

Age-related comb dimensions of the red dwarf honeybee, *Apis florea* F. (Hymenoptera, Apidae) in the plains of Karnataka, India

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ABSTRACT: Studies were conducted to determine the variations in the dimensions of comb parameters of dwarf honeybee, *Apis florea* F. in relation to comb age. The comb parameters viz comb perimeter, mean comb area, brood, pollen, honey, crest size, and comb thickness were varied with comb age and were found to be relatively high in middle-aged combs compared to old and young combs. Furthermore, the observations showed that the width and depth of the comb wax cells also differed significantly with comb age and maximum size of cell width and depth were found in middle-aged combs.

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KEY WORDS: Brood, middle-aged combs, comb parameters, wax comb

INTRODUCTION

The nests are crucial for the expansion and well-being of insect colonies (Kronauer and Libbrecht, 2018; Gruter, 2020). Combs of the nests are used for food storage and brood rearing for several generations (Taha *et al.*, 2021). Furthermore, nests offer a centralized framework for social interaction, enabling individuals to plan their actions in a way that benefits the colony as a whole (Ireland and Garnier, 2018). *Apis florea* F. (Hymenoptera, Apidae) is renowned for its unique nest architecture and tiny colony size. The feral colonies of *A. florea* build single, exposed combs vertically attached to one or many thin branches of bushes, trees (Hepburn and Hepburn, 2011; Nagaraja, 2020) and piles of dried sticks (Ramyarani and Nagaraja,

2024). The construction of the crown is one of the species-specific characteristics found in *A. florea* (Oldroyd and Pratt, 2015). The crown of the nest was found above the twig acts as a honey storage area and serves as a platform for communication dancing by foragers (Oldroyd and Wongsiri, 2006). Honey bee comb changes colour over time, from whitish to brown and later into darker (Karihaloo *et al.*, 2013). Combs become darker and stiffen with successive brood rearing (Zhang *et al.*, 2010; Smith *et al.*, 2021). The long-term use of combs in beekeeping results in an accumulation of cocoons, pollen, and propolis in the cells. As the comb ages, the cell walls become thicker and the internal cell diameter decreases (Karihaloo *et al.*, 2013).

The comb of *A. florea* is a visual treat to know

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how workers build cells with different diameters, small cell sizes for rearing workers, and large cell sizes for drones (Boes, 2010). The comb architecture of cavity-nesting honeybee species viz *A. mellifera* (Crane, 1999; Ruttner, 1988) and *A. cerana* (Deodikar *et al.*, 1977) has been well reported. The comb age affects brood survivorship and colony growth of the nests (Taha, 2021). Rinderer *et al.* (1996) reported the changes in the width of worker and drone cells of *A. mellifera*. Naturally constructed bee combs initially build circular-shaped cells but quickly change to a rounded hexagonal shape (Nazzi, 2016). Information on changes in comb construction is required to know the collective activities of bees engaging in small-scale repetitive tasks, executed by many individuals, which collectively can lead to a multi-purpose structure for the benefit of the colony. The present study aimed to determine the dimensions of comb and wax cell parameters with comb age.

MATERIALS AND METHODS

Studies on comb dimensions of *A. florea* were carried out in and around Bengaluru Rural district (13°27'90" N; 77°23'78" E) India. The study region was rich with agricultural and horticultural crops including different forest bee flora (Ramyarani and Nagaraja, 2024). The colonies of *A. florea* found in a 10 km² radius area on the branches of small trees, thorny bushes, shrubs, hedges, and dried pile sticks at heights between 1 to 3 meters were selected for the study. The bees were removed carefully with a soft brush on both sides of the comb. Each comb of the *A. florea* was photographed using a DSLR camera (Canon EOS 600D with a Nikon 18-55 lens with a settings aperture of 5.6 and a shutter speed: 1/125sec of ISO-100) to measure the comb and cell dimensions. The combs of *A. florea* combs were marked on the day of initiation of comb building after a few days of their arrival at new sites. The combs (N=45) were collected safely without any damage at different age intervals and were categorized into young combs (up to 2 months old) (Free and Williams, 1974), middle-aged combs (2 to 4 months old), and old-aged combs (4 to 6-months old - Fig. 1). The comb metrics viz comb perimeter, and comb area

of each comb were determined by transferring the comb outline onto a transparent plastic sheet. Later, this outline was scanned to produce a digital image. This format was used as an input for the public domain software program "ImageJ". The area occupied by the brood, pollen, and honey on both sides of the comb was measured. The crest size above and below the substratum and the worker and drone comb thickness were also recorded with the help of digital calipers followed by ImageJ software (Rasband, 1997).

To evaluate the differences in cell width and cell depth of worker, drone, pollen, and honey cells concerning comb age, five close-up pictures of the different positions of each comb were taken. The width of cells was measured by wall-to-wall dimensions using ImageJ software (Buawangpong *et al.*, 2014). Similarly, the cell depth of each cell was recorded by a digital caliper. These individual cell-type measurements were used to calculate the means and standard error. The obtained data from the study was analyzed using SPSS software version 22.0. One-way Analysis of variance (ANOVA) was performed to assess differences between comb age and dimensions of different comb parameters. Furthermore, a pairwise comparison with Tukey's HSD test was employed to test the significant differences between cell width and cell depth.

RESULTS AND DISCUSSION

The combs of *A. florea* are incredibly small with hexagon-shaped cells. The young combs were generally creamy white. However, the middle-aged combs were yellowish and the old combs were darkish yellow (Fig. 1). The comb dimensions of 15 each of young, medium, and old combs of *A. florea* colonies showed variation among the ages (Table 1). The observations showed that the comb perimeter of *A. florea* was varied and the maximum comb perimeter size of 41.42 ± 4.84 cm was recorded in middle-aged combs and the minimum size of the comb perimeter of 26.5 ± 4.29 cm was recorded in young combs. The area of comb, brood, pollen and honey was recorded in young, middle-aged and old combs of *A. florea*. The observations revealed that the comb parameters like brood area (297.6 ± 41.53) and comb perimeter (41.42 ± 4.84) in middle-aged

Table 1. Comb dimensions, area of brood and storage of cells in young, middle and old combs of *Apis florea*

Comb Parameters	Comb age		
	Young	Middle	Old
Comb perimeter (cm)	26.5 ± 4.29	41.42 ± 4.84	38.07 ± 4.38
Mean comb area (cm ²)	275.42 ± 21.74	352.01 ± 39.19	345.6 ± 49.41
Brood area (cm ²)	238.85 ± 20.25	297.6 ± 41.53	293.33 ± 33.51
Pollen area (cm ²)	7.78 ± 3.78	19.16 ± 4.34	35.6 ± 3.64
Honey area (cm ²)	36.57 ± 3.95	48.1 ± 18.24	51.33 ± 9.97
Crest size above and below (cm)	3.32 ± 0.65	6.75 ± 2.65	10.36 ± 3.65
	5.17 ± 0.55	9.17 ± 2.83	14.17 ± 3.55
Worker Comb thickness (mm)	15.3 ± 1.95	17.30 ± 2.0	19.51 ± 2.08
Drone Comb thickness (mm)	Nil	Nil	25.22 ± 2.07

N=45; Values are mean ± standard deviation

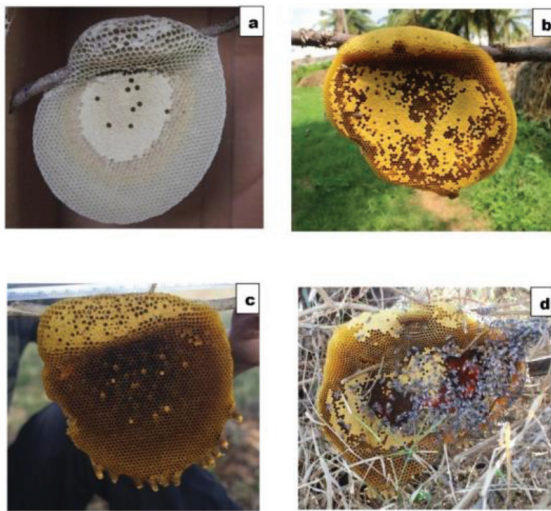


Fig. 1 The young (a), middle-aged (b) and old (c, d) combs of *Apis florea*

combs were greater followed by old combs. The mean comb area (cm²) of young and old combs, was found to be 275.42 ± 21.74 and 345.6 ± 49.41, the brood area at 238.85 ± 20.25 and 293.33 ± 33.51, the pollen at 7.78 ± 3.78 and 35.60 ± 3.64, and the honey at 36.57 ± 3.95 and 51.33 ± 9.97, respectively. The crest size of the bee comb was varied and was relatively larger in old combs compared to young and middle-aged combs. The crest size of *A. florea* combs found above and below the substratum in young, middle-aged and old combs were 3.32 ± 0.65cm (above substratum) and

5.17 ± 0.55 (below substratum), 6.75 ± 2.65cm (above substratum) and 9.17 ± 0.2.83cm (below substratum), and 10.36 ± 3.65cm (above substratum) and 14.17 ± 3.55cm (below substratum) respectively. Furthermore, the thickness of worker and drone combs was larger in old combs and was found to be 19.51 ± 2.08 and 25.22 ± 2.07mm respectively (Table 1).

The cell width of worker brood, pollen and honey cells was recorded in young, middle-aged and old combs of *A. florea*. The observations showed that the cell width of worker brood, pollen and honey cells decreased significantly ($p < 0.05$) with the increase in age of the combs. The maximum cell width of worker brood, pollen and honey cells recorded were 1.91 ± 0.25mm, 1.89 ± 0.30mm and 1.94 ± 0.28mm in young combs. Likewise, in old combs, the minimum cell width size of worker brood, pollen and honey cells recorded were 1.34 ± 0.19, 1.24 ± 0.13 and 1.54 ± 0.20mm respectively (Fig. 2).

The cell depth of worker brood, pollen and honey cells was recorded in young, middle-aged and old combs of *A. florea*. The observations showed that, the cell depth of worker brood, pollen and honey cells were significantly ($p < 0.05$) increased with the increase in age of the combs. The maximum cell depth of worker brood, pollen and honey cells recorded were 8.73 ± 0.14mm, 18.46 ± 0.23mm and



Fig. 2 Cell width of worker, pollen, and honey cells in young, middle and old-aged combs of *A. florea*. Bars having the same letter do not differ significantly between different aged combs at $P < 0.05$ (ANOVA followed by Tukey's test); $N = 20$ cells

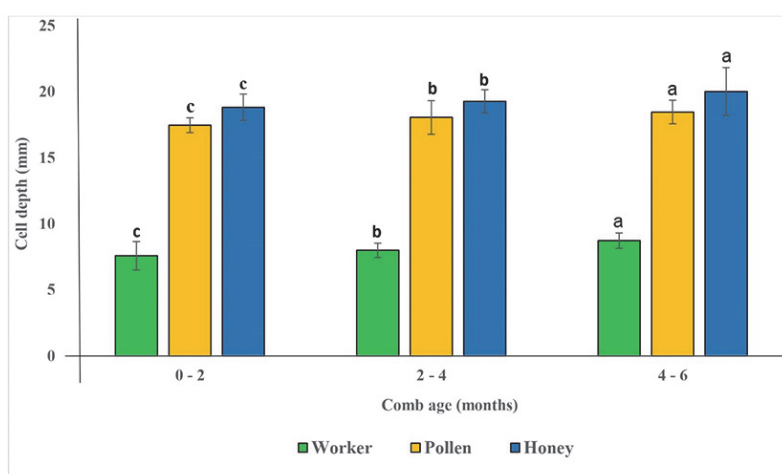


Fig. 3 Cell depth of worker, pollen and honey cells in between different aged combs of *Apis florea*. Bars having the same letter do not differ significantly at $P < 0.05$ (ANOVA followed by Tukey's test) $N = 20$ cells

20.02 ± 0.46 mm in old combs. The minimum size of cell depth was found in young combs and was measured to be 7.58 ± 0.27 mm, 17.4 ± 0.14 mm, and 18.8 ± 0.25 mm respectively (Fig. 3).

The combs of honeybees are double-sided hexagonal structures suited ideally for storing food and housing larvae, reducing building materials and space. The observations on the color of *A. florea* combs showed whitish in the beginning later into golden brown followed by darkish brown. Similar observations were made by Karihaloo *et al.* (2013) in the combs of *A. mellifera*. Taha and El-Sanat (2007) opined that colour changes were due to the accumulation of a large number of contaminants absorbed in the wax over time. Nonetheless, the

combs become more fragile and tenuous with increased comb age (Hepburn and Kurstjens, 1988). Brood rearing for several generations made combs darker, the cell walls thickened and the cells became smaller (Winston, 1987; Taha *et al.*, 2010).

The dimensions comb parameters of *A. florea* showed variation with the age of the combs. The maximum comb perimeter was found in middle-aged combs in contrast to the minimum comb perimeter in young and old combs. The middle-age combs provide greater strength to rear the young ones and accommodate their products by enhancing the comb perimeter. In relation to comb age, the areas containing brood, pollen, and honey varied, with the largest areas being found in young and

middle-aged combs, followed by old combs. The findings of our study are consistent with the observations of Abdellatif (1965) who found better brood areas in young and middle-aged combs. The smaller cells in older combs have a negative impact on egg-laying responses by the queen (Berry and Delaplane, 2001), which may eventually result in a lower production of honeybee workers and drones. Free and Winder (1983) found that old wax combs might contain pheromones of brood, which could inhibit the oviposition by queens. The larger pollen area was found in colonies with new combs and was due to the large number of workers collecting pollen during brood-rearing season (Berry and Delaplane, 2001). The crest size of the bee comb was varied and was relatively larger in old combs compared to young and middle-aged combs. The crown is integral to the structural integrity of the nest and supports the protective curtain of worker bees that shields the nest from the elements (Dyer and Seeley, 1991). Furthermore, the thickness of the drone comb was greater than the worker comb.

The cell width of worker brood, pollen and honey cells of *A. florea* were significantly ($p < 0.05$) decreased with the increase in age of the combs. The maximum cell depth of worker brood, pollen and honey cells was recorded in young combs and the minimum cell width in old combs. This is due to the accumulation of debris and each time a larva pupates it spins a silken cocoon that remains in the cell (Shawer *et al.*, 2020). The diameters of the cells become narrow as the age of the comb increases due to the accumulation of silk and faecal materials produced by the developing larvae (Hepburn and Kurstjens, 1988). The study concludes that the middle-aged combs of *A. florea* are more efficient in rearing brood and storing honey and pollen. Furthermore, larger cell width and depth found in young and middle-aged combs pave the way for the development of healthy colonies. Nonetheless, construction of the new combs by abandoning older combs in *A. florea* would be a strategy for better establishment of their colonies.

ACKNOWLEDGMENTS

The first author would like to thank Bangalore University, Bengaluru, India for providing Dr. B.R.

Ambedkar's research fellowship towards the Ph.D. program. Authors appreciate the encouragement received from Dr. B.P. Harini, Professor and Chairperson, Department of Zoology, Bangalore University, Bengaluru, India.

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