks were fitted with Gaussian functions, using a “non-linear least squares fitting”, in the spectroscopy software Grams Suite 9.3 (Thermo Fisher Scientific Inc., USA). The crystallinity $X_c$ was calculated using Eq. (1):

$$X_c(\%) = \frac{I_c}{(I_c + I_a)} \times 100,$$

where $I_c$ and $I_a$ are the total intensities from the crystal and amorphous phases, respectively.

The crystal strain ($\epsilon_c$) along the c-axis direction at each bulk strain ($\epsilon$) was evaluated from the change in the 2θ position of the 002 meridional reflection. The $\epsilon_c$ was defined as shown in Eq. (2).

$$\epsilon_c(\%) = \frac{(d_{002}(\epsilon) - d_{002}(initial))/d_{002}(initial)}{100} \times 100$$

The crystalline orientation index was evaluated using the difference (180–FWHM)/180, where FWHM is the full width at half maximum of the azimuthal angular distribution of the WAXD equatorial reflection.

SAXS data analysis. For all the cases of SAXS analysis, air scattering was subtracted from the original 2D-SAXS pattern. The meridional or equatorial q-profiles were scanned from the meridional and equatorial lines with an azimuthal angle range of ±10°, where q is the scattering vector, defined as $(4\pi/\lambda)\sin \theta$, where $\lambda$ is the wavelength of the X-ray beam used. Thus, the obtained 1D-q-profile $I(q)$ vs. q plot was replotted as a Kratky plot ($I(q)/q$ vs. q plot) to enhance the peak features more evidently.

The one-dimensional electron density correlation function $K(z)$ between a periodic structure made of phases I and II, which gives an electron density distribution $\eta(z)$, as depicted in Fig. 3c, was calculated from the SAXS data. The correlation function $K(z)$ is defined as shown in Eq. (3)37

$$K(z) = \int q^2 I(q) \cos(2\pi qz) dq$$

$Q, L_0, d_{100}$, and $d_{110}$, shown in Fig. 3e, f denote the invariant, long period, core diameter of I domain, and mean thickness of phase I, respectively. The strain ($\epsilon_{100}$) in the long period was estimated using Eq. (4).

$$\epsilon_{100}(\%) = \left(1 - \frac{L_0}{L_{100}} \right) \times 100$$
The thickness of the nanofibril bundle was evaluated from the broadening of the equatorial SAXS peak, using the Scherrer Eq. (5)\(^\text{10}\): \[ d = \frac{K \lambda}{\beta \cos \theta} \] (5) where \( d \) is the thickness of the nanofibril bundle, \( \lambda \) is the wavelength of the incident X-ray beam (CuK\(\alpha_1 \) 0.15418 nm radiation), \( \beta \) is the angle of the considered reflection, \( \beta \) is the line broadening at half-maximum intensity of the considered reflection, and \( K \) is a dimension-less shape factor (Scherrer factor). For the shape factor \( K \), a value of 0.9 was used. The \( \beta \) was corrected for the instrumental broadening, which was estimated by measuring the powder diffraction profile of silver behenate, on the basis of the Eq. (6). \[ \beta = \sqrt{\beta_{	ext{inh}}^2 - \beta_{	ext{def}}^2} \] (6)

Data availability

The raw sequence data of the next generation sequencer have been deposited in the DNA Data Bank of Japan (DDBJ) Centre (https://www.ddbj.nig.ac.jp/index-e.html) and can be accessed via accession numbers, DRA007344 (short-reads), and DRA007345 (long-reads). The same raw sequence data is available from the Open Science Framework: https://doi.org/10.17605/OSF.IO/7GYSU. Data supporting the findings of this study are available from the authors upon reasonable request.

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Author contributions
All the authors (T.Y., T.T., K.T., A.J., and T.K.) contributed equally to the work and manuscript preparation.

Additional information

Patent applications have been filed for the findings described in this publication (PCT/JP2017/023839 and PCT/JP2017/037327). While the research was done in an academic/institutional environment, since then the material has been taken up by a company to develop for commercialisation.

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