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Original Article

The significance of *Apis cerana cerana* (Hymenoptera: Apidae) gnawing off the old brood cells

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Abstract – *Apis cerana cerana* has the biological characteristic of gnawing off the old brood cells which reared multiple generations of workers. This study investigated the internal structure of newly built, old, and semi-rebuilt brood cells and their effects on the morphological development of workers to understand the significance of *Apis cerana cerana* gnawing off the old brood cells. The results showed that there was no significant difference among the three diameters (at the top, middle, and bottom positions) of newly built or semi-rebuilt brood cells ($P > 0.05$), but these changed within the old brood cells ($P < 0.05$). The top, middle, or bottom diameters of the newly built and semi-rebuilt brood cells were significantly larger than those of the old brood cells ($P < 0.05$), but were almost the same between the newly built and the semi-rebuilt brood cells ($P > 0.05$). The weight and base thickness of the cocoon were significantly greater in the old brood cells than those in the semi-rebuilt brood cells ($P < 0.05$). Importantly, the birth weight, body length, and the tested six external morphological indices did not show a significant difference between the newly built and semi-rebuilt brood cells ($P > 0.05$) but were significantly larger than those of old brood cells ($P < 0.05$). The size of the brood cell and the external morphology of the workers showed a positive correlation. This study highlights the significance of *Apis cerana cerana* gnawing off the old brood cells providing a reference for its scientific rearing.

brood cell-gnawing characteristics / semi-rebuilt brood cell / cocoon / worker morphology

1. INTRODUCTION

Honey bees secrete beeswax from wax glands to build the nest (Hepburn et al. 1991), where the colony conducts various life activities (Winston 1987). The nest consists of one or more combs that are arranged in parallel to each other in a vertically downward manner with a certain interval (Hepburn 1986). Cells, the basic unit of the comb, are used to cultivate bees and store honey and pollen (Budathoki and Madge 1987). The color of the newly built comb is white or light yellow, which deepens with the storage of nectar and pollen (Free and Williams 1974). Also, physiological activities such as defecation, spinning, and molting make the cell color darker and change the volume and internal shape of the cells (Jay 1963, 1964; Hepburn and Kurstjens 1988). Bee species of the genus *Apis* have the characteristics of reusing the same cell to cultivate multiple generations of workers. This leads to the accumulation of feces and cocoons increasing the thickness of cell walls (Berry and Delaplane 2001). The average wall thickness of the newly built cells of the Western honey bee

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is 88 μm, which after use for 5 months, 1 year, and 2 years increases to 120, 246, and 297 μm, respectively (Zhang et al. 2010). Although the house workers clean up the cell residues after the leaving of newly emerged workers, the cleaning is not perfect (Yu et al. 2010). Also, beeswax can easily absorb the color constituents in brood cells, which upon substance accumulation changes comb color from white/light yellow to dark brown or even black (Taha and Manosur 2010).

With the increasing rearing generations, the comb changes from a single-phase substance to a composite phase substance with a significant increase in its weight (Zhang et al. 2010). A study showed that the weight of the Western honey bee comb positively correlates with comb age. The average weight of a newly built comb is 1.69 g/sq inch, which during the age of 1–7 years increases to 2.13, 3.84, 5.69, 6.13, 6.73, 7.53, and 8.51 g/sq inch, respectively (Shawer et al. 2020). Likewise, the structure of brood cells changes. By comparing the molds of newly built and old brood cells, the researchers found that the accumulation of cocoons and excrement in the cells changed the three rhombus cell bases into hemispheres (Hepburn et al. 2007). Also, compared with the newly built brood cell, the diameter and volume of the old brood cell were significantly reduced. The average diameter size and volume of the newly built cell of Western honey bees were 6.04 mm and 0.318 mL, respectively. The average diameter sizes of Western honey bee cells after the use for 1–6 years changed to 5.90, 5.73, 5.65, 4.95, 4.88, and 4.81 mm, respectively, and the average cell volume changed to 0.246, 0.234, 0.223, 0.212, 0.205, and 0.183 mL, respectively (Al-Kahtani and Taha 2021).

Studies showed a significant correlation between the structure of the brood cell and the morphological characteristics of the workers; i.e., the cell size significantly affects the body size of workers (Alfalah et al. 2012; AL-Kahtani 2018). The decrease in the volume of old brood cells significantly reduces their nutrient storage capacity affecting the growth of larvae, and then not-so-developed larvae untimely enter the non-feeding prepupal period (Volosevich and Kulzhinskaya 1953; Abdellatif 1965). Therefore, the birth weight of Western honey bee workers reared in fresh cells (114.89 mg) is significantly greater than that developed in old brood cells (88.05 mg) (Taha et al. 2021). The birth weight of workers is an important indicator of their developmental status and reflects the size of their external morphology (Shawer et al. 2020). A study showed that the external morphological sizes of Western honey bee workers are inversely related to the comb age. The average proboscis lengths of the newly emerged workers reared in 1–4-year-old comb were 6.10, 5.94, 5.55, and 5.30 mm, respectively; the average length of the forewing was 9.05, 9.03, 9.00, and 9.00 mm, respectively, and the average length of the 3rd tergite was 2.30, 2.25, 2.10, and 2.05 mm, respectively (AL-Kahtani 2018). Overall, the sizes of the morphological organs of workers reared in old cells were reduced, which negatively impact the foraging ability of workers and colony productivity (Al-Fattah et al. 2021; Taha et al. 2021). In addition, the old brood cells negatively impact the lifespan of workers and colony strength (Dizaji et al. 2008). Studies showed that a queen prefers egg-laying in fresh brood cells as the sealed brood area is significantly larger in fresh combs than that in old combs (Berry and Delaplane 2001; Al-Fattah et al. 2021). A large sealed brood area offers strong potential for colony development, which is one of the key conditions for beekeepers to obtain high yields (Fathy 1997; Taha and Al-Kahtani 2013).

The long-term use of combs negatively impacts worker morphology, colony strength, and production performance. Although Hu et al. (2021) found that *Apis cerana cerana* has the behavioral characteristics of gnawing off old brood cells, the significance of this phenomenon remains unclear. We speculate that this achieves the purpose of cleaning the old brood cells ensuring enough cell space for the development of workers. Accordingly, in this study, we (1) observed the behaviors of *Apis cerana cerana* gnawing off the old brood cells, (2) measured the structural characteristics of brood cells and cocoons, (3) analyzed the birth weight and external morphological characteristics of newly emerged workers, and (4) compared the data to reveal the significance of this phenomenon. The results provide a basis for keeping *Apis cerana cerana* by formulating reasonable comb replacement strategies.

2. MATERIALS AND METHODS

2.1. Observation of *Apis cerana cerana* gnawing off the old cells

Ten 1-year-old combs (with wax foundation) of *Apis cerana cerana* were collected and put into ten respective colonies. The comb gnawing state was recorded at an interval of every two days, which continued until the colony stopped gnawing off the old combs and began to excrete wax to build a new one. At this time, the area of the gnawed comb was measured.

2.2. Collection of old combs

We collected two types (six each) of old combs with the wax foundation of *Apis cerana cerana*. In type I, the cell walls of the brood-rearing area have been largely gnawed but the cell bases remain intact and the gnawed area is > 100 cm^2. In type II, the area of gnawed cell walls is scattered in the brood-rearing area, and the remaining brood cells which have yet not been gnawed are the critical cells to be gnawed soon after one or two generations of workers (see supplementary material Figure S1).

2.3. Establishment of experimental colonies to construct different types of combs

Using the Langstroth standard 10-frame hive, nine experimental colonies of *Apis cerana cerana* were established. The initial strength of each colony was four full frames, with newly mated healthy queens. Firstly, the experimental colonies were divided into three groups, each with three colonies. Secondly, two frames with wax foundations were put into each hive of the first group to get newly built combs. No treatment was done for the second group. Thirdly, two type I combs were added to each hive of the third group for the construction of semi-rebuilt combs. Here, a semi-rebuilt comb consists of the semi-rebuilt cells comprising of new walls and the old bases (Figure 1a, b). Finally, three newly built combs, three old combs of type II, and three semi-built combs were used for rearing workers.

2.4. Preparation of cell molds to measure the sizes of cell structure

The remaining three newly built combs, three old combs, and three semi-rebuilt combs were used for making the cell molds. First, we measured the brood comb thickness (BC_T) (the depth of the cell on both sides of the comb, thickness of the wax foundation, and base of the cocoon (newly built cells have no cocoon) all contribute to the comb thickness) with a Vernier caliper (accuracy ± 0.02 mm) by randomly selecting 20 brood areas in each comb. Then, 90 brood cells were randomly selected from each comb to make

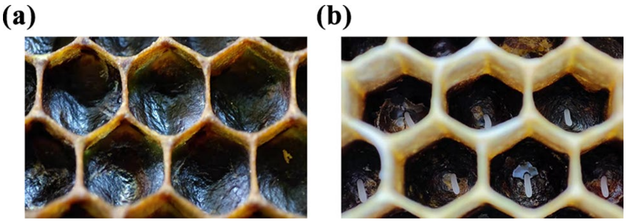

Figure 1. Construction of semi-rebuilt brood cells of *Apis cerana cerana*. **a** Type I comb cells; **b** construction of cell walls on the cell bases of type I combs.

cell molds (Yang et al. 2021). Accordingly, the height and width of the cell mold were used as the cell depth (C_D) and cell diameter dimensions, respectively (see supplementary material Figure S2a). The average widths of the three directions at both ends and the middle of the cell mold were taken as the cell top, bottom, and middle diameters (C_{TD}, C_{BD}, and C_{MD}), respectively (see supplementary material Figure S2b). Cell volume (C_V) was calculated based on density formula.

2.5. Measurement of cocoon characteristics

The comb pieces (5 × 5 cm; length × width) were placed in a solar wax melter to completely melt the beeswax. Then, a single cocoon was collected (see supplementary material Figure S3) using tweezers and weighed (C_w) with an electronic balance. Next, the cocoon was cut along the longitudinal axis with dissecting scissors, and the base thickness of the cocoon (C_{BT}) was measured using a video microscope. Thirty cocoons were randomly selected from each comb for measurement.

2.6. Measuring the external morphological characteristics of the newly emerged workers

The sealed newly built, old, and semi-rebuilt combs were moved to a constant temperature (35 °C ± 0.1 °C) and humidity (75% ± 0.1%) incubator until workers emerged from the cells. Thirty newly emerged workers were randomly selected from each colony. Birth weight (B_W), body length (B_L), proboscis length (P_L), length of the right forewing (W_L), tibia length (T_L), the total length of 3rd and 4th tergite ($T3 + 4_L$), length of 3rd sternite ($S3_L$), and length of the wax plate (WP_L) on 3rd sternite were measured as the external morphological characteristics of the workers (Ruttner 1988). We used an electronic balance and a Vernier caliper to measure the birth weight and body length of workers respectively. The ruler was set to 0.01 mm and a video microscope was used to take pictures of the various tissues and organs. The external morphological indices of workers (see supplementary material Figure S4) were measured with the insect morphological measurement software. The flowchart of the experimental steps is shown in Figure 2.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA; https://www.graphpad.com/scientific-software/prism/). One-way ANOVA test was performed on the indicators with normal distribution and homogeneity of variances, such as brood comb thickness, cell volume, body length, length of the forewing, length of the wax plate, and length of 3rd sternite. The post hoc Tukey test was used to estimate the significance of the difference between the data means. Welch's ANOVA test was performed on indicators to confirm a normal distribution but with unequal variances, such as cell depth, cell top, middle and bottom diameters, birth weight, proboscis length, tibia length, and the total length of the 3rd and 4th tergite. The post hoc Games-Howell test was used to estimate the significance of the differences between the data means. The weights of the base thickness of the cocoon in the old and semi-rebuilt brood cells were compared by *t*-test. Pearson correlation analysis was performed to find correlations between the brood comb cell, cocoon structural characteristics, and morphological characteristics of workers. Significance level $\alpha = 0.05$. Statistical values are expressed as mean ± standard error.

3. RESULTS

3.1. Observing the pattern of *Apis cerana cerana* gnawing at the old combs

Apis cerana cerana gnaws the old comb in two main ways (see supplementary material Figure S5). The first pattern is to completely remove the old brood cells, including the cell bases. Under appropriate conditions, workers can secrete beeswax to build new cells. In the second pattern, only the cell walls are removed

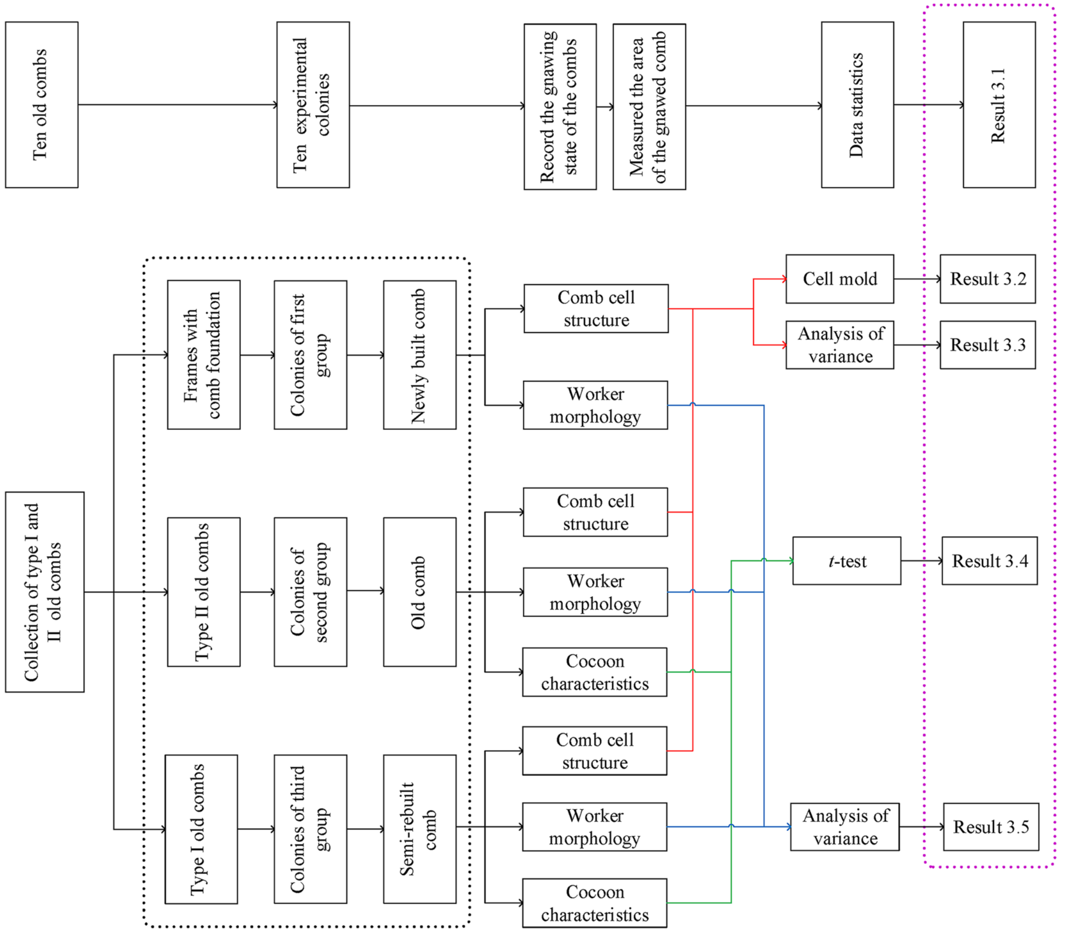

Figure 2. A flowchart shows the experimental steps. The black dotted box represents the process of colony establishment and comb building. The purple dotted box indicates the corresponding results. The red, blue, and green lines highlight the difference in structural characteristics of different brood cells, morphological characteristics between workers, and cocoon characteristics.

leaving the cell bases with a small amount of cocoon. Then, the workers secrete beeswax to make a semi-rebuilt brood cell composed of the new walls and the remaining base. The average gnawed area in the first (n = 3) and second (n = 7) patterns were 41.67 cm^2 ± 3.48 cm^2 and 104.29 cm^2 ± 12.72 cm^2, respectively.

3.2. Shapes of different brood cell

The main shape of the newly built and semi-rebuilt brood cells was a hexagonal prism, while the diameter of the old brood cell gradually decreased from the top to the bottom, resulting in a frustum of a pyramid shape. The individual walls of the newly built brood cell are in the form of a right trapezoid, and three rhombic surfaces are combined to form the cell base. The wall of the old brood cell is surrounded by six trapezoids, and the base shape is a hemisphere. The wall of the semi-rebuilt brood cell is surrounded by six rectangles, and the base shape is also a hemisphere (see supplementary material Figure S6).

3.3. Comparison of structural characteristics of newly built, old, and semi-rebuilt comb brood cells

3.3.1. Comparison of the thickness, cell depth, and volume of newly built, old, and semi-rebuilt brood combs

There were significant differences in the thickness of newly built, old, and semi-rebuilt brood combs. The results of multiple comparisons showed that the average thickness of the old brood comb was significantly larger than that of the newly built and semi-rebuilt brood combs ($P < 0.05$). However, the average thickness of the newly built and semi-rebuilt brood combs was not significantly different ($P > 0.05$) (Table I).

There was no significant difference in the depth of different types of brood cells ($P > 0.05$). However, there were significant differences in the volumes of different brood cells. The results of multiple comparisons showed that the average volume of newly built brood cells was significantly higher than that of the old and semi-rebuilt brood cells ($P < 0.05$). Notably, the average volume of the semi-rebuilt brood cell was also significantly higher than that of the old brood cells ($P < 0.05$) (Table I).

3.3.2. Comparison of cell diameters at different positions in the same brood cell

We found no significant difference in the diameter of the newly built or semi-rebuilt brood cell in different positions (top, middle, and bottom). However, there were significant differences in the diameters of the old brood cell in different positions. The results of multiple comparisons showed that the average diameter of the top position was significantly larger than that of the middle and bottom positions ($P < 0.05$). Notably, the average diameter of the middle position was also significantly larger than that of the bottom position ($P < 0.05$) (Table II).

3.3.3. Comparison of cell diameters at the same position between different cells

There were significant differences in the same position diameters (top or middle or bottom) of different brood cells. The results of multiple comparisons showed that the average top or middle or bottom diameter of the newly built and semi-rebuilt brood cells was significantly larger than that of the old brood cells ($P < 0.05$). There was no significant difference between the average top

Table I Comparison of the thickness, cell depth, and volume of newly built, old, and semi-rebuilt brood combs

Index	Statistical analysis	Statistical value	Degree of freedom	Cell type			*P*-value
				Newly built	Old	Semi-rebuilt	
Brood comb thickness/mm	One-way ANOVA test	F = 81.38	2, 177	20.51 ± 0.07 B	21.77 ± 0.09 A	20.67 ± 0.07 B	$P < 0.0001$
Cell depth/mm	Welch's ANOVA test	W = 2.416	2, 532.6	10.78 ± 0.02 A	10.72 ± 0.02 A	10.75 ± 0.02 A	$P = 0.0902$
Cell volume/mm^3	One-way ANOVA test	F = 181.6	2, 807	190.80 ± 0.57 A	173.47 ± 0.64 C	188.34 ± 0.84 B	$P < 0.0001$

The same capital letters in the same row represent no significant differences ($P > 0.05$), and different capital letters represent significant differences ($P < 0.05$)

Table II Comparison of cell diameters at different positions in the same brood cell

Brood cell type	Statistical Analysis	Statistical value	Degree of freedom	Diameter			*P*-value
				Top	Middle	Bottom	
Newly built	Welch's ANOVA test	$W = 0.4831$	2, 535.0	4.62 ± 0.01 A	4.62 ± 0.01 A	4.62 ± 0.01 A	$P = 0.6178$
Old	Welch's ANOVA test	$W = 68.34$	2, 518.7	4.53 ± 0.01A	4.48 ± 0.01 B	4.35 ± 0.01 C	$P < 0.0001$
Semi-rebuilt	Welch's ANOVA test	$W = 0.2996$	2, 532.4	4.62 ± 0.01 A	4.61 ± 0.01 A	4.61 ± 0.01 A	$P = 0.7412$

The same capital letters in the same row represent no significant differences ($P > 0.05$), and different capital letters represent significant differences ($P < 0.05$)

or middle or bottom diameter of the newly built and semi-rebuilt brood cells ($P > 0.05$) (Table III).

3.4. Comparison of cocoon weight and base thickness between old and semi-rebuilt brood cells

The results of the independent sample *t*-test showed that the average cocoon weight of the old brood cell (57.90 mg ± 1.24 mg) was significantly higher than that of the semi-rebuilt brood cell (2.69 mg ± 0.10 mg) (t = 44.43; df = 178; $P < 0.0001$) (Figure 3a). Similarly, the average base thickness of cocoon of old brood cell (0.96 mm ± 0.03 mm) was significantly larger than that of semi-rebuilt brood cell (0.14 mm ± 0.01 mm) (t = 27.36; df = 178; $P < 0.0001$) (Figure 3b).

3.5. Comparison of morphological characteristics of newly emerged workers

We found significant differences in the birth weight, body length, proboscis length, the length

Table III Comparison of cell diameters at the same position between different cells

Index	Statistical analysis	Statistical value	Degree of freedom	Cell type			*P*-value
				Newly built	Old	Semi-rebuilt	
Cell top diameter/mm	Welch's ANOVA test	$W = 44.89$	2, 512.3	4.62 ± 0.01 A	4.53 ± 0.01 B	4.62 ± 0.01 A	$P < 0.0001$
Cell middle diameter/mm	Welch's ANOVA test	$W = 138.9$	2, 516.2	4.62 ± 0.01 A	4.48 ± 0.01 B	4.61 ± 0.01 A	$P < 0.0001$
Cell bottom diameter/mm	Welch's ANOVA test	$W = 179.9$	2, 502.8	4.62 ± 0.01 A	4.35 ± 0.01 B	4.61 ± 0.01 A	$P < 0.0001$

The same capital letters in the same row represent no significant differences ($P > 0.05$), and different capital letters represent significant differences ($P < 0.05$)

of the right forewing, tibia length, the total length of the 3rd and 4th tergite, length of the wax plate, and the 3rd sternite of newly emerged workers reared in different brood cells. The results of multiple comparisons showed that the above parameters of newly emerged workers reared in newly built or semi-rebuilt brood cells were significantly higher than those reared in old brood cells ($P < 0.05$). However, the same indices of newly emerged workers reared in newly built or semi-rebuilt brood cells showed no significant differences ($P > 0.05$) (Table IV).

3.6. Percentage change in the respective index of semi-rebuilt brood cells relative to newly built or old brood cells

Compared with the newly built cells, the thickness of brood comb with the semi-rebuilt cells increased by 0.78% along with an increase in weight and base thickness of cocoons; however, the top position cell diameter did not change. Notably, other indices showed only a slight decrease between 0.22 and 2.69% (Table V). Conversely, compared with the old cells, the brood comb thickness, weight, and base thickness of cocoons of semi-rebuilt cells decreased by 5.05, 95.35, and 85.42%, respectively; meanwhile, other indices showed an increase between 0.28 and 24.79% (Table V).

3.7. Correlation analysis between the brood cell, cocoon structural characteristics, and morphological characteristics of newly emerged workers

We found a significant correlation between brood comb thickness, brood cell structure, cocoon structure, birth weight, and morphological indices of workers ($P < 0.05$) (see supplementary material Figure S7). The brood comb thickness, cocoon weight, or cocoon base thickness showed a negative correlation with the cell volume, top, middle, and bottom diameters of the cell, birth weight, and morphological indices of workers ($P < 0.05$). The cell volume or top, middle, and bottom diameters of the cell showed a positive correlation with the birth weight and morphological indices of workers ($P < 0.05$). There was no correlation between brood cell depth with brood comb thickness, cell volume, top, middle, and bottom diameters of the cell, cocoon structure, or morphological indices of workers ($P > 0.05$).

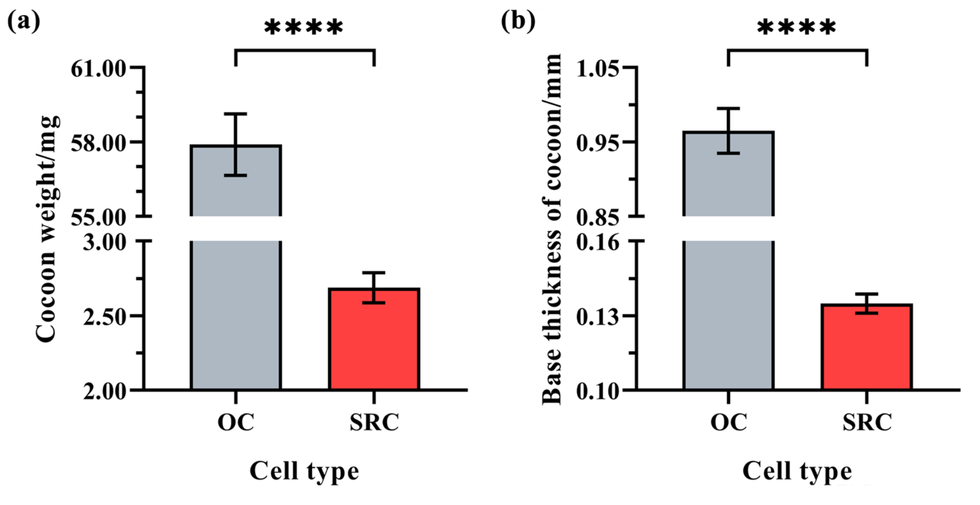

Figure 3. Comparisons of **a** weight and **b** base thickness of cocoon from old and semi-rebuilt brood cells. Four asterisks (****): $P < 0.0001$.

Table IV Comparison of birth weight and morphological characteristics of newly emerged workers reared in different brood cells

Index	Statistical analysis	Statistical value	Degree of freedom	Cell type			*P*-value
				Newly built	Old	Semi-rebuilt	
Birth weight/mg	Welch's ANOVA test	$W = 187.1$	2, 173.1	90.27 ± 0.93 A	70.39 ± 0.69 B	87.84 ± 1.00 A	$P < 0.0001$
Body length/mm	One-way ANOVA test	$F = 25.11$	2, 267	11.92 ± 0.03 A	11.62 ± 0.03 B	11.87 ± 0.03 A	$P < 0.0001$
Proboscis length/mm	Welch's ANOVA test	$W = 17.44$	2, 175.8	5.51 ± 0.04 A	5.17 ± 0.05 B	5.48 ± 0.04 A	$P < 0.0001$
Length of the right forewing/mm	One-way ANOVA test	$F = 58.46$	2, 267	8.16 ± 0.03 A	7.73 ± 0.03 B	8.14 ± 0.03 A	$P < 0.0001$
Tibia length of the hind leg/mm	Welch's ANOVA test	$W = 36.13$	2, 174.4	3.12 ± 0.01 A	2.96 ± 0.01 B	3.07 ± 0.02 A	$P < 0.0001$
Total length of 3rd and 4th tergite/mm	Welch's ANOVA test	$W = 68.05$	2, 174.2	3.80 ± 0.02 A	3.59 ± 0.01 B	3.77 ± 0.02 A	$P < 0.0001$
Length of the wax plate/mm	One-way ANOVA test	$F = 15.65$	2, 267	1.28 ± 0.01 A	1.22 ± 0.01 B	1.27 ± 0.01 A	$P < 0.0001$
Length of the 3rd sternite/mm	One-way ANOVA test	$F = 87.38$	2, 267	2.70 ± 0.01 A	2.53 ± 0.01 B	2.69 ± 0.01 A	$P < 0.0001$

The same capital letters in the same row represent no significant differences ($P > 0.05$), and different capital letters represent significant differences ($P < 0.05$)

4. DISCUSSION

4.1. Characteristics of *Apis cerana cerana* gnawing away the old comb

The physiological behaviors such as defecation, ecdysis, spinning, and cocoon construction lead to the accumulation of substances in brood cells (Jay 1963), while bees repeatedly use the same cell to rear the workers of successive generations. The increasing number of generations, cocoons, and other substances cannot be completely removed by workers and accumulate in brood cells altering the internal cell structure such as the reduction in volume (Hepburn et al. 2007). In addition to genetic factors, a larger brood cell helps rear a larger worker and vice versa (Baudoux 1934; Pensieri 1934; Mcmullan and Brown 2006). Moreover, the small size workers are not conducive to the growth of the colony (Glushkov 1956; Kotelkov and Blashkin 1957). Interestingly, *Apis cerana cerana* has evolved with the biological characteristic of gnawing off the old brood cells to balance the negative impact of the old brood cells on colony growth. This strategy ensures the proper morphology of newly emerged workers. In this study, we found that *Apis cerana cerana* gnawed only the wall of old brood cells leaving the base with a small amount of cocoon. Subsequently, workers secrete

Table V The percentage change in the cell structure and morphological characteristics of workers reared in semi-rebuilt cells *vs* newly built or old cells

Indexes			Brood cell type	
Part	**Parameter**	**Abbreviation**	**Newly built**	**Old**
	Brood comb thickness	BC_T	0.78%	−5.05%
	Cell depth	C_D	−0.28%	0.28%
Comb cell	Cell volume	C_V	−1.29%	8.57%
	Cell top diameter	C_{TD}	0.00%	1.99%
	Cell middle diameter	C_{MD}	−0.22%	2.90%
	Cell bottom diameter	C_{BD}	−0.22%	5.98%
Cocoon	Cocoon weight	C_W	/	−95.35%
	Cocoon base thickness	C_{BT}	/	−85.42%
	Birth weight	B_W	−2.69%	24.79%
	Body length	B_L	−0.42%	2.15%
	Length of proboscis	P_L	−0.54%	6.00%
Worker morphology	Length of forewing	W_L	−0.25%	5.30%
	Tibia length of hind leg	T_L	−1.60%	3.72%
	Total length of 3rd and 4th tergite	$T3 + 4_L$	−0.79%	5.01%
	Length of wax plate	WP_L	−0.78%	4.10%
	Length of 3rd sternite	$S3_L$	−0.37%	6.32%

beeswax-based substances on the old bases to build semi-rebuilt brood cells composed of new walls (Figure 1). This may be due to the beekeepers providing a wax foundation to the colony for quick construction of the comb with a regular and tidy surface. Overall, the process reduces the labor intensity of the workers and economizes the production of beeswax.

4.2. Structure of three types of brood cells

The base of the semi-rebuilt brood cell is not composed of three rhombuses like in the newly built brood cell but is similar to the old brood cell. Both old and semi-built brood cell bases are hemispheres but their curvature is different. Any cross-section of the main shape of the brood cell enclosed by the walls is hexagonal. The side length of the cell is proportional to the diameter (Svečnjak et al. 2019). We found no significant difference in the diameters at the top, middle, and bottom positions of the newly built or semi-rebuilt brood cell. Also, there was no significant difference in the length of each side of the hexagon at the top, middle, and bottom positions, indicating that the main shape of the newly built or semi-rebuilt brood cell remains a hexagonal prism. However, the diameter of the old brood cell gradually decreases from the top to the bottom and so does the hexagonal side length of the old brood cell. Hence, the main shape of the old brood cell is a frustum of a pyramid. Through the combination of geometric shapes, the walls of the newly built, semi-rebuilt, and old brood cells are composed of six right trapezoids, six rectangles, and six trapezoids, respectively.

4.3. Changes in brood comb thickness and cell structure

During the capping period, the mature larvae roll up and down spinning along the inner cell wall (Jay 1964). After each generation, workers will leave a layer of the cocoon in the cells. The layers and weight of the cocoon increase with the rearing generations (Zhang et al. 2010; Al-Kahtani and Taha 2021). Since the newly built or semi-rebuilt brood cells have not been used to rear workers, their walls have no cocoon attached. Therefore, for the diameter at the same position, the old brood cell is significantly smaller than the newly built or semi-rebuilt brood cell. Some studies showed that the accumulation of excreta mainly occurs at the cell base (Jay 1963). Therefore, occupying more bottom space, the weight of the cocoon at the cell base is higher than on the cell wall. Consequently, the diameter of the old brood cell gradually decreases from the top to the bottom altering the cell shape from the original hexagonal prism to frustum of a pyramid. With the age, the volume of the old brood cell also decreases. However, we found no significant difference in the depth of the three types of brood cells. This indicates that the reduction of old brood cell volume is mainly due to the reduction of cell diameters. Moreover, the cocoon at the old brood cell base is thicker; therefore, to ensure the required depth for the normal development of broods, workers secrete wax to raise the height of the old brood cell wall, which makes the old brood comb thicker. Although workers clean the base before building the semi-rebuilt brood cell, there is always a small amount left of the cocoon and other substances decreasing the volume of semi-rebuilt brood cells, albeit, only a little less than the new ones. Workers also raise the walls of semi-rebuilt brood cells to ensure the required depth. However, this action increases the thickness of the semi-rebuilt brood comb slightly higher than that of the newly built brood comb.

4.4. Changes in the morphological characteristics of workers

The individual foraging ability of workers, a key factor affecting colony productivity, is affected by their birth weight, body size, and morphological organ size (Shawer et al. 2020). The morphological size of workers positively correlated with the size of brood cells. It is suggested that workers get less nutriment in old brood cells with reduced volume, and are forced to prematurely enter the prepupal period (Abdellatif 1965). In this study, we found no significant difference in the depth of the different brood cells; however, the body length of workers emerging from old brood cells was significantly smaller than those emerging from newly built and semi-rebuilt brood cells. This is because the bottom size of old cells is significantly reduced, which causes space stress shortening the body length of workers.

5. CONCLUSIONS

Apis cerana cerana gnaws off the old brood cell in two ways either by completely removing the brood cell or only the old brood cell wall leaving the base. Workers build new walls on the old cell bases containing a small amount of cocoon to form semi-rebuilt brood cells. The diameter and volume of the semi-rebuilt brood cell and the morphological size of the cultivated workers are almost the same as of the newly built brood cell but significantly larger than the old brood cell. Brood cell size significantly affects the birth weight, body length, and external morphological indices of newly emerged workers. By gnawing off the old brood cells and making the semi-rebuilt brood cell, *Apis cerana cerana* maintains the cell volume and morphological size of workers, which may positively affect the development of the colony.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

Conceptualization, Wenzheng Zhao and Kun Dong; methodology, Qingxin Meng and Shunhua Yang; performed the experiments, Qingxin Meng, Shunhua Yang, Rong Huang, Linfu Yang, and Lijie Xun; analyzed the data and prepared the figures, Yakai Tian, Xueyang Gong, Jianming Wang, and Haiou Kuang; writing–original draft preparation, Qingxin Meng; writing–review and editing, Qingxin Meng, Shunhua Yang, Wenzheng Zhao, and Kun Dong. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY

The preprocessed data that support the findings of this study are available from the corresponding author upon reasonable request.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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