Hierarchical, multilayered cell walls reinforced by recycled silk cocoons enhance the structural integrity of honeybee combs

Kai Zhang, Huiling Duan, Bhushan L. Karighaloo, and Jianxiang Wang

*State Key Laboratory for Turbulence and Complex Systems and Department of Mechanics and Aerospace Engineering, College of Engineering, Peking University, Beijing 100871, China; and School of Engineering, Cardiff University, Queen's Buildings, The Parade, Cardiff CF24 3AA, United Kingdom

Edited by Grigory Isaakovich Barenblatt, University of California, Berkeley, CA, and approved April 8, 2010 (received for review October 21, 2009)

We reveal the sophisticated and hierarchical structure of honeybee combs and measure the elastic properties of fresh and old natural honeycombs at different scales by optical microscope, environmental scanning electron microscope, nano/microindentation, and by tension and shear tests. We demonstrate that the comb walls are continuously strengthened and stiffened without becoming fragile by the addition of thin wax layers reinforced by recycled silk cocoons reminiscent of modern fiber-reinforced composite laminates. This is done to increase its margin of safety against collapse due to a temperature increase. Artificial engineering honeycombs mimic only the macroscopic geometry of natural honeycombs, but have yet to achieve the microstructural sophistication of their natural counterparts. The natural honeycombs serve as a prototype of truly biomimetic cellular materials with hitherto unattainable improvement in stiffness, strength, toughness, and thermal stability.

Natural honeycombs are used to store honey and pollen, and to rear the brood. By contrast to most insects and birds, bees construct their nests from their own secretions. The comb cells are constructed from wax secreted by worker bees. Then fertilized eggs are deposited in these cells. The eggs develop into larvae, which surround themselves with silk cocoons before their pupation (1). After the pupae have metamorphosed into bees and left the cells, the worker bees cover this silk with wax. Thus, the comb becomes a composite material with usage (2). In addition to structural functions, the honeycomb is an important clue to the nestmate recognition (3) and to understand the evolution of honeybees (4). The age of honeycombs affects the honeybee growth and brood survivorship (5). Thus, whilst the honeybee comb is a most studied natural cellular structure that has long fascinated mathematicians, physicists, and biologists (8–18), it was not known until recently why the bees built the combs out of hexagonal cells (13). The mechanical properties of beeswax and the cell walls of the combs of African honeybees, *Apis mellifera Ligustica*, which are the most popular variety for beekeeping in the world, have yet to achieve the microstructural sophistication of their natural counterparts.

**Results**

We studied two-day-old fresh (10 combs), five-month-old (6 combs), one-year-old (10 combs), and two-year-old (6 combs) honeycombs of the Italian honeybees, *Apis mellifera Ligustica*, which are the most popular variety for beekeeping in the world. The mass fraction of silk cocoons in the walls of the fresh honeycomb consists of small wax grains whose size varies from 500 nm to 1.5 μm (Fig. 2B). The thickness of the honeycomb wall can be divided into two parts: an inner part corresponding to the fresh honeycomb constructed by the worker bees, and an outer additive part generated during the use of the honeycomb. In contrast to the fresh honeycomb, the additive part of the old honeycomb wall exhibits a layered structure (Fig. 3B). We show the detailed structure of the one-year-old comb at different scales in Fig. 3, and note that the five-month-old and two-year-old combs exhibit similar features. The thickness of one layer, measured on specimens from all old combs is 2.45 ± 0.81 μm irrespective of the age. Fig. 3C–E clearly show that the additive part of the old wall is a composite material consisting of wax reinforced with silk. The diameter of the silk is about 2.92 ± 1.12 μm and they are embedded in the wax in a mostly random, with an occasional regular arrangement. The mass fraction of silk cocoons in the walls of one-year-old honeycomb is 33.4%. The silk of honeybee larvae cocoons is an alpha-helical fibroin in which the micelles form a four-stranded array of twisted coils with the major axis parallel to the silk axis (25, 26). We measured the indentation moduli of the silk in the axial and transverse directions by nanoindentation using a 50 nm indenter. The indentation modulus in the fiber axis of the silk is 7.05 ± 0.56 GPa, whereas the indentation modulus perpendicular to the fiber axis measured by indenting the longitudinal surface of the silk, as shown in Fig. 3E, is 3.62 ± 0.26 GPa. Fig. 3D shows the cross

### Authors' Contributions

K.Z. and H.D. designed research; K.Z., H.D., B.L.K., and J.W. performed research; K.Z. analyzed data; and B.L.K. and J.W. wrote the paper.

The authors declare no conflict of interest.

This article appears as part of a PNAS Direct Submission.

1To whom correspondence should be addressed. E-mail: Karighaloo@cardiff.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.0912066107/-/DCSupplemental.
section of several silks. The hierarchical structure of the one-year-old honeycomb at nano-, micro-, and macroscales is shown in Fig. 4. The honeybee silk can be further studied at the molecular level, as has been done by Keten et al. (24) for silkworm and spider silks. They found that the stiffness, strength, and toughness of these β-sheet nanocrystals with weak transverse hydrogen bonds can be improved by nanoconfinement.

We also measured the variation in the in situ indentation modulus across the thickness of the wall of the one-year-old honeycomb by indenting the cross section of the wall (Fig. 5). It exhibits a pronounced gradient between the interior and the exterior of the cell wall. It is low, 0.57 ± 0.08 GPa, around the middle region of the one-year-old wall (which is equal to that of the fresh wall), and increases rapidly to 1.43 ± 0.14 GPa toward the exterior regions of the wall. These regions are the composite layers deposited during the use of the honeycomb. Thus, the wall is progressively stiffened by recycling the silk cocoons.

As the walls of both the fresh and old combs are made of wax grains and are thus heterogeneous at the microscale, we have measured their indentation modulus at the mesoscale (i.e., at the scale of the wall as a whole) by microindentation with a 400 μm spherical indenter (27), and their elastic modulus by direct tension (Fig. 6A). Both tests confirmed an increase in the stiffness of comb wall with age. The direct tension test also provided the tensile strength of the wall, and the strain at maximum load. They all show an increase up to the age of 5 months from the fresh state (Fig. 6B) but practically no increase thereafter.

As the indentation and elastic moduli are approximately related as $E_i = E/(1 - \nu^2)$, we estimate the Poisson ratio of the fresh wall to be 0.313. The elastic modulus of walls of the Italian honeybee fresh and old combs at the mesoscale are appreciably larger than those of the fresh and old honeycomb wall specimens of the African honeybees Apis mellifera scutellata (2) measured by tensile tests at 9.1 and 29.9 MPa, respectively. The most likely reason for this is the differing stiffness requirements of the combs; the African bees are smaller and produce and store less honey than the larger Italian bees (14). On the other hand, the substantial difference between the elastic moduli of the Italian honeycomb wall measured at the micro- and mesoscales is due to incomplete fusion of the wax grains (27). As fusion of the wax grains needs heating, it seems the bees do not expend energy to fuse the wax grains to stiffen and strengthen the new wall when there is no need for it, but instead do it by coating as the need arises during its use.

**Discussion**

Bees construct honeycombs with the hexagonal cell prism axis aligned at an angle 13° to the ground, as shown in Fig. 4 and Fig. S1 (14). Each comb consists of two back-to-back sides with the cells in each side being nearly perpendicular to the surface dividing the two sides (14). As the cells are connected to form an array, and as their depth is much shorter than the height of the comb, the ensemble of the cells can be treated as a deep cantilever beam with a very short span. Under the transverse loading from the weight of honey, pollen, the brood, and of the comb itself, the deformation of such an extremely short cantilever beam is primarily governed by its macroscopic shear rigidity (28); i.e., the out-of-plane shear modulus of the honeycomb as a whole. We measured the macroscopic out-of-plane shear modulus of the fresh and old combs (SI Text) to be 0.72 ± 0.09 MPa (fresh), 2.19 ± 0.30 MPa (five-month-old), 2.71 ± 0.24 MPa (one-year-old), and 3.59 ± 0.39 MPa (two-year-old), as shown in Fig. 6B. Thus, the shear stiffness of the comb has increased more than three-fold in one year and four-fold in two years. From the previously measured tensile elastic modulus of the wall at the mesoscale and the estimated Poisson’s ratio, we can estimate first the shear modulus of the wall using the formula $\mu_s = 0.5E/(1 + \nu)$, and then the shear modulus of the comb using the formula (11) $\mu_{s,\text{comb}} = \mu_s L/L$, where $t$ denotes the thickness of the wall, and $L$ the length of the side of the hexagonal cell. In this manner, we estimate the macroscopic shear modulus of the combs to be 1.01 (fresh), 1.43 (five-month-old), 3.15 (one-year-old), and 4.18 MPa (two-year-old), as shown in Fig. 6B. These estimates are in good agreement with the experimental measurements (27).

![Fig. 1. Honeycomb walls at different ages.](image-url)

![Fig. 2. Fresh honeycomb walls.](image-url)
agreement with the above experimental data, again confirming that the shear stiffness of the comb has increased more than three-fold in one year and four-fold in two years.

The macroscopic shear test also provided the nominal shear strength and the shear strain at maximum load of the comb as a whole, as shown in Fig 6B. The shear strength and the strain at maximum load of the comb continue to increase with age; the shear strength has increased about four-fold in one year and five-fold in two years. The shear strain at maximum load of the one-year-old comb is about 1.5 times higher than that of the fresh comb. In view of the potential stress concentration at the corners near the clamped end of the comb, we also simulated the shear tests of the fresh and one-year-old combs using the finite element method (FEM) (SI Text). The shear strains at the maximum load for the fresh and one-year-old combs as a whole are 4.3% and 7.0%, respectively, as shown in Fig S2, suggesting a strain concentration factor near the corners of around 2 at both ages.

The reason that the bees need to stiffen and strengthen the comb without becoming fragile can be explained by performing a finite element analysis of the comb. We calculated the stress and strain fields in the fresh and old combs using the linear elastic finite element model at 25 °C (SI Text). For the fresh comb, under the weight of honey and worker bees, the maximum normal stress and the corresponding strain along the axis of the cell were found to be 72 kPa and 0.05%, respectively. These are well below the tensile strength (1.1 MPa) and the corresponding strain (0.65%) of the wall that we measured at 25 °C (Fig 6A). The computed maximum nominal out-of-plane shear stress (0.11 kPa) and the corresponding shear strain (0.04%) in the fresh comb are also well below the nominal shear strength (11 kPa) and the corresponding shear strain (2.2% or 4.3% if one allows for strain concentration at the clamped end) at 25 °C (Fig 6B). These results indicate that the fresh comb can safely carry the weight of honey and bees.

It is known however (2) that the temperature inside a honeybee comb can fluctuate from 25 °C to 45 °C. It is also known (2) that fresh wax wall of an African honeybee comb softens when the temperature rises from 25 to 45 °C losing its elastic modulus by a factor of 3.5 and its tensile strength by an order of magnitude, whereas those of an old comb wall that contains 34% silk cocoons by mass are considerably less sensitive to an increase in temperature. Given the fact, that the mass fraction of silk cocoons in the walls of Italian honeybee combs is practically equal to that in the African honeybee comb, it can be assumed that a similar

---

**Fig. 3.** One-year-old honeycomb at different scales. (A) Top view. (B) ESEM image of a cross section of the cell wall. (C) Optical image of the surface of a peeled layer. (D) 3D microstructure of the cell wall cross section. (E) Silk with indentations.

**Fig. 4.** Hierarchical structure of one-year-old honeycomb at macro-, micro- and nanoscales.
temperature dependence prevails in the Italian honeybee combs. We have examined the effect of the viscoelastic nature of the fresh beeswax on the stress and strain fields in the wall of the fresh comb. The finite element method and an appropriate viscoelastic model were used to calculate the stress and strain fields in the fresh comb at 45 °C (SI Text). We found that as a result of creep deformation the maximum out-of-plane shear strain in a fully laden fresh comb has reached 1.9% (Fig S1D); i.e. higher than the shear strain at the maximum load of the fresh comb (1.5%) at 45 °C (SI Text). Thus, a temperature increase inside the comb from 25 °C to 45 °C would result in the collapse of a fully laden fresh comb. That this does not actually happen is because the comb walls are continuously reinforced by silk coccoons during its use.

The old comb walls that contain 34% silk coccoons by mass are practically insensitive to temperature fluctuations (2). Finite element calculations (SI Text) show that the maximum out-of-plane shear strain in the one-year-old comb under the weight of honey and bees is only 0.014% (Fig S1E), which is well below its shear strain at the maximum load of 7.0% (Fig S2B). Thus, even if there is some decrease in the shear modulus and strain of the one-year-old comb with increasing temperature, the comb will still have a sufficient margin of safety against collapse.

Engineering lightweight cellular materials are indispensable to modern industry. Remarkable efforts have been made to improve their performance (29). However, the properties of conventional man-made porous or cellular media including honeycombs with homogeneous walls are bounded by two inherent constraints. First, the overall stiffness of a porous medium cannot exceed that of the solid wall; second, the coefficient of thermal expansion (CTE) of a porous medium is always equal to that of the wall material, irrespective of the microstructure of the medium (30). These two intrinsic constraints impose important restrictions on the engineering application of porous materials. The low stiffness has to be compensated by a large size to maintain structural rigidity and stability; the invariability of the CTE is a disadvantage for maintaining a stable shape in an environment with a varying temperature such as in outer space, and it may result in severe stress concentrations and failure due to the mismatch of the thermal expansions of abutting materials in a structural component. A cellular solid that truly mimics the microstructure of natural honeycombs, in particular the nonhomogeneity of its walls, will overcome these restrictions and thus provide a remarkable degree of design flexibility. For cellular solids with a honeycomb cell structure with straight walls, the latter can be stiffened and strengthened by a judicious choice of the geometric and mechanical properties of a coating material in much the same manner as above in an old honeycomb (Fig 7A). For example, the overall out-of-plane shear modulus of a cellular solid with aligned coated cylindrical pores in a hexagonal configuration is (31) \( \mu_{w,c} = \mu_m [1 - f + (1 + f)B]/[1 + f + (1 - f)B] \), where \( \mu_m \) is the shear modulus of the matrix material, and \( f \) is the porosity, as shown in Fig. 7B. The parameter \( B \) is defined as \( B = t_{coating}/(R t_{matrix}) \), where \( t_{coating} \) and \( t_{matrix} \) denote the thickness and shear modulus of the coating layer, and \( R \) is the radius of the pores. When \( B \) is larger than the critical value \( B_{crit} = 1 \), the shear modulus of the cellular material will exceed that of the matrix material from which it is made (i.e., \( \mu_{w,c} \geq \mu_m \)) irrespective of the porosity \( f \). However, when \( f \) is large, as in a honeybee comb, and the effectiveness of coating can be better described by the ratio \( \mu_{w,c}/\mu_m \) of the shear modulus \( \mu_{w,c} \) of the coated cellular solid to that of the uncoated cellular solid \( \mu_{w,nc} = \mu_m (1 - f)/(1 + f) \) (Fig 7C). It is seen that the larger the porosity, the more effective the coating, even with \( B < 1 \). Other elastic constants of the cellular solids with aligned pores can also be tailored via pore surface coating (31). The coating technique is applicable to pores irrespective of their size; it can range from nm to mm (31). However, as the coating parameter \( B \) depends on the ratio \( t_{coating}/R \), the coating layer will have to be much thinner for nanopores than for macropores to give the same stiffening effect.

As the CTE of cellular solids are coupled with their overall stiffness (32), the coating will thus make the overall CTE of the cellular solids tunable. Stiff, strong, and lightweight porous materials with tunable CTE are vital for high-precision optical devices and sensors whose properties must not degrade as the temperature varies (33, 34). In particular, they are ideal
Materials and Methods

All honeycombs used in this study were collected from the Bee Research Institute of Chinese Academy of Agricultural Sciences. The macrostructure of the honeycombs was examined by an optical microscope and the microstructure by an environmental scanning electron microscope. The mass fraction of the silk cocoons was obtained after dissolving the beeswax. The indentation modulus was measured using a Tribolindenter with a standard 50 nm Berkovich diamond tip or a sapphire spherical (400 μm radius) tip.

Macroscopic tensile tests of slabs cut from the comb walls and shear tests of whole combs were conducted in a MicroTester at a displacement rate of 0.1 mm/min. At least 10 (5) specimens each from the fresh and old honeycombs were tested in tension (shear).

For aerospace applications in an environment with large temperature fluctuations.

Acknowledgments. We thank Xin Yi and Fangwei Si for contributions to calculation and experiments. This work was supported by the National Natural Science Foundation of China Grants 10525209, 10872003, and 10932001; the China–United Kingdom Science Network Scheme of the Chinese Scholar- ship Council; and The Royal Society, London, the Foundation for the Author of National Excellent Doctoral Dissertation of China Grant 2007B2.
Supporting Information

Zhang et al. 10.1073/pnas.0912066107

SI Text

SI Materials and Methods. All honeycombs used in this study were collected from the Bee Research Institute of Chinese Academy of Agricultural Sciences. The macrostructure of the honeycombs was observed by an optical microscope. The uniform part of the cell wall was measured after cutting off the top 1 mm of the cell, which is bulbous (typical of the two-day-old (i.e., fresh), five-month-old and one-year-old combs) or tapered (typical of the two-year-old comb) (Fig. 1). To improve the accuracy of measurements, the thickness of the walls was also measured on the uniform part of sections cut along the axial direction of the cells (Fig. 1). We cut flat slabs from the cell walls and embedded them in epoxy. Then, we cut samples by an ultratome (LKB-2088) during which they were cooled by liquid nitrogen. These samples had smooth cross sections. We used them to examine the microstructure of the honeycomb by an environmental scanning electron microscope (ESEM, Quanta 200 FEG). For nonmetallic specimens, the use of an environmental SEM helps to avoid the accumulation of electric charge on the surfaces. The pressure of the gas in the chamber of ESEM however needs to be adjusted according to the conductive properties of the specimen. To obtain high quality images, the pressure of 0.8 Torr was chosen in our study. The temperature of the microscope stage was kept at 22 °C. To characterise the microstructure further, we carefully peeled thin layers (2–3 μm) off the old honeycomb wall using tweezers and examined them in a transmission light optical microscope (Olympus IX71).

We also weighed a rectangular slab (27 × 7 cells in size) of a one-year-old comb before soaking it first in hot water (100 °C), followed by aceton, and finally acetone to dissolve the beeswax. We then weighed the dried residue to obtain the mass fraction of the honeycomb at different ages. Before the nanoindentation test, we first checked the possible influence of the loading rate on the measurements on the fresh and one-year-old walls. We carried out indentation tests by using the same load and different loading rates 200 μN/s, 375 μN/s, 500 μN/s, 750 μN/s, 1000 μN/s, and found that they virtually had no influence on the results. As mentioned above, both the multicycle testing method and the force control mode were used. The maximum loads were set to 1000 μN for the fresh comb and 4000 μN for the one-year-old comb, respectively, resulting in the maximum indentation depth of about 3 μm for both combs.

According to the rule that the indentation depth should be limited to <10% of the film thickness (1), the load functions were characterized by the following: a constant loading/unloading rate of 50 μN/s, and a constant hold time at the peak load of 0.5 s prior to unloading to 40% of the peak load. The peak load was 300 μN for the silk (200 μN for the fresh comb wall), and only the readings with the maximum depth of indentation for the silk under 150 nm (100 nm for fresh comb wall), which is well below 10% of the diameter of the indented silk specimens (or the thickness of the fresh comb wall), were retained. At least 10 indentations at different locations were made. A different loading function, in which the peak load was 800 μN, with the loading/unloading rate of 200 μN/s and the hold time of 0.5 s, was used to determine the variation of the indentation elastic modulus across the wall thickness of the one-year-old honeycomb (maximum indentation depth of 1 μm). Five indentations were made in each layer. Samples from four different one-year-old combs were tested. The nanoindentation measurements with the 50 nm Berkovich diamond tip were carried out in an ambient condition at 28 ± 2 °C and 50 ± 5% relative humidity.

The microindentation with a sapphire sphere (400 μm radius) was carried out to measure the microindentation moduli of the honeycomb wall. The data obtained by nanoindentation (50 nm indenter) reflect an individual wax grain property rather than the bulk mechanical property of the wall, whereas microindentation (400 μm indenter) gives an average property of an ensemble of nearly two thousand wax grains, including the weak interfaces and pores. Before microindenting the fresh and old walls, we had measured the indentation moduli of the fresh wall by nanoindentation on the cross sections and longitudinal surfaces, and found them to be nearly the same, thus confirming the isotropy of the wax structures. It is well known that for isotropic materials, the indentation modulus $E_I$ is related to other elastic constants by (1) $1/E_I = (1 - \nu^2)/(E_s + (1 - \nu^2)/E_r)$, where $E_s$, $E_r$, $\nu$, and $\nu_i$ are the elastic moduli and Poisson’s ratios of the indented specimen and of the indenter, respectively. As the elastic modulus of the indenter is much larger than that of the honeybee comb specimen, the preceding relation may be approximated by $E_s \approx E_r/(1 - \nu^2)$. As the wall of the fresh comb is isotropic, this relation was used to estimate its Poisson’s ratio by substituting the microindentation modulus $E_I$ and the elastic modulus $E_r$ obtained from the tensile test. The Poisson ratio for the fresh comb is 0.313. The wall of the old comb is an anisotropic medium due the silks. Nanoindentation technique has also been used to measure the indentation moduli of anisotropic biomaterials (2–6), although for anisotropic media, the indentation modulus is a combination of the moduli in all directions (2, 3, 6), which does not follow the above simple relation between the elastic moduli and the indentation moduli for the isotropic media. Therefore, we also estimated the Poisson ratio of the old comb walls using another theoretical method independent of the measurements of the old comb walls. By regarding the old comb walls as fiber-reinforced composites, we can estimate the Poisson ratio of the old comb walls theoretically using the Mori–Tanaka scheme,
which is known to be accurate for stiff fiber-reinforced composites. In this way, we got the theoretical estimates as 0.319 (five-month wall), 0.326 (one-year wall), 0.327 (two-year wall).

Microindentations were made on the longitudinal surfaces of the walls to ensure larger contact areas. A load profile with the peak load of 1000 μN, the loading/unloading rate of 200 μN/s, a hold period of 0.5 s, was applied to the fresh honeycomb. For the five-month-old honeycomb, the load profiles had the peak load of 4000 μN with the loading/unloading rate of 400 μN/s and hold time of 0.5 s, and the load profile for the one-year-old and two-year-old combs had the peak load of 6000 μN with the loading/unloading rate of 600 μN/s and hold time of 0.5 s. The maximum indentation depth for the walls of the fresh and old honeycombs was about 2.4 μm, which is well below 10% of the thickness of the indented wall specimens. Ten microindentations each were made on the fresh and old honeycomb walls. The microindentation measurements with the 400 μm sapphire tip were carried out in an ambient condition at 23 ± 2°C and 30% relative humidity.

Tensile tests of slabs cut from the comb walls were conducted in a MicroTester (Instron 5848) at a displacement rate of 0.1 mm/min, and at least 10 specimens each were tested from the fresh and old honeycombs. The slabs from the fresh comb were approximately 6.4 mm × 3.1 mm in size, and those from the old combs 5.9 mm × 2.8 mm. The macroscopic shear properties of the combs were measured on rectangular specimens approximately 8 × 15 cells in size, cut from the fresh and old combs. The specimens were bonded between two aluminum plates and the simple shear loading was realized by clamping and pulling the offset ends of the plates in the MicroTester. The nominal shear strength is the ratio of the maximum load to the entire surface area of the comb and not just that of the solid material. At least five specimens each were tested in simple shear from the fresh and old honeycombs at a displacement rate of 0.1 mm/min. The tensile and shear tests were performed at 23 ± 2°C and a relative humidity 11 ± 1%.

Finally, the fresh and one-year-old combs fully laden with honey and bees were analyzed by the finite element method (FEM) using the commercial package ANSYS with the Solid Element 185. Beeswax behaves like a viscoelastic material whose mechanical properties vary with temperature and time. Our nanoindentation tests on the walls of the fresh and one-year-old combs show no obvious influence of the loading rate on the mechanical properties of the comb walls at 28 ± 2°C (see above). Moreover, the honeybee silk does not exhibit noticeable viscoelasticity at 23 ± 2°C (7). These results suggest that at 25°C the fresh and one-year-old combs can be considered to behave elastically. Furthermore, the typical stress-strain curves obtained from tensile tests on the comb walls exhibit a distinct linear elastic behavior. Thus, for all the FEM analyses at 25°C, we have calculated the stress and strain fields in the fresh and old combs using the linear elastic finite element model, along with the measured elastic constants of the walls.

It is known (2) however that fresh wax wall of the African honeybee comb softens when the temperature rises from 25 to 45°C, losing its elastic modulus by a factor of 3.5 and its tensile strength by an order of magnitude, whereas those of an old comb wall that contains 34% silk cocoons by mass are considerably less sensitive to an increase in temperature. Given the fact, that the mass fraction of silk cocoons in the walls of Italian honeybee combs is practically equal to that in the African honeybee comb, it can be assumed that a similar temperature dependence prevails in the Italian honeybee combs. Thus, the elastic tensile and shear moduli of the fresh comb wall are assumed to reduce from 156 MPa and 59.41 MPa at 25°C to 44.6 MPa and 16.98 MPa at 45°C, respectively, while the moduli of the old comb wall remain unchanged. However, the viscoelastic behavior will determine the properties of the fresh comb at 45°C for which a viscoelastic model is needed. It has been reported that mixtures of paraffin and the beeswax have the same time dependence of the elastic properties (8). Moreover, it is widely accepted (9) that viscous effects are observed only in the shear modulus, but not in the volumetric deformation of semicrystalline polymers. For the preceding two reasons, we have used the Prony model to obtain the shear relaxation modulus of beeswax $G(t)$ as a function of time $t$ on the basis of the data from the dynamic shear test on the Cerita® wax (paraffin wax) (9). The normalized shear relaxation modulus for the paraffin wax at 45°C is $G(t)/G(0) = 0.131468 + 0.29698 e^{-0.007368 t} + 0.3219 e^{-0.027677 t} + 0.1142 e^{-0.04071 t} + 0.049368 e^{-4.4212 t} + 0.083384 e^{-36.853} t$ where the unit of the time $t$ is second and $G(0)$ is the initial (elastic) shear modulus at time $t = 0$ when the load is first applied, which for the fresh comb wax is 16.98 MPa. We calculated the stress and strain fields in the wall of the fresh comb at 45°C by the FEM during a period of 10 minutes under a constant load resulting from the weight of the honey and the worker bees.

The FEM computation was performed for a comb area with (horizontal) × 15 (vertical) cells. The comb is filled with honey (density = 1400 kg/m³) and covered by 26 worker bees (mass of each bee about 0.1 g) such that the total load is approximately 0.301 N. The load on each cell is 2.51 mN and is distributed along the cell span. The comb is modeled as a short cantilever (span of cell = 10 mm) with the back faces of the cells clamped, as shown in Fig. S1A. In view of potential stress concentration at the clamped end, the finite element mesh on the back part of each cell was finer than the rest of the cell (the smallest size of element was 10 × 40 × 125 μm), as shown in Fig. S1B. To avoid any free surface effects, we use the maximum stresses and strains of the innermost cell unit (1 × 15). At 45°C, the creep deformation increases with time for about 10 minutes and remains almost constant thereafter. Because both the maximum displacement and shear strain occur at the bottom of the comb, the displacement along the direction of the load and the out-of-plane shear strain distribution in the bottom central cell of the fresh comb at 45°C are shown in Fig. S1 C and D. The out-of-plane shear strain in the bottom central cell of the one-year-old comb is shown in Fig. S1 E.

We also simulated the shear tests of the fresh and one-year-old combs using the FEM and the displacements at the maximum load measured in the tests (0.22 mm for the fresh comb and 0.34 mm for the one-year-old comb). The maximum shear strain of the fresh comb is found to be 4.3% at 25°C and 1.5% at 45°C. The maximum shear strain of the one-year-old comb is always 7% irrespective of the temperature. The corresponding out-of-plane shear strain distributions in the innermost cells (1 × 15) of the fresh and one-year-old comb at 25°C are shown in Fig. S2 A and B. Note that apart from the corners near the clamped end where there is a strain concentration, the maximum shear strain away from these corners is roughly one half the values reported above (see Fig. S2 A and B). In other words, the strain concentration factor near the clamped corners is around 2.

Fig. S1. Fresh and one-year-old combs fully laden with honey and bees were analyzed by the FEM. (A) A comb with 8×15 cells is modeled. The back of the comb is clamped. (B) One cell of the comb with the load distributed along its span (10 mm). (C) Displacement distribution in the direction of load in the bottom cell of the innermost cell unit (1×15) of fresh comb at 45 °C. Unit is mm. The maximum displacement is 39.4 μm when the temperature is increased to 45 °C and held constant for ten minutes. (D) Out-of-plane shear strain distribution for the bottom cell in the innermost cell unit (1×15) of fresh comb at 45 °C. The maximum out-of-plane shear strain reaches 1.9% after ten minutes and remains almost constant thereafter. (E) Out-of-plane shear strain distribution for the bottom cell in the innermost cell unit (1×15) of one-year-old comb at 25 °C. The maximum out-of-plane shear strain reaches 0.014%.

Fig. S2. Shear tests of the fresh and one-year-old combs at 25 °C were simulated by using the FEM. (A) Out-of-plane shear strain distribution in the innermost cell unit (1×15) of fresh comb under the displacement at the maximum load (0.22 mm). The maximum shear strain of the fresh comb is 4.3% near the corners, but otherwise 2.2%. (B) Out-of-plane shear strain distribution in the innermost cell unit (1×15) of one-year-old comb under the displacement at the maximum load (0.34 mm). The maximum shear strain of one-year-old comb is 7% near the corners, but otherwise 3.4%.