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ORIGINAL RESEARCH ARTICLE

Comb age significantly influences the emergency queen rearing, morphometric and reproductive characteristics of the queens

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ABSTRACT

A study on the impact of comb age on the number of emergency queen cells and ripped queen cells was conducted. Also, we compared morphometric and reproductive characteristics of queens reared in combs aged 1, 2, 3, and 4 years. The highest number of emergency queen cells was constructed in combs aged 1 year on the 2nd day after dequeening. No more queen cells were constructed after the 5th day of dequeening. The highest numbers of successful queen cells and emerged queens were obtained from the new combs. The amount of royal jelly (RJ)/queen cell, the weight of the newly emerged queen, and the queen cell size significantly decreased with increasing the age of the comb. Compared with the newly emerged queens from combs aged 1 year, the queens from combs aged 3 and 4 years exhibited significantly lower values for antenna length, mandibular gland area, forewing area, number of hamuli, area of the 3rd and 4th abdominal tergites, abdomen length, number of ovarioles/ovary, ovariole length and diameter, and spermathecal size. Significant positive correlations between the queen cell size and RJ yield/queen cell, queen body weight, and all queen characteristics except for the hindwing area were found. It can be concluded that the number of reared queens and their morphometrics and reproductive characteristics were significantly dependent on the age of the combs. The queen's body size can be used as an indicator of the queen's quality.

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Comb; honey bee; queen; queen cells; ovarioles; royal jelly; spermatheca

Introduction

The honey bee queen is the most important individual in the colony because its function of laying eggs maintains and increases the population of the colony. The beekeepers always need queens for compensation for the lost ones, the establishment of new colonies, and the replacement of the old queens (Taha, 2013). Queen rearing is influenced by several factors such as rearing season (Elenany & Abdallah, 2016; Shawer et al., 2021; Taha, 2005), colony strength (Abd Ai-Fattah et al., 2003b), and food supply (Cengiz et al., 2019; Taha, 2000, 2005). Additionally, the rearing technique (Abd Ai-Fattah et al., 2003a; Adgaba et al., 2019; Dhaliwal et al., 2019), age of grafted larva (Cengiz et al., 2019; Dhaliwal et al., 2019; Mahbobi et al., 2012; Okuyan & Akyol, 2018; Ustadi et al., 2022), and subspecies of honey bee (Elenany & Abdallah, 2016) have been recorded as important factors influence the quality of the queen. The queen is the depositary of all inherited characteristics of the species acquired through her progenitors and through the acquisition of sperm from males at the time of mating (Baer et al., 2016).

The queens of honey bees can be reared in limited numbers from the normally built queen cells or commercially in comparatively larger numbers by using an artificial queen-rearing method (Adgaba et al., 2019; Cengiz et al., 2019; Dhaliwal et al., 2019; Taha, 2005). Many beekeepers worldwide still use the emergency queen-rearing method for queen production to use in the requeening and division of colonies. The quality of the queen has been reportedly correlated with its size or weight (Amiri et al., 2017; Delaney et al., 2011; Kahya et al., 2008; Taha, 2005, 2013). However, the size and weight of the queen are positively correlated to the size of the queen cell (Taha, 2005; Shawer et al., 2007), so, emergency queen rearing should be applied in new combs.

According to Al-Kahtani and Taha (2021b) and Taha et al. (2021), there are numerous beekeepers worldwide who still use combs for about 4–5 years. This practice has resulted in a decline in the inner cell diameter (Al-Kahtani & Taha, 2021b; Shawer

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et al., 2020), brood survivorship, colony growth (Abd Al-Fattah et al., 2021; Dizaji et al., 2008; Taha et al., 2021; Taha & Al-Kahtani, 2020), body weights of colony individuals (Al-Kahtani & Taha, 2021b; Shawer et al., 2020; Taha et al., 2021), the performance of the honey bee colony (Taha et al., 2021; Taha & Al-Kahtani, 2020), amount of RJ/queen cell (Taha et al., 2021), and honey yield (Al-Kahtani & Taha, 2021b; Dizaji et al., 2008; Taha et al., 2021; Taha & Al-Kahtani, 2020; Taha & El-Sanat, 2007).

The size of the cells in the old combs is smaller than that in the new combs (Al-Kahtani & Taha, 2021b; Shawer et al., 2020). We may hypothesize that there may be a significant impact of comb age on the quality of queens from the emergency queen cells. Here we aimed to investigate the influence of comb age on the efficiency of emergency queen rearing. Also, the influence of comb age on the body size, morphometrics, and reproductive characteristics of queens from the emergency queen cells was studied.

Materials and methods

Study area

The study was conducted at the apiary of the Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt, during the late winter of 2022. Kafrelsheikh lies at longitude 30° 56′ 45″ E, latitude 31° 6′ 42″ N, and an altitude of 17 m above sea level. The following meteorological factors: air temperature, wind velocity, rainfall, and relative humidity were obtained from Sakha agro-meteorological station, Sakha, Kafrelsheikh, Egypt and can be found in the supplementary table (Table S1).

Experimental colonies

One month before the beginning of the experiment, the oxalic acid vaporization method was applied for Varroa treatment in all apiary colonies. Thirty-two honey bee colonies of the same population size (eight combs for each) headed by young sister open-mated queens were used in this experiment. The colonies were divided into four groups (eight colonies for each). The eight combs in each hive had been replaced by only six combs (a bee bread comb on one side, a honeycomb on the other side, and the experimental combs in the middle). The experimental combs were obtained from the Faculty of Agriculture's apiary. Marked frames with wax foundation were added to the colonies in late winter 2018, 2019, 2020, and 2021, to obtain combs aged 1, 2, 3, and 4 years (supplementary Figure S1), respectively in late winter 2022. To avoid the effect comb position, the experimental combs were arranged according to their ages as follows: 1, 2, 3, and 4 years (group 1); 2, 3, 4, and 1 year (group 2); 3, 4, 1, and 2 years (group 3); and 4, 1, 2, and 3 years (group 4), then the queens were left to lay eggs in the combs. All colonies were supplied with 1 L sugar syrup (1: 1) every 3 days until all queen cells were sealed.

Rearing procedure and measurements

The queens were removed from the colonies (brood chambers) after egg laying in all combs. The daily number of the constructed queen cells in each comb was recorded from the 2nd day of queen removal until the construction of new queen cells stopped. Ten queen cells containing larvae aged 4 days were selected from each group to collect the royal jelly (RJ) using a woody collection tool. The collected RJ was weighted and the mean yield of RJ (mg)/queen cell was calculated. One day before queen emergence, the queen cells were recounted to estimate the number of the ripped queen cells, and then they were caged on the combs. The number of emerging queens was recorded. Thirty-two newly emerged queens from each comb age were used to determine the fresh body weight (mg) using an electrical balance after chilling. The size of the queen cell was measured using a medical syringe and distilled water by calculating the amount of water used to fill the queen cell (Taha, 2005). Also, the depth and the diameter of the queen cells near their bases were measured. The queens were dissected; body appendages, head, and mandibular glands were removed and measured using the Scan Photo technique, as described by Shawer et al. (2021). An HP scanner at a high resolution of 1200 dpi was connected to a personal computer, and the samples were scanned. After taking the images, they were saved on the computer and measured using a Photoshop software program (Adobe Photoshop CS5). The antenna length, head area, mandibular gland area, area of the right fore and hind wings, the number of hamuli on the right hind wings, the 3rd and 4th abdominal tergites areas, abdomen length, and diameter were measured.

Thirty-two queens from each comb age were used to determine the number of ovarioles per ovary, and the diameter of spermatheca. The queens were dissected in a physiological saline solution, the tergites were removed, and the ovaries were taken after removing the tracheae. Each right ovary was placed alone in xylol for ten minutes and washed with tap water. A drop of Puri (1931) medium (10 mL distilled water, 5 mL glycerin, 3 mL glacial acetic acid, 70 g chloral hydrate, and 8 g Gum acacia) was dripped upon it for 1 min. After that, the specimen was washed with tap water several times to remove

all chemical materials, and then another drop of Puri's medium was dripped. This process was repeated four times to get rid of the connective tissues. The ovary was put in a small petri dish filled with distilled water, and the ovarioles were cut into bundles. The number of ovarioles/ovaries was counted under a binocular. The length and diameter of the ovariole were measured. The spermatheca was removed and the diameter was measured. The spermathecal size was determined depending on the spherical shape (Snodgrass, 1956), using the following formula: Size $= 4/3\ \pi r^3$

Where $\pi = 3.14$ and $r = 1/2$ diameter of spermatheca.

Statistical analysis

The differences between the comb ages for the tested parameters were tested by one-way analysis of variance (ANOVA), which indicated significant differences among the ages of combs. The normality in 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. The ANOVA was used to assess differences among the ages of combs tested *via* the PROC GLM function in SAS version 9.1 (SAS Institute, 2003). Pearson correlation coefficients between queen cell size and RJ/queen cells, body weight, and morphometric characteristics were determined. The treatment means were compared using Tukey's HSD Post-hoc test.

Results

Data presented in Table 1 show significant ($p < 0.01$) variations in the number of emergency queen cells in the colonies depending on the age of the comb and the time after removing the queen. The highest number of the constructed queen cells was recorded in the new combs on the 2nd day after queen removal. No more queen cells were constructed after the 5th day of dequeening. The total number of constructed queen cells was 15.55, 9.75, 6.00, and 5.25 queen cells/comb for combs aged 1, 2, 3, and 4 years, respectively. The number of ripped queen cells and the number of emerged queens were significantly ($p < 0.01$) influenced by the age of the comb, while the number of ripped queen cells/comb is not a statistically significant difference between combs aged 3 and 4 years (Table 1). The percentage of successful queen cells was 93.25, 92.31, 79.17, and 61.90% of the constructed queen cells for combs aged 1, 2, 3, and 4 years, respectively (supplementary Table S2). The largest numbers of ripped queen cells (14.50 queen cells) and emerged queens (14.25 queens) were obtained from the combs aged 1 year. The emergence percentages were 98.28, 94.44, 94.74, and 61.54% of the ripped queen cells for combs aged 1, 2, 3, and 4 years, respectively (supplementary Table S2).

Data illustrated in Figure 1 show that the amount of RJ/queen cell, the weight of the newly emerged queen, queen cell size, queen cell depth, number of ovarioles/ovary, and spermathecal size were significantly ($p < 0.01$) influenced by the age of the comb, meanwhile, the weight of the newly emerged queens from combs aged 3 and 4 years, and queen cell depth and spermathecal size from combs aged 2 and 3 years showed no significant difference. The highest weight of RJ/queen cell (265.00 mg/queen cell) and weight of the newly emerged queen (179.57 mg) were obtained from combs aged 1 year vs. 103.25 mg/queen cell and 144.71 mg from combs aged 4 years, respectively (Figure 1). A significant ($p < 0.01$) decrease in queen cell size (0.81 vs 0.66 cm^3), queen cell depth (2.64 vs 1.90 cm), and queen cell diameter (0.94 vs 0.79 cm) occurred in comb age 1 year vs comb aged 4 years (Figure 1).

Except for abdomen diameter ($p < 0.05$) and hindwing area ($p > 0.05$), the remained 10 of 12 examined morphometric characteristics significantly ($p < 0.01$) differed among the tested comb ages (Table 2). Compared with the queens from combs aged 1 year, queens from combs aged 3 and 4 years exhibited significantly ($p < 0.01$) lower values for antenna length, head area, area of the mandibular gland, forewing area, number of hamuli, area of the 3rd and 4th abdominal tergites, abdomen length, ovariole length and diameter. The same trend was observed with the queens from combs aged 2 years for the area of the mandibular gland, forewing area, number of hamuli, area of the 3rd and 4th abdominal tergites, abdomen length, ovariole length and

Table 1. Effect of comb age on the number of the constructed queen cells and number of emerged queens.

Comb age (years)	Days after dequeening					Total No. queen cells	No. ripped queen cells/comb	No. emerged queens/comb
	1	2	3	4	5			
1	0.00	10.75 ± 0.37^{a}	2.75 ± 0.74	1.80 ± 0.20^{a}	0.25 ± 0.19	15.55 ± 1.21^{a}	14.50 ± 1.17^{a}	14.25 ± 1.07^{a}
2	0.00	5.75 ± 0.98^{b}	1.50 ± 0.22	2.00 ± 0.31^{a}	0.50 ± 0.38	9.75 ± 1.07^{b}	9.00 ± 10.14^{b}	8.50 ± 1.04^{b}
3	0.00	4.00 ± 0.77^{b}	1.00 ± 0.54	0.75 ± 0.37^{b}	0.25 ± 0.19	6.00 ± 0.17^{c}	4.75 ± 1.09^{c}	4.50 ± 0.60^{c}
4	0.00	2.50 ± 0.28^{c}	1.25 ± 0.25	1.00 ± 0.00^{b}	0.50 ± 0.28	5.25 ± 0.03^{d}	3.25 ± 1.10^{c}	2.00 ± 0.41^{d}
Sig.	NS	**	NS	**	NS	**	**	**

Values are the mean ± standard error. The means of each column followed by the different letters are significantly different at $p < 0.05$. ** and NS indicate $p < 0.01$ and $p > 0.05$ (insignificant differences), respectively.

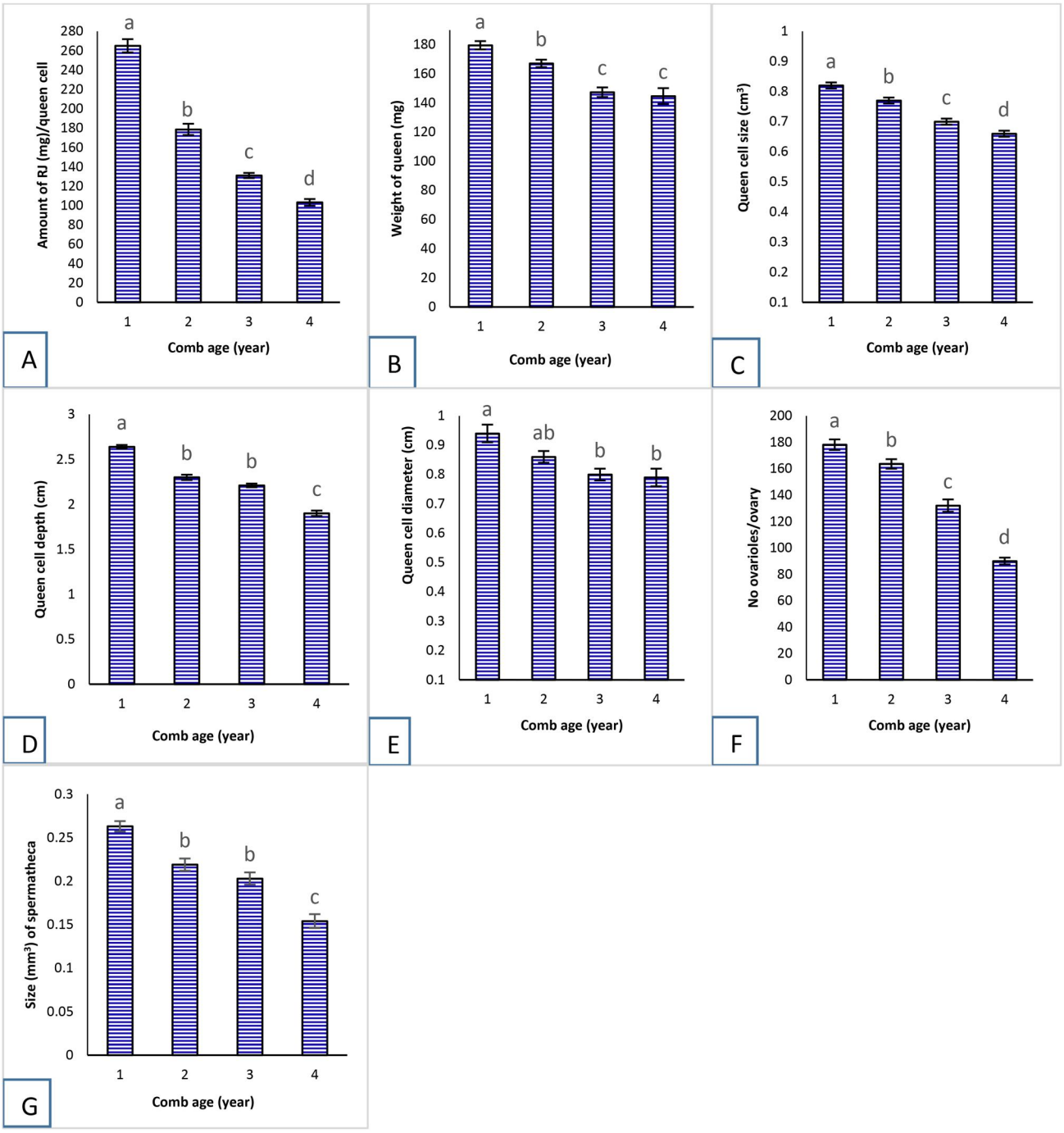

Figure 1. Effect of comb age on the amount of RJ/queen cell (A), the weight of the newly emerged queen (B), queen cell size (C), queen cell depth (D), queen cell diameter (E), the number of ovarioles/ovary (F), and size of the spermatheca (G). Different letters above the bars indicate a significant ($p < 0.05$) difference.

diameter. On the other hand, the hindwing area did not significantly differ among queens from combs of different ages.

Data presented in Table 3 show significant positive correlations ($r = 0.41$–0.89, $p < 0.05$–0.001) between the queen cell size, queen body weight, and all queen characteristics except for the hindwing area. Also, Significant positive correlations ($r = 0.40$–0.94, $p < 0.05$–0.001) were found between the amount of RJ/queen cell, queen body weight, and all queen characteristics. Contrary, Significant negative correlations ($r = -0.44$ to -0.96, $p < 0.01$) were found between the age of the comb and queen cell size, amount of RJ/queen cell, queen body weight, and all queen characteristics except for the hindwing area.

Discussion

The naturally constructed queen cells include emergency queen cells, supersedure queen cells, and swarming queen cells. The honey bee workers have built the supersedure and swarming queen cells in the presence of the queen (Taha, 2000), while the emergency queen cells have been constructed when

Table 2. Effect of comb age on the measurements of some morphometric characteristics and number of hamuli of the newly emerged queen of the honey bee.

	Age of comb (years)				
Parameters	1	2	3	4	Sig.
Antenna length (mm)	4.33 ± 0.05^{a}	4.18 ± 0.10^{ab}	3.98 ± 0.08^{ba}	3.77 ± 0.11^{c}	**
Head area (mm^2)	11.70 ± 0.67^{a}	10.85 ± 0.62^{ab}	10.20 ± 0.31^{ab}	9.87 ± 0.38^{b}	**
Mandibular gland area (mm^2)	3.55 ± 0.14^{a}	3.24 ± 0.03^{a}	2.52 ± 0.13^{b}	2.19 ± 0.17^{b}	**
Forewing area (mm^2)	18.73 ± 0.37^{a}	18.30 ± 0.41^{a}	16.73 ± 0.49^{b}	16.53 ± 0.47^{b}	**
Hindwing area (mm^2)	10.79 ± 0.61	10.50 ± 0.41	9.85 ± 0.13	9.80 ± 0.46	NS
No. hamuli	20.82 ± 1.11^{a}	19.66 ± 0.16^{a}	18.25 ± 0.94^{b}	17.00 ± 0.50^{b}	**
3rd tergite area (mm^2)	29.76 ± 0.54^{a}	27.08 ± 0.51^{b}	25.38 ± 0.44^{c}	25.11 ± 0.26^{c}	**
4th tergite area (mm^2)	25.82 ± 0.13^{a}	23.01 ± 0.49^{b}	19.77 ± 0.57^{c}	19.35 ± 0.40^{c}	**
Abdomen length (mm)	12.31 ± 0.13^{a}	10.16 ± 0.26^{b}	8.66 ± 0.37^{c}	8.29 ± 0.18^{c}	**
Abdomen diameter (mm)	4.97 ± 0.04^{a}	4.88 ± 0.04^{ab}	4.62 ± 0.22^{ab}	4.26 ± 0.24^{b}	*
Ovariole length (mm)	6.56 ± 0.22^{a}	4.71 ± 0.18^{b}	3.87 ± 0.12^{c}	3.82 ± 0.08^{c}	**
Ovariole diameter (mm)	0.074 ± 0.002^{a}	0.056 ± 0.001^{b}	0.040 ± 0.001^{c}	0.030 ± 0.001^{d}	**

Values are the mean ± standard error. The means of each row followed by the different letters are significantly different at $p < 0.05$. **, *, and NS indicate $p < 0.01$, $p < 0.05$, and $p > 0.05$ (insignificant differences), respectively.

the queen is lost (Hatch et al., 1999; Taha, 2013; Tofilski & Czekonska, 2004). In this study, all combs were in the same colony (the nutritional, environmental, and meteorological factors are similar across all combs), and the eggs were from the same queen, so the impacts should have been the same regardless of the comb age where the queen cells were constructed. Therefore, the differences should have been related to the age of the comb. The number of emergency queen cells was higher in the new combs. The highest number of queen cells was constructed on the 2nd day after queen removal, and then a sharp decrease in the number of constructed queen cells occurred until it fully stopped after the 5th day of dequeening. These results confirm those obtained by Hatch et al. (1999) and Tofilski and Czekonska (2004) who found that workers of honey bees rear emergency queens from brood through 1–5 days of dequeening. The decline of queen cells after the 2nd day was due to the increased pheromone responsible for the suppression of queen rearing (Free, 1987; Melathopoulos et al., 1996) and on the 6th day of dequeening due to the pheromone and absence of the young larvae. The low number of constructed queen cells, low percentage of ripped queen cells, and low % emergence in old combs may reflect the problem in the wax comb including the content of harmful elements (Taha et al., 2010).

The amount of RJ/queen cell has been affected by many factors, e.g., nectar and pollen resources (Chen et al., 2002; Taha et al., 2009), season (Gue, 1998; Taha, 2005; Taha et al., 2021), age of grafted larvae (Cengiz et al., 2019; Ustadi et al., 2022), feeding the colonies (Wytrychowski et al., 2013), rearing technique (Dedej et al., 1998), and harvest time post grafting (Al-Kahtani & Taha, 2020a, 2020b). Here all these factors in addition to the larvae and nurse workers are all the same, so the variations in the yield of RJ/queen cells can be related to the age of the comb. This may be due to the size of the queen cell which is correlated to the size of the comb cell which has been influenced by the age of the comb (Al-Kahtani & Taha, 2021b; Shawer et al., 2020). Based on combs aged 1 year, the amount of RJ/queen cells declined by 32.60, 50.57, and 61.04% for combs aged 2, 3, and 4 years, respectively (supplementary Table S3). Significant positive ($p < 0.001$) correlations between the amount of RJ/queen cell and queen cell size ($r = 0.84$), queen cell depth ($r = 0.87$), and queen cell diameter ($r = 0.60$) were found. These results confirm the findings of Taha et al. (2021) who recorded the superiority of new combs in RJ yield/queen cells. In the current study, the queen cell size was negatively significantly correlated ($r = -0.89$, $p < 0.001$) with the age of the comb. In this concern, a significant positive correlation has been found between the yield of RJ/queen cell and queen cell size (Taha, 2005; Taha et al., 2021).

In the current study, all combs were located in one colony, so the availability of pollen and nectar did not differ. In comparison to the newly emerged queen from the comb aged 1 year, the newly emerged queens from combs aged 2, 3, and 4 years showed a significant decline in their body weight and we suggest this decline was associated with the variations in queen cell size that were negatively affected by the age of the comb. In comparison to the queen from comb aged 1 year, the weight of queens from combs aged 2, 3, and 4 years decreased by 6.93, 17.93, and 19.41%, respectively (supplementary Table S3). We found a significant positive correlation ($r = 0.76$, $p < 0.001$) between the queen body weight and queen cell size which was found to be negatively correlated ($r = -0.89$, $p < 0.001$) with the age of the comb. In one previous study, the queen's body weight has been altered by the age of the comb (Taha et al., 2021).

Okuyan and Akyol (2018) reported that the body weight, head, thorax, and abdomen lengths of the queen bees may support their productivity. The morphometrical characteristics of a queen including

Table 3. Pearson correlation coefficients for queen cell, RJ/queen cell, body weight and morphometric characteristics of the honey bee queen.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Queen cell size	0.81**																		
2. Queen cell depth	0.48**	0.57**																	
3. Queen cell diameter	0.84**	0.87**	0.60**																
4. Royal jelly weight/queen cell	0.76**	0.74**	0.58**	0.79**															
5. Body weight	0.51**	0.65**	0.41*	0.58**	0.51**														
6. Antenna length	0.62**	0.72**	0.55**	0.73**	0.73**	0.43**													
7. Mandibular gland area	0.41*	0.30	0.30	0.42*	0.41*	0.01	0.45**												
8. Head area	0.49**	0.57**	0.46**	0.53**	0.53**	0.46**	0.40*	0.24											
9. Forewing area	0.22	0.32	0.33	0.35*	0.23	0.28	0.11	0.13	0.50**										
10. Hindwing area	0.50**	0.55**	0.58**	0.57**	0.73**	0.33	0.58**	0.17	0.48**	0.29									
11. Hamuli number	0.70**	0.77**	0.56**	0.86**	0.65**	0.58**	0.57**	0.27	0.43**	0.31	0.46**								
12. Tergite$_3$ area	0.77**	0.76**	0.56**	0.89**	0.78**	0.59**	0.69**	0.32	0.48**	0.28	0.62**	0.84**							
13. Tergite$_4$ area	0.82**	0.82**	0.46**	0.91**	0.77**	0.53**	0.70**	0.39*	0.56**	0.24	0.49**	0.77**	0.79**						
14. Abdomen length	0.43**	0.31	0.34*	0.40*	0.34*	0.44**	0.16	0.16	0.24	0.06	0.23	0.39*	0.38*	0.26					
15. Abdomen diameter	0.83**	0.81**	0.48**	0.82**	0.70**	0.49**	0.69**	0.55**	0.44**	0.11	0.50**	0.67**	0.73**	0.77**	0.34*				
16. Spermathecal size	0.84**	0.78**	0.44**	0.81**	0.73**	0.53**	0.76**	0.39*	0.48**	0.19	0.61**	0.58**	0.73**	0.75**	0.35*	0.81**			
17. No. ovarioles/ovary	0.79**	0.78**	0.60**	0.88**	0.76**	0.54**	0.72**	0.40*	0.47**	0.18	0.53**	0.70**	0.85**	0.80**	0.38*	0.77**	0.73**		
18. Ovariole length	0.89**	0.88**	0.61**	0.94**	0.78**	0.61**	0.75**	0.39*	0.57**	0.26	0.62**	0.79**	0.86**	0.87**	0.39*	0.83**	0.88**	0.87**	
19. Ovariole diameter	−0.89**	−0.91**	−0.58**	−0.96**	−0.81**	−0.63**	−0.80**	−0.44**	−0.55**	−0.31	−0.62**	−0.80**	−0.88**	−0.88**	−0.43**	−0.88**	−0.92**	−0.85**	−0.96**
20. Comb age																			

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

antenna length, head area, mandibular gland area, forewing area, the number of hamuli, area of the 3rd and 4th abdominal tergites, and abdomen length were significantly decreased by increasing the age of the comb. Compared to the queen from comb aged 1 year, the values of morphometrical characteristics declined by 8.08–29.60% in the queen from comb aged 3 years and by 11.75–38.31% in the queen from comb aged 4 years. Significant negative correlations ($r = -0.34$ to -0.78, $p < 0.01$) were found between all morphometrical characteristics of the queen except for the hindwing area and the age of the comb. On the contrary, the queen morphometric characteristics were positively correlated ($r = 0.35$–0.94, $p < 0.05$–0.001) with the amount of RJ in the queen cells. These results are in harmony with those of Njeru et al. (2017) who found that the availability of RJ in the queen cell had great effects on the morphometrical characteristics of the queen. In addition, the morphometrical characteristics were significantly correlated ($r = 0.44$–0.96, $p < 0.05$–0.001) with the body weight (supplementary Table S3). Relatively similar results have been found by Taha (2005) for queens and by Al-Kahtani and Taha (2014, 2021a) for workers.

Compared to the newly emerged queen from comb aged 1 year, the newly emerged queens from combs aged 2, 3, and 4 years showed a significant decrease in the number of ovarioles/ovary (8.14, 25.90, and 49.48%, respectively), ovariole length (28.21, 41.01, and 41.77%, respectively), ovariole diameter (24.32, 45.95, and 59.46%, respectively), and spermathecal size (16.73, 22.81, and 41.44%, respectively) (Supplementary Table S3). These reductions were related to the decline that occurred in body weight ($r = 0.70$–0.78). These results confirm the findings of Taha (2005) who detected significant positive correlations between body weight and the number of ovarioles/ovary and spermathecal size.

Conclusions

It can be concluded that the number of emergency queen cells, number of emerged queens, and queen morphometric and reproductive characteristics were significantly influenced by the age of the combs. The queen's body size can be used as an indicator of the queen's quality. Not only the age of the comb is important, but also the functions of the combs during their lifetime could affect the emergency queen rearing and their morphometric and reproductive characteristics. Moreover, in the future, we would like to evaluate the accumulated materials and their impacts on the morphometric and reproductive characteristics of the queen.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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