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Seasonal Variations in the Organization and Structure of Apis cerana cerana Swarm Queen Cells

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Abstract

This paper describes the organization and structure of the swarm queen cells of Apis cerana cerana in spring, summer, and autumn in Kunming, Yunnan Province, China. We measured the following indices to reveal the organization rule of swarm cells: number of swarm cells built by each colony during different seasons; the shortest distance between two adjacent swarm cells on the comb; distance between swarm cell base and bottom bar of movable frame. We revealed the swarm cells structural characteristics using the following indicators: maximum diameter of swarm cell, the length between mouth and bottom of swarm cell, depth between maximum diameter and bottom of swarm cell, and the ratio of maximum diameter to depth between maximum diameter and bottom of swarm cell. Regarding seasonal differences, results indicated a significant variation in the distance between the swarm cell base and the bottom bar of the movable frame. Still, no such effect was observed in the shortest distance between two adjacent swarm cells. The maximum swarm cell diameter was not considerably influenced either, while the distance between the maximum diameter and the bottom of the swarm cell had substantial variation. The detected ratio of the maximum diameter to the depth between the maximum diameter and the bottom of the swarm cell indicated seasonal changes in the bottom shape of the swarm cell. This study clarifies the temporal and spatial distribution and structure of swarm cells of A. c. cerana. It establishes the basis for predicting the time and position of appearing swarm cells, thus allowing for a more precise determination of the shape and size of queen-cell punch and the ideal position of a cell cup on the bar of queen cup frames in artificial queen rearing.

Introduction

Swarming is a strategy of colony reproduction in honey bees characterized by the parent colony separating into one or more offspring colonies, with at least one queen bee and a proportion of worker bees in each new colony. The swarming process includes two stages: 1) drone cell building to rear drone broods (Free, 1967; Avitabile & Kasinskas, 1977), and queen cell building to rear queen bees (Walrecht, 1961); 2) the queen bee of the parent colony leaves the original colony with half of the colony worker bees and drones, and chooses a new location as a new home (Winston, 1987). In the first stage, when the drone broods emerge as adult drones from the drone cells, workers begin to build queen cell cups that are different from the hexagonal cells used to rearing workers or drone broods. These queen cell cups are always hung freely along the bottom of the comb, are vertically oriented, and open downward (Simpson, 1959; Lensky & Seifert, 1975).



The queen lays eggs in the queen cell cups, which hatch into queen larvae within three days, while the worker bees secrete royal jelly to feed them. This reproductive behavior ensures that queen larvae continue to develop and grow appropriately in their cells. As queen larvae grow in body size, the worker bees increase the diameter and length of the queen cells (Simpson, 1959). After five days, the worker bees seal the cell mouth with beeswax to form a sealed queen cell when the queen's larval stage is complete. Then, the queen larva will proceed to the next stage of its development, the pupal stage, thus making the total development time eight days (Jay, 1962). At the end of this period, the emerging queen bites off the cap of the queen cell and starts its adult stage (Jay, 1963).

Some studies have shown that honey bee swarming generally exhibits clear seasonality. Among the western honey bee species, the natural swarming of Apis mellifera ligustica begins in spring and ends in autumn. This period was reported to start at the end of May and end at the beginning of August in southern England (Simpson, 1959); according to Gary and Morse (1962), the time of swarming of A. m. ligustica in the northern United States usually occurred in May and June. In contrast, it fell from May to September in the central New York (Ithaca) area (Burgett & Morse, 1974, Fell et al., 1977). The number of queen cell cups of A. m. ligustica varies considerably among different colonies each year, and the factors governing the number of queen cups per colony have not been well-defined. This number for A. m. ligustica colonies in Aberdeen, Scotland, ranged from four to forty-five (mean twenty-four) in 1961, from seven to sixty-two (mean twenty-two) in 1962, from one to eleven (mean four) in 1963, and from one to forty-eight (mean fourteen) in 1964 (Allen, 1965). In Maryland, USA, the number of queen cell cups had obvious seasonal variation; queen cups were present from April throughout the summer season, but their numbers were greatest from late May to early July (Caron, 1979). The shape of the A. m. ligustica swarm cell varies considerably, is about 20-30 mm in length (Robinson, 1982), and its diameter from the base to the mouth gradually decreases. Its wall is thick with its surface carved into wrinkles, resembling a peanut pod, and the opening is always downward (Hamdan, 2010). The natural swarming of eastern honey bee species, such as Apis cerana cerana or Asiatic honey bee (also known as Chinese honey bee, from now on is referred to as Chinese bee), also begins in spring and ends in autumn. This period for A. c. cerana in the Fujian Province of China was shown to occur in spring from March to May. With 3-7 swarm cells produced in each colony, the swarm cells were stout and straight in shape (Liu, 1964). In addition to the period from March to May, the natural swarming of the same subspecies also occurred in summer from June to July and in September in Guizhou Province, while the swarm cell numbers were not determined (Xu, 1987). In Guangdong Province, A. c. cerana natural swarming was reported to occur in spring with 3-9 swarm cells per colony and six cells on average from March to April, with 3-7 (average of 5) swarm cells in each colony in summer from May to June, and no known swarming events in autumn (Liu & Lai, 1990).

The phenomenon of natural swarming, which is one of the inherent biological characteristics of honey bee colonies, may cause economic losses to beekeepers if the swarm is not found and captured in time. As natural swarming has obvious characteristics, such as seasonality or preparation process from breeding drones to rearing queens, beekeepers can use these characteristics to predict swarming in the bee colony and take appropriate beekeeping management measures to prevent the associated economic losses. Kunming is an important apiculture development area in Yunnan Province, one of the main beekeeping areas within China with a high density of Chinese honey bee colonies. Natural swarming that often occurs in these colonies is difficult to predict, which brings many difficulties to the management of the bee colony. Therefore, this study aimed to determine the natural swarming pattern of the Eastern honey bee, A. c. cerana, in three different seasons (spring from March to May, summer from June to August, and autumn from September to November) in Kunming, Yunnan Province, China, and to reveal the temporal and spatial patterns of organization of swarm cells and their structure. Accordingly, we propose the following hypotheses: Hypothesis 1: In Kunming, Yunnan Province, the natural swarming phenomena of Chinese honey bee colonies occur in spring, summer, and autumn; Hypothesis 2: The position of swarm queen cells show a seasonal difference; Hypothesis 3: There is a seasonal difference in the structure of swarm queen cells. The following variables were measured in the spring, summer, and autumn of 2020 to test these hypotheses: (1) the number of swarm cells built within A. c. cerana colonies; (2) the position of swarm cells on the comb; (3) the structure and size of swarm cells. The results will provide a basis for predicting the time and position of swarm cell production and help determine the shape and size of queen-cell punch and the position of cell cup on the bar of the queen cup frames in artificial queen rearing.

Materials and Methods

Statistics of swarm queen cup numbers in colonies

The experiment was carried out from February to November 2020, investigating 40 colonies in the spring, 47 in the summer, and 51 in the autumn. According to the beekeeping requirements of each season, certain highquality virgin queens were selected and retained to replace old queens, or single colonies were artificially divided into several colonies. Some of these newly established colonies were selected as investigation colonies for the next season. The number of swarming queen cups was determined every seven days in all three seasons to obtain N_{sp} for spring, N_{su} for summer, and N_{au} for autumn. The shortest distance (SD) measured between two adjacent swarm cells on each comb was SD_{sp} for spring, SD_{su} for summer, and SD_{au} for autumn. In addition, the distance between the swarm cell base and the bottom bar (**DBB**) of the movable frame was measured to give DBB_{sp} for spring, DBB_{su} for summer, and DBB_{au} for autumn. The above indices were used to reveal the organization rule of swarm cells.

Collection of swarm cell shells and preparation of cell molds

Queen cells were fitted with protectors to prevent them from being destroyed by workers. The cell shells were collected immediately once the queens have emerged from their cells (Fig 1a). Crystal glue solution was prepared by mixing epoxy resin, and its curing agent in a volume ratio of 1:1 extracted using a disposable syringe and then injected into the queen cell shells. The amount of crystal glue solution injected into a shell was adjusted to keep the fluid level with the shell mouth. The solution was left to solidify in the shell at room temperature for 12 hours to form a fixed-shape molding reflecting the shape and size of each shell. During the curing process, no effect of the crystal glue solution on the shape or volume of shells was observed, including shell expansion or shrinkage due to heat; shells retained their original shape and structure.

Measurement index of queen cell mold

The queen cells containing the mold were soaked in hot water at 90 °C to melt the beeswax on the queen cell surface to obtain clean queen cell molds. The acquired queen cell molds had above 80 HD hardness, which did not deform in 90 °C hot water. Therefore, the shape and size of obtained queen cell molds resembled the actual structure and size of collected

queen cells (Fig 1b). Finally, the maximum diameter of queen cell molds was measured using Vernier calipers (accuracy \pm 0.01 mm) to obtain \mathbf{D}_{sp} for spring, \mathbf{D}_{su} for summer, and \mathbf{D}_{au} for autumn. The length of queen cell molds, i.e., the distance from the cell mouth to the bottom of the cell, was also measured to obtain \mathbf{L}_{sp} for spring, \mathbf{L}_{su} for summer, and \mathbf{L}_{au} for autumn. The parameters of \mathbf{DP}_{sp} for spring, \mathbf{DF}_{su} for summer, and \mathbf{DP}_{au} for autumn were measured as the depth between the maximum diameter of the swarm cell mold and the bottom of the swarm cell mold. The above indicators were used to reveal and evaluate the structural characteristics of swarm cells.

Statistical analysis

One-way analysis of variance (ANOVA) was conducted to determine the influence of the season on 1) the shortest distance (SD) between two adjacent swarm cells on the comb in different seasons; 2) the distance (DBB) between the base of swarm cell and the bottom bar of the movable frame; 3) the maximum diameter (**D**) of the swarm cells constructed by colonies; 4) the length (L) of the mouth to the bottom of the swarm cell built by bee colonies; 5) the depth between the maximum diameter and the bottom of the swarm cell (DP) of the colony. The ratio (W) of the maximum diameter (D) to the depth between the maximum diameter and the bottom of the swarm cell (DP) was calculated as W_{sp} for spring, W_{su} for summer, and \mathbf{W}_{u} for autumn, and the shape of the bottom of the swarm cell was determined using these ratios. All data obtained in the experiments were statistically analyzed using the Statistical Analysis System (SAS v8.0) software provided by SAS Institute Inc., North Carolina, USA.

 $\begin{array}{c} (a) \\ (b) \\ (b) \\ (c) \\ (c)$

Fig 1. (a) Swarm cells of the Eastern honey bee, *Apis cerana cerana*. (b) The molds of swarm cells for the Eastern honey bee, *A. c. cerana*. Top, queen cells in spring; middle, queen cells in summer; bottom, queen cells in autumn.

Results

Statistics of queen cup numbers in different seasons

Results showed that 95% of colonies produced queen cups in the spring, 44.7% in the summer, and 9.8% in the autumn. During the investigation period, the number of queen cups per colony ranged from 3 to 23, averaging 8.59. In the spring, the summer, and the autumn, the average number of queen cups per colony averaged 8.05 (4-15), 8.52 (3-17), and 13.00 (4-23), respectively.

Seasonal variation of shortest distance (SD) between two adjacent swarm cells on the same comb

The mean shortest distance **(SD)** of two adjacent swarm cells on the comb was 41.73 mm \pm 3.05 mm (n = 191). The results of one-way ANOVA showed that seasons had no significant influence on the shortest distance between two adjacent swarm cells on the comb of the Eastern honey bee, *A. c. cerana* (F = 1.53; df = 2, 188; *p* = 0.22 > 0.05). The mean shortest distance between two adjacent swarm cells on the comb in the spring, the summer, and the autumn was 49.19 mm \pm 4.40 mm (n = 64), 38.70 mm \pm 5.96 mm (n = 56) and 37.40 mm \pm 5.40 mm (n = 71), respectively. The multiple comparisons showed no significant differences between **SD**_{sp}, **SD**_{sp}, and **SD**_{au} (*p* > 0.05) (Fig 2).

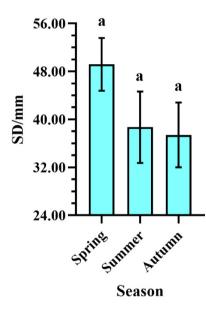


Fig 2. Seasonal variation in the shortest distance (SD) between two adjacent swarm cells on the comb.

Notes: All values in the figures are represented as mean \pm standard error. The same lowercase letter among different columns indicates that the difference is not significant (p > 0.05), while different lowercase letters indicate statistical significance (p < 0.05), the same rule applies to the graphs below.

Comparison of distance (DBB) between swarm cell base and bottom bar of the movable frame

The mean distance (**DBB**) between the swarm cell base and the movable frame's bottom bar was 41.81 mm \pm 1.93 mm (n = 240). The results of Welch's ANOVA test showed that the season had a significant effect on the distance (**DBB**) between the base of the swarm cell and the bottom bar of the movable frame on the comb (W = 21.61; df = 2, 151.4; p < 0.05). The mean distance between the base of the swarm cell and the bottom bar of the movable frame on the comb was 56.82 mm ± 3.88 mm (n = 88), 28.86 mm ± 1.82 mm (n = 70), and 36.76 mm ± 2.74 (n = 82) mm in the spring, the summer and the autumn, respectively. The results of multiple comparisons showed that **DBB**_{su} was significantly higher than **DBB**_{su} and **DBB**_{au} (p < 0.05), whereas the difference between **DBB**_{su} and **DBB**_{au} was insignificant (p > 0.05) (Fig 3).

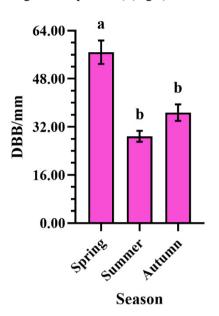


Fig 3. Seasonal variation in distance (DBB) between base of swarm cell and bottom bar of movable frame.

Comparison of maximum diameter of swarm cells among seasons

The average maximum swarm cell diameter was 9.54 mm \pm 0.04 mm (n = 144), ranging from 8.20 mm to 11.19 mm. The results of one-way ANOVA showed no significant seasonal effect on the maximum diameter of natural swarm cells (F = 1.22; df = 2, 141; p = 0.30 > 0.05). The average maximum diameter of swarm cells in the spring, the summer, and the autumn was 9.60 mm \pm 0.06 mm (n = 55), 9.56 mm \pm 0.08 mm (n = 46), and 9.45 mm \pm 0.06 mm (n = 43), respectively. The results of multiple comparisons showed that the differences among **D**_{sp}, **D**_{su}, and **D**_{au} were not significant (p > 0.05) (Fig 4).

Comparison of length between mouth and bottom of swarm cell

The average length of swarm cells was 17.38 mm \pm 0.14 mm (n = 144), ranging from 13.58 mm to 23.91 mm. The Welch's ANOVA test results showed a significant seasonal effect on the length of *A. c. cerana* natural swarm cells (W = 10.19; df = 2, 93.95; *p* < 0.05). The average length of swarm cells in the spring, the summer, and the autumn was 17.77 mm \pm 0.25 mm (n = 55), 17.69 mm \pm 0.21 mm (n = 46), and 16.55 mm \pm 0.20 mm (n = 43), respectively. The results of multiple

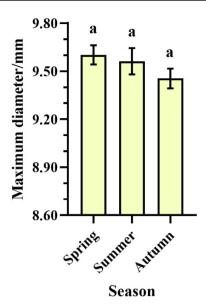


Fig 4. Comparison of maximum diameter of swarm cells among seasons.

comparisons showed significantly smaller L_{au} than L_{sp} and L_{su} values (p < 0.05), while the difference between L_{sp} and L_{su} (p > 0.05) was insignificant (Fig 5).

Comparison of depth between maximum diameter and bottom of swarm cell (DP)

The average depth between the maximum diameter and the bottom of swarm cells (**DP**) was 4.74 mm \pm 0.05 mm (n = 144). The results of Welch's ANOVA test showed that the season significantly affected the depth between the maximum diameter and the bottom of *A. c. cerana* swarm cells (W = 33.35; df = 2, 90.81; *p* < 0.05). In the spring, the summer, and the autumn, the average depth between the maximum diameter and the bottom of swarm cells was 5.12 mm \pm 0.08 mm (n = 55), 4.68 mm \pm 0.10 mm (n = 46), and 4.32 mm \pm 0.06 mm (n = 43), respectively. Multiple comparisons showed

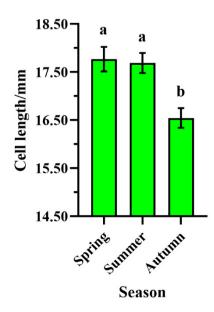


Fig 5. Comparison of length between mouth and bottom of swarm cell.

that the differences among \mathbf{DP}_{sp} , \mathbf{DP}_{su} , and \mathbf{DP}_{au} all reached a significant level (p < 0.05) (Fig 6).

Calculation of ratio (W) of maximum diameter (D) to depth between maximum diameter and bottom of swarm cell (DP)

The mean ratio (**W**) of maximum diameter (**D**) to depth between the maximum diameter and the bottom of swarm cells (**DP**) was 2.05 ± 0.02 (n = 144), ranging from 1.47 to 3.55. The average ratio was 1.89 ± 0.02 (n = 55), 2.08 ± 0.04 (n = 46) and 2.21 ± 0.04 (n = 43), with ranges of 1.47-2.29, 1.57-2.80 and 1.70-3.55 in the spring, the summer and the autumn, respectively.

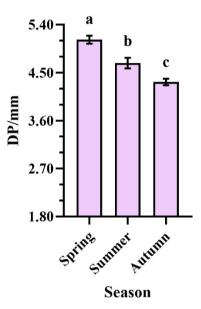


Fig 6. Comparison of depth (DP) between maximum diameter and bottom of swarm cell.

Discussion

Seasonality of natural swarming of Chinese bee

It was established in the present study that, in Kunming, Yunnan, China, the natural swarming of the Eastern honey bee, *A. c. cerana* began in early March and ended in early November, which corresponds to obvious seasonality with spring, summer, and autumn swarming periods.

Concerning the temporal swarming pattern of the Chinese bee, natural swarming was found to occur mainly in spring, followed by summer, and in a few colonies in autumn. The number of queen cells in colonies in spring and summer was the same, but significantly less than in autumn. Therefore, our first hypothesis was confirmed.

In both the spring and the summer, the average number of queen cups per colony was eight, while that in the autumn was thirteen. The number of swarm cells in the autumn was higher than in the spring, or the summer, which may have occurred as the temperature in the autumn was falling, and the daily temperature variation was large. Especially after August, the weather gradually turned cooler. Therefore, the number of swarm cells in the autumn was more conducive to selecting a high-quality virgin queen and thus improved the mating success rate (Zhou, 1995; Yang et al., 2020). Liu and Lai (1990) found out that the average number of queen cups constructed by *A. c. cerana* in spring and summer in Guangzhou, Guangdong Province was six and five, respectively. Our survey results for Kunming, Yunnan Province (with eight queen cups in both spring and summer) were lower, most likely due to the climate differences between regions. Some studies have reported significant differences in the number of queen cells produced between Chinese bee colonies, with the same colony strength in Beijing and Fuzhou, which cities have significantly different climates (Yang, 1974; Yang et al., 1981; Liu, 1993).

The spatial position of natural swarm cells

The shortest distance between two adjacent swarm cells on the comb is defined as the minimum distance to keep between two adjacent cell cups of beeswax on the bar of queen cup frames in artificial queen rearing (Liu, 1962, 1966; Song, 1987; Li, 1993). Our results showed that this distance was not affected by the season, suggesting that beekeepers should keep a minimum of 42 mm distance regardless of the season. Honey bee queen cells are purposefully built outside the normal comb area to accommodate the larger queen (Simpson, 1959, Lensky & Seifert, 1975, Hepburn et al., 2014). These cells hang freely along the bottom of the comb, are vertically oriented, and open downward (Seeley & Morse, 1976). The position of the queen cups on the comb is measured by the linear distance between the base of the queen cup and the bottom bar of the movable frame. In our study, the distance between the queen cup and the bottom bar of the frame was longer in the spring and the autumn. In the summer, when the temperature is higher, the queen cell is placed closer to the bottom bar of the frame, where heat easily dissipates, favoring the development of the queen. Previous studies have indicated that placing artificial cell cups near the beehive entrance can improve the survival rate during artificial queen rearing in summer (Gong & Ning, 1992; Bi & Li, 2003; Yang et al., 2020).

In summary, the rules of swarm cells' temporal and spatial organization revealed no seasonal difference in the shortest distance between two adjacent swarm cells and a seasonal difference in the linear distance between the swarm cell base and the bottom bar of the movable frame. The linear distance from the bottom bar of the movable frame was short in the summer and long in the spring and the autumn. To predict the time and position of the swarm cells occurring in the colony, more than 90% of the bee colonies will produce swarm cells in spring, over 40% of the bee colonies will produce swarm cells in summer, and less than 10% of the bee colonies will produce swarm cells in autumn. In general, swarm cells are located on the lower edge of the comb, and they are closer to the bottom bar of the movable frame in summer when compared with spring and autumn. Accordingly, our second hypothesis was supported.

Therefore, to prevent natural swarming in the colony, we recommend beekeepers take appropriate management steps every five to seven days in spring in all bee colonies. As for the summer and autumn, beekeepers could perform these steps in bee colonies with more than five frames to promote colony strength.

Structure of natural swarm cell

In this paper, the structure of swarm cells was quantified for the Eastern honey bee (A. c. cerana). Indices of maximum diameter, length, depth from maximum diameter to bottom of the cell, and the ratio of maximum diameter to depth between maximum diameter and bottom of the cell, were measured to determine the actual structure of swarm cells. The data provide parameters for defining the structure of artificial queen cups. No seasonal variation was shown in the maximum diameter of swarm cells. Still, seasons significantly impacted the cell length and depth between the maximum diameter and the bottom of the swarm cell. Therefore, our third hypothesis was partially confirmed. Since the swarm cell length values in the spring and the summer were significantly longer than in the autumn, the body length of queens reared by beekeepers in spring and summer may be larger than that of queens raised in autumn. Here we pose the question of whether the depth of artificial queen cups should be determined according to the season and whether it is necessary to use different sizes of queen-cell punches in beekeeping at different times of the year. Answering these questions requires further experimental verification.

The bottom shape of a natural swarm cell

The mean ratio (W) of the maximum diameter (D) to the depth between the maximum diameter and the bottom of A. c. cerana swarm cells (**DP**) was 2.05 ± 0.02 . The mean ratios in the spring, the summer, and the autumn were 1.89 ± 0.02 (1.47-2.29), 2.08 ±0.04 (1.57-2.80), and 2.21 ±0.04 (1.70-1.57)3.55), respectively. These results show that the bottom shape of the swarm cell was a vertical semi-ellipsoid in the spring, a hemisphere in the summer, and a horizontal semi-ellipsoid in the autumn. Herein, the average maximum diameter of the swarm cell was 9.54 mm \pm 0.04 mm, which was greater than that reported by Fang et al. (1995) (9.10 mm) or Ren et al. (2012) (9.02 mm). The average depth between the maximum diameter and the bottom of the swarm cell was $4.74 \text{ mm} \pm 0.05$ mm, which was smaller than the result of Fang et al. (1995) (6.08 mm) or Ren et al. (2012) (6.41 mm). Whether this is explained by regional differences or by different ecotypes of A. c. cerana remains a subject of further studies. However, it is worth noting that the study area of Fang et al. (1995) was Fuzhou, Fujian Province, and the swarm cells built by A. c. cerana of Fujian were of the southern Chinese bee population. Ren et al. (2012) study area was Chongqing Municipality, and swarm cells built by A. c. cerana of Chongqing were of the central Chinese bee population. Meanwhile, the experimental

area of this study was Kunming, Yunnan Province, and the swarm cells built by *A. c. cerana* of Yunnan were of the bee population of Yun-Gui Plateau.

Based on the findings of the current study, we suggest that Chinese beekeepers in Kunming make different shapes of queen-cell punches according to seasonal differences. In artificial queen rearing in spring, the shape of the queen-cell punch should be a vertical semi-ellipsoid, while in summer and autumn, it should be a hemispherical and a horizontal semi-ellipsoid, respectively. The diameter of the queen-cell punch should be no less than 8.0 mm and no more than 9.5 mm. The depth of artificial queen cups in spring, summer, and autumn is suggested to be 5.1 mm, 4.7 mm, and 4.3 mm, respectively. The queen cup frame should be equipped with three bars, and each bar should de equipped with 8-9 cups, The distance between two adjacent cups on the bar should be 47-53mm.

Conclusions

This paper established that the natural swarming of the Eastern honey bee A. c. cerana in Kunming, Yunnan Province, begins in early March and ends in early November, corresponding to spring, summer and autumn, and showed obvious seasonality. The shortest distance between two adjacent swarm cells on the comb showed no seasonal variation, while the distance between the base of the swarm cell and the bottom bar of the movable frame differed among seasons. Although the time of year was not a factor influencing the maximum diameter of swarm cells, it did affect swarm cell length, the depth between the maximum diameter to the bottom of the swarm cell, and the shape of the bottom of the swarm cell. Therefore, it is concluded that both the temporal and spatial organization of swarm queen cells of the Chinese bee exhibit seasonal variations. Based on the findings, recommendations were made to enhance current beekeeping practices to lessen the losses caused by natural swarming.

Authors' Contribution

K.D.: Conceptualization, visualization, writing-review and editing S.Y.: Conceptualization, methodology, investigation, formal analysis, visualization, writing-original draft preparation, writing-review and editing

K.D.: Methodology, investigation,

D.Z.: investigation,

X.G.: investigation,

Y.L.: investigation,

W.Z.: investigation, writing-review and editing

All authors have read and agreed to the published version of the manuscript.

Disclosure statement

We declare no conflict of interest and no competing or financial interests.

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