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SHORT NOTE

Age components of queen retinue workers in honeybee colony (*Apis mellifera*)

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Abstract
It's known that elaborate age is closely associated with polyethism in honeybee colonies, and the circle composed of queen retinue workers is a usual phenomenon in honeybee colonies. In this study, we showed that the age-bracket of retinue workers is 2-23d, but mainly 6-18d by marking newly hatched workers in two colonies.

Honeybees are widely acknowledged as a model insect for the studies of social organization, social behaviour and neurology (Robinson et al., 2005; He et al., 2015; Liu et al., 2015). It is known that the age-dependent division of labor in honeybee colonies is one of the most typical features of this social insect (Robinson et al., 2005). Newly hatched workers undergo the task of cleaning during their first few days (Kryger et al., 2000). Later, they begin to consume protein-rich pollen. During the age of 5-15d, their hypopharyngeal glands fully develop and most of them play the role of nursing the queen and larvae (Pan et al., 2013). Then, they begin to build comb by secreting wax, cleaning the hive and compacting pollen. After 15-20 days of age, some workers begin to undertake tasks that are out of the nest whilst other workers remain inside to hive to act as receivers and distributors (Kryger et al., 2000). However, this division of labor is not immutable. Environmental changes can accelerate, delay, or even reverse the transition of nurse bee and forager bee (Robision 1992; Huang & Robision 1996). Furthermore, polyethism in honeybee colonies might be affected by genotypical variability (Page & Robinson 1991; Kryger et al., 2000; Wang et al., 2012).

The queen retinue pheromone (QRP) released by a honeybee queen attracts workers that we name queen retinue workers to surround beside her (Slessor et al., 2005). Within the hive, it can be easily observed that there is a circle consisting of queen retinue workers, however, little is known about them as few studies have been performed on them. In this study, we intend to understand how the age-bracket of queen retinue workers is determined by marking newly hatched workers.

Two honeybee (*Apis mellifera*) colonies (colony A and B) were maintained at the Honeybee Research Institute, Jiangxi Agricultural University, Nanchang, China (28.46 °N, 115.49 °E). Both colonies consisted of a new comb for the queen to lay eggs, and also a honey comb. Additionally, we placed extra brood combs, which contain sealed honeybee brood that will emergence during the night, into the queen spawn controller at 5 PM (all apertures of the queen bee spawn controller were too small for workers to pass through). Three hundred newly hatched workers were labeled with dyes at 9AM the next day. Following this, they were put into their natal colonies. In total 6000-8000 worker bees were present in

each observation hive. We used colors to represent their date of birth and identify their age. Every two days, we marked workers with different color. In total we marked bees ten times and then began recording videos to monitor their behaviour.

We recorded the queen's behavior and the forming process of the queen retinue workers' circle in an open transparent awning at 9:00-11:30AM and 2:00-4:30PM. In order to minimize the impact of frequent interruptions on colonies, we began recording 2 days after bees were placed into the hive. We then continued recording until there were no more marked bees around the queen, a process which took about 20 days. After recording, we counted the number of marked workers in each color by watching the videos. We were then able to come to a conclusion regarding the age-bracket of queen retinue workers. A frame from a video is shown in Fig 1. We analyzed the differences in the number of retinue workers of each age in the two colonies with the Z-test (SPSS Statistics 17.0).

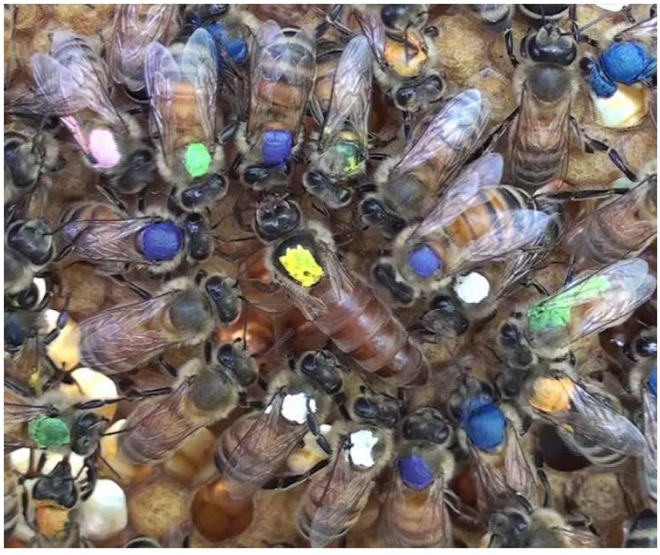

Fig 1. A circle composed of queen retinue workers surrounding a feeding queen. Different colors on worker bee thoraxes stands represent different ages.

It was easy for us to see a circle composed of queen retinue workers when the queens were laying eggs, standing still or being fed in their respective colonies during the recording period. The footage showed that there were many queen retinue workers within the age-bracket of 2-23 days. Additionally, there was only one worker at the age of 29d in colony A, and three 28d workers and one 30d worker in colony B (Fig 2). However, we deduce that this data should be disregarded for the reason that the number of queen retinue workers aged over 23d old was negligible.

In addition, Fig 3 showed that two spots (ages 6 and 8) in colony A and four spots (ages 6, 8, 16 and 18) in colony B were higher than branch line (the number stands for the age of marked workers in colonies). It is known that some workers aged 5-15d play the role of nurse to feed the queen and small larvae (Pan et al., 2013). After 15-20 days of age, some workers begin to undertake tasks that are outside of

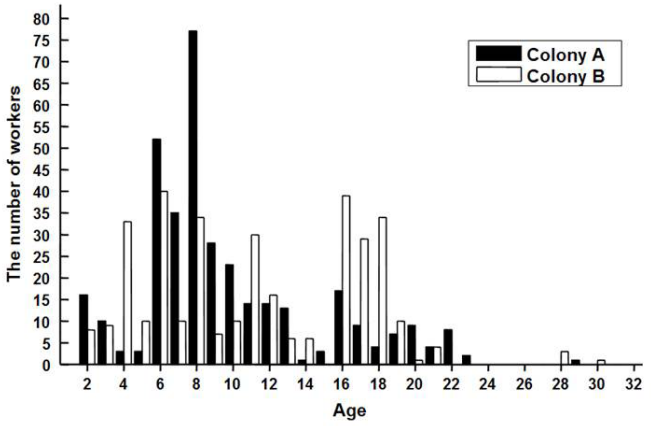

Fig 2. The number of queen retinue workers at each age. Most queen retinue workers were within the age-bracket of 2-23d.

the nest (Kryger et al., 2000). Thus, we indicate that the age-bracket of queen retinue workers is 2-23 day, but mainly 6-18 day. The honeybee queen releases QRP to encourage queen retinue workers to feed and groom her, and also to acquire and distribute her pheromone messages to other workers throughout the colony (Keeling *et al.,* 2003).

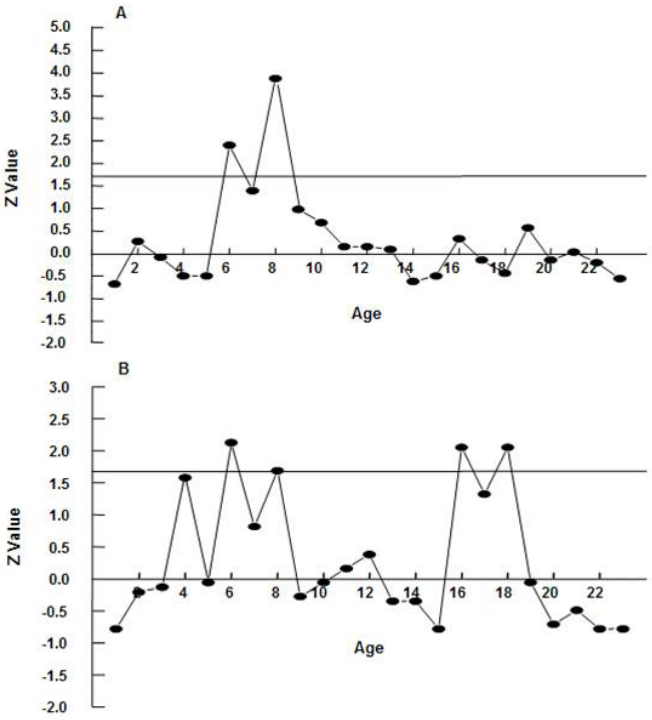

Fig 3. The Z values of same aged queen retinue workers in Colony A and B. The Z-values of two spots in colony A and four spots in colony B were higher than 1.65.

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