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The thermal properties of beeswaxes: unexpected findings

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SUMMARY

Standard melting point analyses only partially describe the thermal properties of eusocial beeswaxes. Differential scanning calorimetry (DSC) revealed that thermal phase changes in wax are initiated at substantially lower temperatures than visually observed melting points. Instead of a sharp, single endothermic peak at the published melting point of 64°C, DSC analysis of *Apis mellifera* Linnaeus wax yielded a broad melting curve that showed the initiation of melting at approximately 40°C. Although *Apis* beeswax retained a solid appearance at these temperatures, heat absorption and initiation of melting could affect the structural characteristics of the wax. Additionally, a more complete characterization of the thermal properties indicated that the onset of melting, melting range and heat of fusion of beeswaxes varied significantly among tribes of social bees (Bombini, Meliponini, Apini). Compared with other waxes examined, the relatively malleable wax of bumblebees (Bombini) had the lowest onset of melting and lowest heat of fusion but an intermediate melting temperature range. Stingless bee (Meliponini) wax was intermediate between bumblebee and honeybee wax (Apini) in heat of fusion, but had the highest onset of melting and the narrowest melting temperature range. The broad melting temperature range and high heat of fusion in the Apini may be associated with the use of wax comb as a free-hanging structural material, while the Bombini and Meliponini support their wax structures with exogenous materials.

Key words: Apidae, wax, differential scanning calorimetry, heat of fusion, melting point, thermal properties.

INTRODUCTION

The wax of eusocial bees is an endogenously produced multicomponent, single-phase material which, when formed into comb, protects the developing brood, stores food, serves as an intermediary in communication (Breed, 1998; Sandeman et al., 1996) and has important thermoregulatory properties (Hepburn, 1986). The family Apidae contains the eusocial bumblebees (Bombini), stingless bees (Meliponini) and honeybees (Apini), as well as subfamilies of primarily solitary bees. During nest construction, bumblebee wax and stingless bee wax is typically mixed with other substances, such as plant resins and pupal silk (Wille and Michener, 1973), and structures formed from these wax mixtures typically do not bear substantial weight. In contrast, honeybee (Apini) wax is formed into one or more free-hanging, heavily weighted combs and is not mixed with foreign substances. Propolis (resinous substances obtained from plants), however, is used to seal openings in the hive and in other places throughout the nest (Strehle et al., 2003). Although the thermal characteristics of beeswaxes can affect important structural properties such as stiffness, strength and toughness depending on temperature variations in the environment, the thermal properties of this important biological material have not been adequately investigated using contemporary techniques.

Eusocial bees display a wide range of natural history traits and nesting ecologies, and the waxes they produce are exposed to different environmental conditions. Bumblebees are primarily found in temperate regions and are the dominant pollinators in arctic and alpine zones. Perhaps because of the low temperatures and lack of resources in winter, most bumblebees do not build large, perennial nests, but follow an annual life cycle. In contrast, stingless bees build perennial colonies, are restricted to the tropics and do not experience the extreme climatic conditions found in the temperate zones. Finally, some honeybee species are native to tropical habitats while others have distributions that span both the tropics and the temperate zone. The Apini are often divided into three groups, the dwarf, giant and cavity-nesting honeybees, whose nesting ecologies differ. As their name implies, the cavity-nesting honeybees build nests in cavities such as a hollow tree or rock overhang, usually with more than one hanging comb. Both dwarf and giant honeybees build a single exposed comb, but giant honeybee nests are much larger and bear proportionally greater weight. The many differences in Apoid nesting ecology may correspond to differences in wax thermal characteristics, but no previous studies have examined this relationship.

Previous investigations of beeswax mechanical and thermal properties have focused primarily on *Apis mellifera* (Linnaeus) wax and its composition. Utermark and Schicke (Utermark and Schicke, 1963) and Tulloch (Tulloch, 1980) reported melting transitions between 61 and 63°C for *A. mellifera* wax using traditional methods. Timbers et al. (Timbers et al., 1977) examined *A. mellifera* wax with modern thermal analysis methods and found a melting transition that peaked at 68°C, while Southwick (Southwick, 1985) found the thermal conductivity of *A. mellifera* wax to be 0.36×10^{-3} cal (cm s °C)$^{-1}$. Tulloch (Tulloch, 1980) provided baseline information on the chemical makeup of *A. mellifera* wax, and subsequent chemical studies of *A. mellifera* wax have largely supported his findings (Aichholz and Lorbeer, 1999; Aichholz and Lorbeer, 2000). Hepburn (Hepburn, 1986) provided a comprehensive synthesis of knowledge concerning *Apis* wax but since the publication of this book, only two studies of *A. mellifera* wax mechanical properties have been published. Morgan et al. (Morgan et al., 2002) tested only *A. mellifera* wax. Buchwald et al. (Buchwald et al., 2006) found that different honeybee subfamilies produce wax with different mechanical properties, and also found that these differences correspond to differences in nesting ecology. Even fewer studies focus on waxes of other bees. Blomquist et al. (Blomquist et al., 1985) analyzed the chemical composition of *Trigona (Trigonisca) buyssoni* (Fabricius) and *Trigona (Trigonisca) atomaria* (Ducke) waxes, Milborrow et al. (Milborrow et al., 1987) analyzed *Trigona australis* (Smith), and Koedam et al. (Koedam et al., 2002) examined the wax of *Melipona bicolor* (Lepeletier).

The primary components of beeswaxes include alkanes, fatty acids and long-chain esters (Tulloch, 1980). Each of these classes is represented by numerous substances, and the mixture is made even more complex by the presence of many other compounds in low concentration (Aichholz and Lorbeer, 1999; Aichholz and Lorbeer, 2000). In addition to hydrocarbons, proteins are present in beeswax and are probably added when wax scales are manipulated in the bees' mouths (Kurstjens et al., 1985; Kurstjens et al., 1990).

Because silk fibers, remnant bee parts and plant resins are often also found incorporated into beeswax, raw beeswax can be appropriately described as a composite material. Beeswax without these additions, however, is best described as a multicomponent material that may or may not be multiphasic (Callister, 2007). It is important to note that multicomponent materials (alloys) melt over a temperature range rather than at a specific temperature (Callister, 2007). Melting curves (thermograms) are often obtained *via* differential scanning calorimetry (DSC), and can be used to assess the purity of a polymeric sample by the characteristics of the melting peak (Turi, 1997). The incorporation of contaminants into an otherwise pure material typically broadens the melting transition and depresses the onset of melting. Although the thermal properties of many engineering materials are well characterized, the thermal behavior of biological materials has received comparatively little attention (Utermark and Schicke, 1963) (but see Lorinczy, 2004).

DSC is a thermal analysis technique that allows quantitative characterization of phase transitions, such as melting. Melting represents a primary phase transition in crystalline materials due to absorption of thermal energy; for polymeric materials that typically melt over a temperature range, the onset of melting may not be instantaneously reflected in externally apparent changes. DSC enables determination of the range of temperatures over which melting occurs as well as the amount of energy associated with the melting transition, i.e. the heat of fusion (Aboul-Gheit, 1997; Turi, 1997).

Using DSC, we investigated the thermal properties of waxes produced by bees in the three eusocial bee tribes: Bombini, Meliponini and Apini. Although bees in these groups share many physiological and ecological characteristics, they also exhibit important differences, including the differences in nesting ecology discussed above. For this study, we tested two hypotheses: (1) that classical melting point studies have not adequately represented the complete nature of beeswax thermal behavior, and (2) that the thermal properties of the waxes are more similar within a taxonomic group than between groups.

MATERIALS AND METHODS

Waxes

We examined 41 honeybee, six stingless bee and two bumblebee samples. Samples of *A. mellifera* and *Bombus rufocinctus* (Cresson) wax were collected from the University of Colorado apiaries and the University of Colorado Mountain Research Station, respectively. All other *Apis* waxes were collected in Malaysia, Indonesia, China and the Philippines. When possible, samples from multiple colonies of the same species were collected and mean values of their phase-transition properties were calculated. The chemical composition of honeybee wax can change with age (Frohlich et al., 2000). To avoid these effects, we collected waxes randomly with respect to age of comb to distribute any age effects among all samples. The *Bombus impatiens* (Cresson) wax samples came from colonies purchased from Koppert Biological Systems (Romulus, MI, USA). Meliponine waxes were collected in Costa Rica or were provided by Vera Imperatriz Fonseca from Brazilian populations. We used only portions of comb containing pure wax, devoid of any foreign particles, but small quantities of propolis may have made its way into our analysis (Strehle et al., 2003).

Differential scanning calorimetry

Thermal analyses were carried out using a Perkin Elmer DSC 7 differential scanning calorimeter (Waltham, MA, USA). We calibrated for temperature and heat flow using high purity indium as a standard. Dry nitrogen was employed as the purge gas through the DSC cell, and a refrigerated intracooler provided sub-ambient cooling to establish uniform initial test conditions. Individual wax samples (~5.5 mg) from each colony were placed in sealed aluminium pans. Temperature scans in all experiments began with the samples held for 1.5 min at 5°C followed by a heating cycle at a rate of 10°C min^{-1} to a temperature of 85°C. Testing over this temperature range excluded non-wax materials from the analysis, as these materials begin melting at much higher temperatures (>100°C). The results from each run were plotted with heat flow (mW) as a function of temperature (°C), and DSC system software was used to obtain the onset, major peak and end of the melting transition, while heat of fusion was calculated by standard methods (Fig. 1). The melting point range is defined as the end temperature of the melting transition minus the onset.

Data analysis

We compared melting properties between three tribes in the family Apidae (Bombini, Meliponini and Apini) as well as between subfamilies in the Apini (dwarf, giant and cavity-nesting honeybees). No comparisons with the tribes Bombini or Meliponini were made due to low sample size and lack of variance (one sample per species was collected). Data were analyzed using commercial statistical analysis software (JMP 5.1, SAS systems) with analyses of variance (ANOVA) and Tukey–Kramer honestly significant difference (HSD).

RESULTS

Thermal characteristics

The DSC thermograms revealed a different, more accurate picture of the melting properties of Apine waxes. For example *Apis* species wax melts between 61 and 63°C as measured by capillary tube methods (Bisson et al., 1940; Tulloch, 1980), whereas DSC measurements clearly indicated a broad transition with the onset of melting observed at approximately 40°C (Fig. 1). In general, thermograms of the Apini revealed two or three overlapping peaks in which the dominant peak (based on the contribution to the total

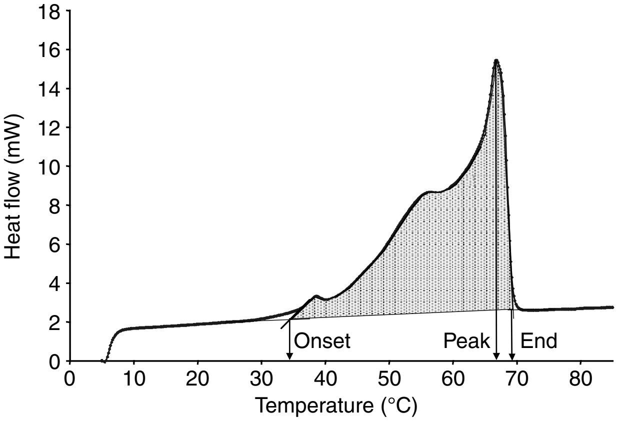

Fig. 1. Representative Apini wax thermogram (*Apis andreniformis*) that defines the four parameters listed in Table 2: onset, peak and end of melting temperature, as well as the heat of fusion. The last is proportional to the area under the melting curve (gray) divided by the sample mass.

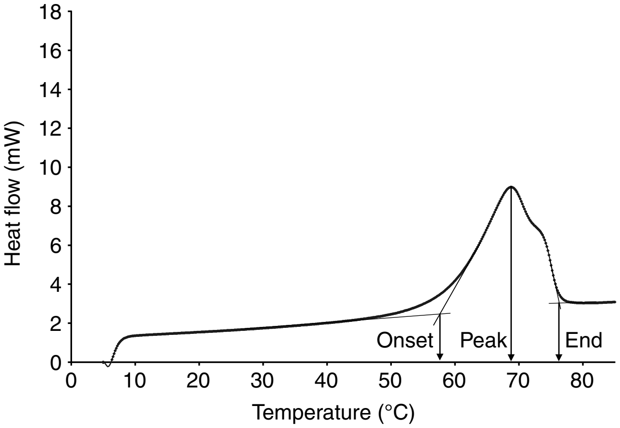

Fig. 2. Representative Meliponini thermogram (*Melipona quadrifasciatta*).

heat of fusion) appeared at the high end of the temperature range. In contrast, the melting transition for Meliponini wax displayed two closely overlapping peaks (Fig. 2) and that for Bombini wax showed two widely separated peaks (Fig. 3). These peaks probably represent the melting of distinct phases, each of which could be single or multicomponent in nature. Indeed, the endpoints of the melting transitions encompass the melting points of many of the major components, i.e. fatty acids and wax esters, found in beeswax (Table 1).

Thermal properties among tribes

The DSC thermograms differed among tribes (Figs 1–3, Table 2). For the onset of melting (initial departure from the baseline), the tribes ranked, from highest temperature to lowest temperature: Meliponini > Apini > Bombini (ANOVA: $F_{2,48}$=157.9, P<0.0001), with all tribes different from each other (Tukey–Kramer HSD, P<0.05 for each pair tested). The same trend was found with respect to the end of the melting transition (ANOVA: $F_{2,48}$=74.4, P<0.0001), with all tribes different from each other (Tukey–Kramer HSD, P<0.05 for each pair tested). The temperature range of the phase change was greatest in the Apini, followed by the Bombini and Meliponini (ANOVA: $F_{2,48}$=85.1, P<0.0001), although Bombini and Meliponini were not significantly different from each other in *post hoc* tests (Tukey–Kramer HSD for Meliponini and Bombini, P>0.05; all other comparisons, P<0.05). The energy required for melting as reflected in the heat of fusion was highest for Apini followed by Meliponini and Bombini (ANOVA: $F_{2,47}$=21.4, P<0.0001), although Meliponini and Bombini were not significantly different from each other in *post hoc* tests (Tukey–Kramer HSD for Meliponini and Bombini, P>0.05; all other comparisons, P<0.05). The temperature of the major thermogram peak was highest in the Meliponini, somewhat lower in the Apini, and substantially lower in the Bombini (ANOVA: $F_{2,48}$=48.1, P<0.0001) with all tribes different from each other (Tukey–Kramer HSD, P<0.05 for each pair tested). See Table 2 for a summary of these results.

Thermal properties within tribes

We analyzed wax samples from 49 subspecies in total, but only within the tribe Apini did we analyze multiple colonies within each subspecies [except for *Apis dorsata binghami* (Cockerell), for which we obtained only one wax sample]. Therefore, we only compared the wax melting properties of subspecies within the tribe Apini.

For the honeybee waxes, all melting properties varied significantly among species (onset of melting: ANOVA: $F_{6,33}$=10.6, P<0.0001; end of melting: ANOVA: $F_{6,33}$=14.6, P<0.0001; melting range: ANOVA: $F_{6,33}$=9.33, P<0.0001; heat of fusion: ANOVA: $F_{6,33}$=5.36, P<0.0006; major peak of melting curve: ANOVA: $F_{6,33}$=20.8, P<0.0001).

When grouped by nesting behavior, we found significant differences among the dwarf honeybees, giant honeybees and cavity-nesting honeybees for the measurements of melting transition onset (ANOVA: $F_{2,36}$=8.88, P<0.0007), end of melting (ANOVA: $F_{2,36}$=16.6, P<0.0001) and major peak of melting thermogram (ANOVA: $F_{2,36}$=24.0, P<0.0001). *Post hoc* testing revealed that giant honeybee wax was less than dwarf and cavity-nesting honeybee wax for these three measurements, while dwarf and cavity-nesting honeybees were not different from each other (Tukey–Kramer HSD). The three groups did show significantly different heats of fusion (ANOVA: $F_{2,36}$=13.0, P<0.0001) with the cavity-nesting honeybee waxes exhibiting a higher mean than the other subgenera, while the dwarf and giant honeybee waxes were not different from each other for this measurement (Tukey–Kramer HSD). The melting point ranges were not significantly different among subgenera (ANOVA: $F_{2,36}$=0.67, P=0.516). See Fig. 4 and Table 2 for a summary of subgeneric comparisons.

Table 1. Published melting point ranges for representative Apine waxes and typical wax components

Sample	Melting-point range (°C)	Reference
Bombus rufocinctus	35–45	(Tulloch, 1970)
Trigona australis	58–60	(Milborrow et al., 1987)
Trigona buyssoni	58–59	(Blomquist et al., 1985)
Trigona atomari	58–59	(Blomquist et al., 1985)
Apis mellifera	64.4	(Bisson et al., 1940)
	63–65	(Tulloch, 1980)
	68 (peak)	(Timbers, 1977)
Pentacosane	53–56	MSDS
Pentatriacontane	73–75	MSDS
Palmitic acid	61–64	MSDS
Behenyl palmitate	52–53	MSDS
Palmityl palmitate	55–56	MSDS

MSDS, Material Safety Data Sheet.

Table 2. Thermal properties of beeswaxes analyzed using differential scanning calorimetry

Species	Colonies tested	Onset (°C)	End (°C)	Range (°C)	Peak (°C)	Heat of fusion (mJ mg^{-1})
Apini						
Dwarf honeybees						
Apis andreniformis Smith	2	34.6±0.92	69.0±0.42	34.3±1.33	67.0±0.33	145.1±1.70
Apis florea Fabricius	5	40.1±1.10	69.2±0.40	29.1±1.22	66.7±0.37	117.9±11.83
Giant honeybees						
Apis dorsata binghami Cockerell	1	37.9	67.5	29.7	64.9	149.9
Apis dorsata dorsata Fabricius	11	35.2±0.50	66.5±0.22	31.3±0.57	63.5±0.29	128.1±11.32
Cavity-nesting honeybees						
Apis cerana cerana Fabricius	4	37.1±0.05	69.4±0.23	32.3±0.25	67.7±0.18	152.3±1.60
Apis cerana indica Fabricius	12	37.4±0.32	70.5±0.46	33.1±0.39	68.6±0.46	168.8±0.99
Apis mellifera Linnaeus	4	40.4±0.72	67.0±0.50	26.6±0.93	64.6±0.67	170.7±4.48
Apis nigrocincta Smith	2	37.4±0.25	69.6±0.41	32.2±0.67	67.6±0.25	165.8±1.74
Mean ± s.e.m.		37.3±0.36	68.6±0.30	31.3±0.41	66.3±0.36	148.5±4.57
Bombini						
Bombus impatiens Cresson	1	34.2	55.2	21.0	50.2	33.7
Bombus rufocinctus Cresson	1	29.4	51.9	22.5	48.5	78.7
Mean ± s.e.m.		31.8±2.42	53.5±1.67	21.8±0.75	49.4±0.83	56.2±22.47
Meliponini						
Plebia schrottkyi Friese	1	53.9	73.7	19.8	67.7	85.9
Melipona quadrifasciata Lepeletier	1	57.7	76.7	19.0	68.9	75.4
Nanotrigona testaeicornis Iratim	1	69.0	85.3	16.3	79.4	n.a.
Plebia quadripunctata Lepeletier	1	58.4	76.0	17.7	71.9	67.8
Trigona angustula Latreille	1	57.2	75.0	17.8	67.9	75.4
Melipona beecheii Bennett	1	55.8	72.7	16.9	67.8	101.2
Mean ± s.e.m.		59.2±2.57	77.4±2.06	18.1±0.60	71.1±2.19	76.1±3.73

When multiple colonies were tested, the mean ± s.e.m. is given.

DISCUSSION

Here, we report the melting properties for the waxes of several *Apis*, *Bombus*, *Melipona*, *Nanotrigona*, *Plebia* and *Trigona* species for the first time. Additionally, we found that previously published results using classical melting point methods give an incomplete picture of the thermal behavior of beeswaxes. Indeed, the full transition behavior obtained *via* DSC provides a more complete, if somewhat more complex, view of melting in comparison with data previously reported in the literature. With these limitations in mind, the significance of the data generated in the current study is best addressed in terms of the two hypotheses stated in the Introduction.

Hypothesis 1: classical melting point studies do not adequately represent the complete nature of beeswax thermal behavior

DSC thermograms provide quantitative characterization of the melting transition from onset through to completion. For multicomponent materials such as beeswax, the number of peaks and their location on the curve reflect the nature of the chemical components (e.g. esters and fatty acids) comprising the sample. Materials with similar relative abundances of these chemical components will display similar DSC thermograms. DSC analysis improves on previous studies of beeswax melting properties by providing a more complete understanding of this surprisingly complex material. For example, the multiple peaks evident in the thermograms such as that shown in Fig. 3 suggest that the wax is not appropriately represented as a single-phase material. In addition, complete thermogram data provide a more robust basis for quantitative comparisons among samples.

DSC studies indicate that beeswax melts over a relatively wide temperature range, with the onset of melting occurring at a temperature well below the temperature at which melting is first observed visually. For example, *Apis* species wax begins melting at approximately 37°C although capillary tube measurements describe the melting point onset at about 61°C. Honeybees actively maintain ambient nest temperatures between 34 and 35°C *via* evaporative cooling and other methods (Seeley and Heinrich, 1981). Not only are the animals themselves sensitive to temperatures above 35°C (Hepburn, 1986) but also the wax that forms their nest actually begins to melt at these temperatures, as evidenced by the DSC thermograms. The low onset of melting also illuminates previous findings regarding the mechanical properties of honeybee waxes. Hepburn et al. (Hepburn et al., 1983) found that the weight of wax comb loaded with honey, pollen and larvae would exceed the yield strength of beeswax above 40°C. This is due to the fact that strength properties significantly decrease as

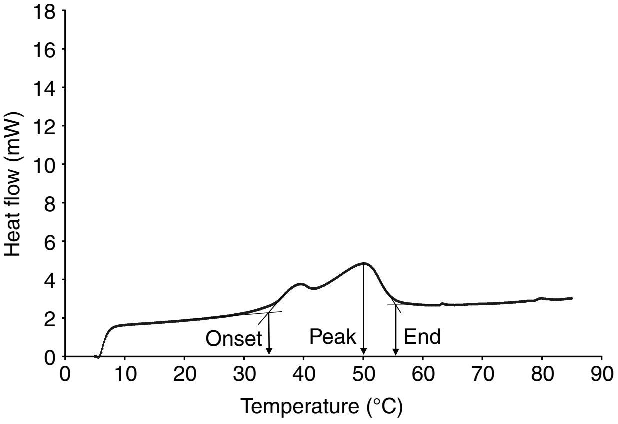

Fig. 3. Representative Bombini thermogram (*Bombus impatiens*).

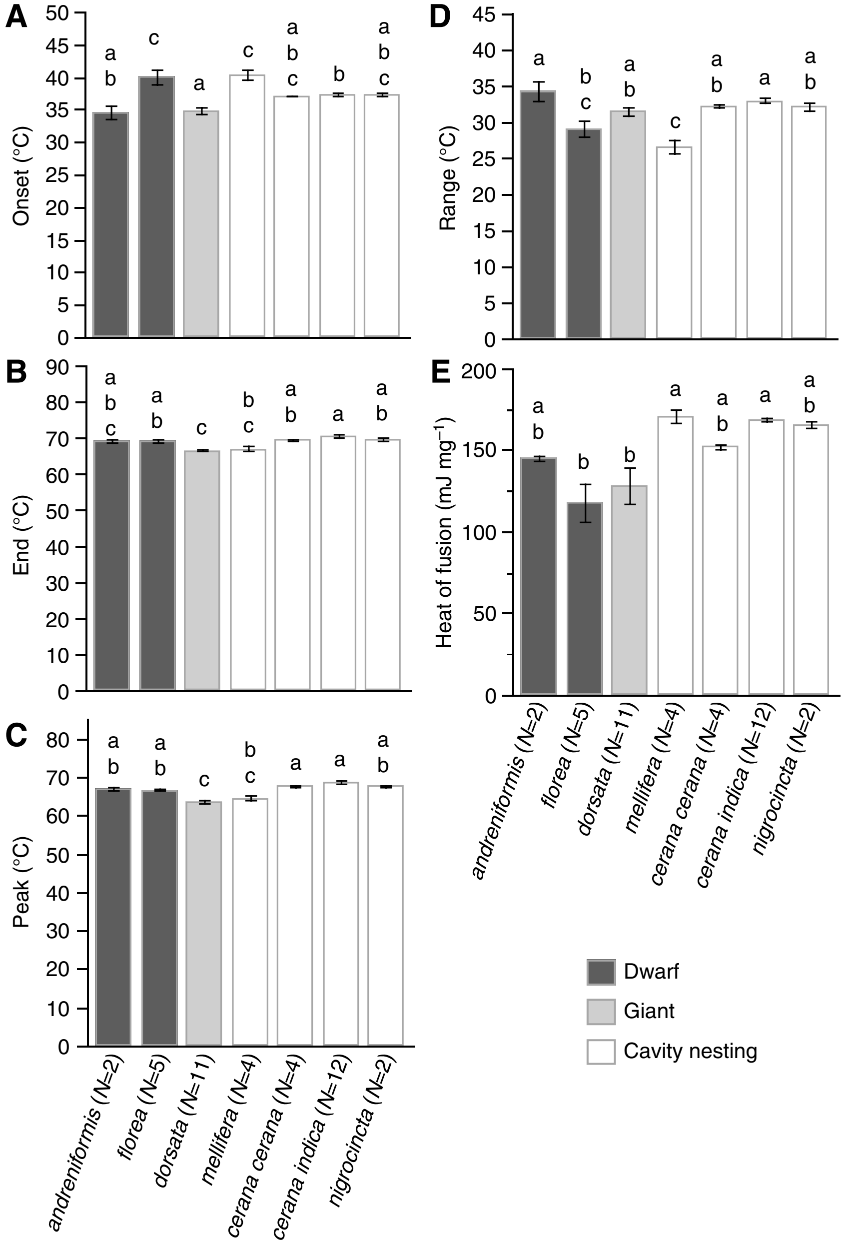

Fig. 4. Melting properties of seven *Apis* subspecies (means ± s.e.m.): melting onset (A), end of melting (B), major melting peak (C), melting range (D), and heat of fusion (E). Letters above bars denote statistical groupings: subspecies with no letters in common are different from each other. Species are also grouped by subgenus as dwarf, giant or cavity-nesting honeybees.

material temperature increases and the sample reaches the onset of melting.

Hypothesis 2: the thermal properties of the waxes are more similar within taxonomic group than between groups

Published capillary melting points for beeswax and characteristic beeswax components are listed in Table 1. Waxes with higher melting points probably contain relatively higher amounts of saturated compounds, polar compounds, higher molecular weight compounds, or all three. Well-known physico-chemical phenomena affect the thermal properties of multicomponent materials such as beeswax. Mixing a pure component with even trace amounts of another component lowers the melting point of the mixture (Callister, 2007). Additionally, solvents absorbed into wax may result in significantly lower wax melting points. Alkenes, which are present in beeswax and are liquid at ambient temperatures, could serve this function.

Our DSC results correspond well with published melting ranges for meliponine and bombine waxes (Table 1). They are less consistent with published melting points for *A. mellifera* waxes (Table 1), suggesting that the complexity of the wax mixture in *Apis* results in unique thermal and physical characteristics (Buchwald et al., 2006). One previous study used DSC to analyze *A. mellifera* wax (Timbers et al., 1977); our thermogram for this species corresponds well to these findings, but the authors did not quantify the heat of fusion or other relevant melting characteristics. The clear advantage of DSC analysis lies in the ability to quantify thermal characteristics that are not apparent in capillary melting point tests or other less precise methods. To this end, Fourier transform infrared (FTIR) spectroscopy may also be a promising method for investigating wax thermal characteristics (Musser and Kilpatrick, 1998).

For both bumblebees and honeybees, we found a close correlation between internal nest temperature and the onset of wax melting as revealed by DSC. Bumblebees exposed to ambient temperatures as low as 5°C keep their brood temperatures between 29 and 33°C (Richards, 1973), and we determined the onset of melting for Bombini wax to average 32°C. Similarly, honeybees maintain nest temperatures between 30 and 35°C to avoid impaired development or death, and we found the onset of melting for honeybee waxes to be between 34 and 40°C. For both these tribes, the onset of wax melting closely coincides with the upper limit of ambient nest temperatures. We wish to note, however, that our investigation of bumblebee wax is derived from a small sample size and consequently the results should be viewed as preliminary.

Beeswax comprises hundreds of chemicals in at least nine principal compound families (Aichholz and Lorbeer, 1999), and multicomponent materials typically display depressed melting points compared with the melting points of their components in pure form (Callister, 2007). The need to maintain nests at specific temperatures for proper brood development has probably served as a selective pressure that kept the onset of melting above these thresholds. Very little information on stingless bee nest temperatures is available, but even without active thermoregulation their nests are rarely exposed to temperatures above 38°C, as they are well insulated and restricted to the tropics. The high onset of melting exhibited by meliponine waxes may be a response to other selective pressures, including the mixing of foreign materials with endogenous wax in stingless bee nest construction.

Despite differences in melting properties among honeybee species, all DSC thermograms within this group displayed a marked similarity with respect to the shape of the melting curves; all curves departed from the baseline between 34 and 40°C, contained two or three closely overlapping peaks of increasing magnitude, and returned to the baseline between 67 and 70°C. Similarly, DSC thermograms of bumblebee waxes all displayed two relatively distinct peaks with melting ranges from 31 to 53°C, while stingless bee waxes all showed two very closely overlapping peaks at much higher temperatures. The differences in the wax thermal properties of Apine tribes correspond well with differences in nesting ecology and nest construction. Honeybees are unique among the social insects in using essentially unmodified wax for nest construction. Even though *Apis* cocoons remain in the comb after brood emergence, these cocoons are not essential to the structural integrity of the comb, as comb can bear the full weight of a brood and food before the first brood enters the pupal stage. In contrast, bombine waxes are softer and cannot bear as great a load (R.B., personal observation). These waxes are used in conjunction with silk, plant resins and gums (propolis) in bumblebee nests to form a strong composite material. The silk in *Bombus* wax has a very high melting point, and its phase transition behavior was not examined. Similarly, stingless bees also incorporate a variety of materials, such as propolis, mud, feces and plant fibers, into their nest construction (Wille and Michener, 1973). These additives probably affect the thermal properties of Meliponine and Bombine nest-building materials, but their individual contributions have yet to be quantified. The DSC thermograms for all species were remarkably similar in shape within each tribe and remarkably different between tribes (Figs 1–3). Although we found significant differences for all thermal properties when comparing bee tribes, we tested only two bumblebee waxes and six stingless bee waxes out of the hundreds of species found globally. Our results should therefore be viewed more as a difference among the species tested than a generalization of differences between tribes. Further work would include better representation of the waxes of more bumblebee, stingless bee and honeybee species.

Within the genus *Apis*, comparisons of melting parameters were significantly different when all species were considered. The species examined can be grouped according to size, nesting ecology and phylogenetic relationship (Michener, 2000) into three groups: dwarf, giant and cavity-nesting honeybees. When grouped this way, we found that dwarf honeybee wax and cavity-nesting honeybee wax displayed a higher onset and end of melting temperature, as well as peak melting temperature, than the giant honeybees. Nest temperatures of *A. dorsata* fluctuate between 30 and 33.5°C [see references in Mardan and Kevan (Mardan and Kevan, 2002)], while those of *A. mellifera* rarely fluctuate beyond 34–35°C (Seeley and Heinrich, 1981). Perhaps because giant honeybees must keep their nests at a lower temperature than other species, their wax experiences different selective pressures and is thus able to accommodate a lower melting temperature than the cavity-nesting or dwarf honeybees. Results from a comprehensive study of the mechanical properties of honeybee waxes show similar trends, with giant honeybee wax displaying a different yield strength and stiffness value compared with both dwarf and cavity-nesting species (Buchwald et al., 2006). In the current study, the heat of fusion was higher in cavity-nesting honeybees than in dwarf or giant honeybees, indicating that it takes more energy per unit mass to melt their wax. Honeybee waxes all share a high level of complexity in their chemical makeup, but different species have different relative abundances of certain chemicals or principal compound classes (Aichholz and Lorbeer, 1999). Perhaps differences in wax chemical composition are responsible for the differences in heat of fusion. Our analysis included a few representative species from each nesting group. Although we did find significant differences between nesting types, we view our results as suggestive; there are additional species of *Apis* in each nesting type, and further analyses should include supplementary species. Moreover, inadequate representation in some groups precludes our analysis from correcting for issues of phylogenetic independence. Further research with more species would better address these issues as well.

Bees in the family Apidae represent a large group of highly derived species. These flying insects all use endogenously produced waxes to construct elaborate nests where resources are stored and young are reared. This study has revealed quantitative differences in the melting properties of representative Apoid species that are themselves interesting. However, when examined in the context of nesting ecology and known differences in wax mechanical properties, a wider perspective emerges in which we see how evolution can shape a material that is produced endogenously and subsequently used externally for structure and function. In addition, this work confirms the advantages of using quantitative engineering techniques to address important issues regarding evolution and ecology.

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