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Original article

Possibility to change the body size in worker bees by a combination of small-cell and standard-cell combs in the same nest

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Abstract – The aim of the study was to investigate the impact of the combination of the colony type (kept on small-cell or standard-cell combs) and the width of worker comb cells (small-cell or standard-cell combs) on the body weight and morphometric traits of worker bees. The values of morphometric parameters of worker bees changed within a substantially lower range than the width of their rearing cells. This indicates that the worker body size is relatively constant, and manipulation with the cell width is not a good method for modeling the body size of workers. The reduction in the thorax weight was proportional to the decrease in the comb cell width, and this part of the body proved to be most susceptible to weight reduction caused by the use of small-cell combs. The rearing of workers in small-cell combs in the colony kept on standard-cell combs resulted in an increase in the value of the fill factor (thorax width to cell width ratio). The relatively constant body size of workers in combination with the use of small-cell combs resulting in an increase in the fill factor may be one of the determinants of increased resistance of the insects to *Varroa destructor*. The values of the morphometric traits commonly used for identification of honeybee subspecies, i.e., the length of the fore wing, the sum of the widths of 3rd and 4th tergites, and the proboscis length, were inconsiderably altered vs. the changes in the comb cell width, which confirms their high suitability for identification of honeybee subspecies.

Apis mellifera **/ small-cell comb / worker bees / morphometric traits**

Abbreviations

SMComb	Small-cell section combs
STComb	Standard-cell section combs
SMCol	Colony on small-cell combs
STCol	Colony on standard-cell combs
SD	Standard deviation
n. s.	Not significant

1. INTRODUCTION

Social insects display task-related division of labor. In some species, division of labor is related to differences in body size, and worker caste members display morphological adaptations suited for particular tasks (Couvillon and Dornhaus 2009). Such a morphological division of labor is observed, e.g., in termites (Noirot and Pasteels 1987), ants (Mertl and Traniello 2009), and bumblebees (Couvillon and Dornhaus 2009). For example, in the bumblebee *Bombus impatiens*, morphological polymorphism is a result of poorer nutrition of larvae in the peripheral nest zones (Couvillon and Dornhaus 2009). Compared to the social insects mentioned above, the body size in honeybee workers varies within a very narrow range (Sauthier et al. 2016). This is probably a result of good care of larvae, especially during the full season when bee colonies are strong (large numbers of nurse bees), and rearing worker bees in honeycombs with low cell size variability, which is additionally

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reduced by the use of an artificially produced wax foundation.

Wax foundation is a thin plate with embossed cell bottoms. It is made of beeswax or plastic. Bees build combs on wax foundations. In modern apiary management, the use of a wax foundation is a standard, especially in developed countries.

The introduction of the wax foundation usually resulted in an increase and standardization of the width of worker comb cells (McMullan and Brown 2006). Currently, the width of standard worker cells is usually in the range of 5.40–5.50 mm. In natural nests of European dark bees (*Apis mellifera mellifera*), the width of worker comb cells was found to vary between 4.90 and 5.10 mm (Cowan 1904). At present, 4.90-mm-wide comb cells are referred to as "small cells" (McMullan and Brown 2006), whereas approximately 5.50-mm-wide cells are considered standard. However, the notion of the small or standard width/size of comb cells varies between different regions of the world, where bees with different genotypes are reared (Table I).

Investigations of colony nests built without the wax foundation conducted by Maggi et al. (2010) in South America demonstrated a considerable variation in the comb cell width ranging from 4.17 to 8.07 mm even in the same colony. Worker and drone brood was reared in 4.17–6.86 mm and 5.05–8.07 mm wide cells, respectively. This range includes all cell sizes reported in the studies mentioned above. It cannot be ruled out that such a large variation in the width of comb cells in the same bee colony influences its biology by modifying the morphological and physiological traits of the insects. Some of these assumptions were considered, and the width of comb cell width was increased when the artificially manufactured wax foundation was introduced. It was assumed that the body size of worker bees would increase proportionally to the increase in comb cells (Grout 1937), which would be accompanied by a rise in productivity, as larger worker bees can theoretically carry heavier pollen and nectar loads (Sauthier et al. 2016). The belief that the size of the worker's body changes proportionally to alterations in the cell width still persists (Ruttner 1988, 1992; McMullan and Brown 2006). Erickson et al. (1990) claimed that alterations of the comb cell width induced changes in the worker body size without selection and breeding. However, contradictory conclusions were formulated by McMullan and Brown (2006) and Seeley and Griffin (2011). McMullan and Brown (2006) found that a 7–8% reduction of comb cell width resulted in an only 1% decrease in the head and thorax width. Similar results were obtained by Seeley and Griffin (2011). This indicates that the bee body size is relatively constant, and the effect of the cell width on these parameters is lower than previously assumed (McMullan and Brown 2006; Seeley and Griffin 2011).

There are only few fragmentary reports in the apidological literature on the presence or

Table I. Width of comb cells regarded as small and standard in different countries

Country	Cell size [mm]		Paper
	small	standard	
Brazil	4.50–4.60	4.90–5.10	Message and Goncalves (1995)
	4.84	5.16 and 5.27	Piccirillo and De Jong (2003)
The USA	4.90	5.40	Ellis et al. (2009)
	4.90	5.30	Berry et al. (2010)
	4.82	5.38	Seeley and Griffin (2011)
Ireland	4.91	5.48	Coffey et al. (2010)
New Zealand	4.70, 4.80, 4.90 and 5.00	5.40	Taylor et al. (2008)

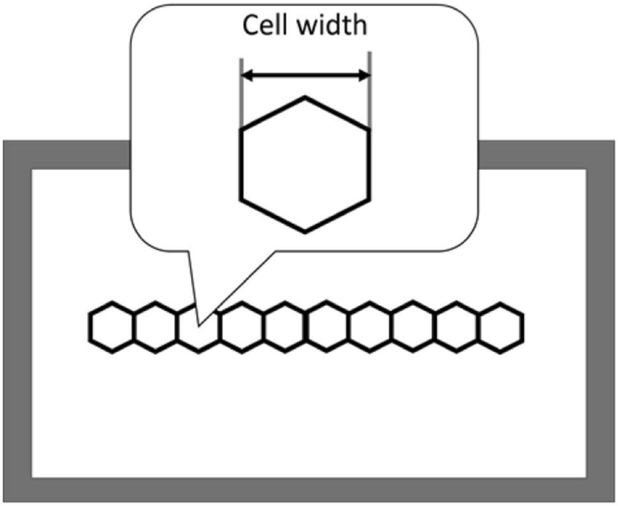

Figure 1. Site and method of measuring cell width in section combs

magnitude of the impact of the wax foundation-assisted standardization and change in the comb cell width on the morphological traits of worker bees. Therefore, the aim of the study was to investigate the effect of the combination of the foster colony (kept on small-cell or standard-cell combs) and the width of worker comb cells (small-cell or standard-cell combs) on the weight and morphometric traits of worker bees.

2. MATERIALS AND METHODS

2.1. Rearing workers

The experiment was carried out in the apiary of the University of Life Sciences in Lublin (Poland; 51.224039N–22.634649E) in 2019. Section frames with a 1.09 dm^2 comb (115 × 95 mm) were constructed. Six frames were equipped with a small-cell foundation (cell width 4.90 mm), and another six frames had with a standard-cell foundation (cell width 5.50 mm). All section frames with the small-cell foundation were drawn out in one colony kept on small-cell combs, and section frames with the standard-cell foundation were drawn out in one colony kept on the standard-cell combs. After the cells were built on the wax foundation, the section combs were removed from the colonies, and the cell width was measured in each. In the central part of section comb, the widths of 10 adjacent cells contacting with vertical side walls were measured (Figure 1). Each of the 10 cells was measured separately (McMullan and Brown 2006). The measurements were made on photographs with the use of the Multi Scan digital image analysis system v. 14.02 (segment measurement option) supplied by Computer Scanning System II, Warsaw.

In the last week of June, three strong bee colonies kept in Dadant Blatt hives headed by naturally mated Buckfast sister-queens were selected in the apiary. We selected Buckfast bee colonies for the study due to their effective adaptation to living on small-cell combs in the apiary of the University of Life Sciences in Lublin. In each colony, workers populated one brood chamber (10 frames; 435 × 300 mm) and three honey supers (10 frames; 435 × 150 mm). Two of these colonies were kept on standard-cell combs and one was kept on small-cell combs. To rear sister-workers, standard-cell section combs (STComb) and small-cell section combs (SMComb) were placed alternately at 12-h intervals in the space cut out in center of the brood comb located in the center of the brood chamber in one of the colonies kept on the standard-cell combs (Figure 2). In each section comb, the queen was trapped in a frame cage made of a queen excluder for oviposition. Section combs with laid eggs were transferred into the other colonies, which served as brood-rearing foster colonies. The foster colony kept on the standard-cell combs (STCol) and that kept on the small-cell combs (SMCol) were equipped with 3 STComb (standard-cell section combs) and 3 SMComb (small-cell section combs) each. STComb and SMComb were placed in the frame in the center of the first honey super above the brood chamber. After 19 days in the foster colony, each section comb was placed in a separate mesh frame cage and transferred into an incubator (34.5 °C and 60% RH), where it was kept until emergence of workers. Approximately 100 workers from each section comb were placed in a separate cage and left in the incubator (26 °C and 60% RH) for 7 days to allow their chitin cuticle to harden. During this time, the workers were fed with an aqueous solution of saccharose (1:1). The four groups of workers obtained corresponded to the combination of the comb type and the foster colony type: bees reared in small-cell combs in colonies kept on small-cell combs (SMComb + SMCol), reared in small-cell combs in colonies kept on standard-cell combs (SMComb + STCol), reared in standard-cell combs in colonies kept on standard-cell combs (STComb + STCol), and reared in standard-cell combs in colonies kept on small-cell combs (STComb + SMCol).

2.2. Measurement of weight and morphometric evaluation of workers

The measurements involved 15 workers from each group of each section comb (Ruttner 1988, 1992). On each worker the following steps of analysis was procedure: (1) anesthesia with ethyl acetate; (2) measurement of the thorax width and length and the abdomen length on the dorsal side using an Olympus SZX16 stereoscopic microscope; (3) weighing whole bees, dissection of the main body parts (head, thorax with legs and wings, and abdomen), and weighing each

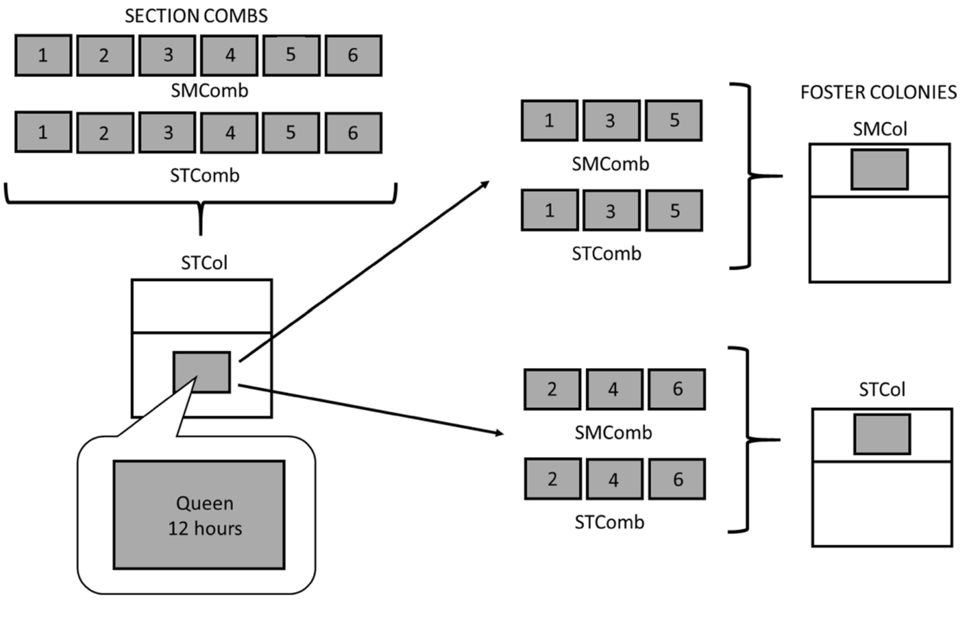

Figure 2. Transfer of the section combs from the single parent colony to the two foster colonies. SMComb, small-cell section combs; STComb, standard-cell section combs; SMCol, foster colony on small-cell combs; STCol, foster colony on standard-cell combs

part separately; (4) measurement of the width and height of bee heads placed on glass slides using an Olympus SZX16 stereoscopic microscope; (5) dissection of the proboscis, the right fore wing, and the 3rd and 4th abdominal tergites and transfer thereof onto glass slides; and (6) measurement of body parts (see point 5) using an Olympus SZX16 stereoscopic microscope. The following linear measurements were obtained: proboscis length, fore wing length and width, cubital vein distances a and b, and 3rd and 4th abdominal tergites width (Ruttner 1988, 1992). The cubital index was calculated as the distance a/b ratio as proposed by Goetze (Ruttner 1988). The percentage fill factor was calculated by dividing the thorax width by the comb cell width and multiplying the result by 100 (McMullan and Brown 2006; Seeley and Griffin 2011).

We checked whether the differences, expressed as a percentage and calculated vs. the STComb + STCol group, between the total body weight and the weight of the main body parts and the values of linear morphometric traits of workers from the other three groups from the comb type-foster colony type combination (SMComb + SMCol, SMComb + STCol, STComb + SMCol) corresponded to the percentage of the differences in the cell width between the small-cell section combs and standard-cell section combs.

2.3. Statistical analysis

The results were analyzed statistically using Statistica software version 13.3 (2017) for Windows, StatSoft Inc., USA. The data distribution was analyzed with the Kolmogorov-Smirnoff test. The widths of cells in the small-cell section combs ($n = 60$) and in the standard-cell section combs ($n = 60$) were compared with Student's *t* test for independent samples. The effect of the section comb on the width of cells in the small-cell section combs and standard-cell section combs was analyzed with a one-way analysis of variance. The effect of the comb type (SMComb, $n = 90$, and STComb, $n = 90$) regardless of the foster colony type, the effect of the foster colony type (SMCol, $n = 90$, and STCol, $n = 90$) regardless of the comb type, and the interactions between these factors were assessed with a two-way analysis of variance with interaction. The Tukey HSD test was used for comparison of the means in the four worker groups corresponding to the comb type-foster colony type combinations (SMComb + SMCol, $n = 45$; SMComb + STCol, $n = 45$; STComb + STCol, $n = 45$; STComb + SMCol, $n = 45$).

3. RESULTS

3.1. Comb cell width

The width of cells in the small-cell section combs was significantly lower (4.93 ± 0.02 mm; $n = 60$) than in the standard-cell section combs (5.59 ± 0.03 mm; $n = 60$; $p < 0.001$ — Student's *t* test). There was no significant effect of the section comb on the width of cells in the small-cell section combs or in the standard-cell section combs ($F_{5,54} = 0.34$, $p = 0.884$; $F_{5,54} = 0.11$, $p = 0.988$, respectively).

3.2. Weight of the body and its main parts

Irrespective of the comb type, the foster colony type did not exert an effect on the total body weight and the weight of the main body parts (body weight SMCol = 110.09, STCol = 108.20; head weight SMCol = 10.29, STCol = 10.19; thorax weight SMCol = 39.81, STCol = 40.82; abdomen weight SMCol = 59.99, STCol = 57.20) (Table II). Except for the thorax weight, the total body weight and its other main parts were higher in the workers reared in SMCol (colony kept on the small-cell combs) than in STCol (colony kept on the standard-cell combs) (Table II). Regardless of the foster colony type, the total body weight and the weight of the main body parts were higher in workers reared in STComb (Table II), with significance of the total body weight, the thorax weight, and the abdomen weight (body weight SMComb = 104.32, STComb = 113.97, $p < 0.01$; head weight SMComb = 10.12, STComb = 10.36; thorax weight SMComb = 38.37, STComb = 42.27, $p < 0.001$; abdomen weight SMComb = 55.83, STCol = 61.35, $p < 0.05$). The effect of the comb on the total body weight, the head weight, and abdomen weight was significantly dependent on the foster colony type (comb × colony interaction; $p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively). In the worker groups representing the comb type and foster colony type combination, worker bees reared in the STComb + SMCol combination had the highest total body weight and the weight of its main parts, with the exception of the thorax (Figure 3). The highest thorax weight was found in workers reared in the STComb + STCol variant.

3.3. Linear measurements of the head, thorax, and proboscis and the fill factor value

The foster colony type exerted a significant effect on the values of all linear measurements: the head, thorax, and proboscis (Table III). Significantly higher values of these parameters were always obtained in the STCol worker bees (Table III). In turn,

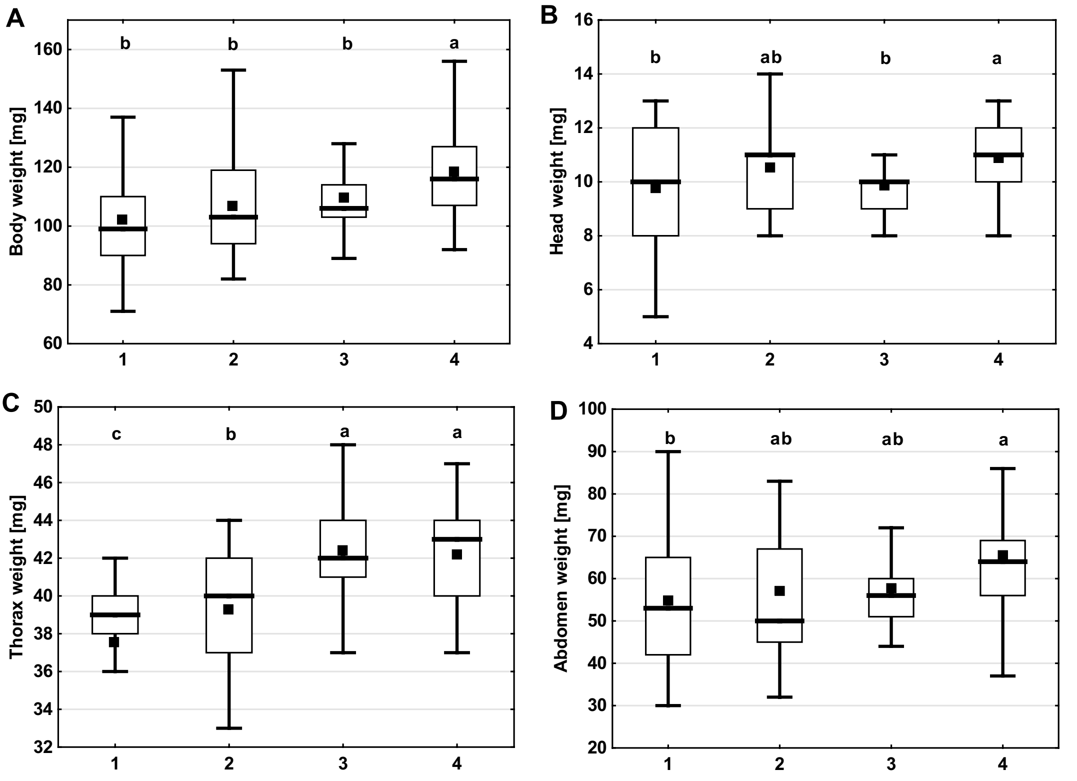

Figure 3. Values of total body weight and weight of the main parts of the body: **A** body weight, **B** head weight, **C** thorax weight, **D** abdomen weight. (1) SMComb + SMCol, workers reared in small-cell combs in colonies kept on small-cell combs (n = 45); (2) SMComb + STCol, workers reared in small-cell combs in colonies kept on standard-cell combs (n = 45); (3) STComb + STCol, workers reared in standard-cell combs in colonies kept on standard-cell combs (n = 45); (4) STComb + SMCol, workers reared in standard-cell combs in colonies kept on small-cell combs (n = 45). The boxes indicate the data between the 25 and 75% quartiles including the median (black line); the whiskers represent the minimum and maximum values; the black squares represent the mean; (**a**), (**b**), (**c**) — the differences in the values of the traits between the comb type-foster colony type combinations are significant at $p \leq 0.05$

the comb type exerted a significant effect on the head width and height, the thorax width, and the fill factor (Table III). The STComb workers were characterized by a greater head width and height ($p < 0.01$) and thorax width ($p < 0.01$), whereas a higher value of the fill factor ($p < 0.01$) was noted in the group of the SMComb workers. The effect of the comb type on the head height, proboscis length, and fill factor value was significantly dependent on the foster colony type (comb × colony interaction; $p < 0.001$, $p < 0.001$, and $p < 0.05$, respectively). In the groups of insects from the comb type-foster colony combination variants, the STComb + STCol workers had the highest values of the head width and height and the thorax width and length (Figure 4). In turn, the longest proboscis and the highest fill factor value were noted in the SMComb + STCol worker bees (Figure 4).

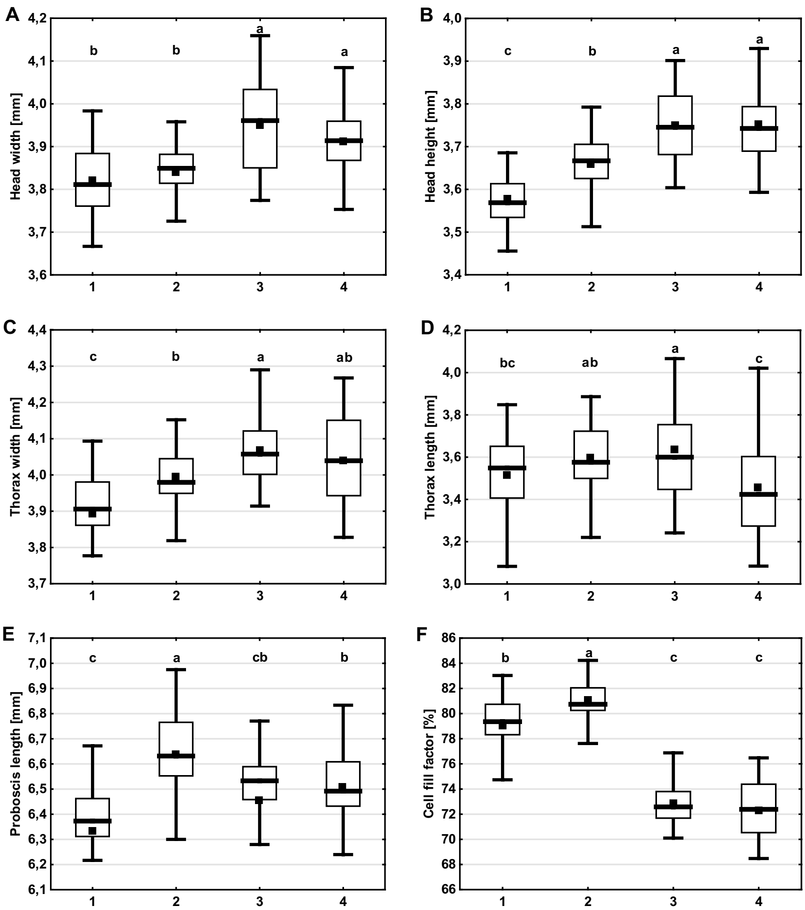

Figure 4. Values of linear measurements of the head, thorax, and proboscis and cell fill factor values: **A** head width, **B** head height, **C** thorax width, **D** thorax length, **E** proboscis length, **F** cell fill factor. (1) SMComb + SMCol, workers reared in small-cell combs in colonies kept on small-cell combs ($n = 45$); (2) SMComb + STCol, workers reared in small-cell combs in colonies kept on standard-cell combs ($n = 45$); (3) STComb + STCol, workers reared in standard-cell combs in colonies kept on standard-cell combs ($n = 45$); (4) STComb + SMCol, workers reared in standard-cell combs in colonies kept on small-cell combs ($n = 45$). The boxes indicate the data between the 25 and 75% quartiles including the median (black line); the whiskers represent the minimum and maximum values; the black squares represent the mean; (**a**), (**b**), (**c**) — the differences in the values of the traits between the comb type-foster colony type combinations are significant at $p \leq 0.05$

3.4. Linear measurements of the fore wing and its elements and the cubital index value

The foster colony type had a significant effect on the length and width of the fore wing and distance b of cubital vein (Table IV). Significantly higher values of these parameters were recorded in the STCol group ($p < 0.01$). In turn, the comb type exerted a significant effect on the fore wing length and distance b of cubital vein ($p < 0.01$). The STComb workers were characterized by significantly higher values of these parameters (Table IV). The effect of the comb on the fore wing length was significantly depend on the foster colony type (comb × colony interaction; $p < 0.001$). In the groups of workers from the comb type-foster colony type combination, the highest value of the parameters were usually noted in the STComb + STCol variant, with the exception of distance a of cubital vein and the cubital index (Figure 5).

3.5. Linear measurements: abdomen, 3rd and 4th abdominal tergites and the sum of the widths of 3rd and 4th tergites

The foster colony type had a significant effect only on the width of the 3rd tergite, which was greater in the STCol workers (Table V). The comb type significantly influenced the width of 3rd and 4th tergites and the sum of their widths. Significantly higher values of these parameters were always found in the STComb workers ($p < 0.01$). The effect of the comb was significantly depend on the foster colony type only in the case of the sum of the widths of 3rd and 4th tergites (comb × colony interaction, $p < 0.01$). In the groups of workers from the comb type-foster colony type combinations, the abdomen length was similar, and the highest 3rd and 4th tergite width and sums of the 3rd and 4th tergite widths were noted in the STComb + SMCol and STComb + STCol workers (Figure 6).

3.6. Percentage changes in comb cell width, total body weight, and the weight of the main body parts, linear morphometric traits, and fill factor

The percentage decrease in the total body weight of the SMComb + SMCol workers, compared to the STComb + STCol group (7.1%), was slightly lower than the percentage decrease in the cell width in the case of SMComb and STComb (11.8%) (Table VI). The decrease in the total body weight was mainly determined by the decrease in the thorax weight, which was similar to the decrease in the cell width, i.e., 11.6% and 11.8%, respectively. A 1.4% and 4.8% decline was noted in the head weight and the abdomen weight, respectively. The reduction of most of the linear morphometric parameters was substantially lower than the decrease in the comb width and ranged from 2.6% (abdomen length) to 4.5% (head height). Slightly stronger reduction was noted in the case of distance b of cubital vein (11.1%). The lower distance b of cubital vein value was accompanied by a significant 8.0% increase in the cubital index value. The proboscis length decreased only slightly (by 1.9%), whereas the abdomen length increased by 2.6%.

Regardless of the foster colony type, the decrease in the comb cell width in the SMComb group vs. the STComb workers resulted in a significant increase in the fill factor (from 8.5% to 11.3%), with the highest increase in the SMComb + STCol variant (by 11.3%). The head weight in the workers from the SMComb + STCol and STComb + SMCol groups increased significantly by 6.5% and 9.9%, respectively, compared to those from the SMComb + SMCol and STComb + STCol groups.

The proboscis length seems to be weakly depended on the comb type-foster colony type combination, as its values changed in a small range from − 1.9 to 2.9%. In all groups, the range of the changes in the cubital index value was similar (from 7.1 to 8.4%).

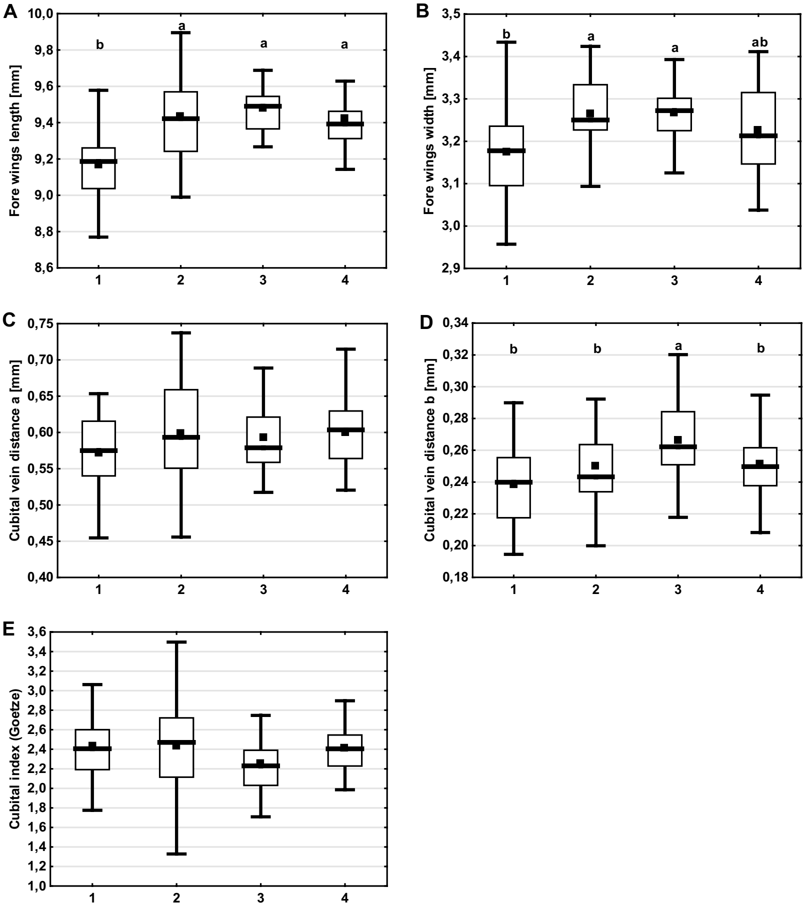

Figure 5. Values of linear measurements of the wing and its elements and cubital index values: **A** fore wings length, **B** fore wings width, **C** cubital vein distance a, **D** cubital vein distance b, **E** cubital index (Goetze). (1) SMComb + SMCol, workers reared in small-cell combs in colonies kept on small-cell combs (n = 45); (2) SMComb + STCol, workers reared in small-cell combs in colonies kept on standard-cell combs (n = 45); (3) STComb + STCol, workers reared in standard-cell combs in colonies kept on standard-cell combs (n = 45); (4) STComb + SMCol, workers reared in standard-cell combs in colonies kept on small-cell combs (n = 45). The boxes indicate the data between the 25 and 75% quartiles including the median (black line); the whiskers represent the minimum and maximum values; the black squares represent the mean; (**a**), (**b**) — the differences in the values of the traits between the comb type-foster colony type combinations are significant at $p \leq 0.05$

4. DISCUSSION

The increase and standardization of the width of honeycomb cells introduced in the last century was prompted by a belief that the size of the worker bee body would grow proportionally to the increase in the cell size (Goetze 1933; Grout 1937). As reported by Ruttner (1988), the body size gain in workers changed correspondingly to the alterations in the width of cells where they were reared. Furthermore, Erickson et al. (1990) found that changes in the comb cell width were able to change the worker body size without selection and breeding. Our investigations demonstrated that the decline in the body weight (by 7%) of the SMComb + SMCol bees compared to the body weight of the STComb + STCol workers was significantly lower than the reduction in the width of the SMComb cells compared to the STComb cells

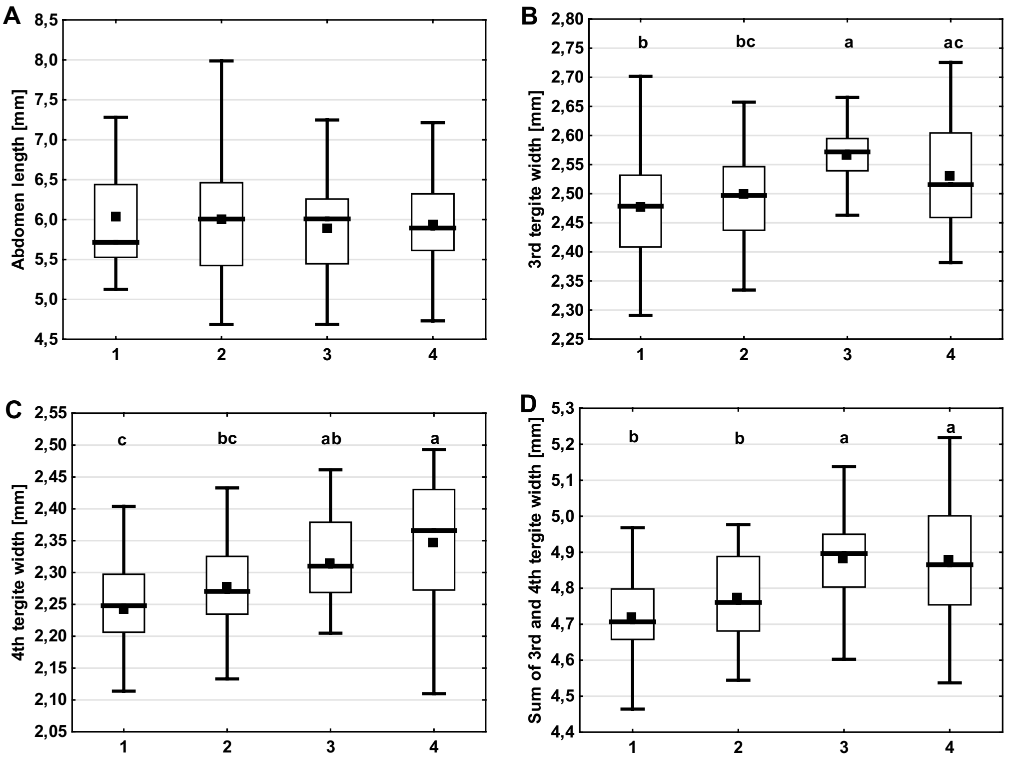

Figure 6. Values of linear measurements of the abdomen and 3rd and 4th abdominal tergites and the sum of the widths of 3rd and 4th tergites [mm]: **A** abdomen length, **B** 3rd tergite width, **C** 4th tergite width, **D** sum of the widths of 3rd and 4th tergites. (1) SMComb + SMCol, workers reared in small-cell combs in colonies kept on small-cell combs ($n = 45$); (2) SMComb + STCol, workers reared in small-cell combs in colonies kept on standard-cell combs ($n = 45$); (3) STComb + STCol, workers reared in standard-cell combs in colonies kept on standard-cell combs ($n = 45$); (4) STComb + SMCol, workers reared in standard-cell combs in colonies kept on small-cell combs ($n = 45$). The boxes indicate the data between the 25 and 75% quartiles including the median (black line); the whiskers represent the minimum and maximum values; the black squares represent the mean; (**a**), (**b**), (**c**) — the differences in the values of the traits between the comb type-foster colony type combinations are significant at $p \leq 0.05$

(by 11.8%). However, the decrease in the total body weight was mainly determined by loss of thorax weight (by 11.6%), which was proportional to the decrease in the cell width. In turn, the range of changes in most of the linear morphometric parameters ranged from 2.6 to 4.5%. These values were within or only slightly different from the range of seasonal changes, i.e., 2–3% (Ruttner 1992). The value of parameters regarded by Ruttner (1992) as a measure of the bee body size, i.e., the length of the fore wing and the sum of the widths of 3rd and 4th tergites, ranged from − 0.5% to − 3.4% and from 0.0% to − 3.3%, respectively. The resistance of these features and proboscis length to changes in the width of comb cells, manifested by a small range of changes, regardless of the comb type-foster colony type combination, confirms their great suitability for assessment of the size of worker bee body and identification of the honeybee subspecies.

Similar results to the findings of our study were obtained by McMullan and Brown (2006) and Seeley and Griffin (2011) and Michailoff (1927). In the 1920s, the latter researcher compared worker bees reared in worker cells to those reared in drone cells. The rearing in the drone cell, which was by 39% wider than

Table II. Effect of the foster colony irrespective of the comb and effect of the comb irrespective of the foster colony on the mean values of the total body weight and the weight of the main body parts [mg]

Traits	Foster colony impact		Comb impact		Interaction: comb × colony
	SMCol	STCol	SMComb	STComb	
Body weight	110.09 $n=90$ SD ± 18.87	108.20 $n=90$ SD ± 15.19	104.32** $n=90$ SD ± 17.41	113.97** $n=90$ SD ± 15.44	$p<0.01$
Head weight	10.29 $n=90$ SD ± 2.00	10.19 $n=90$ SD ± 1.34	10.12 $n=90$ SD ± 2.04	10.35 $n=90$ SD ± 1.27	$p<0.001$
Thorax weight	39.81 $n=90$ SD ± 4.16	40.82 $n=90$ SD ± 3.14	38.37** $n=90$ SD ± 3.78	42.27** $n=90$ SD ± 2.39	n. s.
Abdomen weight	59.99 $n=90$ SD ± 17.18	57.20 $n=90$ SD ± 14.20	55.83* $n=90$ SD ± 16.91	61.36* $n=90$ SD ± 14.12	$p<0.05$

SMCol foster colony on small-cell combs, *STCol* foster colony on standard-cell combs, *SMComb* small-cell comb, *STComb* standard-cell comb, *SD* standard deviation. *The impact of the comb is significant at $p \leq 0.05$; **the impact of the comb is significant at $p \leq 0.01$; *n. s.* not significant.

the worker cell, resulted in a mere 10% increase in the worker body weight and increased the proboscis length by 5%, the sum of the widths of 3rd and 4th tergites by 4.37%, and the fore wing length by only 2.69%. Thus, the substantially larger difference in cell the width (39%) than that observed in our study (11.8%; small vs. standard cell) contributed to only slightly greater changes in the morphometric traits. In the present study, in the SMComb + SMCol group vs. the STComb + STCol group, the percent decline in the parameters was as follows: 7.1% in the body weight, 1.9% in the proboscis length, and 3.3% in the sum of the widths of 3rd and 4th tergites and 3.3% in the fore wing length.

Similar to the results reported by McMullan and Brown (2006) and Seeley and Griffin (2011), our investigations indicate a relatively constant body size in the worker bees, with a lower effect of the comb cell width on this parameter than assumed previously (Ruttner 1988). This implies that, in the case of small cells, the free space between the pupa and the walls of the cells is reduced, which is associated with the lower value of the decline in the bee body size than that of the cell size. The cell is then more tightly filled by the pupa, which may restrict reproduction of *V. destructor*. This mechanism has been observed in Africanized bees (Piccirillo and De Jong 2003) and African *A. m. capensis* (Martin and Kryger 2002), which naturally build small-cell combs. As suggested by Martin and Kryger (2002), the tighter filling of cells by the pupa contributes to increased mortality of *V. destructor* males due to difficulties in movement of the mite in the cell, as the pupa thorax fills the cell lumen tightly. Additionally, movements of the pupa in the tight cell may contribute to damage to the developmental forms of the parasite (Donze and Guerin 1994), which seem highly susceptible to damage due to their non-chitinized cuticle.

In the present study, the fill factor was significantly influenced by both the width of the cells of section combs and the width of the cells of the other combs in the nest, more specifically, by the body size of nurse bees from the foster colony associated with the width of cells in which they had been reared. Therefore, it can be assumed that the sizes of the body parts exert an impact on the physiological characteristics of the worker. This was confirmed by the higher

Table III. Effect of the foster colony irrespective of the comb and effect of the comb irrespective of the foster colony on the mean values of linear measurements of the head, thorax, and proboscis [mm] and cell fill factor values [%]

Traits	Foster colony impact		Comb impact		Interaction: comb × colony
	SMCol	STCol	SMComb	STComb	
Head width	3.87* $n=90$ SD ± 0.09	3.89* $n=90$ SD ± 0.10	3.83** $n=90$ SD ± 0.08	3.93** $n=90$ SD ± 0.09	n. s.
Head height	3.66** $n=90$ SD ± 0.12	3.70** $n=90$ SD ± 0.09	3.62** $n=90$ SD ± 0.07	3.75** $n=90$ SD ± 0.09	$p<0.001$
Thorax width	3.97** $n=90$ SD ± 0.16	4.03** $n=90$ SD ± 0.11	3.94** $n=90$ SD ± 0.14	4.05** $n=90$ SD ± 0.11	n. s.
Thorax length	3.48** $n=90$ SD ± 0.20	3.61** $n=90$ SD ± 0.21	3.55 $n=90$ SD ± 0.21	3.55 $n=90$ SD ± 0.23	n. s.
Proboscis length	6.42** $n=90$ SD ± 0.24	6.55** $n=90$ SD ± 0.27	6.49 $n=90$ SD ± 0.27	6.48 $n=90$ SD ± 0.26	$p<0.001$
Cell fill factor	75.65 $n=90$ SD ± 4.38	76.93 $n=90$ SD ± 4.59	80.03** $n=90$ SD ± 2.92	72.55** $n=90$ SD ± 2.04	$p<0.05$

SMCol foster colony on small-cell combs, *STCol* foster colony on standard-cell combs, *SMComb* small-cell comb, *STComb* standard-cell comb, *SD* standard deviation. *The impact ot the foster colony is significant at $p \leq 0.05$; **the impact of the foster colony and the impact of the comb are significant at $p \leq 0.01$; *n. s.* not significant.

values of the vast majority of morphometric traits in the STCol than SMCol workers, which was particularly evident in the SMComb + STCol combination and yielded the highest fill factor reported in the literature (81%). The body size was probably influenced by the more abundant food supply provided to the larvae by the STCol workers, whose larger head promoted better development and enhanced the efficiency of hypopharyngeal glands. This result confirms the observation that workers reared in STComb feed larvae more efficiently (Willem et al. 2006). It is possible that such a mechanism also functions in colonies with natural nests where the variability in worker cell sizes (4.17–6.86 mm) (Maggi et al. 2010) results in simultaneous presence of different-sized workers that had been reared in cells with different widths during the period of maximum colony development. An advantage of such a natural nest arrangement may be the highest fill factor value in the SMComb + STCol worker group shown in the present study. The increased fill factor may be one of the determinants of limitation of *V. destructor* reproduction, which seems to be confirmed in the study conducted by Maggi et al. (2010).

The results of the present study suggest a question whether the changes in the worker body size, and more specifically in morphological traits caused by the different widths of comb cells where they were reared, can increase the non-reproductive division of labor in the bee colony. Such an increased non-reproductive division of labor is observed in ant species with morphologically different worker sub-castes — morphological polyethism (Mertl and Traniello 2009). The occurrence of morphological sub-castes in a honeybee colony may be favored by the considerable variability in the width of comb cells in natural nests (Maggi et al. 2010). So far, research

Table IV. Effect of the foster colony irrespective of the comb and effect of the comb irrespective of the foster colony on the mean values of linear measurements of the wing and its elements [mm] and cubital index values

Traits	Foster colony impact		Comb impact		Interaction: comb × colony
	SMCol	STCol	SMComb	STComb	
Fore wings length	9.29** $n=90$ SD ± 0.24	9.45** $n=90$ SD ± 0.18	9.30** $n=90$ SD ± 0.25	9.45** $n=90$ SD ± 0.16	$p < 0.001$
Fore wings width	3.20** $n=90$ SD ± 0.11	3.27** $n=90$ SD ± 0.08	3.22 $n=90$ SD ± 0.11	3.25 $n=90$ SD ± 0.09	n. s.
Cubital vein distance a	0.59 $n=90$ SD ± 0.05	0.60 $n=90$ SD ± 0.07	0.58 $n=90$ SD ± 0.07	0.60 $n=90$ SD ± 0.04	n. s.
Cubital vein distance b	0.24** $n=90$ SD ± 0.03	0.26** $n=90$ SD ± 0.03	0.24** $n=90$ SD ± 0.03	0.26** $n=90$ SD ± 0.03	n. s.
Cubital index (Goetze)	2.42 $n=90$ SD ± 0.33	2.34 $n=90$ SD ± 0.42	2.43 $n=90$ SD ± 0.43	2.34 $n=90$ SD ± 0.32	n. s.

SMCol foster colony on small-cell combs, *STCol* foster colony on standard-cell combs, *SMComb* small-cell comb, *STComb* standard-cell comb, *SD* standard deviation. **The impact of the foster colony and the impact of the comb are significant at $p \leq 0.01$; *n. s.* not significant.

of the honeybee has been focused on age polyethism. This is probably related to the high morphological similarity of worker bees, which is additionally increased by the standardization of the comb cell width by the widespread use of the wax foundation. It cannot be ruled out that,

Table V. Effect of the foster colony irrespective of the comb and effect of the comb irrespective of the foster colony on the mean values of linear measurements of the abdomen and 3rd and 4th abdominal tergites and the sum of the widths of 3rd and 4th tergites [mm]

Traits	Foster colony impact		Comb impact		Interaction: comb × colony
	SMCol	STCol	SMComb	STComb	
Abdomen length	5.98 $n=90$ SD ± 0.67	5.94 $n=90$ SD ± 0.73	6.02 $n=90$ SD ± 0.72	5.91 $n=90$ SD ± 0.59	n. s.
3rd tergite width	2.50* $n=90$ SD ± 0.10	2.53* $n=90$ SD ± 0.07	2.48** $n=90$ SD ± 0.09	2.54** $n=90$ SD ± 0.07	n. s.
4th tergite width	2.29 $n=90$ SD ± 0.10	2.29 $n=90$ SD ± 0.08	2.26** $n=90$ SD ± 0.08	2.33** $n=90$ SD ± 0.09	$p < 0.01$
Sum of the widths of 3rd and 4th tergites	4.80 $n=90$ SD ± 0.17	4.85 $n=90$ SD ± 0.12	4.74** $n=90$ SD ± 0.13	4.91** $n=90$ SD ± 0.13	n. s.

SMCol foster colony on small-cell combs, *STCol* foster colony on standard-cell combs, *SMComb* small-cell comb, *STComb* standard-cell comb, *SD* standard deviation. *The impact of the foster colony is significant at $p \leq 0.05$; **the impact of the comb is significant at $p \leq 0.01$; *n. s.* not significant.

Table VI. Percentage changes in comb cell width, total weight, and weight of the main body parts, and morphometric parameters in three groups of workers reared in colonies with different comb type-foster colony type combinations vs. workers reared in standard-cell combs in a colony kept on standard-cell combs (STComb + STCol)

Traits	Groups: comb type-foster colony type combinations		
	SMComb + SMCol	SMComb + STCol	STComb + SMCol
Cells width	−11.8	−11.8	0.0
Body weight	−7.1	−2.7	+7.8
Head weight	−1.4	+6.5	+9.9
Thorax weight	−11.6	−7.5	−0.6
Abdomen weight	−4.8	−0.8	+11.3
Head width	−3.3	−2.8	−1.0
Head height	−4.5	−2.4	0.0
Thorax width	−4.4	−2.0	−0.7
Thorax length	−3.3	−1.1	−4.7
Proboscis length	−1.9	+2.9	+0.9
Fill factor	+8.5	+11.3	−0.7
Wing length	−3.4	−0.5	−0.6
Wing width	−2.8	−0.3	−1.2
Distance a of cubital vein	−3.4	+1.7	+1.7
Distance b of cubital vein	−11.1	−7.4	−7.5
Cubital index (Goetze)	+8.0	+8.4	+7.1
Abdomen length	+2.6	+2.0	+0.9
3rd tergite width	−3.5	−2.7	−1.6
4th tergite width	−3.0	−1.3	+1.7
Sum of 3rd + 4th tergite width	−3.3	− 2.3	0.0

SMComb + SMCol, workers reared in small-cell combs in colonies kept on small-cell combs; SMComb + STCol, workers reared in small-cell combs in colonies kept on standard-cell combs; STComb + SMCol, workers reared in standard-cell combs in colonies kept on small-cell combs.

besides age polyethism, the bee colony exhibits elements of morphological polyethism, which may represent a compromise between specialization and behavioral flexibility. In social bees, physiological and behavioral differences between large and small worker bees in the same colony have been observed in bumblebees (Spaethe and Weidenmüller 2002; Worden et al. 2005). Perhaps the answer to the above question will be provided by the research conducted as part of our grant: "Elucidation of the phenomenon of behavioral overdominance of honeybee colonies kept on two types of combs with standard- and small-cell size," no. 2018/31/B/NZ9/02480, financed by National Science Center, Poland.

5. CONCLUSIONS

The change in the value of the morphometric parameters of the worker bees was not proportional to the change in the width of the comb cells where they were reared. These traits were found to change less substantially than assumed previously; therefore, changing the comb cell width is not a good approach for modeling the body size of worker bees.

In terms of the changes in the weight of the main worker body parts, the thorax weight was most susceptible to reduction resulting from rearing in small cell combs. The decrease in the thorax weight was proportional to the decline in the comb cell width.

Feeding larvae reared in small-cell combs by worker bees reared in standard cell combs, which was possible due to the presence of both small and standard cell combs in the nest of the same bee colony, resulted in an increase in the fill factor value.

The relatively constant body size of worker bees and the use of small cell combs for brood rearing, which increase the fill factor, may be one of the determinants of an increase in resistance to *Varroa destructor*.

The value of morphometric traits that are commonly used for identification of honeybee subspecies, i.e., the fore wing length, proboscis length, and sum of the widths of 3rd and 4th tergites, changed only inconsiderably vs. the changes in the width of the worker comb cells where they were reared. The low dependence of these traits on changes in the comb cell width confirms their high suitability for identification of honeybee subspecies.

AUTHOR CONTRIBUTION

PD concept, research, discussion, writing. GB research, discussion, writing. KO research concept in apiary research, discussion, writing. PD, GB, KO acceptance of manuscript last version.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

DECLARATIONS

Consent for publication All authors have approved the manuscript for submission.

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REFERENCES

Berry, J.A., Owens, W.B., Delaplane, K.S. (2010) Small-cell comb foundation does not impede Varroa mite population growth in honey bee colonies. Apidologie. **41**, 40-44. https://doi.org/10.1051/apido/2009049

Coffey, M.F., Breen, J., Brown, M.J.F. McMullan, J.B. (2010) Brood-cell size has no influence on the population dynamics of *Varroa destructor* mites in the native western honey bee, *Apis mellifera mellifera*. Apidologie. **41**, 522-530. https://doi.org/10.1051/apido/2010003

Couvillon, M.J., Dornhaus, A. (2009) Location, location, location: larvae position inside the nest is correlated with adult body size in worker bumblebees (Bombus impatiens). Proc. R. Soc. B (2009) **276**, 2411–2418. https://doi.org/10.1098/rspb.2009.0172

Cowan T.W. (1904) The honeybee: its natural history, anatomy and physiology. Houlston and Sons, London.

Donze, G., Guerin, M.P. (1994) Behavioral attributes and parental care of Varroa mites parasitizing honeybee brood. Behav. Ecol. Sociobiol. **34**, 305-319. https://doi.org/10.1007/BF00197001

Ellis, A.M., Hayes, G.W., Ellis, J.D. (2009) The efficacy of small cell foundation as a varroa mite (*Varroa destructor*) control. Exp. Appl. Acarol. **47**, 311-316. https://doi.org/10.1007/s10493-008-9221-3

Erickson, E.H., Lusby, D.A., Hoffman, G.D., Lusby, E.W. (1990) On the size of cells: speculations on foundation as a colony management tool. Glean. Bee. Cult. **118**, 173-174.

Grout R.A. (1937) The influence of the size of brood cell upon the size and variability of the honeybee (*Apis mellifera* L.). Iowa Agric. Exp. Sta. Bull. **218**(19), 260-280.

Goetze G (1933) Einfluß der Wabenzellengröße auf die Größe der Bienen. Archiv. f. Bienen. **4**, 137-140.

Maggi, M., Damiani, N., Ruffinengo, S., De Jong, D., Principal, J., Eguaras, M. (2010) Brood cell size of *Apis mellifera* modifies the reproductive behavior of *Varroa destructor*. Exp. Appl. Acarol. **50**, 269-279. https://doi.org/10.1007/s10493-009-9314-7

Martin, S.J., Kryger, P. (2002) Reproduction of *Varroa destructor* in South African bees: does cell space influence Varroa male survivorship?. Apidologie. **33**, 51-61. https://doi.org/10.1051/apido:2001007

McMullan, J.B., Brown, M.J.F. (2006) The influence of small-cell brood combs on the morphometry of honeybees (*Apis mellifera*). Apidologie. **37**, 665-672. https://doi.org/10.1051/apido:2006041

Mertl, A.L., Traniello, J.F.A. (2009) Behavioral evolution in the major worker subcaste of twig-nesting Pheidole (Hymenoptera: Formicidae): does morphological specialization influence task plasticity? Behav. Ecol. Sociobiol. **63**, 1411-1426. https://doi.org/10.1007/s00265-009-0797-3.

Message, D., Goncalves, L.S. (1995) Effect of the size of worker brood cells of Africanized honey bees on infestation and of the ectoparasitic mite *Varroa jacobsoni* Oud. Apidologie. **26**, 381-386. https://doi.org/10.1051/apido:19950503

Michailoff AS (1927) Über den Zusammenhang zwischen den Umfang der Bienenzelle und dem Umfang des Bienenkörpers und seiner Teile. Archiv. f. Bienen **8**, 313-321.

Noirot, C., Pasteels, J.M. (1987) Ontogenetic development and evolution of the worker caste in termites. Cell. Mol. Life Sci. **43**, 851–952. https://doi.org/10.1007/BF01951642.

Piccirillo, G.A., De Jong, D. (2003) The influence of brood comb cell size on the reproductive behavior of the ectoparasitic mite *Varroa destructor* in Africanized honey bee colonies. Genet. Mol. Res. **2**(1), 36-42.

Ruttner F (1988) Biogeography and taxonomy of honeybees. Springer Verlag, Berlin Heidelberg New York

Ruttner F (1992) Naturgeschichte der Honigbienen. Ehrenwirth Verlag, München

Sauthier, R., I'Anson Price, R., Grüter, C.h. (2016) Worker size in honeybees and its relationship with season and foraging distance. Apidologie. **48**, 234–246. https://doi.org/10.1007/s13592-016-0468-0

Seeley, T.D., Griffin, S.R. (2011) Small-cell comb does not control Varroa mites in colonies of honeybees of European origin. Apidologie. **42**, 526-532. https://doi.org/10.1007/s13592-011-0054-4

Spaethe, J., Weidenmüller, A. (2002) Size variation and foraging rate in bumblebees (Bombus terrestris). Insect. Soc. **49**, 142–146

Taylor, M.A., Goodwin, R.M., McBrydie, H.M., Cox, H.M. (2008) The effect of honey bee worker brood cell size on *Varroa destructor* infestation and reproduction. J. Apic. Res. **47**(4), 239–242. https://doi.org/10.1007/s13592-018-0610-2

Willem, J., Boot, J.N.M., Allsopp, C., Allsopp, M. (2006) Differential feeding of larvae affects caste differentiation in *Apis mellifera*. Proc. Neth. Entomol. Soc. Meet. **17**, 63-69. https://doi.org/10.1007/s00265-003-0666-4

Worden, B.D., Skemp, A.K., Papaj, D.R. (2005) Learning in two contexts: the effects of interference and body size in bumblebees. J. Exp. Biol. **208**, 2045–2053. https://doi.org/10.1242/jeb.01582