The Journal of Experimental Biology 209, 3984-3989
Published by The Company of Biologists 2006
doi:10.1242/jeb.02472

Interspecific variation in beeswax as a biological construction material

Robert Buchwald[1,*], Michael D. Breed[1], Alan R. Greenberg[2] and Gard Otis[3]

[1]Department of Ecology and Evolutionary Biology and Institute for Behavioral Genetics, [2]Department of Mechanical Engineering, University of Colorado at Boulder, Campus Box 427, Boulder, CO, 80309-0427, USA and [3]Department of Environmental Biology, University of Guelph, Guelph, ON, N0B 2J0, Canada

*Author for correspondence (e-mail: buchwald@colorado.edu)

Accepted 8 August 2006

Summary

Beeswax is a multicomponent material used by bees in the genus *Apis* to house larvae and store honey and pollen. We characterized the mechanical properties of waxes from four honeybee species: *Apis mellifera* L., *Apis andreniformis* L., *Apis dorsata* L. and two subspecies of *Apis cerana* L. In order to isolate the material effects from the architectural properties of nest comb, we formed raw wax in to right, circular cylindrical samples, and compressed them in an electromechanical tensometer. From the resulting stress–strain curves, values for yield stress, yield strain, stress and strain at the proportional limit, stiffness, and resilience were obtained. *Apis dorsata* wax was stiffer and had a higher yield stress and stress at the proportional limit than all of the other waxes. The waxes of *A. cerana* and *A. mellifera* had intermediate strength and stiffness, and *A. andreniformis* wax was the least strong, stiff and resilient. All of the waxes had similar strain values at the proportional limit and yield point. The observed differences in wax mechanical properties correlate with the nesting ecology of these species. *A. mellifera* and *A. cerana* nest in cavities that protect the nest from environmental stresses, whereas the species with the strongest and stiffest wax, *A. dorsata*, constructs relatively heavy nests attached to branches of tall trees, exposing them to substantially greater mechanical forces. The wax of *A. andreniformis* was the least strong, stiff and resilient, and their nests have low masses relative to other species in the genus and, although not built in cavities, are constructed on lower, often shielded branches that can absorb the forces of wind and rain.

Key words: *Apis*, honeybee, yield, strength, stiffness, resilience, wax, beeswax.

Introduction

A central prediction in the science of biological materials is that evolution will have finely tuned materials to fit the structural demands they regularly incur (Hallgrimsson and Hall, 2005). We explore this question by comparing waxes from closely related species of honeybees that have differing nesting habits. Most animals construct their nest using either plant or inorganic materials; if endogenous products are used, their role is usually restricted to providing a matrix that supports or adheres pieces of material acquired from the environment. By contrast, honeybees, members of the genus *Apis*, stand out by constructing nests that consist almost exclusively of beeswax, a mixture of glandular products produced endogenously by the bees. We tested the mechanical properties of beeswaxes across four species of *Apis* that differ in their nesting habitats and whose nests consequently incur different environmental forces.

The metabolic and anatomical processes relating to beeswax production have been well characterized (Blomquist et al., 1980; Cassier and Lensky, 1995; Hepburn et al., 1991). These come primarily from studies of *Apis mellifera* Linnaeus, but seem to apply generally to bees in the genus *Apis*. Relatively young adult bees secrete wax scales from specialized glands on the ventral surface of their abdomen. Although wax scales are a metabolic product, the effect of diet on the chemical composition of wax scales is unknown. Wax scales are then collected and manipulated by adult bees using their mandibles, and secretions from the mandibular gland or salivary glands are added (Kurstjens et al., 1985). The processed wax is then used in comb construction, eventually forming the familiar hexagonal-shaped cells. Young bees are reared in the comb and nutritional stores in the form of honey and pollen are stockpiled in the comb's periphery. Combs of *A. mellifera* must be strong enough to hold the many kilograms that a comb full of honey and larvae can weigh (Ruttner, 1988). Comb wax also has important thermal properties that facilitate heat retention in *A. mellifera* nests (Hepburn et al., 1983). Finally, comb wax plays an important role as the source of nestmate recognition pheromones for honeybees (Breed, 1998; Breed et al., 1998; Brockman et al., 2003).

Beeswax is a complex multicomponent material, consisting primarily of alkanes, wax esters and free fatty acids (Tulloch, 1971; Tulloch, 1980; Aichholz and Lorbeer, 1999; Aichholz and Lorbeer, 2000). Changes in the relative amounts of these classes of compounds in the wax should result in corresponding changes in yield strength, stiffness (Kotsiomiti and McCabe, 1997) and resilience (Gibbs, 2002), but these properties of wax are relatively unexplored. Beeswax exhibits both elastic and plastic properties (Shellhammer et al., 1997). In the elastic region, deformation is nonpermanent; when an applied load is released, the material returns to its original shape. However, if the applied load exceeds a critical value, the material enters a plastic region, where deformation becomes permanent.

The mechanical properties of a structure are the result of interactions between its architectural properties and the properties of the material itself. For instance, the strength, stiffness and resilience of a bridge beam are dictated by both its I-shaped cross section and the inherent characteristics of the steel used in its construction. Similarly, honeybees form beeswax into a tightly packed hexagonal shape, and the mechanicial characteristics of a comb are therefore the sum of interactions of architectural features such as the hexagonal design, the thickness of walls and the depth of the cells, with the material properties of raw wax.

For our study, we removed the issue of architecture in order to focus on the mechanical properties inherent in the wax. The architectural characteristics of comb vary among *Apis* species, largely because of variation in overall sizes of honeybee workers among species. A reasonable initial hypothesis is that the mechanical properties of waxes produced by members of the genus *Apis* do not vary among species; that interspecific differences in comb are entirely due to architectural variation. Alternatively, selection may have acted on the wax phenotype, as well as the architectural phenotype; in this case material and structure would interact to produce species-specific properties of the nest. In other words, natural selection could have acted mainly on nest architecture, or on both architecture and material, in shaping the properties of honeybee nests to the environments in which different species in the genus live.

The few published studies of the mechanical properties of beeswax have examined only wax from *A. mellifera*. Hepburn et al. pressed comb wax from the walls of drone cells onto a polyester shim with a heated spatula (Hepburn et al., 1983). These shims were attached to an extensometer in a water bath, and extension tests were conducted over a range of temperatures. Measures of yield strain (amount of strain required to cause permanent deformation) increased linearly with increasing temperature, whereas yield stress (amount of stress required to cause permanent deformation) decreased linearly. Relative workability and absolute workability, both measures of system energy, which would probably be referred to as toughness today, decreased dramatically as temperature increased.

In another study (Kurstjens et al., 1985), six preparations of *A. mellifera* wax were used: virgin wax scales (freshly secreted wax, not yet incorporated into the comb) and comb wax were untreated, chloroform-extracted, or sheeted (glued onto shims of polyester film). Measures of yield stress, yield strain and stiffness (the rate of change of stress per unit strain) were obtained from tension tests in a custom-built extensometer. All tests were performed at room temperature (23°C), except one in which virgin wax scales were tested over a range of temperatures from 25°C to 45°C. Scale wax was as strong as comb wax but less stiff and less distensible.

The mechanical properties of *A. mellifera* wax were measured using both compressive and tensile extension tests over a range of temperatures (Morgan et al., 2002). Morgan et al. found that measures of yield stress and stiffness agreed well, whether tested in compression or extension, especially at room temperature and above. Additionally, yield stress and stiffness decreased linearly with increasing temperature and increased logarithmically with increasing strain rate.

The honeybee genus, *Apis*, is thought to comprise nine species (Engel and Schultz, 1997; Alexander, 1991). The species used in the current study are representative of all three honeybee size types – the more basal 'dwarf' honeybees (subgenus *Micrapis*), the large, or 'giant' honeybees (subgenus *Megapis*) and the medium-sized, cavity-nesting honeybees (subgenus *Apis*). *Apis andreniformis* Smith is a dwarf honeybee found in the old world tropics (Wongsiri et al., 1997). It builds relatively small nests, approximately 10 cm×10 cm, that comprise a single, exposed, vertically hanging comb, usually attached to a small branch with wax completely covering the substrate, often low to the ground and protected by dense vegetation. Also found in the old world tropics, the giant honeybee, *Apis dorsata* Fabricius, builds exposed nests of a single vertical comb, as well. *Apis dorsata* nests are much larger than those of the *Micrapis*, however; a single comb may be 2 m long by 1 m across and may weigh several kilograms, and their nests are usually built high in the canopy attached to the underside of large tree branches. Our remaining species, *A. mellifera* and *Apis cerana* Fabricius, both build nests of multiple combs in a cavity such as a hollow tree or cave. The sizes of these species' nests are restricted by the location in which they build their nests; although comb size varies widely, the total comb area is usually intermediate between *A. andreniformis* and *A. dorsata* (Michener, 1974).

We examined the inherent mechanical properties of honeybee waxes, independent of architecture, for these four representative species; we also compared two subspecies of *A. cerana*, *A. cerana cerana* and *A. cerana japonica*. Stress and strain at the proportional limit, and yield point as well as the stiffness and resilience of these waxes were measured and compared in order to test the hypothesis that wax differs in mechanical properties among these species, even once the structural effects of comb geometry was removed by melting and molding.

Materials and methods

Comb wax of *Apis mellifera* L. was collected in the spring of 2002 from research colonies at the University of Colorado, Boulder, CO, USA. Wax from colonies of the other *Apis* species were obtained from colleagues working in East and Southeast Asia. In total, we obtained wax samples from 18 *A. mellifera*, 5 *A. andreniformis*, 15 *A. cerana cerana*, 20 *A. cerana japonica* and 21 *A. dorsata nests*, each with a mass of 5–10 g. In order to remove the effects of architecture, waxes were slowly melted and non-wax components, such as bee parts and silk, were removed by filtering. Any remaining plant resins and pollen were removed from the wax by warm water extraction. Pure wax was melted and then poured into a cylindrical mold and allowed to cool. Once solidified, wax was removed from the mold and sectioned into identical circular cylinders (17.8 mm×8.1 mm; length×diameter). The approximate 2:1 aspect ratio was chosen to achieve an appropriate balance between buckling (high ratios) and end effects (low ratios).

The cylindrical specimens (N=81) were placed in an Instron 5800 universal electromechanical test system (Grove City, PA, USA) and compressed at a constant rate of 10 mm min^{-1} (Fig. 1) at 23°C. We performed these tests in compression rather than extension to simplify our experimental apparatus and because previous work has shown no difference between mechanical properties tested in either direction (Morgan et al., 2002). The applied load and displacement of the upper platen were measured throughout the test and then converted into stress–strain values. Engineering stress (MPa) is calculated as the applied force divided by the initial cross-sectional area of the cylindrical specimen whereas engineering strain (%) is calculated as the change in specimen length divided by the initial specimen length multiplied by 100.

From the specimen stress–strain curves, the following mechanical parameters were obtained: stress and strain at the proportional limit, stress and strain at the yield point, stiffness and resilience. As indicated in Fig. 2, the proportional limit represents the point at which the stress–strain curve departs from linearity, the yield point occurs at the initial maximum stress, and the stiffness is given by the slope of the stress–strain curve. Both proportional limit and yield stress can be employed to indicate the transition from elastic (recoverable strain) to plastic (non-recoverable strain) behavior. Resilience (energy/volume) is defined by the area under the stress–strain curve in the elastic region and represents the amount of energy needed to deform the specimen to the specified value of strain. In the present case we chose to equate resilience with the proportional limit in order to minimize uncertainty in the area calculation. Given the nature of the stress–strain curves, this is undoubtedly a somewhat conservative convention since resilience values could also reasonably be associated with the yield point.

Statistical comparisons were made using ANOVA (JMP 5.1, SAS Institute).

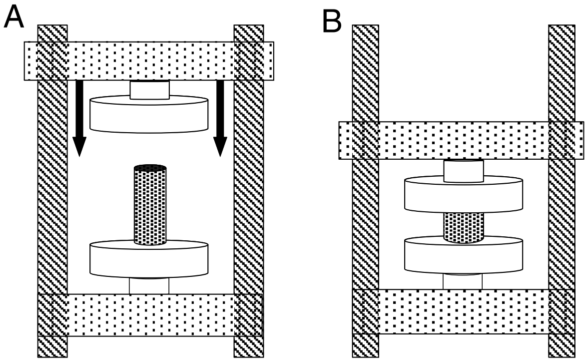

Fig. 1. Schematic representation of wax sample before (A) and after (B) a compression test.

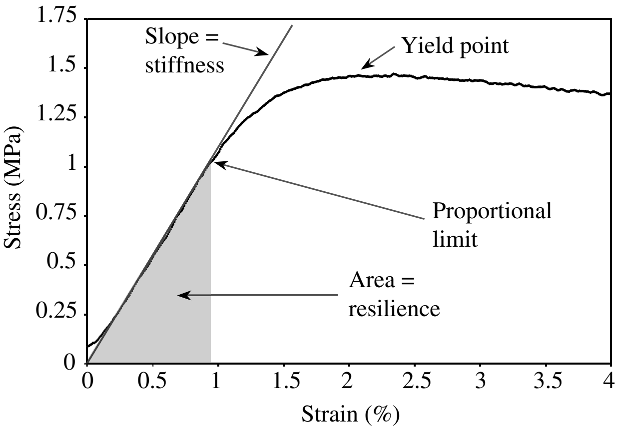

Fig. 2. Sample output from a wax compression test. Strain is on the abscissa, calculated as the change in length divided by the original length of the sample, and has no units. Stress, in MPa, is on the ordinate and is calculated as the force applied to the sample divided by its cross-sectional area. The proportional limit is defined as the point where the curve leaves linearity and the sample moves from elastic to plastic deformation. The yield point is the point of local maximum stress that the sample can withstand. Resilience is defined by the area under the curve at the proportional limit and is a measure of the energy that the sample can absorb before deformation is permanent.

Results

The comparative mechanical properties of the waxes are shown in Fig. 3. The results indicate that the stress at the proportional limit and the yield point varied significantly as a function of species, whereas the corresponding strain values did not. Measures of stiffness also varied significantly among some species but not others, while resilience, a derived measure, did not discriminate the waxes.

Apis dorsata wax was the strongest wax tested. This wax reached the proportional limit and ultimately yielded at a higher stress than other waxes. *Apis cerana cerana* wax was the next strongest, followed by that of both *Apis cerana japonica* and *Apis mellifera*. Finally, the wax of *Apis andreniformis* could withstand the least stress at the yield point and at the proportional limit. Both variables vary significantly among species: yield stress (ANOVA, $F_{4,74}$=39.894, P<0.0001;

Fig. 3A), proportional limit stress (ANOVA, $F_{4,74}$=12.717, P<0.0001; Fig. 3C).

Although we found *A. dorsata* wax to be significantly stiffer than the other waxes, no clear trend was observed when comparing these less-stiff waxes to each other (ANOVA, $F_{4,74}$=7.479, P<0.0001; Fig. 3E).

For resilience, *A. dorsata* wax segregated with the *A. cerana* waxes as the most resilient group, whereas *A. andreniformis* wax was the least resilient and the *A. mellifera* wax intermediate (ANOVA, $F_{4,74}$=9.352, P<0.0001; Fig. 3F).

The other measures less clearly differentiated the waxes. *Apis cerana japonica* wax showed a higher yield strain than the waxes of *A. andreniformis*, *A. dorsata* and *A. mellifera*, with *A. cerana cerana* wax being the same as the latter group (ANOVA, $F_{4,74}$=4.871, P=0.0015; Fig. 3B). Strain at the proportional limit did not differ significantly among the waxes (ANOVA, $F_{4,74}$=1.120, P =0.3535; Fig. 3D) and we found relatively high variances for all groups for this measure.

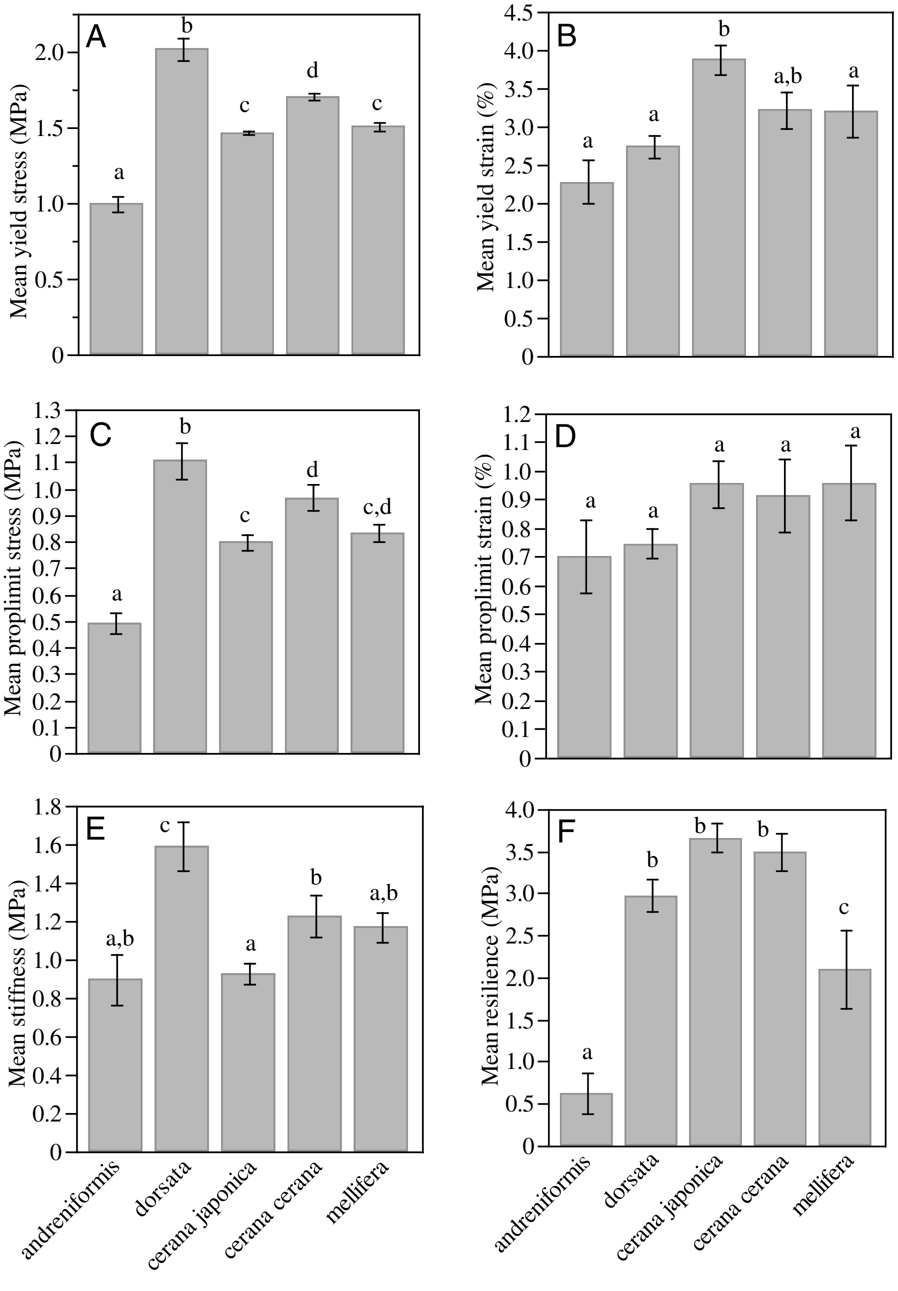

Fig. 3. Comparisons among the waxes of five honeybee (*Apis*) subspecies for six mechanical measures: yield stress (A), yield strain (B), stress at the proportional limit (C), strain at the proportional limit (D), stiffness (E) and resilience (F). Bars represent means ± s.e.m. Different letters indicate statistically different groups.

Discussion

Honeybee waxes are strong yet resilient materials. Although very similar in appearance and composition, the waxes of different honeybee species differ in their inherent mechanical properties. This finding supports the alternative hypothesis, that selection has acted on the composition of wax, as well as on nest architecture. Indeed, all of the mechanical parameters evaluated, with the exception of strain at the proportional limit and resilience, differed significantly among species. It is important to note that these findings reflect differences in the inherent material characteristics. By contrast, structures composed of these waxes could evidence a rather different set of comparative outcomes depending upon the specific geometry of the structures. Our most important finding is that wax mechanical properties vary among *Apis* species even when the wax is experimentally formed into cylinders, so that our measures reflect the waxes' material properties independent of its geometry. For example, the stiffness and strength of a beam will depend upon the stiffness and strength of the particular material as well as the dimensions of the beam cross section. Therefore, given similar geometry, waxes with higher measures for material properties will have correspondingly higher measures for structural properties. Indeed, the results of this study indicate that the wax structures that must bear the greatest loading have the highest yield stress and stiffness. Although the species of honeybees in this study all use their wax in a similar fashion, each is subject to a unique set of environmental and natural history circumstances, which are reflected in their different mechanical properties.

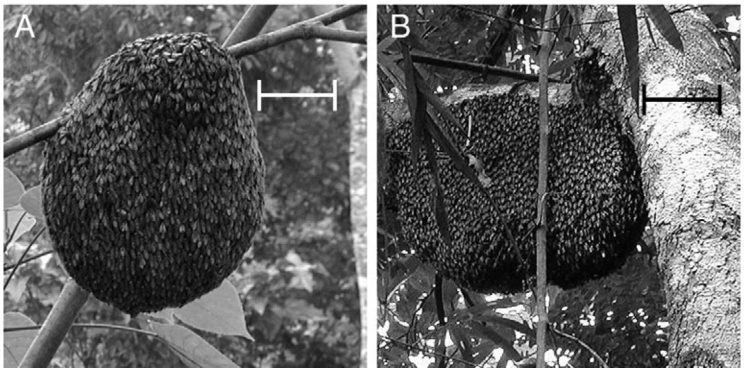

Fig. 4. Photograph of a dwarf honeybee nest (A) and giant honeybee nest (B) in Southern China. Note how the dwarf honeybee nest completely surrounds the branch to which it is attached instead of hanging from the bottom of the branch like the giant honeybee nest and those of other honey bee species. Also note the differences in size; scale bars 10 cm (A), 50 cm (B). Photo: Deng Xiao-bao.

The giant honeybees, represented in this study by *Apis dorsata*, build a single large comb that must support the weight of the entire colony, including immature bees, adults and stored food resources, whereas cavity nesting species such as *Apis mellifera* and *Apis cerana* build multiple combs over which the total weight is distributed. Additionally, *A. dorsata* combs are often located high above the ground where they are exposed to wind and debris, especially in storms (Michener, 1974). The combs of *A. mellifera* and *A. cerana* would usually be free from such forces. Perhaps selection in response to these factors has resulted in the beeswax of *A. dorsata* being stronger and stiffer than beeswax of other honeybee species in order to create more robust structures. The dwarf honeybees, here represented by *Apis andreniformis*, also build exposed nests, but their waxes were less strong and less stiff than the other waxes. Compared to the other species examined, these bees have much smaller combs (Ruttner, 1988), and their honey stores, located in cells affixed directly to the nesting substrate, exert little force on the brood comb (Fig. 4A). Additionally, their nests are usually located among dense vegetation attached to small branches that can bend in the wind. Perhaps reduced wind speeds at these heights and a relatively small colony mass have relaxed the selection pressure for stiff waxes, allowing *A. andreniformis* colonies to function with less stiff wax.

Interestingly, there were marked differences between the two subspecies of *A. cerana*. In pair-wise comparisons, stress at both the proportional limit and the yield point as well as the stiffness of *A. cerana cerana* wax were greater than the wax from *A. cerana japonica*. However, the two waxes did not differ in their resilience or either of the strain parameters. The significance of such differences within the same species is not clear. Perhaps the similarities in resilience and strain responses reflect similar evolutionary pressures on nest construction between populations; however, we do not have the data to test this hypothesis.

The results of this study provide interesting comparisons to earlier work (Hepburn et al., 1983; Kurstjens et al., 1985). The experiments conducted by these researchers utilized wax of a subspecies, *A. mellifera scutellata*, not included in the current study. Hepburn et al. examined the tensile properties of wax from comb cell walls at a range of temperatures (Hepburn et al., 1983). They reported a mean yield stress of approximately 1.3 MPa at 20°C, as compared with 1.5 MPa for *A. mellifera carnica* in this study. However, the values of yield strain were rather different, i.e. ≈32% *versus* 3.2% in this study. These differences most probably reflect differences in the test mode (tension *versus* compression) as well as differences in strain rate (3.19 mm min^{-1} *vs* 10 mm min^{-1}).

Kurstjens et al. examined the stiffness and yield stress for untreated *A. mellifera scutellata* comb wax at 23°C (Kurstjens et al., 1985). Their reported value for yield stress, 1.5 MPa, is the same as that for *A. mellifera carnica* wax in this study. By contrast, their result for stiffness, 4.2 MPa, differed substantially from our value of 1.2 MPa. We note that different engineering materials can exhibit similar values of strength but different values for stiffness.

Although our results for yield stress agree with the findings of Hepburn et al. (Hepburn et al., 1983) and Kurstjens et al. (Kurstjens et al., 1985), our values of yield stress are ≈25% higher than those reported by Morgan et al. (Morgan et al., 2002). Morgan et al. did use a very similar experimental technique – we measured compressive yield stress at similar strain rates. The differences between these studies and that of Morgan et al. (Morgan et al., 2002) may be attributable to the use of a different subspecies of *A. mellifera* used in their study or to differences in the collection or purification techniques of the raw wax [details regarding the subspecies used or the collection and purification methods are not provided (Morgan et al., 2002)].

We focused on the inherent mechanical properties of honeybee waxes. However, the mechanical response to an applied force is different when a material is formed into different geometries. The hexagonal-shape of cells in finished honeycomb contribute substantially to the mechanical properties of comb, and wax in this shape probably differs in interesting ways from the inherent material properties discussed in this study. More needs to be determined about the mechanical properties of wax in its finished comb shape to gain a more complete picture of the mechanical properties of beeswax, and how differences among species relate to differences in their ecologies.

We thank John Coyle for his help with equipment, the Vietnam Bee Research and Development Center in Hanoi for samples of *A. cerana, A. dorsata* and *A. andreniformis* wax, Deng Xiao-bao and Liu Fang-lin for *A. cerana cerana*, *A. andreniformis* and *A. dorsata* wax from Yunnan, China, and Masami Sasaki for *Apis cerana japonica* wax. Funding was provided by the Institute for Behavioral Genetics NIH research traineeship – Biological Sciences, NIH-T32MH016880, PI; Jeanne Wehner and the Chancellor's Teaching Fellowship.

References

Aichholz, R. and Lorbeer, E. (1999). Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography–chemical ionization mass spectrometry I. High-temperature gas chromatography. *J. Chromatogr. A* **855**, 601-615.

Aichholz, R. and Lorbeer, E. (2000). Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionization mass spectrometry II: High-temperature gas chromatography-chemical ionization mass spectrometry. *J. Chromatogr. A* **883**, 75-88.

Alexander, B. A. (1991). Phylogenetic analysis of the genus *Apis* (Hymenoptera, Apidae). *Ann. Entomol. Soc. Am.* **84**, 137-149.

Blomquist, G. J., Chu, A. J. and Remaley, S. (1980). Biosynthesis of wax in the honeybee, *Apis mellifera* L. *Insect Biochem.* **10**, 313-321.

Breed, M. D. (1998). Recognition pheromones of the honey bee. *Bioscience* **48**, 463-470.

Breed, M. D., Leger, E. A., Pearce, A. N. and Wang, Y. J. (1998). Comb wax effects on the ontogeny of honey bee nestmate recognition. *Anim. Behav.* **55**, 13-20.

Brockmann, A., Groh, C. and Frohlich, B. (2003). Wax perception in honeybees: contact is not necessary. *Naturwissenchaften* **90**, 424-427.

Cassier, P. and Lensky, Y. (1995). Ultrastructure of the wax gland complex and secretion of beeswax in the worker honey-bee *Apis mellifera L. Apidologie* **26**, 17-26.

Engel, M. S. and Schultz, T. R. (1997). Phylogeny and behavior in honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* **90**, 43-53.

Gibbs, A. G. (2002). Lipid melting and cuticular permeability: new insights into an old problem. *J. Insect Physiol.* **48**, 391-400.

Hallgrimsson, B. and Hall, B. (ed.) (2005). Variation and variability: central concepts in biology. In *Variation*, pp. 1-9. Boston: Elsevier Press.

Hepburn, H. R., Armstrong, E. and Kurstjens, S. (1983). The ductility of native beeswax is optimally related to honeybee colony temperature. *S. Afr. J. Sci.* **79**, 416-417.

Hepburn, H. R., Bernard, R. T. F., Davidson, B. C., Muller, W. J., Lloyd, P., Kurstjens, S. P. and Vincent, S. L. (1991). Synthesis and secretion of beeswax in honeybees. *Apidologie* **22**, 21-36.

Kotsiomiti, E. and McCabe, J. F. (1997). Experimental wax mixtures for dental use. *J. Oral Rehabil.* **24**, 517-521.

Kurstjens, S. P., Hepburn, H. R., Schoening, F. R. L. and Davidson, B. C. (1985). The conversion of wax scales into comb wax by African honeybees. *J. Comp. Physiol. B.* **156**, 95-102.

Michener, C. D. (1974). *The Social Behavior of the Bees*. Cambridge, MA: Belknap Press.

Morgan, J., Townley, S., Kemble, G. and Smith, R. (2002). Measurement of physical and mechanical properties of beeswax. *Mater. Sci. Technol.* **18**, 463-467.

Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees*. New York: Springer-Verlag.

Shellhammer, T. H., Rimsey, T. R. and Krochta, J. M. (1997). Viscoelastic properties of edible lipids. *J. Food Eng.* **33**, 305-320.

Tulloch, A. P. (1971). Beeswax – structure of esters and their component hydroxy acids and diols. *Chem. Phys. Lipids* **6**, 235-238.

Tulloch, A. P. (1980). Beeswax–composition and analysis. *Bee World* **61**, 47-62.

Wongsiri, S., Lekprayoon, C., Thapa, R., Thirakupt, K., Rinderer, T. E., Sylvester, H. A., Oldroyd, B. P. and Booncham, U. (1997). Comparative biology of Apis andreniformis and Apis florea in Thailand. *Bee World* **78**, 23-35.