Correction

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The authors note that the author name Ryuma Niyama should instead appear as Ryuma Niiyama. The corrected author line appears below. The online version has been corrected.

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Investigation of hindwing folding in ladybird beetles by artificial elytron transplantation and microcomputed tomography

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Ladybird beetles are high-mobility insects and explore broad areas by switching between walking and flying. Their excellent wing transformation systems enabling this lifestyle are expected to provide large potential for engineering applications. However, the mechanism behind the folding of their hindwings remains unclear. The reason is that ladybird beetles close the elytra ahead of wing folding, preventing the observation of detailed processes occurring under the elytra. In the present study, artificial transparent elytra were transplanted on living ladybird beetles, thereby enabling us to observe the detailed wing-folding processes. The result revealed that in addition to the abdominal movements mentioned in previous studies, the edge and ventral surface of the elytra, as well as characteristic shaped veins, play important roles in wing folding. The structures of the wing frames enabling this folding process and detailed 3D shape of the hindwing were investigated using microcomputed tomography. The results showed that the tape spring-like elastic frame plays an important role in the wing transformation mechanism. Compared with other beetles, hindwings in ladybird beetles are characterized by two seemingly incompatible properties: (i) the wing rigidity with relatively thick veins and (ii) the compactness in stored shapes with complex crease patterns. The detailed wing-folding process revealed in this study is expected to facilitate understanding of the naturally optimized system in this excellent deployable structure.

Significance

Hindwings in ladybird beetles successfully achieve compatibility between the deformability (instability) required for wing folding and strength property (stability) required for flying. This study demonstrates how ladybird beetles address these two conflicting requirements by an unprecedented technique using artificial wings. Our results, which clarify the detailed wing-folding process and reveal the supporting structures, provide indispensable initial knowledge for revealing this naturally evolved optimization system. Investigating the characteristics in the venations and crease patterns revealed in this study could provide an innovative designing method, enabling the integration of structural stability and deformability, and thus could have a considerable impact on engineering science.

Author contributions: K.S. and Y.O. designed research; K.S. performed research; K.S., S.N., and R.N. contributed new reagents/analytic tools; K.S., S.N., S.Y., R.N., and Y.O. analyzed data; and K.S. and S.Y. wrote the paper.

The authors declare no conflict of interest.

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operation. The detailed method is explained in Materials and Methods. Fig. 1A shows the hindwing of *C. septempunctata*. The wing basal component is supported by two thick veins: the mediocubital loop (MCL) and radiomedial loop (RML) (8, 9). The positions of the folding lines in the rest position are shown in Fig. 1B. The crease lines can be classified into three types on the basis of their functions. Red lines are the principal transverse fold (PTF) and apical transverse fold (ATF), which fold a wing along the longitudinal direction. Blue lines emerge between the MCL and RML, and fold a wing along the transverse direction. These two types of lines are connected by diamond-shaped crease patterns (green lines). The detailed wing-folding processes are explained with reference to Fig. 1 C–G. After landing, the elytra are first closed before the hindwing folding commences (Fig. 1C). The hindwings are simultaneously aligned backward and protrude out from the closed elytra. Held by the elytron, the wing is slightly folded in the transverse direction (Fig. 1D). Then, the ladybird beetle lifts its abdomen, and the hindwings are pressed into the underside of the elytron. During this process, the characteristic shape of the MCL fits the curve of the ventral surface of the elytron and ark-like mountain folding lines appear beneath the MCL (Fig. 1E). The abdominal lifting movements are observed multiple times. These movements result in the rubbing of the underside of the hindwings and pulling them into the dorsal

![Fig. 1](image)

Fig. 1.  Wing-folding process in a ladybird beetle. (A) Hindwing of *C. septempunctata*. The basal part of the wing is supported by thick two veins: the MCL and RML. (B) Main crease lines of hindwing folding. The crease lines can be classified into three types based on their functions. Red lines are the PTF and ATF, which fold the wing along the longitudinal direction. Blue lines emerge between the MCL and RML and fold the wing along the transverse direction. The movements of these two types of lines are connected by diamond-shaped crease patterns (green lines). Fine lines represent mountain folding lines, and dashed lines represent valley folding lines. The dashed-line square corresponds to the paper model of Fig. 3. (C–G) Schematic representations of the wing-folding process. (C) Elytra are closed ahead of the hindwings, and they are simultaneously aligned backward. (D) Held by the elytron, the wing is slightly folded in the transverse direction and a triangular crease pattern emerges between the MCL and RML. (E) PTF and ATF are first introduced by the inner curve of the elytron and the edge of the elytron with the abdomen (PTF' and ATF'). (F) According to the wing retraction by abdominal movement, the ATF' drifts toward the apex of the wing by abdominal push and the wing is gradually retracted. The position of the PTF' is held by the edge of the elytron. Finally, two transverse folding lines are stabilized into the positions of the PTF and ATF as shown in B. (G) Explanation of the stored shape. The hindwings are folded into a Z-shape on the folding lines of the PTF and ATF, and the diamond-shaped crease pattern emerges in the center of the hindwing.
storage space. For maximum effectiveness of these movements, the tergal plates contain microspicule patches (Assisting Structures for Wing Folding and Fig. S2), which are considered to hold the surface of the hindwings and assist smooth wing folding (2–4). The PTF is introduced by the inner curve of the elytron (PTF in Fig. 1G) and appears at almost the same position as in Fig. 1B. The ATF is initially introduced by the edge of the elytron at the position shown as ATF in Fig. 1E and gradually drifts toward the apex of the wing guided by the abdominal movements (Fig. 1F). Finally, two transverse folding lines (red lines) are stabilized into the positions of the PTF and ATF in Fig. 1B, and the diamond-shaped crease pattern (green lines) emerges in the center of the wing. In the stored shape, the hindwings are folded into a Z-shape on the folding lines of the PTF and ATF as shown in in Fig. 1G (Center). The wing folding is completed within 2.0 s. For better understanding, a high-speed movie of these folding processes is available in Movie S2.

The majority of the hindwing comprises relatively soft membrane. During flights, the hindwing shape is maintained by thick veins. Among them, the anterior margin area (Fig. L4) including the supporting vein 1 (SV1) and SV2 veins and the RML, is hard and considered to confer stiffness and strength to the hindwings. We investigated the deformation of this part in both folded and unfolded states using micro-CT. Fig. 2 shows the 3D shape of the unfolded wing reconstructed by the result of the CT scan. The thicknesses of these main veins have no difference from other areas; however, their cross-sections have a characteristic curved shape similar to the shape of a tape spring (10–13). No special structures, including articulations, thinner parts, or an inner cavity, are observed on the cross-point of the crease lines and veins. Fig. 2B shows the 3D model of the folded hindwing. It is observed that the anterior margin is bent into a cylinder shape with a constant curvature instead of being bent at a sharp angle (white circles in Fig. 2). The bent shapes of these veins are similar to the shapes of the localized folds (10) observed in a tape spring. For better understanding of the shape of the folded wing, a translucent 3D image is available in Movie S3.

Discussion

With respect to the wing venation, two loop-shaped veins (MCL and RML) are a common feature found in many beetle species; however, the MCLs in ladybird beetles have characteristic arc-like shapes. As mentioned above, this shape functions to introduce the crease lines by fitting the ventral curve of the elytron. These steady venations not only confer sufficient strength and stiffness in hindwings but also play an important role for interlocking the action of crease lines. The deploying actions are caused by the skeletonmuscular apparatus of the metathorax (8, 14) and are propagated to the wing apex direction by these thick veins. Therefore, ladybird beetles can interlock the movement of two transverse folding lines and achieve compact wing folding and quick deployment. C. septempunctata has a crease pattern similar to the crease pattern of the other ladybird Aiolocaria sp. reported by Fedorenko (8). According to Forbes (1), Epiplacha borealis (Fabricius) and Anatis 15-punctata (Olivier) also possess similar crease patterns with the minor difference of additional small fold lines in the posterior margin. A diamond-shaped crease pattern similar to the green lines in Fig. 1B was also found in these ladybird beetles and can be regarded as an important feature of their hindwings. Fig. 3A shows the schematic of the folding movement of this pattern. Note that this figure shows the ventral side of the hindwing; therefore, the mountain and valley assignments of folding lines are exchanged from the case of Fig. 1B. These diamond-shaped crease lines have little involvement in the global deformation of the wings but cause the additional fold on the ridge of the central folding line, as indicated by the red circle in Fig. 3A. One possible function of this additional fold is the lock mechanism that helps avoid accidental unfolding caused by wing elasticity. Fig. 3B shows the semi-transparent image of the folded hindwing. As shown in the red circle in this figure, the additional fold of the diamond-shaped crease is fixed by tucking between the MCL and RML (also Movie S3). It can be considered that this tucking functions to maintain the folded shape in the PTF. An additional possible function may be to introduce bistability into the structure. The bistable behavior of the hindwings, which is considered to assist wing transformation and shape locking, has been reported in many previous studies (1–3, 6, 8); however, its detailed mechanisms remain unclear. Considering the structural stability, the crease pattern evident in Fig. 3A has a negative degree of freedom; hence, it is not rigidly foldable in general (15, 16) (Rigid Foldability in Origami Crease Patterns). Therefore, the act of folding and unfolding should be accompanied by elastic deformations. These deformations in nonrigid origami usually emerge as stretching and shrinkage of fold lines, fold line drifts, and out-of-plane deformation of facets. In the case of these diamond-shaped crease lines, out-of-plane deformations are considered

Fig. 2. Three-dimensional models of the hindwings in C. septemunpunctata reconstructed from the results of micro-CT. (A, Upper Left) Unfolded hindwing (from the underside). (A, Lower Right) Slice view on the white line is shown. Compared with other parts, the cross-sections of the main supporting veins (SV1, SV2) have no difference in thickness but have a characteristic curved shape. (B) Folded hindwing. The main supporting veins are bent into a cylindrical shape (white circles) similar to the shape of a tape spring.

Fig. 3. Schematic of the folding movement in the wing central part (from the ventral side of the right hindwing). (A) Diamond-shaped crease lines, which are characteristic of hindwings in ladybird beetles, have little involvement in the global deformation of wings but cause the additional fold on the ridge of the central folding line, as indicated by the black dotted lines and red circles. (B) Translucent image of a folded hindwing in C. septempunctata. The additional fold part (white dotted lines) is positioned between the RML (yellow dotted line) and MCL (green dotted line) (also Movie S3).
the dominant factor. Observing the wing structure, the facets as presented in Fig. 3A are not wholly hard plates but are partially supported by veins; therefore, these facets can readily cause out-of-plane deformation involving some diagonal lines. In fact, it can be confirmed that certain facets in the paper model of Fig. 3A result in out-of-plane deformations during the folding process. This elastic deformation in the folding/unfolding process is considered to impart the bistable behavior to hindwings.

From an engineering standpoint, the most interesting aspect of hindwings in beetles is how they can achieve the compatibility between the deformability (or instability) required for the wing storage and the strength properties (or stability) required for flying. These two properties generally demonstrate a trade-off relationship, and thus are difficult to combine (Crease Patterns and Supporting Structures in Beetle Hindwings). Ladybird beetles have successfully resolved these two conflicting requirements, resulting in the evolution of relatively thick veins with decent strength properties while achieving sufficiently compact wing folding with two folding lines in the longitudinal direction of the wing. It is noteworthy that Fedorenko (17) concluded that ladybird beetles are categorized as one of the most advanced groups within the context of wing folding.

The biggest challenge for ladybird beetles is that they are required to embed the two transverse folding lines (PTF and ATF) on the anterior margin, which acts as the main support structure of the hindwing during flight. Simple articulations or positionally fixed compliant hinges in this area may cause a considerable decrease in the stiffness and strength of the wing. Our results show that ladybird beetles solve this problem by using tape spring-like veins as the main wing-supporting structures. A tape spring is a thin elastic strip with a curved cross-section that is commonly known as a carpenter tape. This structure becomes elastically stable when it is extended and can be stored into a compact form only by elastic folding (10); therefore, it is widely used in the extension booms and hinges of space-deployable structures (11–13). Fig. 4 presents a schematic of the functions of tape spring-like veins in wing folding/unfolding. These veins are stabilized in the unfolded shape and can confer sufficient stiffness for flight (Fig. 4A). Based on finite element analysis that was conducted on a simple shell model of the SV vein obtained from the results of micro-CT scanning, this curved cross-section has about 11-fold greater bending stiffness than flat-shaped veins of the same weight (Finite Element Analysis of the Tape Spring-Like Vein and Fig. S3). If the specific stiffness is the only issue, more effective forms were found in insect wings. For example, wing veins in the dragonfly have a tubular sandwich microstructure (18). It is also reported that the elytra of beetles are constructed with honeycomb-like sandwich structures (19). Besides the structural reinforcement property, tape spring veins have three unique properties enabling wing folding/unfolding in ladybird beetles. First, the veins can be bent at an arbitrary position by forming localized folds during wing storage, and therefore can serve as compliant hinges where appropriate. As shown in Fig. 1D and E, the edge of the elytron is used to initialize this folding (Fig. 4B). A second characteristic of this structure is its ease of moving the bending point (position of the localized fold) along the beam length (Fig. 4C). The revealed folding processes include the drift of the ATF in the basal-apex direction as shown in Fig. 1F. This drifting fold is considered to enable gradual wing retractions. The third function of the tape spring vein is to store elastic energy for wing deployment (Fig. 4D and E). The elastic force caused by the resilience in the localized folds is considered to enable rapid wing deployments in ladybird beetles (Movie S1). With respect to wing-deploying forces, it is reported that some beetles use hydraulic mechanisms that may straighten the hindwings by blood pressure (7). Our micro-CT investigation shows no concrete evidence regarding whether ladybird beetles exhibit similar mechanisms (e.g., cavities in hindwings, inflation of unfolded veins). Further investigations, particularly of the cross-sections of wing veins, by scanning electron microscopy (SEM) and microtomate are warranted to clarify this issue.

The detailed wing-folding mechanism revealed in the present study is expected to facilitate understanding of the optimization process that has developed during the course of evolution, which can elucidate the innovative design method enabling the integration of both structural stability and deformability. Immediate applications may be deployable structures, including space-deployable structures represented by solar array paddles and antenna reflectors of satellites, wings of carrier-based aircrafts, and many articles of daily use with a deforming function (e.g., umbrellas, fans). The relationship between the wing-actuating mechanisms and latest mechanics is similarly interesting. The deformations include elastic behavior in the supports, and therefore can be determined as the compliant mechanism (20, 21). This mechanism is an active research target within the mechanics and microelectromechanical system fields. In regard to the geometry of the crease pattern, Brackenbury (3) and Haas and Wootton (22) proposed a simplified geometrical model based on the characteristic patterns found in most beetles. These studies have recently been considered to gain importance because of the advancement in applied origami (23–27). Combined with these emerging technologies, the wings of beetles will have a great impact on multiscale production in various engineering fields.

**Materials and Methods**

**Transplant Operation.** Specimens used were of the ladybird beetle species *C. septempunctata* (L.) (Coleoptera: Coccinellidae) and were captured in the grass fields of Tokyo, Japan. Each specimen was anesthetized using carbon dioxide gas, and two-thirds of its right apical elytron was excised using scissors. Silicone (Silldeft wash-5X; SHOFU Co., Ltd.) was used to construct the impression of the undersurface of the excised elytron. The curing time was 5 min at 25 °C. The UV-cured resin (Craft arrange clear; CHEMITECH, Inc.) was applied in a thin layer on the impressions using a brush. After curing by UV light, the transparent acrylic elytron was fabricated. These artificial wings were adhered on the apices of the excised wings of the original ladybird beetles using a UV-cured resin. Each transplant operation was completed in ≤1 h to minimize the damage to the hindwings and living beetles.

**Observations by High-Speed Cameras.** The specimens were placed on an acrylic board and were kept in focus by simultaneously moving the board on the table to maintain their position in the frame. High-speed cameras (Phantom V1611, Nobby Tech. Ltd.; VW9000, KEYENCE CORPORATION; and HAS-L2, DITECT Co., Ltd.) were used to acquire slow-motion movies.
wing-deploying scenes (Movie 51) were filmed using 3,000 and 4,000 frames per second (fps), whereas the folding scenes (Movie 52) used 60 fps.

Micro-CT. This study used the microfocus X-ray CT system, inspeXio SMX-100CT (SHIMADZU CORPORATION), which comprises a nenclosure tube-type X-ray generator operating at a voltage of 100 kV. Specimens used were the folded and unfolded hindwings of C. septempunctata vertically mounted on the stage along the wing-length direction. CT images were acquired for each 10-μm length at a resolution of 1,024 × 1,024 pixels and reconstructed into 3D images using the visualization software VGStudio MAX (Volume Graphics GmbH).

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