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Original article

The influence of small-cell brood combs on the morphometry of honeybees (*Apis mellifera*)*

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Abstract – Until the late 1800s honeybees in Britain and Ireland were raised in brood cells of circa 5.0 mm width. By the 1920s this had increased to circa 5.5 mm. We undertook this study to find out if present-day honeybees could revert to the cell-size of the 1800s and to evaluate resulting changes in honeybee morphometry. Seven measurements were made; head width, radial cell length, trachea diameter, cubital index, discoidal shift, bee mass and abdominal markings. The study showed that the colonies of *Apis mellifera mellifera* bees had no apparent difficulty in drawing out the wax and raising brood in the reduced brood cells. Bees reared in these cells were significantly smaller, but this reduction was not in proportion (<20%) to the change in the brood-cell size in contrast to the strongly proportional relationship in other bee strains. Also the ratio of thorax width to cell width ('fill factor') was much larger in the *Apis mellifera mellifera* strain.

***Apis mellifera* / morphometry / cell size / small cell / brood combs**

1. INTRODUCTION

The honeybee species *Apis mellifera* L. is made up of a number of geographical strains or subspecies that have developed largely as a result of natural selection, unhindered by man, in their native regions (Ruttner, 1988a). This changed with the discovery of the concept of 'bee-space' by Langstroth in 1851 and the resulting use of moveable hive frames and wax foundation. According to Cowan (1904) the width of the brood-cell of the Northern European dark bee *Apis mellifera mellifera* at this time was in the range 4.9 to 5.1 mm (Fig. 1).

The period from the late 1800s to the 1920s saw a major change in cell size with the introduction of large commercially produced foundation in the region of 5.5 mm. The apparent reason for this increase (~10%) was to compensate for the cocoon build-up in the cell as well as the culture at the time that "big is good" in agriculture (Baudoux, 1933).

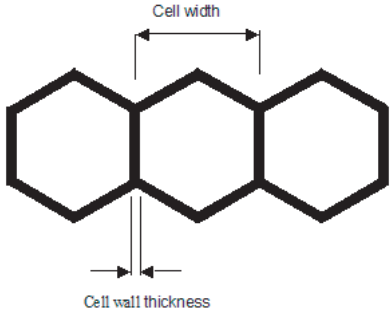

Figure 1. Brood comb showing the measurement of the external cell width across opposite faces of the hexagonal cell. The cell width includes half the width of the cell walls on each side.

In the 1930s, experiments were undertaken by Grout (1937) to identify the influence of even larger brood cells on the size and variability of the emerging bees. Grout concluded

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that while there was some conflict between the results they did show that an increase in the size of the brood cells is accompanied by a corresponding increase in the range of physical measurements. This belief in a corresponding relationship between cell size and bee size is still widespread. Ruttner (1988a) stated "If bees are forced to raise brood in cells of a size other than the specific one – smaller or larger – then the size of emerging bees is changed correspondingly". A quotation from Erickson et al. (1990) also illustrates the position. "These data clearly demonstrate the ease with which a beekeeper can effectively reduce comb cell width in colonies. A corresponding reduction in bee size should follow without selection and breeding". This assumption has appeared in more recent work (Martin and Kryger, 2002).

Here we investigate the ability of colonies of *A. m. mellifera* to draw out and raise workers in the brood-cell size of the 1800s (circa 5.0 mm) and undertake measurements of key morphometry data. We discuss the results in the context of other published work that examined changes in bee morphometry resulting from changes in cell size, and consider possible implications for parasitic mite infestation.

2. MATERIALS AND METHODS

2.1. Preparation of test colonies

The original study was undertaken between April and September 2003 using bee colonies in North County Dublin, Ireland. In mid April 2003, three colonies of bees that had over-wintered in 5 frame nucs on standard-sized brood cells were transferred to full-sized hives, fed 50% sugar syrup and had small-cell foundation progressively added over a period of four weeks. The foundation was 4.9/5.0 mm (nominal) wired Langstroth bees wax (E.H. Thorne Ltd.) sourced in the U.S. and fitted into Modified Commercial hive frames. All three colonies had fully drawn out the six frames of small-cell foundation in the four-week period. Each hive was then made up of six frames of new small-cell brood comb and the balance with frames of standard-cell (5.5 mm nominal) brood comb less than one year old.

2.2. Collecting test bees

On 5th September 2003, two adjacent frames (one standard and one small cell size) in the centre of the brood nest, with emerging brood, were selected from each of the three test bee colonies. The adhering bees were brushed off and each frame was inserted into a separate stainless steel (perforated) frame cage and transferred to an incubator.

At eight-hour intervals over the next 24 h callow bees emerging from the six frames were collected, placed in numbered boxes and stored at –30 °C to await measurement. A maximum of sixty test bees was collected from each frame. We continued to collect and store all the emerging bees for a further 10 days, grouped together on the basis of the two cell sizes (standard and small).

Because bees may gradually adapt to new nesting conditions we repeated the analyses in April 2005, when the standard and small cell-size combs had been present in the same hive for 24 months.

2.3. Cell-size / morphometric measurements

The cell sizes (width) of the brood combs from which the bees emerged were measured. The width was taken as the distance between opposite faces of the hexagonal cell and included half the thickness of the cell wall on each side (Fig. 1). Five samples of 30 cells each were taken for each of the three axes (at 60° spacing), from both sides of each of the six target brood combs. The cell depth was also measured using twenty samples from each of the six target brood combs.

Physical measurements were taken of the test bees. (i) The width of the head was measured using calipers (±0.01 mm). (ii) The diameter of the prothoracic trachea was measured under a stereo microscope (±2 μm) using an ocular grid. The bees were dissected by removing the head and thoracic collar as described by Shimanuki and Knox (2000). The left prothoracic trachea was removed and placed on a double-sided tape attached to a glass slide. The diameter, mid way between the spiracle and the first branch, was calculated by conversion from the measurement of the circumference of the flattened trachea on the sticky tape. (iii) The size of the radial cell on the right forewing was calculated using digitized wing venation data from Beemorph© (2004), the bee wing morphometric analysis program. (iv) The cubital index

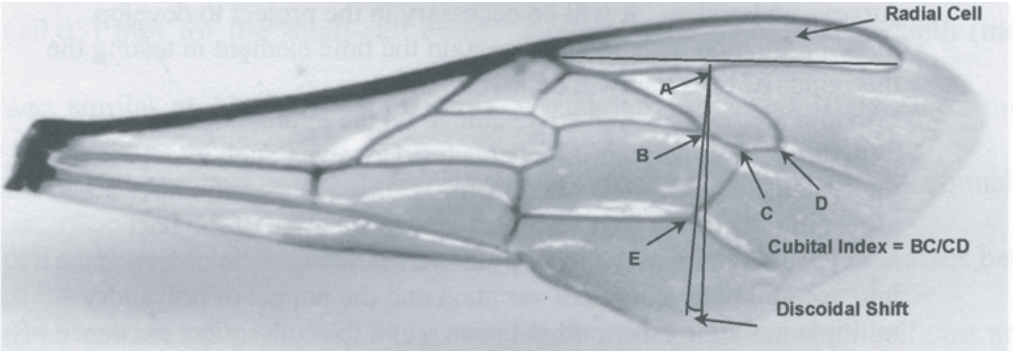

Figure 2. The right forewing of a test honeybee showing the measurement of the cubital index and discoidal shift. These parameters can be used to establish the *Apis mellifera* subspecies of the honeybee. Discoidal shift is the angle (in degrees) between the perpendicular on the radial cell length through node A and the line through node E. A negative value is illustrated.

(Fig. 2) was also measured using the Beemorph© program to give an assessment of the genetic strain of the bee colony (Ruttner, 1988a, b). A mean value less than 1.9 and individual bees under 2.2 are taken as indicative of *A. m. mellifera*. in the German Breeding Regulations (D.I.B., 1986). (v) The discoidal shift (Fig. 2) was also calculated using the Beemorph© program. A negative value is indicative of *A. m. mellifera* while in the case of *A. m. ligustica* it is positive, and positive or zero for *A. m. carnica* (Ruttner, 1988a, b). (vi) The callow bees emerging from the brood frames in the incubator were collected every eight hours and the average mass was obtained on a daily basis for each of the two cell sizes for a period of ten days (13-day period for the April 2005 study). (vii) The colouration of the plates of the abdomen (tergites) and the width of the tomentum were noted using the approach of Ruttner (1988b).

2.4. Statistical analyses

Differences between small-cell and standard-cell bees in biometric traits (i) to (v) (see above) were analysed using a 2-way MANOVA for overall and individual effects, with cell-size and colony as fixed factors. The bee mass and cell-size data were analysed using one-way and two-way ANOVA respectively, the latter being log transformed as the variances were not homogeneous. A chi-square test was used to compare the abdominal colouration of the bees from the two cell sizes.

3. RESULTS

The morphometric data for the experiments carried out in September 2003 and April 2005 are given in Table I.

3.1. Drawing wax and rearing brood

The colonies constructed small-cell comb and reared brood without any apparent difficulty. The small-cell comb was drawn out in a regular manner and had a much smaller variance in cell size than the standard comb (Tab. I).

3.2. Brood cell sizes

Comparing the small-cell comb to the standard-cell comb, there was a significant overall reduction of 8% and 7% in the cell width (mean ± s.d.) for 2003 and 2005 respectively. The average cell depth (mean ± s.d.) was similar at 11.03 ± 0.64 and 11.09 ± 0.61 mm for the standard cells and 10.98 ± 0.62 and 11.03 ± 0.49 mm for the small-cell comb. There was no significant difference in the cell sizes between the three colonies ($F_{[2,174]} = 2.71$, $P = 0.07$).

Table I. Summary of morphometric measurements for all the test honeybees raised from the standard and the small brood cells in (a) September 2003 and (b) April 2005.

Measurements	Standard Cell (mean ± s.d.)	Small Cell (mean ± s.d.)	Size ratio[1]	Significance[2]
Cell size (mm)	5.48 ± 0.12 (n = 90)	5.04 ± 0.03 (n = 90)	0.920	***
(a) September 2003 Study				
Head width (mm)	3.80 ± 0.06 (n = 173)	3.77 ± 0.05 (n = 175)	0.992	***
Radial cell length (mm)	3.46 ± 0.06 (n = 173)	3.40 ± 0.06 (n = 175)	0.982	***
Trachea dia (μm)	191 ± 5.7 (n = 173)	189 ± 5.6 (n = 175)	0.993	*
Cubital index	1.62 ± 0.24 (n = 173)	1.64 ± 0.24 (n = 175)	1.010	n.s.
Discoidal shift (°)	−2.35 ± 2.31 (n = 173)	−1.67 ± 2.63 (n = 175)	0.710	**
Bee mass (g)	0.113 ± 0.003 (n = 10)	0.101 ± 0.004 (n = 10)	0.894	***
(b) April 2005 Study				
Cell size (mm)	5.44 ± 0.13 (n = 90)	5.07 ± 0.08 (n = 90)	0.931	***
Head width (mm)	3.75 ± 0.04 (n = 179)	3.71 ± 0.04 (n = 175)	0.989	***
Radial cell length (mm)	3.40 ± 0.08 (n = 179)	3.35 ± 0.07 (n = 175)	0.986	***
Trachea dia (μm)	185 ± 3.8 (n = 179)	182 ± 3.4 (n = 175)	0.983	***
Cubital index	1.82 ± 0.22 (n = 179)	1.81 ± 0.24 (n = 175)	0.999	n.s.
Discoidal shift (°)	−2.05 ± 2.20 (n = 179)	−2.35 ± 1.81 (n = 175)	1.150	n.s.
Bee mass (g)	0.116 ± 0.004 (n = 13)	0.107 ± 0.003 (n = 13)	0.922	***

[1] Measurements of bees raised in small cells divided by corresponding measurements in standard cells.
[2] Significance of comparison between morphometric measurements of bees raised in small and standard brood cells. * Significance at 0.05, ** Significance at 0.01, *** Significance at 0.001, n.s. not significant.

3.3. Morphometric comparisons

3.3.1. Multivariate analysis

There was an overall cell-size effect (2003: $F_{[5,338]} = 21.61$, $P < 0.001$); (2005: $F_{[5,344]} = 37.66$, $P < 0.001$), and colony effect (2003: $F_{[10,678]} = 19.21$, $P < 0.001$); (2005: $F_{[10,690]} = 15.42$, $P < 0.001$) on the five biometric measurements; head width, radial cell length, trachea diameter, cubital index and discoidal shift.

3.3.2. Univariate analysis

Small cell size caused significant reductions in head width (~1%), radial cell length

(1–2%), tracheal diameter (~1%). There was no effect on cubital index but discoidal shift was significantly greater in 2003 (see Tab. I for statistics). The cubital indices for the three colonies indicated dominant *Apis mellifera mellifera* strains as did the high negative values of discoidal shift for the three colonies.

3.4. Other measurements

There were significant overall reductions of 8 and 11% in the mass per bee for the callow bees that emerged in 2003 and 2005 respectively (Tab. I). All the bees examined had a 'narrow' tomentum width that is consistent with an *A. m. mellifera* strain (Ruttner, 1988b). The bees from all the colonies, except colony 3 (2003 study), had the tergites either 'all black' or with very 'small brown/yellow spots' on the second tergite. In the case of colony 3 (2003 study) 27 of 58 bees from standard cells had a narrow yellow ring on the second tergite while the corresponding data for small-cell bees was 24 of 58 bees, representing no statistical difference (d.f. = 1, $\chi^2 = 0.315$, $P = 0.58$).

3.5. Published data

Data from other studies in the published literature were reviewed to enable a comparison with different honeybee strains in the different parts of the world. Within each of the experiments reviewed there has been a similar percentage change in linear bee measurements across all measurement categories e.g. forewing length, thorax width etc. Hence change in thorax size would be representative of the other measurement categories (Tab. II). It can be seen from the table that the *A. m. mellifera* strain has a different morphometry. The changes in thorax width in the 'Italian' or 'American' bee strains in papers 1, 2 and 3 reflected strongly the changes in cell size (80%, 67% and 120% respectively). This compared with a weak (10%) response in *A. m. mellifera.*

The thorax width is about one third larger in *A. m. mellifera* at circa 4 mm versus circa 3 mm. The thorax width to cell width ratio ('fill factor') varied from 53% to 57% for the 'American' strains compared to 73% to 79% for *A. m. mellifera.*

4. DISCUSSION

Our results are in direct contrast to the changes in bee measurements resulting from changes in brood cell size in 'American' honeybee strains which show a proportional response. In our experiment the small-cell brood combs also resulted in smaller bees, but this reduction in size was clearly not in proportion to the reduction in cell size. Reductions of 7 to 8% in the brood-cell size resulted in reductions in linear bee dimensions in the order of only 1%. While Grout (1937) concluded that changes in brood-cell size and bee dimensions are proportional, this is not consistent with his data. In fact his data show a similar pattern to that in the present study, and would indicate that the strain of bees used in Grout's experiments was likely to be *Apis mellifera mellifera* as this strain was widespread prior to 1940 (Ruttner, 1988a). Our results were consistent between September 2003 and April 2005, indicating not only that our conclusions are reliable, but also that the reduction in bee size caused by smaller cell size is a step change rather than a response that changes over time.

The wing venation and abdominal coloration analyses indicated that the three honeybee colonies were predominantly of the Northern European dark bee strain, *A. m. mellifera*. These colonies had no apparent difficulty in drawing out the wax foundation and raising brood in the smaller comb and this suggests that the cell size of the mid 1800s may still be the 'natural' size for these *A. m. mellifera* colonies. This ease with which the bees adapted to the new comb stands in contrast to the experience in other parts of Europe where bees were moved onto wax foundation with small-sized cells. There are reports of colonies of honeybees in Britain, that are thought to have been on large cells (>5.5 mm) for many decades, drawing out small-cell foundation in a series of rosettes

Table II. Comparison of measurements of brood-cell width and thorax width from published papers.

Published Data	Cell Width (mm)	Thorax Width (mm)	Fill Factor[1] (%)
Paper 1. Spivak and Erickson (1992)			
Apis mellifera (European strain[2])			
Tucson, Arizona			
Commercial comb	5.37	3.02	56
Small comb	5.08	2.89	57
Change (%)	–5.04	–4.3	+1
Paper 2. Jagannadham and Goyal (1980)			
Apis mellifera (Californian strain[2])			
Ladhiana, India			
Large comb	6.00	3.20	53
Normal comb	5.24	2.93	55
Change (%)	–12.7	–8.5	+2
Paper 3. Baudoux (1933)			
Apis mellifera ligustica (Belgian Congo strain[2])			
Large comb	5.55	4.15	75
Normal comb	4.95	3.59	73
Change (%)	–11.2	–13.5	–2
Paper 4. Grout (1937)			
Apis mellifera (Strain not stated)			
Ames, Iowa			
Large comb	5.50	9.66[3]	n.a.
Normal comb	5.19	9.61[3]	n.a.
Change (%)	–5.6	–0.60	
Paper 5. Current paper			
Apis mellifera mellifera			
County Dublin, Ireland			
Standard comb	5.48	4.00	73
Small comb	5.04	3.97	79
Change (%)	–8.0	–0.8	+6

[1] Fill factor is thorax width/cell width expressed as a percentage.
[2] Strain designations as given in the source papers.
[3] Forewing lengths used as a representative measurement as thorax width not available.

across the face of the comb (Dave Cushman, pers. comm.).

It is clear from this study that the morphometry of *A. m. mellifera* honeybees is significantly different in many respects from other *Apis mellifera* strains. The thorax width was about one third larger than that of the 'American' strains probably reflecting Bergmann's rule for the North European strain. Furthermore, the 'fill factor' (thorax width to cell width ratio) varied from 53% to 57% for the 'American' strains compared to 73% to 79% for the *A. m. mellifera* strain in Europe (Tab. II). The latter represents a relatively restricted condition for the developing bee in the brood cell and this is further intensified with the change to small brood cells.

Our results may have implications for parasitism by mites. Since the size of the bee does not reduce pro rata with the cell reduction

there is considerably less space for the developing bee in the small cell for the *A. m. mellifera* honeybees. This may explain low reproduction of the parasitic mite *Varroa destructor* in the cell. (Martin and Kryger, 2002; Piccirillo and De Jong, 2003). In contrast, the small reduction in tracheal diameter is unlikely in itself to affect access or reproduction of female tracheal mites (*Acarapis woodi* width 70 μm) in the trachea.

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Résumé – Influence des rayons de couvain à petites cellules sur la morphométrie des abeilles domestiques (*Apis mellifera*). L'utilisation des feuilles de cire gaufrée dans la seconde moitié du 19e siècle a permis aux apiculteurs de changer la taille des cellules de couvain dans lesquelles les abeilles étaient élevées. En conséquence la largeur des cellules de couvain en Grande-Bretagne et en Irlande est passée d'environ 5,0 mm à environ 5,5 mm dans les années 1920. Nous avons entrepris cette étude pour savoir si les abeilles d'aujourd'hui pourraient construire et élever du couvain dans les cellules de petite taille des années 1800 et pour comparer la morphométrie de ces abeilles avec celles élevées dans des cellules de taille standard. Les abeilles ont été élevées dans 3 colonies contenant un mélange de cadres aux cellules standard et de cadres à petites cellules et l'analyse morphométrique a été faite sur les abeilles émergentes. Les résultats montrent que les abeilles appartenaient à la sous-espèce *Apis mellifera mellifera* et qu'elles n'avaient pas de difficultés à revenir à des cellules de petite taille. Il y a eu une réduction petite mais significative de la taille des abeilles pour un certain nombre de dimensions physiques clés (Tab. I). Pourtant, contrairement à une croyance populaire, les dimensions des abeilles n'ont pas été réduites en proportion de la réduction de la taille de la cellule contrairement à la relation fortement proportionnelle chez certaines autres lignées d'abeilles. Une synthèse des données publiées a confirmé que le « facteur de remplissage » (rapport largeur du thorax/largeur de la cellule) était beaucoup plus grand chez *A. m. mellifera*.

***Apis mellifera* / morphométrie / taille / cellule / rayon de couvain**

Zusammenfassung – Einfluss von Waben mit kleinen Brutzellen auf die Morphometrie von Honigbienen (*Apis mellifera*). Der Gebrauch von künstlichen Mittelwänden seit der zweiten Hälfte des 19. Jahrhunderts ermöglicht es den Imkern, die Größe der Zellen für die Aufzucht der Brut zu variieren. Als Konsequenz nahm die Größe der Brutzellen in England und Irland von etwa 5,0 mm (1920) auf etwa 5,5 mm zu. Wir führten Untersuchungen durch, ob Bienen heutzutage so kleine Zellen wie im 18. Jahrhundert bauen und ob sie darin ihre Brut aufziehen können, und um die Morphometrie dieser Bienen mit denen aus in heutigen Standardzellen aufgezogenen zu vergleichen. Honigbienen wurden in drei Völkern aufgezogen, die eine Mischung aus Waben mit Standardgröße und kleinen Brutzellen besaßen. Es wurde eine morphometrische Analyse der schlüpfenden Bienen durchgeführt. Die Untersuchung ergab, dass die Bienen der Unterart Apis *mellifera mellifera* angehörten und offensichtlich keine Schwierigkeiten hatten, auf die kleine Zellgröße zurückzukehren. Es ergab sich aber eine signifikante Reduktion in der Größe der Bienen bei einer Anzahl von physikalischen Schlüsselmaßen. (Tab. I). Im Gegensatz zur allgemeinen Annahmen reduzierte sich jedoch die Größe der Bienen (<20 %) nicht proportional zur Reduktion der Zellgröße. Das steht im Gegensatz zum streng proportionalen Verhältnis bei einigen anderen Rassen der Honigbienen. Ein Überblick von publizierten Daten bestätigte, dass der "Füllfaktor" (Verhältnis von Thoraxbreite zur Zellbreite) bei der Unterart *Apis mellifera mellifera* deutlich höher war.

***Apis mellifera* / Morphometrie / Zellgröße / kleine Zelle / Brutwaben**

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