

Functional Morphology of the Honey Stomach Wall of the European Honey Bee (Hymenoptera: Apidae)

D. SAMMATARO¹ AND JOSEPH M. CICERO²

Ann. Entomol. Soc. Am. 103(6): 979–987 (2010); DOI: 10.1603/AN09111

ABSTRACT The crop, or honey stomach, of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is invested in cords of muscles that are numerous enough, in both latitudinal and longitudinal directions, to fully enclose and confine the underlying, cuticle-lined epithelium. Although appressed against the inner wall of this enclosure by the crop's contents, the epithelium is largely free of ligations that would immobilize it. It can therefore slide on the inner wall and undergo extensive pleating as needed to conform to the diameter of the enclosure, regardless of the extent of contraction or distention. The two primary components of the epithelial layer, epidermal cells and procuticle, can undergo extreme compression to maintain pleats while enduring the pressure exerted by the volume of crop contents. During engorgement with nectar, the muscular enclosure relaxes to larger and larger diameters. Correspondingly, pleats unfold as needed. During dispensation of nectar in the hive, the muscular enclosure contracts and forces the epithelium to pleat itself again. Pleats are present in even the most grossly distended crops, indicating that capacity is not a limiting factor in the volume of nectar a bee can accumulate during foraging. Individual pleats are appressed, too, indicating that a lubricating, cohering substance occurs between them.

KEY WORDS honey stomach, crop, honey bee, *Apis mellifera*, cuticle

The expandable, collapsible honey stomach, herein referred to as the crop, of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), allows bees to carry loads of nectar and to dispense it into honeycomb cells (Snodgrass 1956) for future consumption. Generally among Insecta, the distensibility of the crop is afforded by a passively stretchable epithelium, whereas contractibility is afforded by an external wrapping of muscles that contracts as needed to expel the contents. The investigation of Brosch and Schneider (1985) of the innervation of the *A. mellifera* crop indicated this to be the case in that species but also that the design managing the volume: size ratio is far more elegant and elaborate.

According to them, and Schreiner (1952), the crop wall consists of three layers. The innermost is referred to as the epithelium with its cuticular intima that faces the lumen, as is typical of the insect stomodeum. The epithelium in turn is externally surrounded by two muscle layers, an inner longitudinal layer and an outermost “circular” layer. Hemolymph fills the spaces between muscle fibers. The authors noted that the epithelium of the empty crop is extremely folded and that unfolding allows for extensive stretching during the filling process. They also report that it is possible to carefully separate the “intima from the muscle

layers,” indicating that the two are free of each other. The cuticle consists of a thin epicuticle and a thick, parabolically elaborated procuticle without pore canals.

During development of techniques to visualize the presence of lactic acid bacteria in the honey stomachs of honey bees (Olofsson and Vasquez 2008) by using scanning electron microscopy (SEM), we found that the morphology of this organ needs much more attention beyond the preliminary light and transmission electron microscopy (TEM) done by Brosch and Schneider (1985). The purpose of our study is to provide illustration and clarification to the construction of the wall of the crop, and the configurations that it assumes when the organ is laden and depleted of nectar. The complexities of the esophagus and proventriculus were not investigated.

Materials and Methods

The abdominal interior was accessed by removing the last two to three abdominal segments and then cutting transversely across the metathorax to sever the esophagus. The crop was revealed by pulling out the ventriculus, removed intact by cutting distad of the proventricular constriction, and collected in filter-sterilized bee buffer [0.9% (wt:vol) NaCl, 0.1% (wt:vol) Tween 80, and 0.1% (wt:vol) peptone] by severing the ventricular apex. The volume was estimated as needed by measuring the longest

¹ Corresponding author: USDA-ARS, Carl Hayden Honey Bee Research Center, Tucson, AZ 85719 (e-mail: diana.sammataro@ars.usda.gov).

² School of Plant Sciences, P.O. Box 210036, University of Arizona, Tucson, AZ 85721.

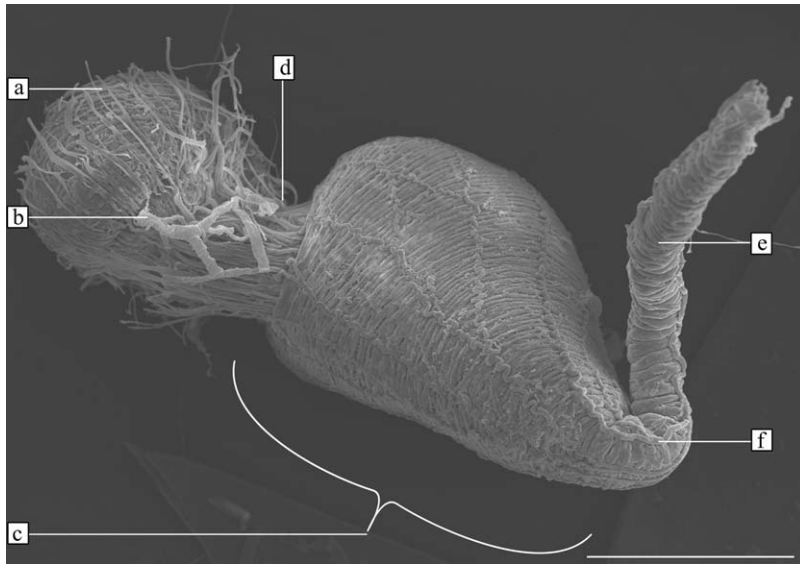


Fig. 1. SEM of a depleted honey bee honey stomach (crop). Lateral view. (a) Apex of severed ventriculus (midgut). (b) Trachea. (c) Ventral surface. This is the shortest distance between proventriculus and esophagus. During engorgement, most of the inflation occurs dorsally, where the opposing face distends outward. (d) Proventricular constriction. (e) Esophagus. (f) Axonal trunk. Scale bar = 500 μ m.

height and widest girth. The adherence of the epithelium to the cuticle was tested by teasing with forceps. All dissections ($n = 100$) were carried out under an SMZ-U dissecting microscope (Nikon, Tokyo, Japan).

For fixation, three to four crops were collected into a tube and processed simultaneously. Each was slit with microdissecting scissors (Electron Microscopy Sciences, Hatfield, PA) to allow contents to exchange with the buffer. The buffer was gradually replaced with 4% formaldehyde, 0.5% glutaraldehyde, and 0.2% picric acid in 0.01 M Na-K phosphate-buffered saline (PBS) (Sambrook and Russell 2001) so that they would not collapse or contact the meniscus. Fixation proceeded overnight, followed by 3×30 -min rinses in PBS, 20 min in 0.1% OsO_4 in PBS, and then crops were rinsed again and dehydrated in a 25, 50, 75, 95, 100% ethanol series. For TEM, crops were infiltrated with 25, 75, 100% LR White resin; polymerized at 55°C overnight; sectioned with an LKB 2128 ultratome (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom), and viewed on a Phillips CM12 equipped with a digital camera. For SEM, dehydrated specimens were critical point-dried, mounted, and viewed with an S3400N Variable Pressure SEM (Hitachi, Tokyo, Japan).

Results

When individuals were depleted of their nectar reserve, the crop was contracted into an urn shape (Fig. 1). When individuals were engorged, the crop was distended to a volume ≈ 10 times larger. Distention was not radially uniform, instead, when full (laden), the esophagus, marking the anterior, and the

proventriculus, marking the posterior, were closely set along one face of the crop (Fig. 2e), whereas the opposing globose shape inflated away from it. This bias also was seen in the depleted crop (Fig. 1).

Dissections indicated that the shorter side of the crop (Fig. 1c) was ventral and corresponded to the concavity of the ventral abdominal exoskeleton, whereas the globose side was dorsal and corresponded to the convexity of the dorsal abdominal exoskeleton, as well as the large space afforded posterior to the acclivous second tergum (Snodgrass 1956: 136, fig. 51). During distention, the midgut (ventriculus) was usually negotiated ventrally, under the inflating crop, so that the dorsal, globose side of the crop could continue to expand posteriorly beyond the position of the proventriculus.

Six layers of tissue were identified. From outer to inner, they were 1) axonal trunks, 2) latitudinal muscle fibers, 3) longitudinal muscle fibers, 4) the epithelium, 5) the procuticle, and 6) the epicuticle. Dissections in buffer indicated that the outer surface of the crop was hydrophobic. This hydrophobicity compromised infiltration of embedment and resulted in artifacts (cf. Fig. 7a).

Axonal trunks were longitudinal in orientation and could be recognized from the underlying muscle fibers by their sparse number, larger girth, and "stitching" pattern produced by growth over one to several latitudinal muscle fibers before intercalating between them (Figs. 1f, 2c, 3a, 4, and 11c). Axonal trunks were variable in both number and extent of ramification from specimen to specimen. Some sponsored several branches, whereas others did not. Each trunk tracked more or less longitudinally until a branching occurred. Branches diverged diagonally from each other and

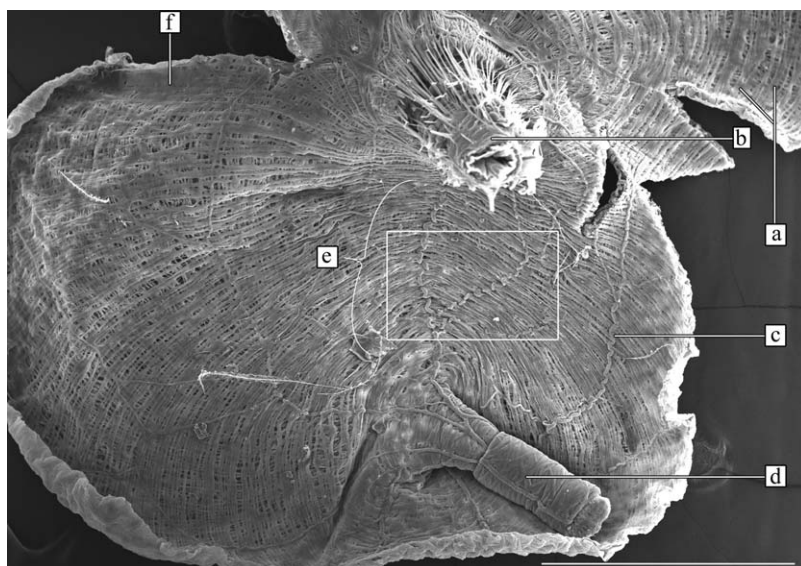


Fig. 2. SEM of a laden honey bee honey stomach cut in half longitudinally and flattened onto the mounting stub. (a) Dorsal-most surface. Latitudinal muscles are fused in the areas pointed to. Refer to Fig. 5. (b) Proventriculus. (c) Axonal trunk. (d) Esophagus. (e) Ventral surface. (f) Fusion of latitudinal muscles also can be seen here and elsewhere. Inset, see Fig. 3. Scale bar = 1.0 mm.

then resumed a longitudinal track. Tracks were seen to span the entire length of the crop or end anywhere between the esophagus and proventriculus.

Latitudinal muscle fibers tracked transversely around the crop. Fibers were fused to each other in some areas (Figs. 2a and f and 5a) and free of each other elsewhere (Fig. 6). Those free of each other had a braided, ankylose appearance due to demarcation of sarcomerel segments.

Longitudinal muscles had a braided appearance also (Fig. 5b). No fusion was indicated in SEM or TEM. All fibers seemed to exist as individual units, free of each other, although some branching was noted.

The epithelium was a cellular layer continuous with the epidermis of the outer body (Figs. 7f, 8b, and 9e). It could be easily peeled away from the two muscle layers with forceps, but the possibility of weak adherence or ligation could not be determined with this

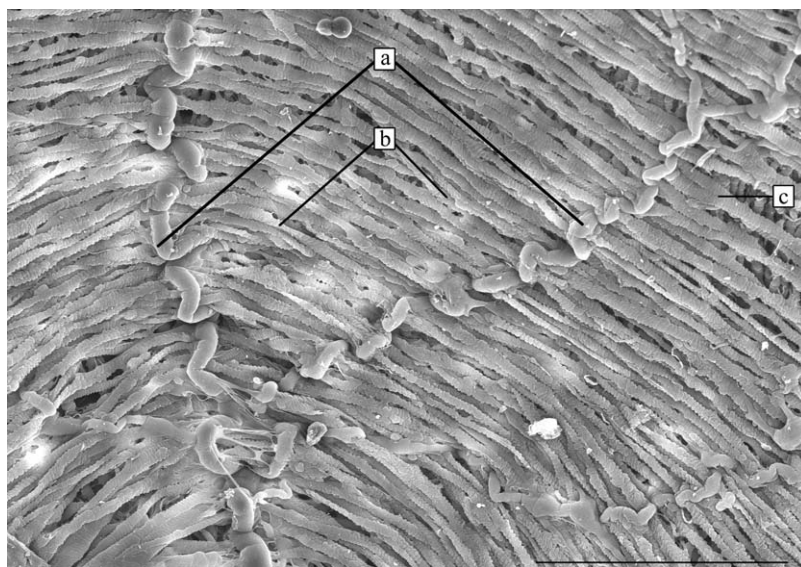


Fig. 3. Close-up of inset in Fig. 2. (a) Axonal trunks. (b) Latitudinal, nonfused muscle fibers. Longitudinal muscle fibers can be seen underneath them. (c) Branching of a latitudinal fiber. Scale bar = 200 μ m.

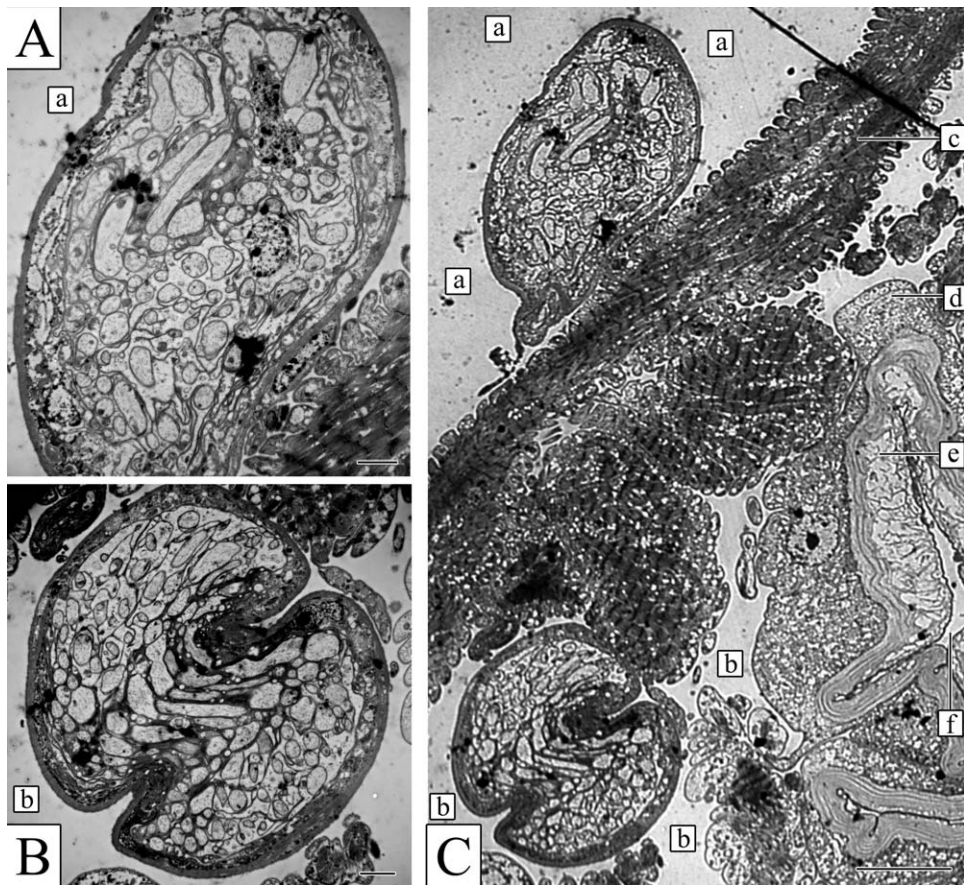


Fig. 4. TEM cross sections of axonal trunks. Refer to Fig. 3a. (A and B) Close-ups of corresponding structures in C. Scale bars = 2 μm (A and B) and 10 μm (C). (a) Bloodspace on the hemolymph side of the crop. (b) Interstitial fluid between latitudinal muscle fiber and epithelium. (c) Latitudinal muscle fiber. (d) Epithelium. (e) Procuticle with electron transparent vesicles. Refer to Fig. 9d. (f) Space between the epicuticles of an outfolded pleat is continuous with the crop lumen.

method. It was thrown into a compound labyrinth of pleats when the crop was minimally filled. Terminology needs to be established. These pleats underwent unfolding as needed during engorgement. Pleats elaborated in both directions, that is, by the infolding and outfolding of the epithelium. Infolding brought basal lamina into opposition (Figs. 9c and 12) whereas outfolding brought epicuticles into opposition (Fig. 10e). Epicuticles and epithelial cells, respectively, always opposed each other.

The procuticle was $\approx 1.0\text{--}4.0\text{ }\mu\text{m}$ in thickness, flexible, compressible, and had several conspicuous ultrastructural features. It was made up of approximately three continuous layers, and the full thickness of each layer was elaborated with one dense row of parabolic lines oriented perpendicular to their laminar plane (Figs. 7h and 8a). Electron transparent vesicles appeared in the procuticle just underneath the epicuticle (Fig. 9d) in some areas but not in others (Fig. 7). These vesicles were not membrane bound. In some areas, noncuticular moieties seemed intercalated into the layers (Figs. 8f and 10c).

The epicuticle was an $\approx 0.1\text{-}\mu\text{m}$ -thick uniform layer with no specialized features (Figs. 7g, 8d, and 10e). In some areas, epicuticle seemed to attenuate, allowing opposing procuticular layers to contact each other and fuse together (Fig. 8f).

The latitudinal and longitudinal muscle fibers formed a cage that completely enclosed the epithelial/intima bilayer. An interstitial fluid appeared between the cage and the bilayer in certain areas (Fig. 7k). The pleats of the epithelium/cuticle bilayer were always tightly appressed to the inner surface of the cage. Contrary to Brosch and Schneider (1985: 336), no indication was found that the epithelium can separate or be mechanically separable from its intima under any conditions.

Tracheal, tracheolar, and neural connections notwithstanding, there were no indications that the bilayer and the ensheathing muscle fibers are attached directly to each other anywhere in the crop except perhaps at the esophageal and proventricular ends. Also, the only indication of coupling of pleats was in areas where opposing procuticles were fused. Otherwise, pleats were able to elaborate and compound

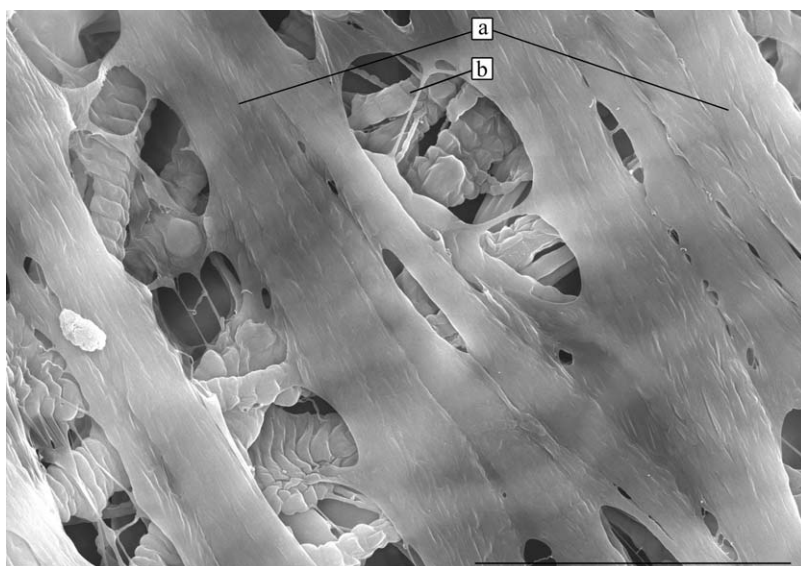


Fig. 5. TEM of crop wall. (a) Latitudinal muscle fibers. Note partial fusion. (b) Longitudinal muscle fibers can be seen underneath this layer, along with fine neural tubes, respiratory tubes, or both. Scale bar = 50 μm .

independently of each other. There was no indication of excessively long pleats that might otherwise singularly absorb the demand for contraction of diameter (Fig. 13e). Instead, pleats seemed to be well distributed over the sphere.

The pleating was multiply compounded at various loci, and pleats were seen to envelope each other (Fig. 7e, inset). Pleats were profuse in certain areas of even the most highly distended crops (Fig. 11b). The epithelial cell layer was seen to attain states of extreme compression in some areas but not in others of any same cross section. Nuclei were seen to maintain

spherical integrity even when the cytosol was highly compressed (Fig. 8c). The thickness of the procuticle, too, was seen to undergo differential compression where it was folded.

An interstitial fluid appeared in the spaces between infolded and outfolded pleats. Spaces within infolded pleats (Fig. 7c and 8e) traced to the epithelium-cage interface, and through the interstitial space between cage fibers (Figs. 6a and 7k) to the hemolymph (Fig. 7i). Interstitial fluid between outfolded pleats traced to the stored nectar in the lumen (Figs. 9a and 10f).

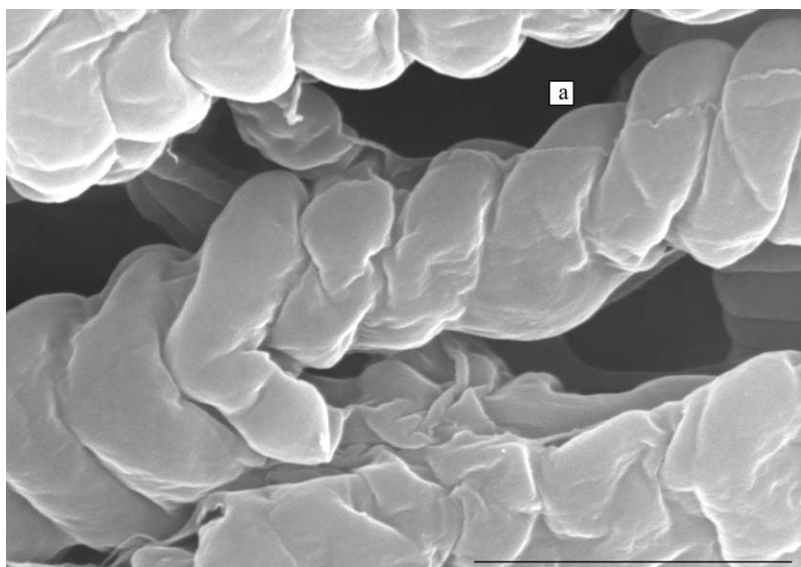


Fig. 6. TEM of nonfused latitudinal muscle fibers, corresponding to those pointed to in Figs. 5b and 7j. (a) Interstitial space. Scale bar = 10 μm .

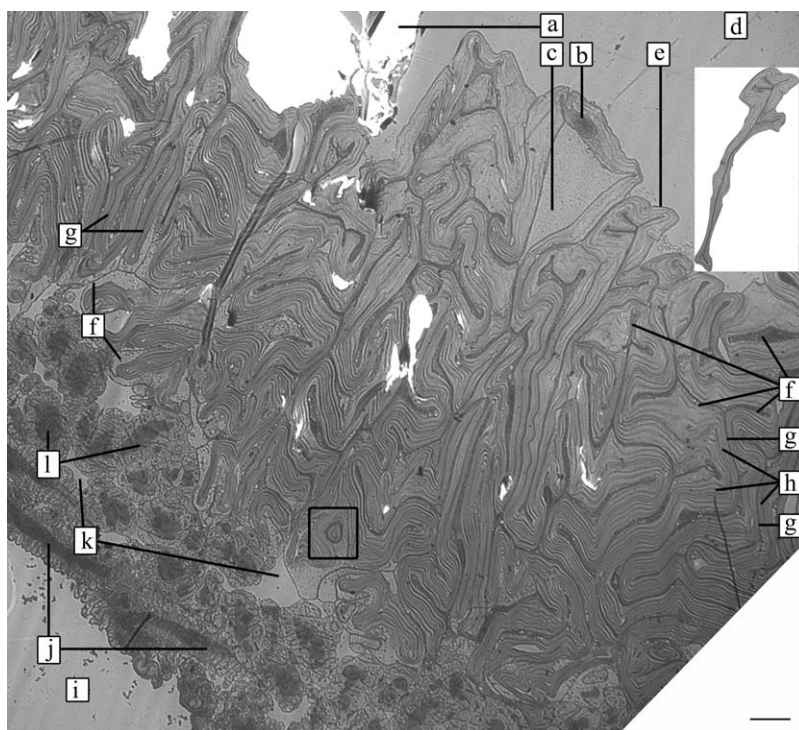


Fig. 7. TEM cross section of the wall of a moderately depleted crop. The hydrophobicity of the outer surface of the crop compromised specimen processing, resulting in uninfiltated areas as indicated. (a) Artifact. (b) Nucleus of one cell of the continuous epithelium. (c) Fluid substance inside an infolded pleat traces to the hemolymph. (d) Crop lumen. (e) This pleat, digitally excised and copied to outset box at right, has circularity, indicating that it is part of a compound pleating that emerges into this cross-section from the z-axis. (f) Points along the continuous epithelium. (g) Points along the continuous epicuticle. (h) Points along the continuous procuticle. (i) Bloodspace external to the crop. (j) Latitudinal muscles. (k) Fluid substance between muscles and epithelium. (l) Longitudinal muscles. Inset, only the pinnacle of this pleat appears in this cross section. The rest of the pleat is elsewhere in the z-axis. See Fig. 8A. Scale bar = 10 μ m.

Longitudinal muscle fibers were never seen to be trapped or intercalated between pleats and pulled inward, out of the spherical continuity of that layer. Similarly, pleats were never seen to have worked themselves between the fibers and out of the confines of the cage.

Discussion

The general design of the honey bee abdomen accommodates the high scalability of the crop's volume. The upper and lower diaphragms blanket all abdominal organs and confine hemolymph between them,

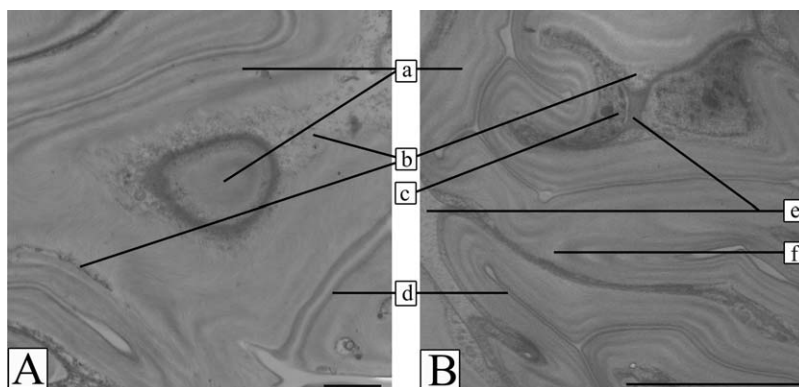


Fig. 8. High magnification TEM of aspects of the crop's epithelium/cuticle bilayer. (A) Inset of Fig. 7. Scale bar = 2 μ m. (B) Fusion of procuticular lamina. Scale bar = 10 μ m. (a) Procuticle. Note parabolic pattern of cuticular elaboration. (b) Epithelium. (c) Nucleus. (d) Epicuticle. (e) Fluid substance between epithelial cells. (f) Confluence of two fused procuticular lamina.

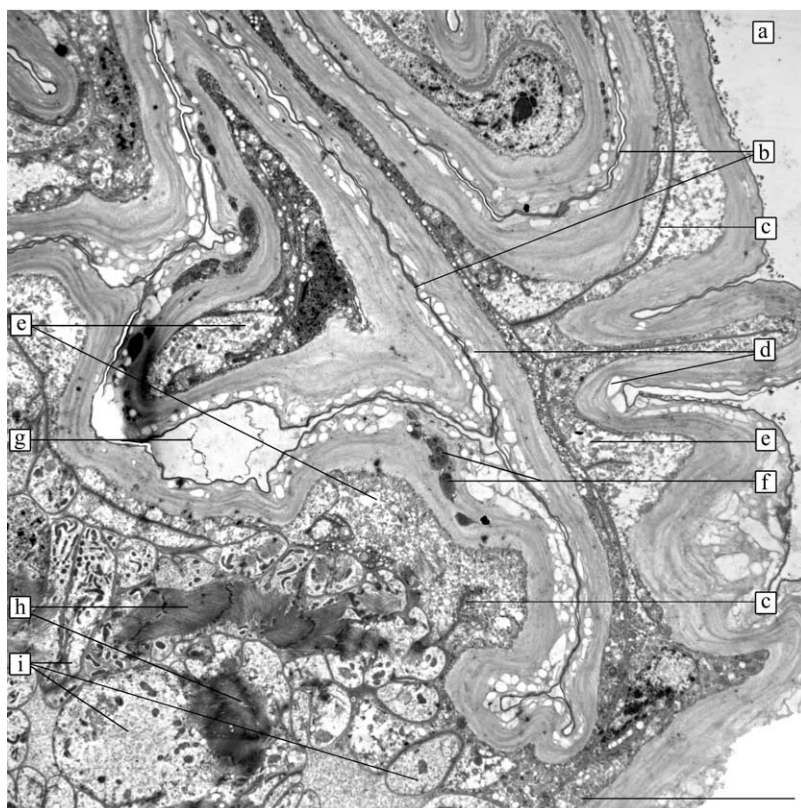


Fig. 9. TEM of crop wall. (a) Crop lumen. (b) Epicuticle. (c) Opposing, tightly appressed, basal lamina of the epithelial cell layer. The interface between this fold traces to the hemolymph. See Fig. 12. (d) Electron transparent vesicles along the apical margin of the procuticle. (e) Epithelium. (f) Intercalated objects. See Fig. 10c. (g) Unidentified structures spanning the luminal space between two opposing epicuticles. They could be an extremely sheer tangential section of another pleat in the z-axis above or below this section. (h) Muscle. (i) Muscle cell cytoplasm. Scale bar = 10 μ m.

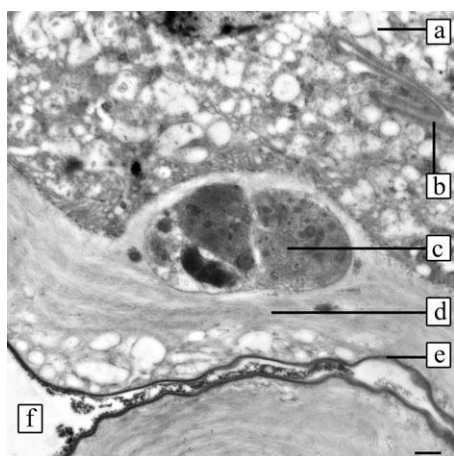


Fig. 10. Close-up of procuticle with intercalated objects pointed to in Fig. 9f. (a) Epithelium. (b) Fold in epithelial basement membrane. (c) Intercalated objects. (d) Procuticle. Note parabolic elaborations. (e) Epicuticle. (f) Interstitial space of this outfolded pleat traces to the lumen. Scale bar = 0.5 μ m.

making available a vacuous hemocoelic space above and below for distention. Furthermore, the petiole channels the esophagus along the ventral abdominal midline so that the ventral crop walls are pressed against the convex exoskeleton, whereas the dorsal crop walls are able to distend dorsally into the free space provided by the acclivous second tergite. The midgut (ventriculus) usually works its way underneath the crop during distention so that maximum space for nectar loads is provided.

The stretchability of the bilayer, the stitching pattern of axon segments, and the number of available pleats affects scalability, too, as well as behavioral considerations. High compressibility of the epithelium and its intima is demonstrated in compound pleats. Such stretchability is reminiscent of the properties of the bilayer of insects in teneral stages. Residual pleating occurs in highly distended crops (Fig. 11b), indicating that the number of available pleats is not a factor in limiting the volume of nectar that can be imbibed. However, the unfolding process may possibly be halted in areas where procuticles are fused. Because pleats are so confluent and intercalated into and upon each other, it is likely that the density of pleats in any given area shifts to other areas during

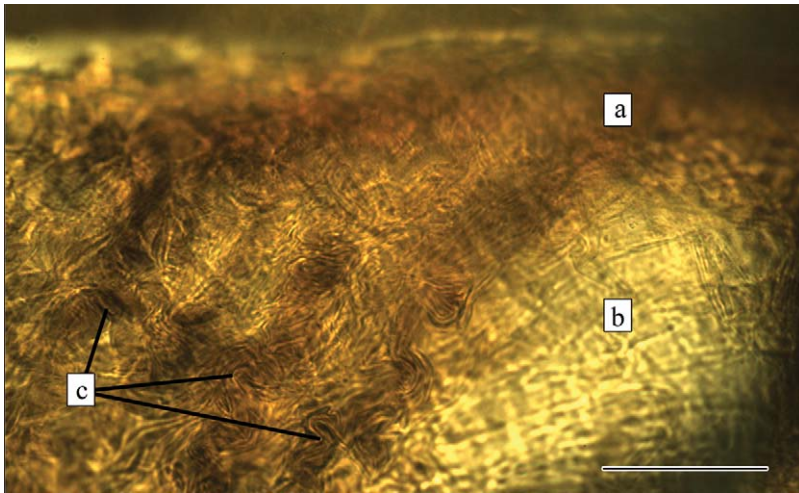


Fig. 11. Transmitted light micrograph of a strongly distended crop showing differentially expanded regions of the underlying epithelial pleats. (a) Dark brown color indicates higher degree of opacity and correlates to extensive pleating available for still further distention. (b) Light brown color indicates lower opacity and correlates to low number of pleats. Pleats are still profuse even in this extremely distended zone. (c) Axonal trunks. Scale bar = 100 μm . (Online figure in color.)

consecutive and/or alternating expansion and depletion of the crop.

The confluence and intercalation of pleats seen in cross section (Fig. 7) is certainly in part due to their

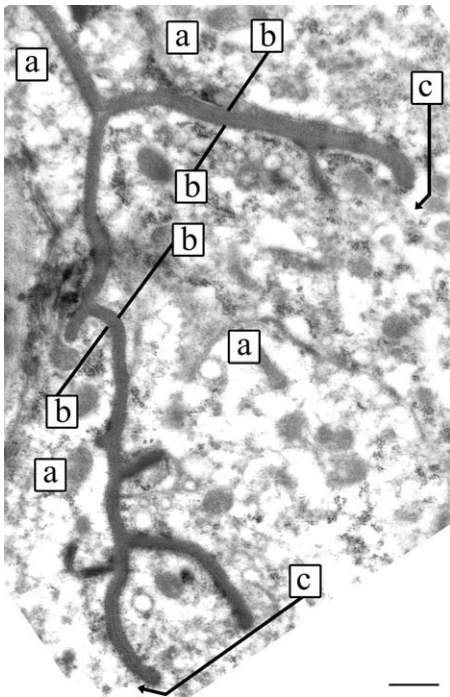


Fig. 12. TEM of epithelial cells, showing greater detail of the basal laminar folds such as those pointed to in Fig. 9c. As a consequence of pleating, the basal lamina is folded over upon itself multiple times. (a) Cytoplasm. (b) Lengths of basal lamina folded over and tightly appressed to each other. (c) Cytoplasm continues around the basal laminar folds. Scale bar = 0.5 μm .

origination from the stomach's spherical configuration. Because of the inversion from a spherical wall to an infolding, each pleat must elaborate into a distorted and roughly cup-shaped fold (Fig. 13f), with strong tendency to compound into subfolds.

Limitations on the length of pleats is indicated. If the epithelium was completely free of the cage, and nothing constrained pleats from continued lengthening, then it is conceivable that the need for contraction could be absorbed by continual involution of one or a few pleats (Fig. 13e). All other epithelial fields would then simply slide into them. Instead, it seems that new pleats are profusely generated throughout during the contraction process. Therefore, there must be a mechanism or mechanisms that anchor the epithelium to the cage at certain loci so that free sliding is constrained. Neural and tracheal connections may cause this anchoring. Also, the friction between pleats may be greater than the friction between the epithelium and the cage. Both would cause spontaneous generation of pleats throughout the sphere.

Although the nectar store exerts outward pressure upon the bilayer and keeps it tightly, spherically, appressed to the inner walls of the cage during expansion, this pressure cannot explain the appressed position of individual pleats or pleat complexes because it is exerted evenly around each one (Fig. 13c). Therefore, another factor is in effect that confines the growing and waning pleats to circumferential positions. The interstitial fluid (Figs. 7c and 8e), present between pleats on the hemolymph side of the bilayer, may not be hemolymph—it may be a substance that lubricates and coheres the pleats simultaneously. Folds of the epithelium can be accompanied by appression of opposing basal lamina that is so tight as to apparently exclude interstitial fluid, but it may still be present as an ultrafine film. Among the general Insecta, molting fluid is secreted from the apical epithe-

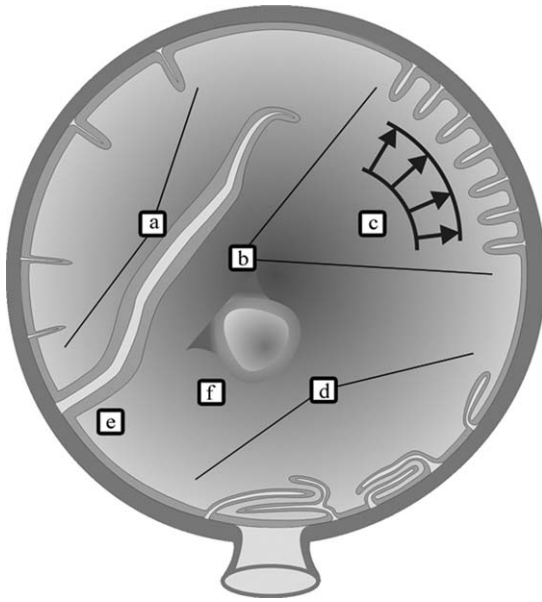


Fig. 13. Model for the basic construction of the honey bee honey stomach. A collapsible, but inelastic sphere is confined inside an elastic balloon. As the balloon contracts, the sphere undergoes involutions (pleats) to compensate. As the sphere expands, unfolding of pleats occurs. (a) Some areas have a low incidence of pleats. (b) Some areas have a high incidence of pleats. (c) Outward pressure exerted by the contents of the inner sphere against the inward pressure of the balloon keeps the sphere appressed to the inner walls of the balloon, but does not influence the attitude of individual pleats. (d) In real life, pleats maintain a position that is tightly, outwardly appressed to the muscle cage as they lengthen and branch into compound arrays. Therefore, something else, thought to be an interstitial, lubricating fluid, is involved in maintaining this attitude. (e) Another factor, thought to be attachment of respiratory and neural tubes, prevents the contracting forces from being absorbed by one or a few large pleats. Instead, relatively short pleats are generated profusely around the circumference, and compound into branches. (f) Pleats emerging from a sphere would be more or less spoon-shaped.

lial/epidermal membranes after apolysis. In contrast, the fluid in question herein coats the basal lamina.

The interstitial fluid between the muscle cage and epithelium (Figs. 6a and 7k) may not be hemolymph either, because the outer surface of the crop is hydrophobic. It may or may not be the same substance as that seen deep in the recesses between pleats (Figs. 7c and 8e); however, as pleats are formed and elongated, their increasing need for interstitial fluid to fill the increasing space must be accounted for. Three possible sources can be suggested that, alone or in

combination, might explain the volumetric relationship between interstitial pleat space and pleat length. 1) Fluid may be drawn in from the muscle cage. 2) Fluid may be secreted from some unknown source. 3) Fluid may be globally redistributed by moving it from pockets to ultrafine interfaces and vice versa.

Schmid-Hempel et al. (1985) have determined that honey bees quit nectar gathering even though their crops are not full, due to flight-cost budgeting. If this is the case, then reserve capacity for emergency long distance foraging is well served anatomically. However, their studies were based on behavioral data and did not include dissections. Furthermore, there is no consideration of the possibility that honey bees may leave the hive for continued foraging with crops that have not been fully emptied. The size of the crop and its capacity for enlargement also might be a necessity during swarming or absconding activity, when bees are carrying all their food reserves in their bodies to establish a new colony. This extra food is needed to activate the wax glands that produce beeswax, the material bees use for their nest.

Finally, the length of fully relaxed muscular cage fibers and axonal trunks, and thresholds of stretch receptors certainly contribute to scalability, but no information is available on these considerations.

Acknowledgments

We thank and acknowledge assistance from G. DeGrandi-Hoffman, Milagra Weiss, and the staff of the Carl Hayden Bee Laboratory, the University of Arizona Imaging Facility, and J. Brown.

References Cited

- Brosch, U., and L. Schneider. 1985. Fine structure and innervation of the honey stomach (crop) of the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae). *Int. J. Insect Morphol. Embryol.* 14: 335–345.
- Olofsson, T. C., and A. Vasquez. 2008. Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honeybee *Apis mellifera*. *Curr. Microbiol.* 57: 356–363.
- Sambrook, J., E. Fritsch, and D. W. Russell. 2001. Molecular cloning. A laboratory manual, 3rd ed. Volume 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Schmid-Hempel, P., A. Kacelnik, and A. I. Houston. 1985. Honeybees maximize efficiency by not filling their crop. *Behav. Ecol. Sociobiol.* 17: 61–66.
- Schreiner, T. 1952. Über den nahrungstransport im darm der honigbiene. *Z. Vgl. Physiol.* 34: 278–298.
- Snodgrass, R. E. 1956. *Anatomy of the honeybee*. Comstock Publishing Associates, Ithaca, NY.

Received 31 July 2009; accepted 23 July 2010.