

Critical Structure for Telescopic Movement of Honey bee (Insecta: Apidae) Abdomen: Folded Intersegmental Membrane

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Abstract

The folded intersegmental membrane is a structure that interconnects two adjacent abdominal segments; this structure is distributed in the segments of the honey bee abdomen. The morphology of the folded intersegmental membrane has already been documented. However, the ultrastructure of the intersegmental membrane and its assistive role in the telescopic movements of the honey bee abdomen are poorly understood. To explore the morphology and ultrastructure of the folded intersegmental membrane in the honey bee abdomen, frozen sections were analyzed under a scanning electron microscope. The intersegmental membrane between two adjacent terga has a Z-S configuration that greatly influences the daily physical activities of the honey bee abdomen. The dorsal intersegmental membrane is 2 times thicker than the ventral one, leading to asymmetric abdominal motion. Honey bee abdominal movements were recorded using a high-speed camera and through phase-contrast computed tomography. These movements conformed to the structural features of the folded intersegmental membrane.

Key words: honey bee, abdomen, folded intersegmental membrane, morphology, telescopic movement

The morphology and ultrastructure of honey bees (*Apis mellifera ligustica*) have attracted increasing attention in the past few decades (Snodgrass 1942; Deseyn and Billen 2005; Levy 2011; Rogers et al. 2013). Nevertheless, the special structures that aid movement in the segmental abdomen are rarely discussed. We previously investigated the asymmetric motion of the segmental abdomen and observed that the folded intersegmental membrane (FIM) contributes to the flexible athletic ability of the honey bee abdomen (Zhao et al. 2015). However, the details of the morphology and ultrastructure of the folded intersegmental membrane distributed in the honey bee's abdomen have not been explored. In addition, the previous work just discussed the function of dorsal (Z-shaped) FIM in the process of unidirectional bending of the honey bee abdomen. Here we further investigate the telescopic movement of the honey bee abdomen and reveal the cooperation of both the Z-shaped FIM and S-shaped FIM in controlling the telescopic movement of honey bee abdomen.

In studying any part of the honey bee body wall, an observer must remember that cuticular protein and chitin, which can be tough and elastic, are the main substance constituting the exoskeleton of a honey bee (Vincent and Wegst 2004; Kucharski et al. 2007). A honey bee has no tough framework of bones such as that of vertebrates; instead, the chitin provides a hard shell so that the muscles can attach to the adjacent body wall. The segmented body

wall of cuticle is divided into plates called sclerites, which connect with each other through the membranes (Vincent and Wegst 2004). In each segment of the body wall, the terga and sterna are furthermore subdivided into smaller plates called tergites and sternites, respectively (Noirot and Quennedey 1974; Guerino and Cruz-Landim 2002).

The membranes connect the tergum and the sternum in a flexible manner; hence, these structures are usually regarded to be morphologically important. The flexible intersegmental membrane is often sufficient and allows each segment to telescope into the one in front of it (Zhao et al. 2015). However, the details of the morphology and ultrastructure of the folded intersegmental membrane distributed in the honey bee's abdomen have not been explored. Structural details of the FIM and the relationship between the FIM ultrastructure and the honey bee abdominal movement are lacking.

In the present study, a description of FIM morphology and ultrastructure as well as an analysis of honey bee abdominal asymmetric motion are provided.

Materials and Methods

Experimental Animals

Our morphological data deal with foraging adult worker honey bees (*A. mellifera ligustica*). All specimens were collected from a single

hive at Tsinghua University, Beijing, China (40° 00' N, 116° 33' E). To avoid chemical contamination, unfed, laboratory-reared specimens were handled using latex gloves. These specimens were cold anesthetized and dissected; their abdomens were fixed in 4% paraformaldehyde for 2 h.

Histological Sections

The dissected abdominal halves were fixed in 2% cold glutaraldehyde and buffered with phosphate buffer at pH 7.3. After fixation, the dissected abdominal halves were dehydrated using an acetone series (50%, 60%, 70%, 80%, 90%, and 100%) for 10 min in each concentration. The specimens were dried in a Balzers CPD 030 Critical Point and then fixed to aluminum stubs with adhesive tape. After this procedure, the specimens were sectioned to 10 μm thickness using a microtome cryostat (LEICA CM1900, Leica, Wetzlar, Germany); the sections were then arrayed on glass slides. The frozen sections (FSs) were stained using hematoxylin–eosin (AMRESCO LLC, 6681 Cochran Road, Solon, America, Fig. 1) and were documented using a Nikon image acquisition system (Eclipse 90i, Nikon, Tokyo, Japan).

Scanning Electron Microscopy

For observations using scanning electron microscopy (SEM; Philips XL30 ESEM, Philips, Amsterdam, Netherlands, Fig. 1), specimens were completely dehydrated with ethanol (100%) over several stages, dried with hexamethyldisilazane, sputter coated with gold (Emitech K500, Emitech, Ashford, United Kingdom), and then fixed on a rotating specimen holder. Specimens were embedded in methacrylate to investigate the FIM ultrastructure. Cross and longitudinal sectioning was carried out, cutting the middle region of the FIM. Methacrylate was then dissolved using xylol. After gradually replacing xylol by acetone, specimens were dried at the critical point and then examined via SEM.

Observation of Honey bee Abdominal Motion

The setup for observing honey bee abdominal motion comprised a positioner and a microscope (Axiostar Plus, Zeiss, Oberkochen, Germany) equipped with a high-speed camera (Phantom M110, Vision Research, Wayne, NJ, United States, up to 2,000 frames per second). The positioner actuated by a servo motor could move up and down with a motion accuracy of 1 μm . A live honey bee was then glued to the fixture, which was connected to the precision positioner via its thorax so that its abdomen could be moved vertically. We filmed the abdominal motion at a frequency of 500 frames per second (see Supp Video S1). Moreover, we observed the motion of the FIM by using in-line phase-contrast computed tomography in X-ray Imaging and Biomedical Application Beamline of the Shanghai Synchrotron Radiation Facility (see Supp Video S2).

Results

Location of the FIM in Honey bees

The honey bee abdomen, which stretches and deforms in response to physiological activities, contains several segments (Fig. 1). For a thorough inquiry in the morphology and ultrastructure of the honey bee abdomen, FSs and SEM were used to investigate morphing behavior (Fig. 1).

Each segment of the abdomen consists of a sclerotized tergum, a sternum, and sometimes a pleurite. The FIM divides the ring-like anterior abdominal sternite from the cuticular ring of the posterior abdominal sternite.

Functional Morphology of Dorsal FIM and Ventral FIM

Functional Morphology of Dorsal FIM

Figure 2 shows the microscopic structure of the intersegmental membranes of the honey bee abdomen in the dorsal side. The FS

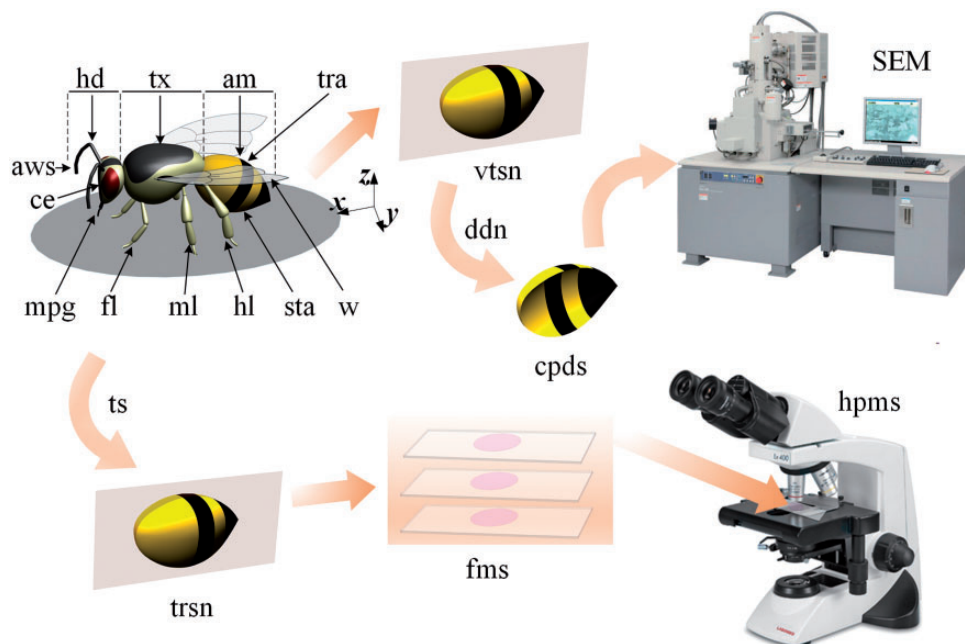


Fig. 1. Materials and methods for observing the morphology and ultrastructure of the honey bee abdomen. Schematic of honey bee orientation and sample extraction methods. A model of *Apis mellifera ligustica* with major body parts labeled. The sample size is 40. hd, head; tx, thorax; am, abdomen; aws, antenna with segments; ce, compound eye; mpg, mouthparts (maxillary palps and galea); fl, foreleg; ml, midleg; hl, hindleg; tra, terga; sta, sterna; w, wing; ts, take samples; trsn, transverse section; vtsn, vertical section; ddn, dehydration; fms, freezing microtome section; cpds, critical point drying samples; hpms, high-power microscope; sem, scanning electron microscope.

images demonstrate that the FIM of the tergum is a bilayer (Fig. 2a). At the end of each tergum, a fraction bends to the inner abdominal space and forms a knoblike protuberance (Fig. 2b). At the portion of bending, a length of the semi-rigid cuticle shield extends along its original direction. It bends back and stores the elastic energy with the elastic deformation of FIM and semi-rigid cuticle, which can be regarded as an elastic buffer structure. The knoblike protuberance is covered by a layer of the membrane labeled as a submarginal ridge. This feature stiffens an apodeme in insects and maximizes the secondary area by being hollow. For example, the wings and elytra are stiffened because of their overall curved shapes. The exoskeleton is further stiffened by ribs, webs, stringers, and flanges made from stiff, sclerotized cuticle, which leads to the production of structures that resemble those of aerospace structures; in some parts, the cuticle even forms sandwich-like structures (Vincent and Wegst 2004). At the tip of the protuberance, a layer of granulated tissue attaches to the cuticle. The terminal part of the protuberance tissue is loosely packed, with the middle being much tighter through a structure analogous to the cancellous and compact bones (Fig. 2b and c).

In the dyed FS specimens, the connection between each segments of the tergum is in the Z shape with the bilayer membrane (Fig. 2c). The transparency of the FIM is also much greater than that of the cuticle tergum; hence, the membrane is probably more flexible than the tergum. The membrane spread over the knoblike protuberance

becomes much thinner in the middle position of the dorsal FIM (Fig. 2c). At the other terminal portion of the FIM, an interspinal structure is attached to the anterior tergum. The structure has numerous cuticular spines, forming a low-lying bumpy texture that is similar to a rack of gear engagement (Fig. 2c).

At the elastic buffer structure of the semi-rigid cuticle shield, a Z-shaped folding structure is formed with the protuberance from part of the semi-rigid cuticle shield (Fig. 3a and b). The radius of the knoblike protuberance is $37.3 \pm 0.6 \mu\text{m}$. The distance between the center of the knoblike protuberance and the inflection point of the posterior tergum is $120.8 \pm 1.3 \mu\text{m}$. The lengths of the long and short sides of the dorsal FIM are 306.4 ± 6.4 and $130.7 \pm 4.2 \mu\text{m}$, respectively. As shown in Fig. 3c and d, the bending angle of the posterior tergum is $60.4^\circ \pm 2.4^\circ$. The thickness of the dorsal FIM is $14.5 \pm 1.2 \mu\text{m}$. However, the thickness of the posterior tergum is $22.6 \pm 2.3 \mu\text{m}$, which is higher than that of the posterior tergum (Fig. 3d).

Functional Morphology of Ventral FIM

Similarly, FS images demonstrate that the ventral FIM in the honey bee abdomen is a monolayer (Fig. 4a and b). The morphology of the ventral FIM is similar to the capitalized letter "S". Hence, we called this structure the S-configuration FIM. At the end of each segment

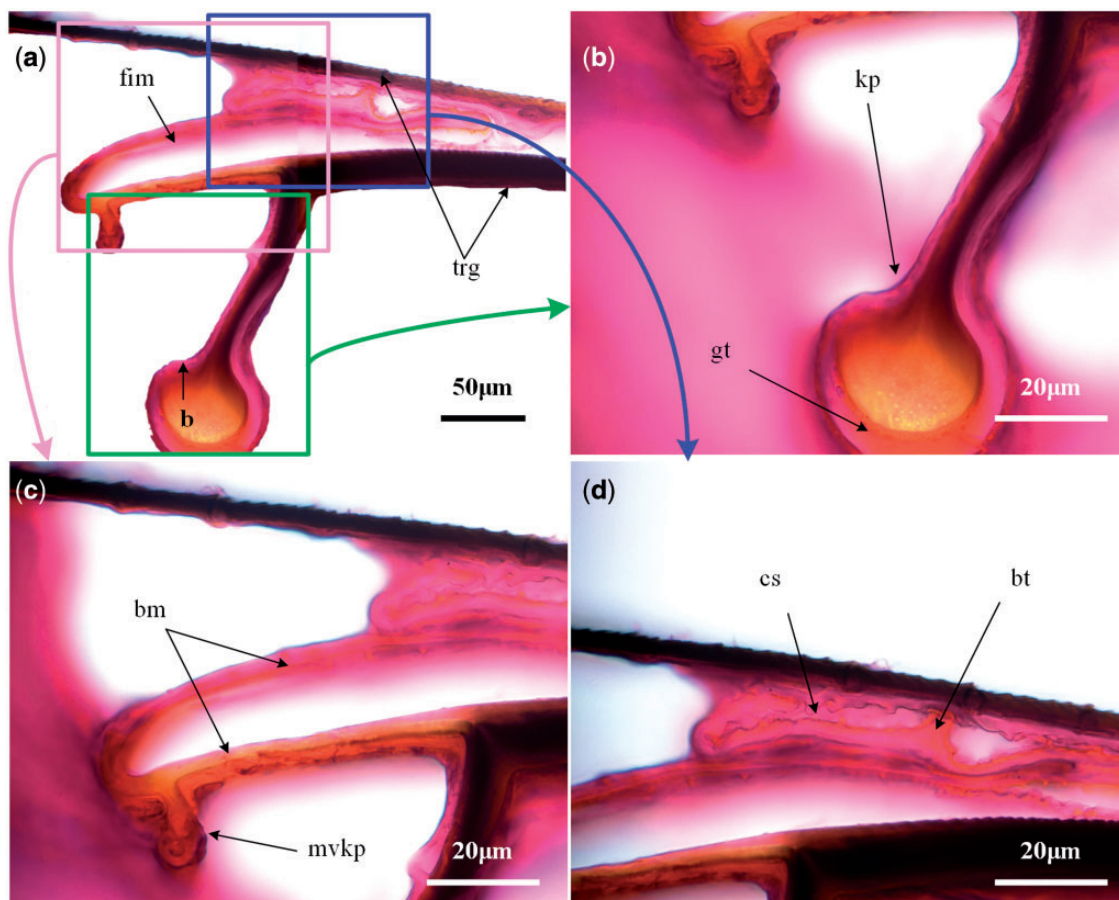


Fig. 2. Histological sections of the dorsal FIM in the honey bee abdomen. (a) Configuration of the dorsal FIMs during FS observation and connection between the third and fourth segments. The membrane between terga has a bilayer structure. Trg, tergum; fim, folded intersegmental membrane; (b) an elastic buffer structure of the knoblike protuberance. kp, knoblike protuberance; gt, granulated tissue. (c) Z-shaped dorsal FIM. bm, bilayer membrane; mvkp, miniature version of the knoblike protuberance. (d) Interspinal structure of the dorsal FIMs in the honey bee abdomen. The tergum connection with the bilayer membrane has a Z-shape. bt, bumpy texture; cs, cuticular spines. The sample size of this observation is 40.

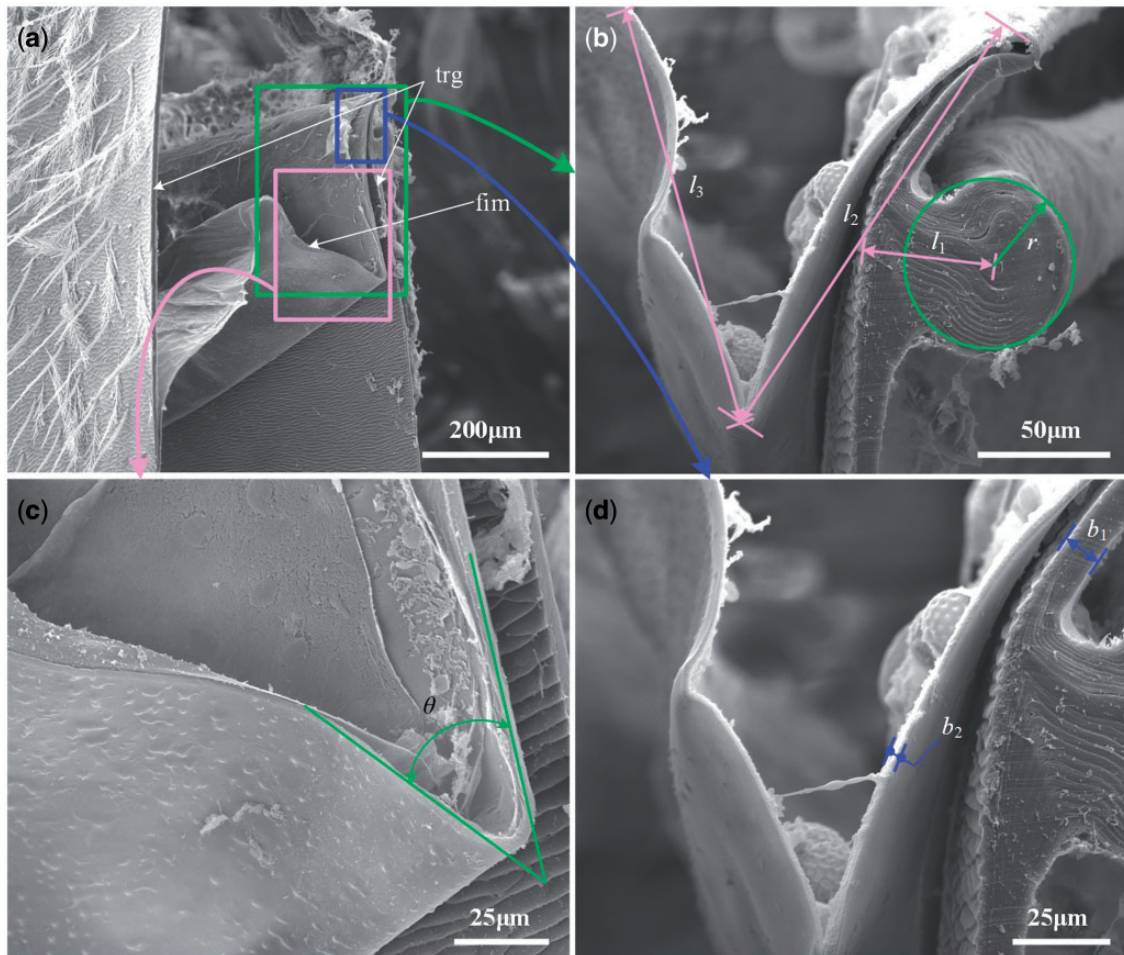


Fig. 3. Cross-section of the dorsal FIM in the honey bee abdomen under SEM. (a) Dorsal FIM configuration under SEM and connection between the third and fourth segments. The membrane between terga has a bilayer structure. Trg, tergum; fim, folded intersegmental membrane; stm, sternum; (b–d) Magnified images of the dorsal FIMs in the honey bee abdomen between the terga and the sterna. The connection of the tergum with the bilayer membrane is in Z-shape. (b) The radius of the knoblike protuberance r is $37.3 \pm 0.6 \mu\text{m}$; the distance between the center of the knoblike protuberance and the inflection point of the posterior tergum l_1 is $120.8 \pm 1.3 \mu\text{m}$; the lengths of the short and long sides of the dorsal FIM l_2 , l_3 are 130.7 ± 4.2 and $306.4 \pm 6.4 \mu\text{m}$, respectively; the lengths of dorsal FIM is l_0 , where $l_0 = l_2 + l_3$. (c) The bending angle of the posterior tergum θ is $60.4 \pm 2.4^\circ$. (d) The thickness of the posterior tergum b_1 is $22.6 \pm 2.3 \mu\text{m}$. The thickness of the dorsal FIM b_2 is $14.5 \pm 1.2 \mu\text{m}$. The sample size of this observation is 40.

of the ventral sternum, a hook-like structure exists, which bends to the inner abdominal space. As shown in Fig. 4c, the hook-like structure has a semi-rigid cuticle shield and bends back for an elastic buffer structure, which is similar to the knoblike protuberance in the dorsal FIM. A sequence of muscle fiber nets connects the outside of the hook with the inner surface of neighboring segment (Fig. 4a). The ventral FIM is attached to the bottom of the hook-like structure. Another terminal of the FIM is connected with the inner surface of the anterior sternum. In addition, many black textures are spread throughout the entire ventral FIM (Fig. 4d). Considering the flexibility and the low intensity of the ventral FIM, we speculated that these black textures contribute to the strength and stiffness of this structure. As the S-shaped FIM appears similar to a flexible rope, it can limit the bending movement of the abdomen.

The microstructure of the ventral FIMs was observed in 3D using SEM to gain further details of the S-configuration (Fig. 5a). Between the two neighboring segments, a membrane-like structure is derived from the muscle tissue in the lower part of the ventral shield, the end of which links to the upper shield, thereby making the abdomen closer to the external environment (Fig. 5a). The outside of hook-like structure contains a rigid cuticle with a membrane structure

attached, whereas the inside of this structure contains a semi-rigid cuticle (Fig. 5a and b). The lengths of the long and short shafts of the hook-like structure are 26.3 ± 1.4 and $12.4 \pm 1.1 \mu\text{m}$, respectively. The distance between the center of the hook-like structure and the inflection point of the posterior sternum is $84.5 \pm 3.7 \mu\text{m}$. The thickness of the long handle of the hook-like structure is $20.7 \pm 1.4 \mu\text{m}$ (Fig. 5b). As shown in Fig. 5c, numerous muscle fibers are attached to the external surface of the hook-like structure. The diameter of each muscle fiber is $20.8 \pm 1.2 \mu\text{m}$ (Fig. 5c). The thickness of the posterior sternum is $12.7 \pm 1.5 \mu\text{m}$. By contrast, the S-configuration FIM with a thickness of $6.8 \pm 1.2 \mu\text{m}$ is much thinner than the posterior tergum (Fig. 5d).

Table 1 shows different measurements on the FIM ultrastructure in the honey bee abdomen and statistical data on FIM sizes in the adjacent abdominal segments.

Comparison of Morphological Parameters between the Dorsal and Ventral FIMs

As shown in Fig. 6a, the honey bee abdomen has six exposed abdominal segments; the FIMs in the dorsal and ventral sides are labeled with

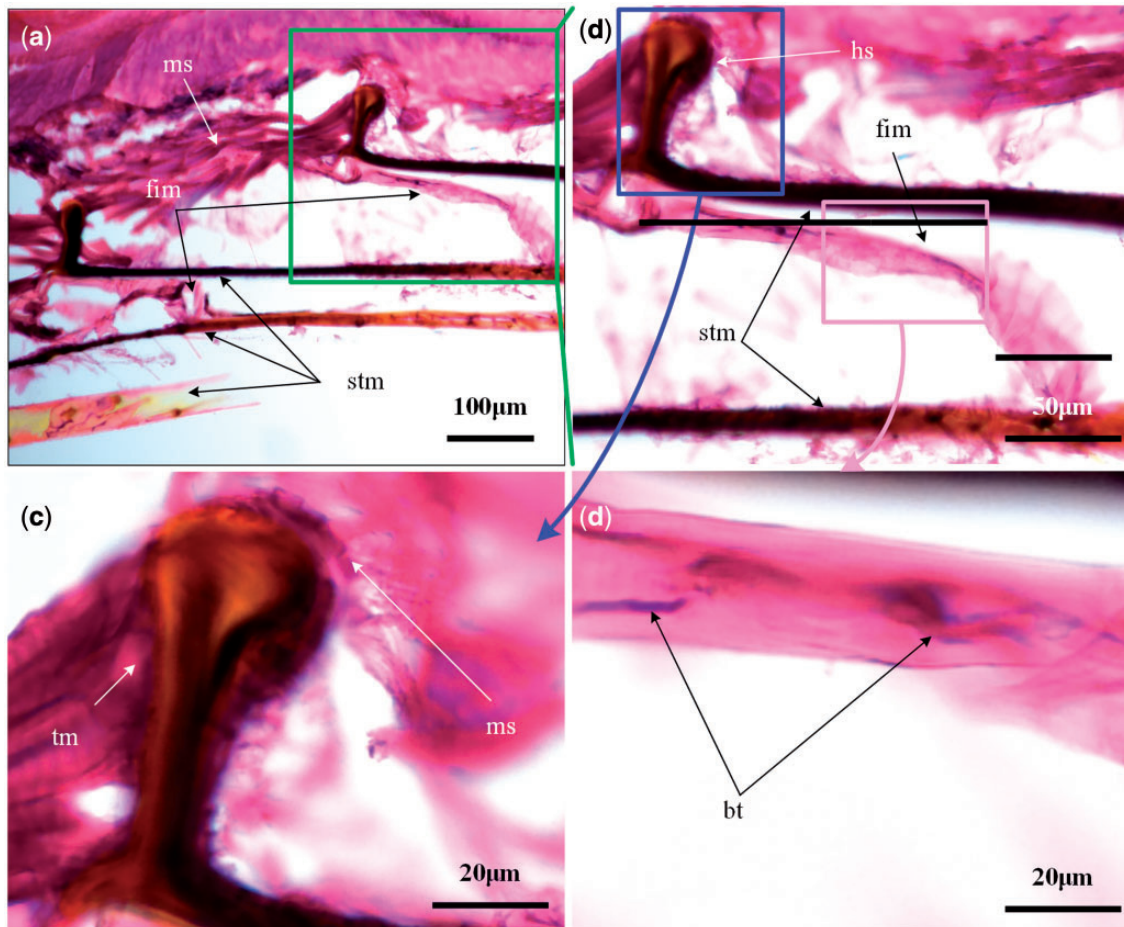


Fig. 4. Histological sections of the ventral FIM in the honey bee abdomen. (a) Internode connection structure in the ventral side of the honey bee abdomen. fim, folded intersegmental membrane; ms, muscle; stm, sternum; (b) Configuration of the ventral FIM under SEM and connection between the third and fourth segments. hs, hook-like structure; (c) Magnified image of the hook-like structure in the posterior sternum of the honey bee abdomen. ms, membrane structure; tm, termination of muscle fibers; (d) Ultrastructure of the ventral FIM. bt, black texture. The sample size of this observation is 40.

different numbers. Observations show that only an intersegmental fold exists in the junction of the dorsal shield, whereas a complex structure exists in the junction of the ventral shield. The length of the dorsal FIM is about double that of the ventral FIM. From the first abdominal segment (AS1) to the sixth abdominal segment (AS6), the FIM lengths initially increase and then decrease. The longest FIMs in the dorsal and ventral sides of the abdomen are DF3 and VF3, with lengths of 437.1 ± 6.4 and 219.5 ± 5.2 μm , respectively (Fig. 6b). The lengths of the bended structure in the dorsal and ventral sides similarly increase and then decrease from AS1 to AS6. The maximum lengths of the bended structure appear in DF3 and VF3 (i.e., 120.8 ± 1.3 and 84.5 ± 3.7 μm , respectively) (Fig. 6c). In addition, the ventral FIM is always thinner than the dorsal FIM in the same abdominal segment. However, the changing trend of the FIM thickness differs from that of the FIM length, with a roughly linear decrease from AS1 to AS6. The maximum FIM thicknesses in the dorsal and ventral sides of the abdomen are 16.3 ± 0.8 and 9.5 ± 1.2 μm , respectively (Fig. 6d). Meanwhile, the maximum thicknesses of the cuticle exoskeletons in DF1 and VF1 are 26.8 ± 2.1 and 15.7 ± 1.8 μm , respectively (Fig. 6e). The thickness of the cuticle exoskeletons also gradually decreases. Until DF5 and VF5, the thickness of the cuticle exoskeletons decreases to 20.9 ± 2.1 and 11.7 ± 1.3 μm , respectively (in Fig. 6e).

Discussion

Numerous types of special structures and insect abilities have been previously observed (Hidetoshi 2003; Herb et al. 2012; Burrows and Sutton 2013), which indicate that a special structure usually corresponds to a specific area of functionality. Compared with our previously work (Zhao et al. 2015) on analysis of Flexion and Extension of honey bee abdomen, we further explore the morphology and ultrastructure of the folded intersegmental membrane in this study. The telescopic movement of the honey bee abdomen is firstly provided by high-speed video images. Through numerous experiments, the morphological parameters of the dorsal and ventral FIMs from the first abdominal segment (AS1) to the sixth abdominal segment (AS6) are provided in detail. The interrelation between the cooperation of the two types FIMs and telescopic movement of honey bee abdomen is revealed.

Through numerous experiments, we observed the telescopic movement of the honey bee abdomen under a high-speed camera. Whether or not the honey bee abdomen bends upward while its thorax is fixed on the panel was investigated. Current results show that the abdomen moves freely, as recorded using a high-speed camera from 0 to 450 ms (Supp Video S1). The terga contour curve lengthens while the sterna contour curve shortens as the abdomen curling process increases from the minimum amount to the maximum

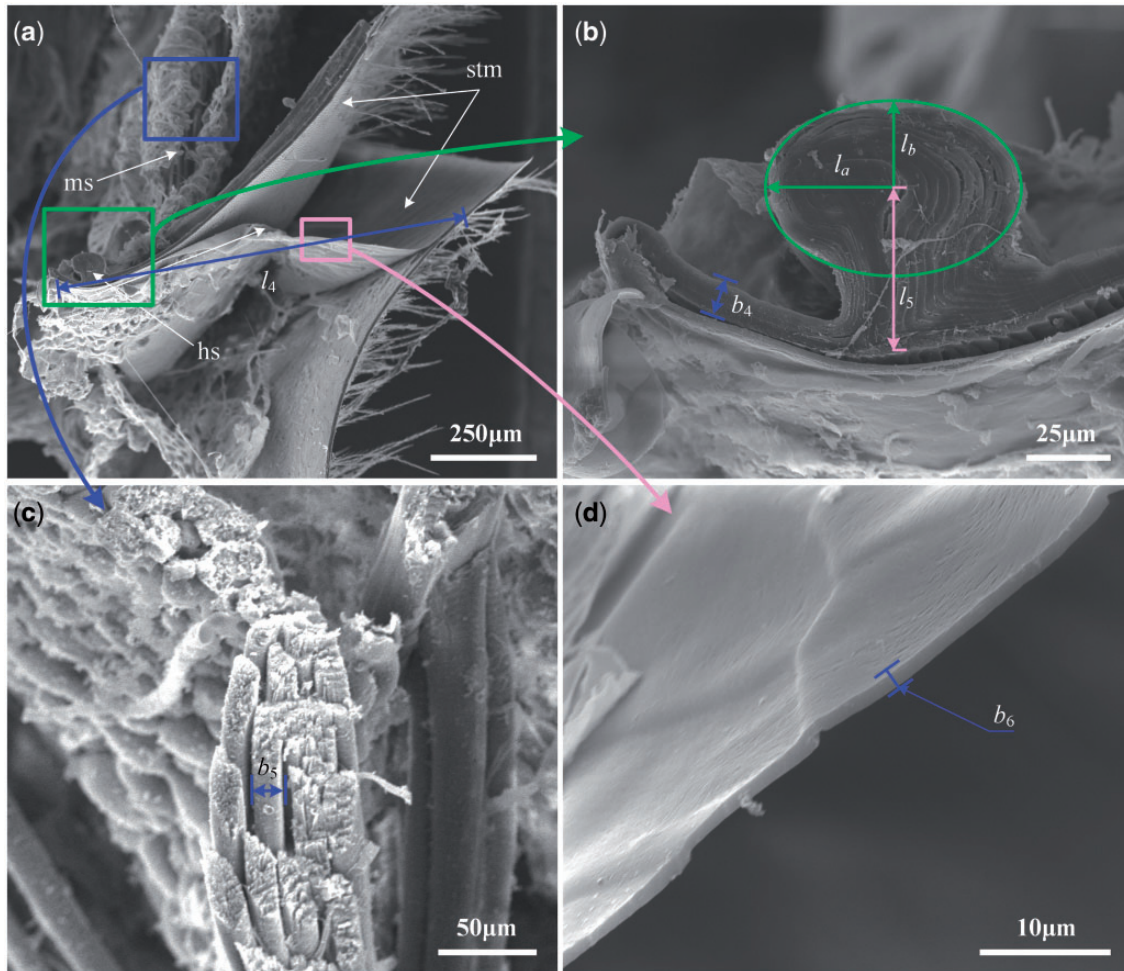


Fig. 5. Cross-section of the ventral FIM in the honey bee abdomen under SEM. (a) Ventral FIM configuration under SEM. The membrane between sterna has only one layer. fim, folded intersegmental membrane; stm, sternum; the lengths of ventral FIM l_4 is $219.5 \pm 5.2 \mu\text{m}$. (b–d) Magnified images of the ventral FIM in the honey bee abdomen. The ventral FIM has an S-shaped connection with the monolayer membrane. (b) The lengths of the long and short shafts of the hook-like structure, l_a , l_b , are 26.3 ± 1.4 and $12.4 \pm 1.1 \mu\text{m}$, respectively. The distance between the center of the hook-like structure and the inflection point of the posterior sternum l_s is $84.5 \pm 3.7 \mu\text{m}$. The thickness of the long handle of the hook-like structure b_4 is $20.7 \pm 1.4 \mu\text{m}$. (c) The diameter of each muscle fiber b_5 is $20.8 \pm 1.2 \mu\text{m}$. (d) The S-configuration FIM thickness. The thickness of S-configuration FIM b_6 is $6.8 \pm 1.2 \mu\text{m}$. The sample size of this observation is 40.

Table 1. Morphological characterization of FIMs in the honey bee abdomen

FIM	Length of FIM (μm)		Length of bended structure (μm)		Thickness of FIM (μm)		Thickness of cuticle exoskeleton (μm)	
	DF	VF	DF	VF	DF	VF	DF	VF
AS1	397.4 ± 5.9	192.9 ± 5.1	103.9 ± 1.5	72.4 ± 3.3	16.3 ± 0.8	9.5 ± 1.2	26.8 ± 2.1	15.7 ± 1.8
AS2	413.4 ± 5.7	206.7 ± 4.6	115.4 ± 2.1	79.3 ± 3.2	15.6 ± 0.7	7.9 ± 1.4	25.2 ± 2.1	14.3 ± 1.3
AS3	437.1 ± 6.4	219.5 ± 5.2	120.8 ± 1.3	84.5 ± 3.7	14.5 ± 1.2	6.8 ± 1.2	22.6 ± 2.3	12.7 ± 1.5
AS4	403.0 ± 6.2	201.4 ± 4.9	110.5 ± 1.6	78.6 ± 3.4	13.3 ± 1.0	6.2 ± 0.9	21.6 ± 2.0	12.1 ± 1.5
AS5	384.7 ± 5.9	187.6 ± 4.9	100.2 ± 1.6	69.2 ± 3.5	12.3 ± 0.8	6.0 ± 0.7	20.9 ± 2.1	11.7 ± 1.3

AS, abdominal segment; DF, dorsal FIM; VF, ventral FIM.
The sample size is 60.

amount. Unidirectional bending of the honey bee abdomen does not influence the internal organization of the abdomen. In addition, unidirectional bending still exists despite the removal of all the internal organizations (Supp Video S2). These results show that the abdominal bending in the sagittal plane of honey bees is asymmetric. These two FIM types that connect adjacent terga and sterna build a deformable sealed structure to provide convenience for the asymmetric bending of the honey bee abdomen.

The dorsal and ventral FIMs have Z-configuration and S-configuration, respectively. Hence, we called them Z–S configuration FIMs. When the free end (B) of the Z-shaped FIM between the adjacent terga meets the lower surface of the pro-tergum, the Z-shaped FIM limits the further movement of the FIM and the terga with the other end (F) of the Z-shaped FIM, pressing to the upper surface of the post-tergum. Meanwhile, the S-shaped FIM is determinedly bent and folded, the progress of which is controlled by abdominal

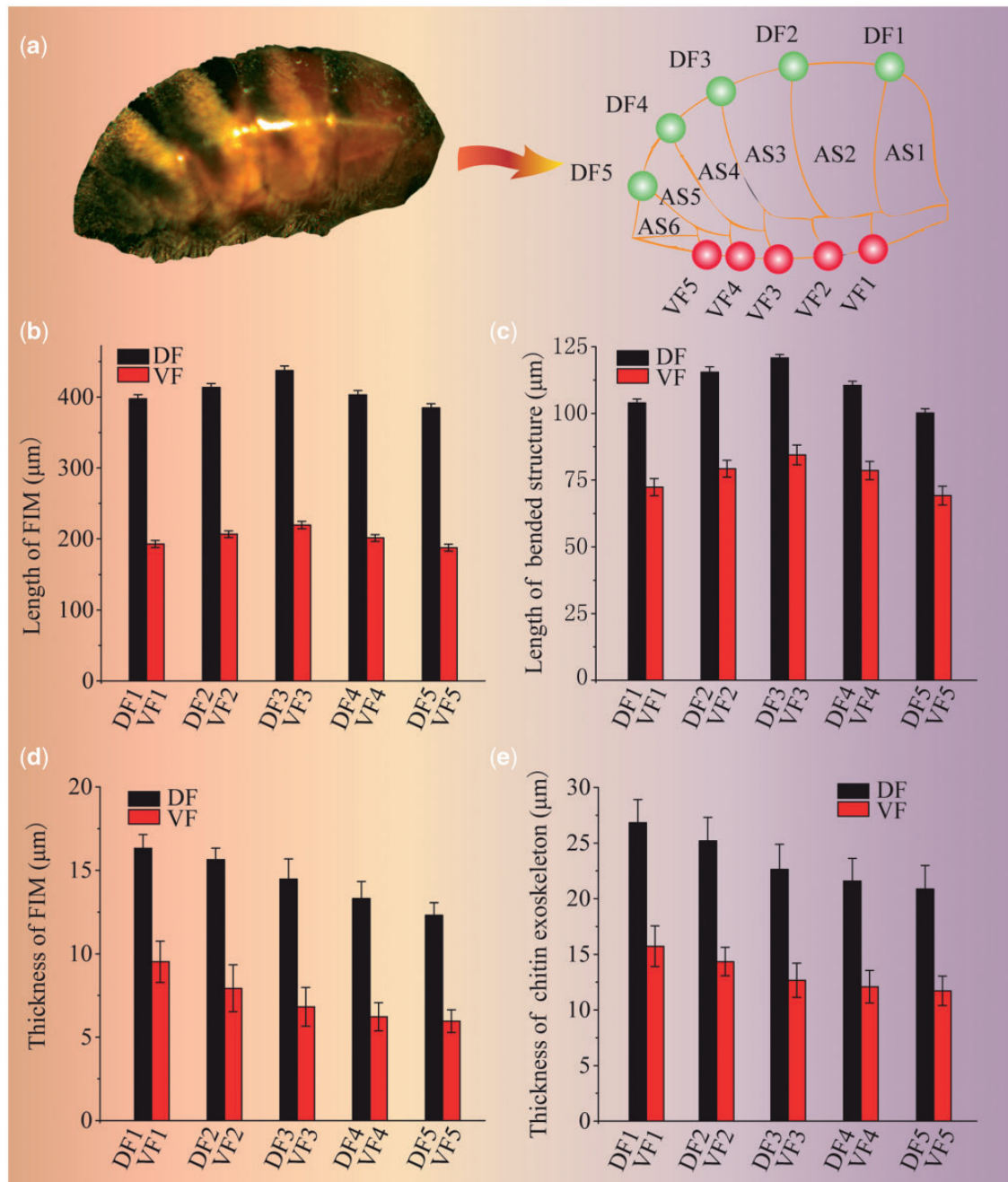


Fig. 6. Comparison of morphological parameters between the dorsal and ventral FIMs. (a) The FIMs in the dorsal and ventral sides are labeled with different numbers. DF, dorsal FIM; VF, ventral FIM; AS, abdominal segment; (b) FIM lengths in the dorsal and ventral sides of the honey bee abdomen. (c) Bended structure lengths in the dorsal and ventral sides of the honey bee abdomen. (d) Changing trend of the FIM thickness in the dorsal and ventral sides of the honey bee abdomen. (e) Chitin exoskeleton thickness from the first abdominal segment to the sixth abdominal segment. The error bars are same with those listed in Table 1. The sample size of honey bees is 40.

muscles and limited by the Z-shaped FIM. At this stage, the honey bee abdomen could not curl up anymore. When the upper muscle in the elastic buffer structure relaxes and the lower muscle between the adjacent sterna contracts, the honey bee abdomen can curl to the ventral side. This phenomenon causes the Z-shaped FIM (B) to break away from the lower surface of the pro-tergum. The Z-shaped folding structure begins to extend, thereby changing the relative location of the two neighboring segments of the dorsal shield. As the terminal end (F) of the Z-shaped FIM meets the lower surface of the pro-tergum, the Z-shaped FIM limits the further movement of FIM

and tergum again. Meanwhile, the S-shaped FIM becomes stretched in a straight-line shape, limiting the abdomen to further bend toward the ventral side. Up to this point, the honey bee abdomen bends to the ventral limit position.

The effect of abdominal behavior on honey bee flight have been received much attention from the researchers (Capaldi et al. 2000; Taylor et al. 2013). However, the mechanism of abdominal behavior has been reported rarely. This study for the telescopic movement of the honey bee abdomen is the first to provide high-speed video images of asymmetric morphing and describe the FIM structure as

Z–S configuration. Comprehensive methods of morphological observation and microstructure imaging were initially used to investigate the relationship between the FIM configuration and the asymmetric morphing of the honey bee abdomen. The special morphology of the FIM in the honey bee abdomen is ideally suited to design a new metamorphic mechanism, particularly a special deformation mechanism, in the future.

Supplementary Data

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

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The authors have declared that no competing interests exist.

References Cited

- Burrows, M., and G. Sutton. 2013. Interacting gears synchronize propulsive leg movements in a jumping insect. *Science* 341: 1254–1256.
- 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 honey bee revealed by harmonic radar. *Nature* 403: 537–540.
- Deseyn, J., and J. Billen. 2005. Age-dependent morphology and ultrastructure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae). *Apidologie* 36: 49–57.
- Guerino, A. C., and C. D. Cruz-Landim. 2002. Ultra-estrutura de glandulas abdominais tegumentares em *Oxaea flavescens* (Hymenoptera, Andrenidae, Oxaeinae). *Iheringia Ser. Zool.* 92: 37–45.
- Herb, B. R., F. Wolschin, K. D. Hansen, M. J. Aryee, B. Langmead, R. Irizarry, G. V. Amdam, and A. P. Feinberg. 2012. Reversible switching between epigenetic states in honey bee behavioral subcastes. *Nat. Neurosci.* 15: 1371–1373.
- Hidetoshi, I. 2003. A reconstruction method of projection image on worker honey bees' compound eye. *Neurocomputing* 52: 561–566.
- Kucharski, R., J. Maleszka, and R. Maleszka. 2007. Novel cuticular proteins revealed by the honey bee genome. *Insect Biochem. Mol. Biol.* 37: 128–134.
- Levy, S. 2011. The pollinator crisis: what's best for bees. *Nature* 479: 164–165.
- Noirot, C., and A. Quennedey. 1974. Fine structure of insect epidermal glands. *Annu. Rev. Entomol.* 19: 61–80.
- Rogers, L. J., E. Rigosi, E. Frasnelli, and G. Vallortigara. 2013. A right antenna for social behaviour in honey bees. *Sci. Rep.* 3: 2045.
- Snodgrass, R. E. 1942. *The skeleto-muscular mechanisms of the honey bee*, 1st ed. Smithsonian Institution, Washington, DC.
- Taylor, G. J., T. Luu, D. Ball, and M. V. Srinivasan. 2013. Vision and air flow combine to streamline flying honey bees. *Sci. Rep.* 3: 2614.
- Vincent, J. F. V., and U. G. K. Wegst. 2004. Design and mechanical properties of insect cuticle. *Arthropod. Struct. Dev.* 33: 187–199.
- Zhao, J., J. Wu, and S. Yan. 2015. Movement analysis of flexion and extension of honey bee abdomen based on an adaptive segmented structure. *J. Insect Sci.* 15: 109. DOI: 10.1093/jisesa/iev089