Movement Analysis of Flexion and Extension of Honeybee Abdomen Based on an Adaptive Segmented Structure

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Abstract. Honeybees (Apis mellifera) curl their abdomens for daily rhythmic activities. Prior to determining this fact, people have concluded that honeybees could curl their abdomen casually. However, an intriguing but less studied feature is the possible unidirectional abdominal deformation in free-flying honeybees. A high-speed video camera was used to capture the curling and to analyze the changes in the arc length of the honeybee abdomen not only in free-flying mode but also in the fixed sample. Frozen sections and environment scanning electron microscope were used to investigate the microstructure and motion principle of honeybee abdomen and to explore the physical structure restricting its curling. An adaptive segmented structure, especially the folded intersegmental membrane (FIM), plays a dominant role in the flexion and extension of the abdomen. The structural features of FIM were utilized to mimic and exhibit movement restriction on honeybee abdomen. Combining experimental analysis and theoretical demonstration, a unidirectional bending mechanism of honeybee abdomen was revealed. Through this finding, a new perspective for aerospace vehicle design can be imitated.

Key Words: honeybee, abdomen, segmented structure, bending mechanism, intersegmental membrane

Materials and Methods

Experimental Animals. The morphological data of this study dealt with adult foraging worker honeybees (Apis mellifera Ligustica). All specimens were collected from a single hive maintained at the intelligent bio-mechanical laboratory of Tsinghua University, Beijing, China (40.00’N, 116.33’E). Only A. mellifera Ligustica identified as those carrying pollen on their hind legs when returning to the hive were collected. No specific permissions were required for these locations or activities, and the field studies did not involve endangered or protected species.

Macroscopic Observation Experiments. Hundreds of honeybees were kept in a 120 cm by 40 cm by 60 cm transparent cage. Few weeks after being captured, the bending process of the honeybee abdomen was captured, from extending position to extreme bending position, with a high-speed camera (Olympus i-SPEED TR, Tokyo, Japan) operating at 1,000 frames per second with a zoom lens (Navitar 12X Zoom, Rochester, NY, USA) (Fig. 1 and Suppl Movie 1 [online only]).

The thorax of the honeybee was kept in a foam with 50 mm by 50 mm by 30 mm monitoring platform. The abdominal movement was not restricted. Three hours after capture, still photographs (using Nikon AF-S DX, Nikon Corp., Tokyo, Japan) of the honeybee bending its abdomen from extending position to extreme bending position were taken (Fig. 3 and Suppl Movie 3 [online only]).

Microstructure Observation Experiments. The abdomen of a deceased bee was cleaned in a methanol solution and naturally dried, and then the FSs were sliced using a cryostat–microtome (Leica CM1900, Nussloch, Germany). These 10-μm-thick sections were dyed through hematoxylin–eosin staining and observed under Nikon image acquisition system (Eclipse 90i, Nikon Corp., Tokyo, Japan).

Honeybees successfully evolved millions of years ago, during which they were gradually endowed with special powerful skills (Snodgrass 1956, Nunes et al. 2014). As a propaganda of several artworks, honeybees can casually and gracefully bend their abdomen (Smith and Hickner 2007), which can rotate upward up to the pedicel. This movement can be observed when the bee is disseminating its aggregation pheromone. An enormous amount of information was accessed to determine whether honeybee abdomen can bend in any direction and an intriguing but less studied feature that it could only bend unidirectionally was discovered.

Previous investigations have shown that honeybee abdomen appeared to consist of six segments, but honeybees actually have seven exposed abdominal segments and nine in the drone (Snodgrass 1956, Esch et al. 2001). Each abdominal segment, except the first one, consists of a tergum and sternum, the former reaching far down on the side of the segment where it carries the spiracle and overlaps the edge of the sternum (Hager and Kirchner 2013, Stead 2013). The abdominal end of the drone is different from that of the worker bee because at least nine segments are visible on the drone’s parts, the last being very much modified and exposed only on the sides and below part (Paar et al. 2012, Clarke et al. 2013). Each sternum is widely underlapped by the one in front of it, and the folded intersegmental membrane (FIM) is reflected from the middle of the dorsal surface or to the anterior edge of the following sternum (Heinrich 1974, Pennisi 2001). Moreover, the transverse line of attachment of the membrane divides the sternum into posterior part, which is a prolonged reduplication underlapping the following sternum (Nachtragall et al. 1998).

These results indicated that the physical abdominal structure of a bee has been qualitatively reported and described. However, the curling of honeybee abdomen has not been elucidated, and no quantitative analysis has been proposed (Finlayson and Lowenstein 1958, Oldroyd and Wongsiri 2006, Nenchev et al. 2007, Headrick and Gordh 2009). In this study, a high-speed video camera was used to capture the curling of honeybee abdomen and to analyze the bending scope and variation of its arc length, not only in free-flying mode but also in the fixed sample. In addition, frozen sections (FSs) and environment scanning electron microscope (ESEM) were used to investigate the microstructure and motion principle of the adaptive segmented abdomen. Combining experimental analysis and theoretical demonstration, a unidirectional bending mechanism of the honeybee abdomen was revealed.
To investigate the bending behavior, the honeybee abdomen was also examined in an ESEM (FEI Quanta 200, FEI Company, Eindhoven, The Netherlands). The samples were observed under low vacuum mode (30 kV), and the resolution of images was about 3.5 nm. Furthermore, the motion of FIM was observed using the in-line phase contrast computed tomography method of X-ray imaging and biomedical application beamline at Shanghai Synchrotron Radiation Facility (see Suppl Movie 2 [online only]).

Results

Curling of Honeybee Abdomen in Free-Flying Mode. Experiments, especially the quantitative analysis, were conducted to study the curling of honeybee abdomen. The abdominal specimens of worker bees of *A. mellifera Ligusticas*, with an average length of 12 mm, were chosen to observe the bending phenomenon of the segments. A high-speed video camera was used to capture the motions of the abdomen (1,000 frames per second). Images of free-flying honeybees were recorded to verify the unidirectional bending of honeybee abdomen using a high-speed video camera during free-flying mode (Fig. 1a–f and Suppl Movie 1 [online only]). As the honeybee landed on the flower, its abdomen curled to the ventral side, landing steadily from 0 ms to 24 ms (white arrow in Fig. 1a–f).

During the initial stage, the honeybee abdomen started to curl downward. The arc lengths on the terga and sterna were 9.72 ± 0.42 and 9.62 ± 0.44 mm, respectively (Fig. 2). The arc length on the terga (Fig. 1b) then decreased to 9.67 ± 0.30 mm at 4 ms. Meanwhile, the arc length on the sterna decreased to 8.73 ± 0.38 mm (Fig. 1b), which means that the abdomen curled to the ventral side. As the time was increased to 12 ms, the arc lengths on the terga and sterna decreased to 5.43 ± 0.21 and 4.01 ± 0.17 mm, respectively. Subsequently, the honeybee abdomen curled to the ventral side from 12 to 24 ms until the arc length on the terga and sterna increased to 10.44 ± 0.16 and 9.65 ± 0.18 mm, respectively. Error bars:

![Fig. 1. Motion observation of honeybee abdomen in free-flying mode. (a–f) High-speed video images of honeybee landing on the flowers (captured at a rate of 1,000 frames per second). Honeybee started landing at 0 ms and steadily landed on the flower at 24 ms.](image)

![Fig. 2. Variation in arc length of the abdomen of free-flying honeybee. The sample size of this experiment is 60. During the initial stage, the honeybee abdomen curled downward, and the arc lengths on the terga and sterna were 9.72 ± 0.42 and 9.62 ± 0.44 mm, respectively. The arc lengths on the terga and sterna then decreased to 9.67 ± 0.30 and 8.73 ± 0.38 mm under the free-flying time of 4 ms, respectively. As the time was increased to 12 ms, the arc lengths on the terga and sterna decreased to 5.43 ± 0.21 and 4.01 ± 0.17 mm, respectively. Subsequently, the honeybee abdomen curled to the ventral side from 12 to 24 ms until the arc length on the terga and sterna increased to 10.44 ± 0.16 and 9.65 ± 0.18 mm, respectively. Error bars: ± 0.42 mm (dorsal side) and ± 0.44 mm (ventral side) at 0 ms; ± 0.30 mm (dorsal side) and ± 0.38 mm (ventral side) at 4 ms; ± 0.46 mm (dorsal side) and ± 0.44 mm (ventral side) at 8 ms; ± 0.21 mm (dorsal side) and ± 0.17 mm (ventral side) at 12 ms; ± 0.21 mm (dorsal side) and ± 0.28 mm (ventral side) at 16 ms; ± 0.26 mm (dorsal side) and ± 0.24 mm (ventral side) at 20 ms; ± 0.16 mm (dorsal side) and ± 0.18 mm (ventral side) at 24 ms.](image)
unidirectional bending of honeybee abdomen during free-flying mode (Fig. 1a–f).

Curling of Honeybee Abdomen in the Fixed Sample. An attempt was made to investigate whether honeybee abdomen bends upward while its thorax is fixed on the panel. For the time being, the abdomen was freely moving, which was recorded using a high-speed camera (Fig. 3a). These images can illustrate a special bending phenomenon.

As shown in Fig. 3a–e, the dynamics bending process of the fixed honeybee abdomen was captured by processing a set of images as the time increased from 0 to 450 ms. Configurations I (Fig. 3b and c) and III (Fig. 3b and e) show dual limit positions where the abdomen stretched to the minimum amount and curled to the maximum amount. Configuration II (Fig. 3b and d) displaces a position between the two limits, where the arc lengths on the terga and sterna were 10.48 and 7.41 mm, respectively. In Configuration I (Fig. 3b and c), the contour curves on the terga and sterna were almost similar in length, indicating that the arc lengths on the terga and sterna were both 8.74 mm. From Configuration I to Configuration III, the contour curve on the terga turned longer, and the arc length increased from 8.74 to 12.43 mm. In contrast, the contour curve on the sterna became shorter, and the arc length shortened from 8.74 to 6.97 mm (Fig. 3b). More importantly, the phenomenon of unidirectional bending of the honeybee abdomen occurred, which did not influence the internal organization of the abdomen. Therefore, unidirectional bending still exists despite removal of all the internal organizations (Suppl Information and Movie 2 [online only]).

There are indeed some differences in the abdominal bending in the high speed free flight, compared with that in the tethered experiment. The deformational degree of abdominal bending in the high speed free flight was much smaller than that in the tethered experiment. Moreover, both the dorsal and ventral arc lengths were shortened (Fig. 2) in contrast to the tethered experiment (Fig. 3) where the dorsal side lengthened and the ventral side shortened. This was due to honeybees’ need to improve their flying stability by slightly adjusting the abdominal bending. However, honeybees bended their abdomens remarkably to flounce off manacle in the tethered experiment. At this point, the dorsal side was lengthened and the ventral side was shortened. All of the results show that abdominal bending in the sagittal plane of honey bees is unidirectional (only towards the ventral side).

FIM Microstructure of Honeybee Abdomen. To further explore the role of the adaptive segmented structure of the honeybee abdomen while curling, FSs and ESEM were used to investigate the segmented structure. The 10 μm FSs exposed the microscopic structure between the segments of abdomen under a high-power microscope. The images of FSs demonstrated that the FIM of tergum is bilayer (Fig. 4a),
whereas the FIM of sternum is monolayer (Fig. 4c). In addition, the material of FIM was more flexible than that of the chitin in abdominal terga and sterna (Nenchev et al. 2007). In the dyed specimens of the FSs, the connection among the segments of tergum was in Z shape with bilayer membrane, whereas the FIM of the sternum was in S shape with monolayer membrane. This structure was called “Z–S” configuration (Fig. 4a–h). These two types of FIMs connected the adjacent terga and sterna to build a deformable sealed structure, thereby providing new clues to examine the mechanism of unidirectional bending.

Furthermore, the microstructure of the connecting parts was observed in 3D view using ESEM to gain some details regarding “Z–S” configuration. As shown in Fig. 4(i–p), the thickness of FIM between the adjacent terga and sterna were 24.63 ± 3.26 and 14.25 ± 2.72 μm, respectively. The overall result showed that when the abdominal muscle connected with the upper tergum drags the lower tergum to the right, the shape of FIM between the adjacent terga changes. Indeed, the terminal of Z-shaped FIM was resisted on the upper tergum, which limits the movement of the lower tergum. Meanwhile, the abdominal muscle connected with the upper sternum drags the lower sternum to the left.

Discussion
A geometrical model was proposed to illustrate the bending behavior of honeybee abdomen (Fig. 5e). As illustrated in Fig. 5a–d, the location of FIM can be recognized in an independent honeybee (Fig. 5a), and this finding can be identified more clearly in cross section (Fig. 5c). The zoom-in images (Fig. 5b and d) of FIM intuitively demonstrated the “Z–S” configuration. With the aim of depicting the bending mechanism, Fig. 5e shows the function of FIM in the process of unidirectional bending of the honeybee abdomen. When the free end of the Z-shaped FIM between the adjacent terga became in contact with the lower surface of the pro-tergum, the Z-shaped FIM locked the position of FIM and terga. For the time being, the honeybee abdomen could not curl upward anymore. In contrast, it can curl to the ventral side, which resulted in the Z-shaped FIM breaking away from the lower surface of the pro-tergum. While the free end of the Z-shape FIM became in contact with the upper surface of the posttergum, the Z-shaped FIM locked the position of the FIM and terga again. Meanwhile, the honeybee abdomen bent to the ventral limit position (Fig. 5e).

Guided by the detailed mechanistic insights into the function of FIM in the process of unidirectional bending, a geometrical model of

![Fig. 4. Microstructure imaging of the connections in honeybee abdomen. (a, c) The configuration of the honeybee FIM in the optical observation of the dyed FS and the connection between the third and fourth segments. The membrane between terga had a bilayer structure, but the one between sterna was monolayered. Trg, tergum; fim, folded intersegmental membrane; stm, sternum; (i, k) 3D structure of the connections between terga and sterna. The connection of tergum is the Z shape with bilayer membrane, whereas the FIM of the sternum is the S shape with monolayer membrane; (b, d, e–h, j, l, m–p) Magnified images of FIMs in honeybee abdomen.](image-url)
the Z–S configuration can be stretched as one four-bar link mechanism with an extendible linkage (Suppl Information [online only]).

The amount of abdominal bending can be measured by the angle $h$ as the function of $a$, $l_3$, which is limited within the geometrical region $a^*$ and $l_3^*$:

$$h = F(a, l_3), \quad a \in a^*, l_3 \in l_3^*. \quad (1)$$

where $\theta$ is the angle between two adjacent abdominal segments, which defines the bending angle of the abdomen, $\alpha$ is the angle between the free end of the Z-shaped FIM and pro-tergum, and $l_3$ is the straight-line distance of S-shaped FIM. The relationship among the three parameters is represented in Fig. 5b–d. The distributed points on the curved surface all satisfied $\theta > 0$, indicating that the abdomen can only curl unidirectionally.

According to the results of the modeling analysis in Fig. 5f, the angle $\theta$ is always larger than $0^\circ$, which means that the abdomen can curl only unidirectionally. The error $\Delta \theta$, which represents the difference between the theoretical angle $\theta$ and the experimental angle $\theta^*$, is defined as $\Delta \theta = |\theta - \theta^*| = |9^\circ34' - 9^\circ17'| = 0^\circ17'$, which validates the theoretical analysis. The images of macroscopic motion captured by high-speed video camera and the results of modeling analysis illustrated the unidirectional bending of the honeybee abdomen, which overturns the traditional view of its curling.

No research had been reported on unidirectional bending of honeybee abdomen until high-speed video images of unidirectional bending of honeybee abdomen were captured and the FIM structure was described as “Z–S” configuration in this study. Previous studies have shown that when bees are restrained for tethered flight preparations, they have very exaggerated abdominal movements (Michelsen et al. 1992, Dornhaus and Chittka 1999, Marco et al. 2008). Although several theories on how the abdomen could control flight exist, no firm tests have been performed to validate these theories or to verify the key role of abdominal movements in flight control (Capaldi et al. 2000, Srinivasan et al. 2000, Luu et al. 2011). In this study, the behavior of
honeybee abdomen was examined to gain insights into the new view of abdominal deformation. The integrated motions of abdomen are sufficient for maintaining stable flight of honeybee by actively adjusting its body shape to control flight (Dyhr et al. 2013, Taylor et al. 2013). However, abdominal movements contain not only the integrated motions of abdomen but also the curling of the abdomen itself. The present findings suggest that the abdomen of a free-flying bee could only bend unidirectionally. Morphological observation and microstructure imaging were first utilized to investigate the relationship between FIM configuration and unidirectional bending. The observed microstructure of FIM indicates the mechanism of unidirectional bending. The special structure of FIM led to the function of locking, which legitimately elaborates two kinds of stable configurations in the bending process. This intriguing feature may provide more evidence with liberal abdominal movements to reveal the active role of the abdomen in flexible strategies for flight control. Future works should focus on the mechanism of curling of the abdomen and its function in free-flying control.

Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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References Cited
Capaldi, E. A., A. D. Smith, J. L. Osborne, S. E. Fahrbach, S. M. Farris, D. R. Reynolds, A. S. Edwards, A. Martin, G. E. Robinson, G. M. Poppy, et al. 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature 403: 537–540.
Clarke, D., H. Whitney, G. Sutton, and D. Robert. 2013. Detection and learning of floral electric fields by bumblebees. Science 340: 66–69.
Dornhaus, A., and L. Chittka. 1999. Evolutionary origins of bee dances.

References Cited
Capaldi, E. A., A. D. Smith, J. L. Osborne, S. E. Fahrbach, S. M. Farris, D. R. Reynolds, A. S. Edwards, A. Martin, G. E. Robinson, G. M. Poppy, et al. 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature 403: 537–540.
Clarke, D., H. Whitney, G. Sutton, and D. Robert. 2013. Detection and learning of floral electric fields by bumblebees. Science 340: 66–69.
Dornhaus, A., and L. Chittka. 1999. Evolutionary origins of bee dances.

Nature 401: 38.
Dyhr, J. P., K. A. Morgansen, T. L. Daniel, and N. J. Cowan. 2013. Flexible strategies for flight control: an active role for the abdomen. J. Exp. Biol. 216: 1523–1536.
Esch, H. E., S. W. Zhang, M. V. Srinivasan, and J. Tautz. 2001. Honeybee dances communicate distances measured by optic flow. Nature 411: 581–583.
Finlayson, L. H., and O. Lowenstein. 1958. The structure and function of abdominal stretch receptors in insects. P. R. Soc. Lond. B. Biol. Sci. 148: 433–449.
Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine. 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature 491: 105–108.
Hager, F. A., and W. H. Kirchner. 2013. Vibrational long-distance communication in the termites Macrotermes natalensis and Odontotermes sp. J. Exp. Biol. 216: 3249–3256.
Headrick, D. H., and G. Gorth. 2009. Anatomy: Head, Thorax, Abdomen, and Genitalia, pp. 11–20. In V. H. Resh and T. C. Ring (eds.), Encyclopedia of insects. Academic Press, Burlington, MA.
Heinrich, B. 1974. Thermoregulation in endothermic insects. Science 185: 747–756.
Luu, T., A. Cheung, D. Ball, and M. V. Srinivasan. 2011. Honeybee flight: a novel ‘streamlining’ response. J. Exp. Biol. 214: 2215–2225.
Marco, R.J.D., J. M. Gurvitz, and R. Menzel. 2008. Variability in the encoding of spatial information by dancing bees. J. Exp. Biol. 211: 1635–1644.
Michelsen, A., B. B. Andersen, J. Storm, W. H. Kirchner, and M. Lindauer. 1992. How honeybees perceive communication dances, studied by means of a mechanical model. Behav. Ecol. Sociobiol. 30: 143–150.
Nachtigall, W., A. Wissel, and D. Elsinger. 1998. Flight of the honey bee. VIII. Functional elements and mechanics of the “flight motor” and the wing joint-one of the most complicated gear-mechanisms in the animal kingdom. J. Comp. Physiol. B. 168: 323–344.
Nenchev, P., I. Zhelyazkova, I. Chakmak, and S. Seven. 2007. Morphological characteristics of the honey bee reared in Strandzha-Sakar, Bulgaria. I. Color of the abdomen, parameters of the proboscis and the wing. J. Anim. Sci. 44: 69–73.
Nunes, T. M., S. Mateus, A. P. Favaris, M.F.Z.J. Amaral, L. G. von Zuben, G. C. Clossoksi, J. M.S. Bento, B. P. Oldroyd, R. Silva, R. Zucchi, et al. 2014. Lopes queen signals in a stingless bee: suppression of worker ovary activation and spatial distribution of active compounds. Sci. Rep. 4: 7449.
Oldroyd, B. P., and S. Wonsiri. 2006. Asian honey bees: biology, conservation, and human interactions. Harvard University Press, Cambridge, MA.
Paar, J., B. P. Oldroyd, and G. Kastberger. 2000. Entomology: giant honeybees return to their nest sites. Nature 406: 475.
Pennisi, E. 2001. Bee Dance reveals bee’s-eye view. Science 292: 1628–1629.
Smith, S. J., and H. Hickner. 2007. Family comedy film—bee movie. (http://en.wikipedia.org/wiki/Bee_Movie) (accessed 24 January 2015).
Snodgrass, R. E. 1956. The reproductive system, pp. 134–167. In L. O. Howard et al. (eds.), Anatomy of the honey bee, Chapter 11. Cornell University Press, Ithaca, NY.
Srinivasan, M. V., S. W. Zhang, J. S. Chahl, E. Barth, and S. Venkatesh. 2000. How honeybees make grazing landings on flat surfaces. Biol. Cybern. 83: 171–183.
Stead, N. 2013. The abdomen: the secret to stabilising flight. J. Exp. Biol. 216: i–ii.
Taylor, G. J., T. Luu, D. Ball, and M. V. Srinivasan. 2013. Vision and air flow combine to streamline flying honeybees. Sci. Rep. 3: 2614.

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