Chapter 13
Conversion of Wax Scales into Comb Wax

Abstract The cyclical changes in cellular organelles and the chemical composition of beeswax precursors found in the haemolymph and gland tissues, closely coincide with age-related wax secretion rates. It is one of the divisions of labour, a coincidence of physiology and behaviour that parallels other polyethisms. The mechanical properties and crystal structure of wax change with chemical additions by honeybees. Intact wax scales contain some non-lipoidal components and differ from comb in lipid composition. The mechanical properties of scale and comb wax vary with temperature. There is a linear relationship between load and elongation in the tensile stress-strain curves to the maximum sustainable load, so that the yield stress coincides with the ultimate strength of the material. New comb wax is an isotropic plastic whose mechanical properties depend on temperature. Larvae introduce silk into the comb in a random array, the addition of which improves the load-carrying capacity of the combs. Over time, the combs become fibre-reinforced composite materials, with properties entirely different from the individual components. Wax scales form as the liquid wax fractions transude from the pore canals onto the surface of the wax mirror, where these small droplets coalesce to form thin layers of wax, and this process continues until a wax scale forms. The relatively crystalline scale is reduced to an amorphous state during cell construction; but, given the warmth of the colony, the physical manipulations of the wax by the bees gradually introduce an ordered texture.

13.1 Introduction

This chapter reviews the cycle of wax secretion in which the cyclical changes of cellular organelles and the chemical composition of beeswax precursors, found in the haemolymph and gland tissues, closely coincide with age-related rates of wax secretion (Rösch 1927; King 1928; Hepburn et al. 1991). It is one of the divisions of labour, and this temporal coincidence of physiology and behaviour parallels other polyethisms, such as colony defense (Whiffler et al. 1988; Breed et al. 1990)

and brood care (Liu 1989; Crailsheim and Stolberg 1989); all are predictable activities correlated with age and cycles of glandular functions.

Some years ago, Robinson (1987) suggested that juvenile hormone was involved in the regulation of physiological processes that are associated with division of labor in honeybees, but the effects of juvenile hormone on behavior were not clear. He went on to test the hypothesis that, juvenile hormone affects worker age polyethism using a chemical analogue of the hormone, methoprene. On the basis of his results Robinson (1987), and subsequently Robinson and Vargo (1998) and Sullivan et al. (2000), claimed juvenile hormone was involved in the control of age polyethism, and that the hormone may regulate a colony's allocation of labour by altering the probabilities of response to tasks. Robinson and Vargo (1998) further stated that their experiments demonstrated that juvenile hormone acts as a "behavioral pacemaker," influencing how fast a worker grows up and makes the transition from nest to foraging activities. Unfortunately, the juvenile hormone analogue, methoprene, is a toxic, insect growth regulator, that was first registered as a conventional chemical pesticide in 1975, and remains so to date (US Environmental Protection Agency 2001). So in the circumstances, all of the attempts to define a role for juvenile hormone in the division of labour, based on methoprene, remain inconclusive.

Attempts to alter this cycle by increasing or decreasing the amounts of juvenile hormone and/or the addition or removal of the corpora allata, had no measurable effect on the onset of wax secretion, its duration, or the amount of wax actually produced for this age cohort (Muller and Hepburn 1994). Juvenile hormone III and the corpora allata do not play a role in regulating the age-related physiology of wax secretion in adult worker honeybees, as neither factor affected either the onset of wax production or the amount of wax produced. Allatectomy of newly eclosed workers did not affect wax production in adult worker honeybees. An experimentally increased juvenile hormone III haemolymph titre, as a result of either a single large injection or by implanting corpora allata from older workers into younger workers, did not affect either the onset of wax production or the mean amount of wax produced. No critical period could be established during which an elevated juvenile hormone III titre would affect the rate of wax secretion. Methoprene, on the other hand, significantly reduced wax secretion. This suggests that methoprene, applied pharmacologically as is done routinely in polyethism studies, is sublethal and poisonous to worker honeybees. Methoprene is a compound which mimics the action of an insect growth regulatory hormone and is used as an insecticide because it interferes with the normal development.

Hepburn and Muller (1988) performed experiments to determine the nature of the cycle of wax secretion. First, using bees of a precisely known age, it was shown that secretion was a continuous process, there was no diel rhythm. This led to a 2 year study of wax secretion in queenright colonies, from which the wax scales of some 11000 bees were recovered and weighed. Wax secretion was parabolically cyclic and related to age: secretion begins at about 3 days after emergence, reaches a peak between 9 and 12 days, and wanes between 18 and 21 days (Hepburn et al.

1991). These data finally filled the gap between the histological observations of Rösch (1927) and the physiology of the secretory cycles.

13.2 Wax Scales

The probable chemical changes in the conversion of wax scales into comb wax, first suggested by Hunter (1792), rest on two pieces of evidence. The first comes from Huber (1814), who found that wax scales dissolved quickly in turpentine, but fragments of comb failed to dissolve completely, leaving many particles of comb wax in the solvent. Moreover, fragments of comb disintegrated and fell to the bottom of a flask of ether, but scales preserved both size and shape and lost only their translucency. These two wonderful little experiments are easily repeated. The second arises, *en passant,* from the work of Lambremont and Wykle (1979), in which scale wax was analysed by thin layer chromatography and the resulting chromatograms lacked any activity in the diester position. However, the presence of this fraction in scale wax has since been confirmed (Schoening 1980; Kurstjens et al. 1985).

The components of the 'salivary' secretions of honeybees are poorly known, and those added to wax during comb-building are totally unknown. However, Heselhaus (1922) suggested that the salivary material added to wax might derive from the postcerebral gland, while others (Örösi-Pal 1957; Cruz Landim 1963, 1967) implicated the mandibular gland. Indeed, Cruz Landim claimed that the secretion of an isolated mandibular gland of some wild bees can dissolve wax. A ketone, 2-heptanone, has been identified from this gland in honeybees by Shearer and Boch (1966), which partially dissolves comb but does not affect scale wax at all.

13.3 Chemical Differences Between Scale and Comb Wax

While one can measure changes in the mechanical properties of wax that result from chemical additions by honeybees, allowance must be made for the changing crystal structure as well. Kurstjens et al. (1985) investigated permutations of scales and comb wax by separately varying crystal texture and chemical composition (to include or exclude a possible protein fraction in the wax—Kurstjens et al. 1990), and then tested these waxes under identical mechanical conditions. This led to two conclusions about the chemical differences of the waxes. First, intact scales must contain some non-lipoidal component. Secondly, wax scales must differ from comb wax in lipid composition as well (cf. Chap. 16).

Kurstjens et al. (1985) tested these inferences with standard techniques used for both the gross analysis of proteins and lipids, and found that both waxes contain proteins and also differ in gross lipid composition. Davidson and Hepburn (1986) then found that some of the changes in lipid composition were associated with the conversion of wax scales into comb wax. Although the total glycerol content of

both waxes remains constant, the diacylglycerols predominate in scales, but are reduced by nearly half in comb wax; on the other hand, the monoacylglycerol pool of comb wax is nearly double that of scale wax. However, this potential reduction in the stiffness of comb is ameliorated by a corresponding saturation of the acylglycerol fatty acids in comb wax. That these transformations enhance the stiffness of comb wax is evidenced by the fact that texturally isotropic and protein-free sheets of comb wax are significantly stiffer than similar specimens made of scale wax (Kurstjens et al. 1985).

The 'saliva' added to the wax scales by bees contains material with probable lipolytic activity that reduces the diglyceride pool of the scale wax with a corresponding increase in the monoglyceride fraction of the comb wax. The combined affects of the crytstallographic and chemical changes on the mechanical properties of the waxes are as follows: (1) scale wax is stronger than comb wax and, although the latter is twice as stiff as scale wax, it is less distensible than the former; (2) the energy to fracture comb wax (an index of the work bees must invest to shape it) is only half that of scale wax over the range of temperatures likely to impinge on the nests of honeybees; (3) the effects of mandibulation by the honeybees are to transform the texturally anisotropic scale wax into isotropic comb wax.

Although the mechanical properties of scales and comb wax vary with temperature (cf. Chap. 14), we have not considered the significance of temperature as such. It has been documented (Hepburn et al. 1983; Hepburn and Kurstjens 1984) that the physical effort required of bees and the mechanical performances of the nest are a superb compromise between bees and material at 35 °C. At the only slightly higher temperature of 40 °C, the mechanical properties of the nest are dangerously compromised, and the bees themselves begin to die in droves. On the other hand, were bees to work at lower temperatures, their construction costs would burgeon with decreasing temperature (cf. Chaps. 11 and 14). All things considered, one wonders why bees did not evolve a more heat-resistant wax. As it is, once the wax scale forms on the wax mirror, it has a melting point of about 65 °C, but the wax precursors were transuded through the pore canals at bee-body temperature, of only 35 °C. However, there is the intriguing reality that honeybee enzymes of begin to denature above 40 °C. The selective processes that led to this compromise must have been extraordinary.

13.4 Maturation of Newly Constructed Combs

Once the wax scales have been fashioned into pristine comb, many more physical and chemical transformations of the nest material occur. The first hint of such changes were observed by Huber (1814); that the very white wax of new combs seemed more brittle than that of the stronger and more pliable yellow combs. He also noted that bees add propolis (whose origin from plants he discovered independently of Hornbostel 1744) to wax, both in bulk and as a surface varnish, which he thought reinforced the combs. There is no dispute about the progressive

Fig. 13.1 Silken 'ghost' cells exhibit clear-cut rhomboids on the bottom outside of *A. m. capensis* cells (Hepburn et al. 2007)

changes in comb colour particularly associated with the rearing of several cycles of brood in the nest. The new, white cells progress through yellow, various shades of brown and finally become a very dark brownish-black.

The above is the usual sequence of colour changes in nests of all species of honeybees that have been examined. However, bees have been known to secrete pink or red wax; the pigment of candy floss collected by foragers in one case (S. Taber, pers comm.), and from dye, Sudan III, fed to bees in a vegetable oil carrier in another case (Örösi-Pal 1956). The blackness is said to derive partially from larval excrements and from propolis, but the chemical identity of such pigments has not been resolved (Chauvin 1962; Tischer 1962). The walls of brood cells also become thicker with continued use and include the exuviae and silken fibres spun by generations of larvae. Very old combs, from which the wax has been extracted, leave behind fairly substantial 'ghosts' of hexagonal cells (Fig. 13.1).

Through some simple chemical studies, Huber (1814) was able to show that the yellow principle was not likely to have come from propolis, a point subsequently confirmed by Jaubert (1927), who identified the component as 1,3-hydroxyflavone and named it chrysine. The origin of chrysine is totally obscure. Vansell and Bisson (1935) believed that the yellow colour of the wax arose through contamination by carotenoids from pollen, a view consistent with the report of Freudenstein (1962), that the combs of caged bees lacking pollen were white and those of bees with access to pollen were yellow. This is a commonplace occurrence. Alternatively, Philipp (1928) and Freudenstein (1932) suggested that it might be a glandular secretion. The occasional occurrence of bright yellow cells in the midst of new white combs with pollen nowhere near, also occurs.

What is the significance of these colour changes in comb? Reasonable hypotheses were formulated by Woog and Yannaquis (1935, 1936a, b) on the basis of their physical studies. They found that yellow and white wax taken from recently constructed combs had the same degree of crystal orientation, far greater than that of brown wax, which had only very slight, or no crystal orientation at all.

Table 13.1 Tensile properties of propolis and beeswax of the African honeybee, *A. m. scutellata* (Hepburn and Kurstjens 1984)

	Temperature (°C)	Yield strain (MPa)	Yield stress (%)	Stiffness (MPa)	Work to yield (MJm^{-1})
Propolis	25	0.26 ± 0.07	38	0.68	0.07
	30	0.16 ± 0.03	23	0.69	0.03
	35	0.12 ± 0.02	17	0.71	0.02
	40	0.08 ± 0.02	16	0.5	0.01
	45	0.03 ± 0.01	14	0.21	0.004
Beeswax	25	1.13 ± 0.08	30	3.77	1.2
	30	0.83 ± 0.06	14	5.93	0.5
	35	0.54 ± 0.08	12	4.5	0.23
	40	0.21 ± 0.03	4.2	5	0.04
	45	0.07 ± 0.01	4	1.75	0.01

However, in samples of white and yellow wax incubated at 38 °C for 3 days, the orientation of crystals in the latter was enhanced, and even more so after 50 °C for only 2 h. They concluded that chrysine somehow accelerates crystal orientation. Brown wax, in contrast, appeared totally different. There was no measurable crystallinity in these propolis-bearing waxes. Woog and Yannaquis (1935, 1936a, b) suggested that the wax-propolis mixtures were more plastic than either the white or yellow wax, and that the solidity of such constructions was augmented by the bees incorporating pupal exuviae into the cell walls. They further argued that such a fusion is highly dependent on good adhesion between the two, such forces being greater than surface effects that might otherwise have led to increases in crystal orientation.

The work of Woog and Yannaquis (1935, 1936a, b) provides ideas for a mechanism by which changes in the cell walls of combs may occur. In any event, other changes in comb have been explored by Hepburn and Kurstjens (1984) who compared the tensile properties of propolis (whose older name 'bee-glue' is actually more appropriate in view of its general use by bees), and of new white comb wax. They found that propolis exhibited an unusual behaviour on tensile deformation; there was a linear relationship between load and elongation from the origin of the curves to the maximum sustainable load. Thus, the yield stress coincided with the ultimate strength of the material. But propolis, like beeswax, is an entirely plastic material in the range of 25–40 °C, so that this linearity was not an elastic one. On yield, propolis was highly ductile and flowed about 200 % before the necking thread finally failed.

The tensile strength of propolis decreased eightfold over the range of temperatures tested, and the yield strain some threefold (Table 13.1). The stiffness remained virtually constant at lower temperatures with a major transition between 35 and 40 °C. The work to yield also decreased with increasing temperatures, as did the ductility of the substance. Over the range of temperatures tested, beeswax was at least four times stronger than propolis. Even so, the combs of bees in warm countries sometimes fail in hot weather (wax and honey were actually observed
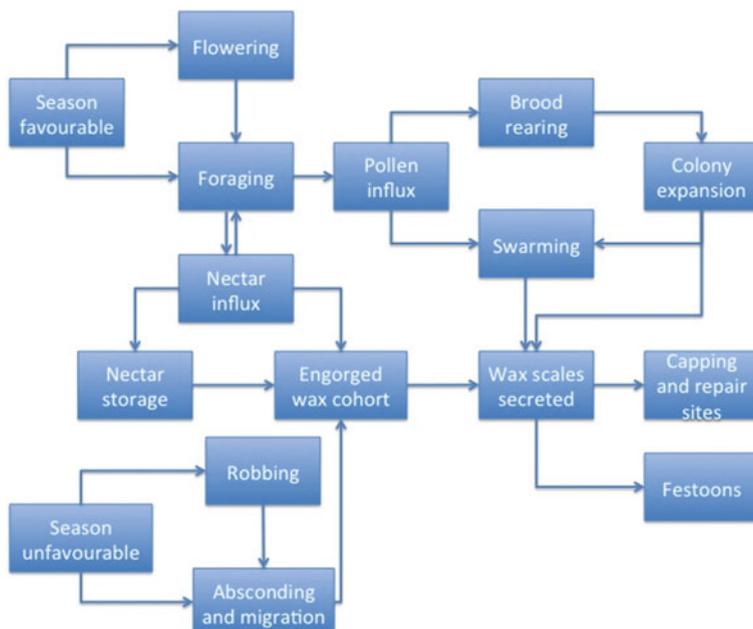
Fig. 13.2 Simplified flow diagram showing the steps involved in the conversion of newly secreted wax scales into new comb, followed by the events associated with the maturation and use of combs (Hepburn 1998)

flowing out of the entrance of hive exposed to the full sun in summer in South Africa (Hepburn unpubl. obs.). Propolis at its strongest is comparable to beeswax at its weakest over the range of temperatures tested that are likely to have an effect on honeybees' nests. These results are certainly consistent with the interpretation of Woog and Yannaquis (1935, 1936a, b) as to the possible effects of propolis in wax, but they do not constitute a direct test of the variables associated with the maturation of combs.

The modifications of comb performance by the presence of proteins and water can now be related to the material properties of combs as they evolve in the nest (Hepburn and Kurstjens 1988). In the course of its development, new comb wax is an isotropic plastic whose mechanical properties depend heavily on temperature. In time, generations of larvae introduce silk into the waxen structure in a random alignment to achieve equal properties in all directions. Thus with use, the comb becomes a fibre-reinforced composite material which exhibits properties entirely different to its individual components. The addition of silk greatly improves the load-carrying capacity of the combs (Hepburn and Kurstjens 1988). Although not a theoretically ideal stiff plate structure (Nachtigall and Kresling 1992), the mature comb is nonetheless a remarkable compromise between technical construction and the biological purposes it serves. A flow diagram showing the conversion of wax scales into comb is given in Fig. 13.2.

13.5 Wax Scales

The wax scales of honeybees are roughly pentagonoid but with rounded corners
and are slightly convex in the surface plane. As such, they are of the same outline
shape as the surfaces of the wax mirror cuticle on which they form (cf. Fig. 12.1);
the most posterior of the four pairs of wax mirrors are somewhat smaller than the
anterior ones (Huber 1814; Dreyling 1903). The wax scales vary in thickness,
depending upon the time they have been developing on the abdomen of the
honeybee (Huber 1814; Dönhoff 1854). They normally range in thickness from
about 200 μm to 500 μm when used by bees (or fall from bees), but extremes of
1000 μm have been observed in *A. mellifera* (von Buttel-Reepen 1915; Jordan
1962) and *A. cerana* (HQ Zheng, University of Zhejiang, pers. comm. and pho-
tomicrograph). Whether scales are laminated has been a controversial point since
Huber (1814) originally asserted that they are. Dreyling (1906) examined the
fracture faces of thick scales that he had broken, and noted the jagged edges of
distinct layers. But because the entire scale readily took up coloured dyes rather
than penetrated the interstices of his apparent layers, he concluded that the scales
were fused. The laminations of scales have also been shown by others (Gwin 1936;
Baldaev 1968; Dietz and Humphreys 1970), but the suggestion that the layers are
fused remains (Coggshall 1953), and, indeed, they are.

  The formation of wax scales, as intimated by Huber (1814), have been observed
microscopically by Philipp (1935), confirmed by Jordan (1962) and more recently
by Cassier and Lensky (1995). The liquid wax fractions transude from the pore
canals onto the surface of the wax mirror, where these small droplets coalesce to
form a first layer of very thin wax. In the next phase of secretion, more droplets
reach the surface of the mirror, lifting the first layer and become attached to it. So
the process continues until several layers have been secreted to form a full wax
scale. Scales usually consist of about three to six laminae by the time a bee uses
them (Jordan 1962), and the older and thicker they become, the greater the extent
of delamination at the edges. Dietz and Humphreys (1970) were able to resolve the
laminae into finer sublayers of about 80 μm in thickness. It is very likely that the
occurrence of real, but fused laminae, reflects 'pulsations' in the rate of wax
secretion (Hepburn and Muller 1988). Indeed, the secretion of wax, at least in
summer, follows a circadian rhythm (Baldaev 1968). As such, the layered nature
of the scale merely reflects the supposition of Brewster (1815) that beeswax, like
rubber, gum arabic and other substances, form by the successive deposition and
induration of thin layers, a point confirmed by the observations of Philipp (1935)
and Cassier and Lensky (1995).

  Ever since Hunter (1792) observed that wax scales are translucent, but new
comb is white and opaque, the idea that comb wax might be a mixture arose.
Huber (1814) noted that worker bees chew and fragment wax scales and add a
frothy substance to them. These facts assured that both mechanical and chemical
changes occur in the conversion of scales into comb. The study of such changes
and how they might come about has been slow and, experimentally, extremely

difficult. But, most importantly, studies of wax texture, or the arrangement of crystallites in relation to temperature, mechanical deformation, pressure and time, have provided much insight into how honeybees convert their minute scales of wax into combs.

Armed with a reasonable description of the gross changes in shape and in crystal texture that occur in the metamorphosis of wax scales into honeycomb, we are left to consider how these and other changes might come about and affect the wax combs as structural nest material. The roles of annealing, pressure, compressive and tensile deformation and time have been identified as important means by which the crystal texture of wax may change. The conceptual inter-relationships of these factors were developed in the papers of Woog and Yannaquis (1935, 1936a, b). They argued, from experimental analogies, that while the relatively crystalline scale is more or less reduced to an amorphous state during cell construction, given the warmth of the colony, the mandibulation of the wax by the bees, and the passage of time, the comb wax gradually becomes more ordered. Time and the warmth of the nest in tempering wax have also been considered by others (Hunter 1792; Kratky 1937; Schmidt 1941; Martin and Lindauer 1966). It is implicit in this argument that the more crystalline the structure, the stronger the material, an assumption that has now been tested experimentally.

13.6 Unnatural Building Materials

Recently, there have been several experiments and observations on the interspecific uses of beeswaxes. The different honeybee species share some homologous neutral lipids; but significant species-specific differences remain (Aichholz and Lorbeer 1999; Phiancharoen et al. 2011). Hepburn et al. (2009) analysed behavioural variation for wax choice in honeybees, calculated the Euclidean distances for different beeswaxes and assessed the relationship of Euclidean distances to wax choice among species. They tested *A. m. capensis*, *A. florea*, *A. cerana* and *A. dorsata* beeswaxes, the plant waxes, Japan wax, candelilla and bayberry and the mineral wax ozokerite. Foundation-like sheets of these waxes were produced and placed in *A. m. capensis*, *A. florea* and *A. cerana* colonies. *A. m. capensis* accepted the four beeswaxes, removed the Japan and bayberry waxes, and 'ignored' the candelilla and ozokerite waxes. *A. cerana* colonies accepted the *A. cerana*, *A. florea* and *A. dorsata* waxes but rejected or ignored the *A. m. capensis*, plant and mineral waxes. *A. florea* colonies accepted the *A. cerana*, *A. dorsata* and *A. florea* wax but rejected that of *A. m. capensis*. Unfortunately we had too little of the plant and mineral waxes to test on these bees in this experiment. In retrospect this might have been predicted on the basis of the Euclidean distances for the beeswaxes, which are also consistent with currently prevailing phylogenies for *Apis* (Raffiudin and Crozier 2007; Koeniger et al. 2011). Despite post-speciation chemical differences in the beeswaxes, they remain largely acceptable interspecifically, while

the plant and mineral waxes are not chemically close enough to beeswax for their acceptance.

This experimental approach was further extended by Hepburn et al. (2010) who worked on the basis that salvaging wax from an abandoned nest and reusing for the construction of a new nest is only known for absconding colonies of the red, *A. florea* (Pirk et al. 2011) and black dwarf honeybees, *A. andreniformis* (Duangphakdee and Wongvilas, pers. comm.). Hepburn et al. (2010) tested whether *A. florea* would preferentially choose to salvage wax from their own, original natal combs over other conspecific combs, and whether they would salvage wax from comb 'facsimiles' of *A. florea* combs fashioned from the combs of *A. cerana, A. dorsata* and *A. mellifera*. In the first experiments, *A. florea* preferences for their own natal combs were significantly greater than for non-natal combs. In the second experiment, *A. florea* did not collect wax from any of the heterospecific combs. It is evident that wax discrimination is very much context-dependent, and that there is considerable genetic variation for the wax-salvaging trait.

Gums, waxes and resins from plants are used as nest materials by many wild bee species (Michener 1974; Roubik 1992), but honeybees primarily restrict the building of their nests to beeswax and propolis. The popular literature occasionally lists the use of various paints, tars and asphalt collected by honeybees and these materials are sometimes recovered in pollen traps. It is also a comment on the flexibility of *A. mellifera* bees to note that, while they readily recover propolis from exposed and used frames and hives, they very rarely salvage wax beyond the confines of the nest in *A. mellifera* (Meyer 1954); however, wax salvage from abandoned nests is commonplace in *A. florea* (Pirk et al. 2011) and *A. andreniformis* (O. Duangphakdee and S. Wongvilas, pers. comm.). Moreover, the many unsuccessful attempts by man to get honeybees to accept foreign materials, such as raw plastics, as the base for their combs, attests to the reluctance of bees to work with unnatural materials (Johansson and Johansson 1971).

Thus it would be of interest, from an evolutionary point of view, to examine the extent of plasticity in honeybee behaviour regarding unusual materials. In this respect, we have the extraordinary results from the experiments by Perret-Maisonneuve (1927). In one instance, he gave *A. mellifera* colonies pieces of pure beeswax as well as dyed or coloured samples of ruberoid, modelling clay, ceresine (a purified ozokerite occasionally used as a substitute for beeswax), resins and beeswax, a mixture of carnauba wax, ceresine and beeswax and various permutations of these substances in different proportions. After a week, he found that comb had been drawn on all these variously coloured substitutes, and yet the piece of pure beeswax was virtually untouched. In a second experiment, Perret-Maisonneuve (1927) prepared a sheet of aluminium covered to a depth of 1 mm with a series of adjacent layers, each of which was composed of the same substitutes as well as the pitch fraction of propolis mixed with wax. Although the bees worked slowly, they constructed comb cells on all of the foreign bases, particularly worked on ruberoid, which they mixed with beeswax. These experimental results were met with some incredulity at the time, and were very soon repeated by Roussy (1929), who confirmed them. The extent to which these kinds of results support a notion of

plasticity in the choice of nest materials would probably be best assessed after the principles of similarity of these different materials to wax have been ascertained.

These observations obviously hold great interest and importance for their eventual application to studies of wax synthesis. It could be expected that the differences in the relative amounts of the major compound families in the waxes would be reflected in the physical and mechanical properties of the waxes (Buchwald et al. 2009). These authors recently reported the results of a comparative study of the mechanical properties of several different beeswaxes (*A. andreniformis, A. cerana, A. dorsata* and *A. mellifera*), and measured, among other things, the relative stiffness and resilience of the waxes. Because the mechanical properties of any structure result from both the intrinsic chemical nature of a material as well as its structural form, it is obviously desirable, but extremely difficult experimentally to work with whole comb specimens. So Buchwald et al. (2006) compromised by eliminating structure and simply measured the behaviour of wax cylinders under compression.

Although compression testing is not biologically appropriate for extrapolation to whole combs, (which are actually tension members with a relatively complex structure), the results of such measurements have heuristic value in trying to relate mechanical behaviour to differences in the major compound families of comb waxes given. Resilience represents the amount of energy required to deform the test material until it begins to fail irrecoverably. Stiffness is simply the rate of change of stress per unit strain. *A. dorsata* wax is significantly stiffer than those of other species. The waxes of *A. cerana* and *A. dorsata* do not significantly differ, but are significantly more resilient than that of the intermediate *A. mellifera,* which in turn is more resilient than that of *A. andreniformis*.

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