

# Young Honeybee (*Apis Mellifera Bandasii*) Queens Perform Better than Old Ones

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## Abstract

A productivity of queen bee depends on several factors. Queen age is one of the most important factors affecting queen's, as well as colony performance. The study was conducted to determine the effects of queen ages on performances of honeybee (*Apis mellifera*) colonies. The queens were reared from local honeybee, *Apis mellifera bandasii* (*A. m. bandasii*); nuclei colonies were formed and then transferred to standard Langstroth hives when all the frames of the nuclei boxes were occupied with brood and adult bees. Performances of the colonies headed by the new queens were evaluated over three years. Brood area, number of queen cell cups started and swarm queen cells constructed, number of empty cells/100 cells/per colony (brood solidness) and honey yield per harvest were determined every active season for three consecutive years. Colonies headed by 1-, 2- and 3-year-old queens produced  $4721.43 \pm 511.85$ ,  $5523.08 \pm 490.07$  and  $3433.82 \pm 413.83$  cm<sup>2</sup> sealed brood, and constructed  $0.07 \pm 0.07$ ,  $2.23 \pm 0.52$  and  $9.90 \pm 1.89$  queen cell cups and swarm cells/colony with  $1.86 \pm 0.38$ ,  $2.40 \pm 1.65$  and  $8.85 \pm 2.52$  empty cells/100 cells/per colony, respectively. Honey harvested from 1- and 3-year-old queen colonies averaged  $13.34 \pm 1.11$  and  $10.50 \pm 2.04$  kg/colony/harvest, respectively. The colonies headed by 1-, and 2-year-old queens had more brood areas, better brood solidity, less number of queen cells but no significant differences in honey production compared to colonies headed by 3-year-old queens. Our results indicate that queen age has a significant effect on colony performances, suggesting that requeening every three years is adequate for our local honeybee colonies.

**Keywords:** *Apis mellifera*, Queen age, Brood rearing, Swarm queen cells, Queen cell cups

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## 1. Introduction

A productivity of queen bee depends on several factors. Queen age is one of the most important factors affecting queen quality (Cengiz et al., 2009; Invernizzi et al., 2006) that influences the brood production, colony growth rate and productivity of the colonies (Akyol et al., 2007). It is commonly accepted that the queen bee maintains its productivity only for a limited time of her lifespan, which may remain from one to four years in different climates (Seeley, 1978). As the queen bees get older and older, their laying ration could decrease and they may lay more unfertilized eggs than young queens. Moreover, when queens get old, they start to produce insufficient queen pheromones (Crane, 1990). The lesser amount of queen pheromones in turn affect queens attraction to workers (De Hazan et al., 1989) and queens' power to inhibit building of swarm queen cells (Lensky and Slabezki, 1981), which can reduce colonies productive performances.

Different studies conducted in temperate climates of England, New Zealand and Poland, revealed that colonies headed by a year old queens have shown better performance than those head by two years old queens (Forster, 1969; Hatjina et al., 2014; Simpson, 1959). Moreover, a study conducted in Israel (under subtropical condition) found that colonies headed by 7 to 10-months-old queens had higher brood areas, constructed less swarm queen cells and produced more honey than those headed by up 20-month-old queens during nectar flow season (Hauser and Lensky, 1994). As a result, yearly requeening of a colony in commercial apiaries has been implemented as a routine practice in countries like Israel (Hauser and Lensky, 1994), as queen's reproductive physiology is greatly affected by the climatic conditions of an area where the honeybee colonies kept (Rangel et al., 2016).

Apart from climatic conditions, the efficient reproductive lifespan of queen bees could be affected by the nectar and pollen flow and resource fullness of an area. To this effect, queens in the areas with constant flow of resources and with a high constant rate of egg laying can get exhausted after year (Furgala and McCutcheon, 1992). Moreover, it has been indicated that queens in the resource rich areas where the environmental conditions allow full activities of the colonies for a longer period of a year, lay eggs rapidly through the whole season (Crane, 1990). Under this situation, the queens may get exhausted soon and become less efficient in heading the large colonies in short period of time as opposed to those queens in longer dearth period areas. Despite all the aforementioned studies on the performances of colonies headed by queens of different ages under different environmental conditions elsewhere, there is no quantitative data on the effects of honeybee queen's age on the performances of Ethiopian local honeybee colonies. Therefore, the aim of this work was to determine the effect of queen's age on the performances of local honeybee colonies, such as brood population, brood solidness, queen cups and swarm queen cells, and honey production under central highland condition of Ethiopia.

## 2. Materials and methods

### 2.1 Description of the study area

The study was conducted at the experimental Apiary of Holeta Bee Research Center in Oromia Regional State of Ethiopia. The apiary is located in central highlands of Ethiopia at 9° 30' N and 38° 30' E, 2450 m above sea level and 30 km west of Addis Ababa. The annual mean temperature of the site is 14 °C with May the hottest month (mean temperature 23.8 °C) and December the coldest month (mean temperature 1.7 °C). The annual mean rainfall is 1060 mm with an average relative humidity of 58%. The vegetation around Holeta is characterized by bush lands, numerous herbaceous and grass floras, scattered trees and planted eucalyptus trees, cultivated oil and horticultural crops.

### 2.2 Establishing experimental colonies

Central highland local honeybee (*A. m. bandasii*) colonies were obtained from local beekeepers around Holeta, and kept in standard Langstroth hives with 10 frames for colony build-up at the apiary of Holeta Bee Research Center during the active season (September to November).

Honeybee queens were reared using grafting method from the stock established during the second breeding season (in May). Five frame nuclei colonies with two frames of emerging bees, two frames of honey and one frame pollen were formed, as described elsewhere (Akyol and Kaftanoglu, 2001). Then, newly hatched virgin queens in queen cages were given to all nuclei colonies. A day later the virgin queens were released from their cages and left for open mating. Three weeks later queens were checked for their egg laying and queens started egg laying were marked on their thorax with number codes. Nuclei colonies were transferred to standard Langstroth hives when the bees occupied all the frames of the nuclei boxes.

Prior to the beginning of the study, colonies were subjectively estimated for their strength based on areas of combs covered by adult bees, brood, nectar and honey, and pollen, as described elsewhere (Delaplane et al., 2013). Then, 15 colonies with similar strength were selected as the experimental colonies. All the necessary management practices were done to the colonies.

### 2.3 Measuring colony performance

The following variables were monitored every active season using an objective mode which uses empirical measures:

**Brood area:** the area occupied by immature worker honeybees (eggs + larvae + capped brood) in colonies was evaluated every 21 days by overlaying a grid pre-marked in 5 cm by 5 cm on each sides of every brood frame and the area covered with the brood was visually summed (Maucourt et al., 2018).

**Brood pattern (solidness):** the degree of worker brood solidity was determined using a piece of cardboard with a rhombus equals in size to 10 × 10 cells was laid over a section of sealed brood and number of empty cells within the section was counted. This measure was repeated on different patches of brood frames to obtain a mean of at least 10 observations (Delaplane et al., 2013).

**Queen cell production:** swarming tendency of the colonies was verified once every two weeks. All brood frames in each colony were carefully inspected and all queen cell cups and swarm queen cells were counted and destroyed to prevent swarming and redundant duplicate observations (Delaplane et al., 2013).

**Honey yield:** harvested honey yield was measured as a difference between weight of honey frames before and after honey extraction. Honey combs remained for a colony as a reserve food were not included in this honey yield estimation.

**Statistical analysis:** statistical tests were conducted using SAS software (version 9.0, SAS Institute Inc.) at the 0.05 level of significance. Variables were analyzed using general linear model (GLM). When a significant effect was observed ( $p < 0.05$ ), the means of the experimental groups were compared using Tukey-Kramer grouping's least significant difference method.

## 3. Results and discussion

### 3.1 The effect of queens' age on brood production

The average brood area of queens with different age groups is depicted in Table 1. We found no significant differences in brood production among 1-, and 2-year-old queens ( $p > 0.05$ ). However, the decline of brood production in colonies headed by older queens were apparent during the third year, when they produced significantly less brood than 1- and 2-year-old queens ( $p < 0.05$ ), indicating that 1- and 2-year-old queens were more prolific than 3-year-old queens. Our result with older queens is in agreement with the previous reports that brood production of a queen decrease with increasing age of the queen (Akyol et al., 2007, 2008; Hatjina et al., 2014; Hauser and Lensky, 1994; Kostarelou-Damianidou et al., 1995), suggesting that colonies headed by young queens had a greater brood area compared to colonies headed by old queens. Thus, the current result indicates that queen age may be among the important elements that affect brood production performance of a colony and also needs due attention during making management decisions based on whether a queen is judged to be good or failing.

### 3.2 The effect of queens' age on construction of queen cups and swarm queen cells

The average number of queen cups and swarm queen cells constructed in colonies headed by 3-year-old queens was 4.46 times higher than 2-year-old queens, and 141.43 times more than 1-year-old queens (Table 1). This results reveal that colonies headed by old queens constructed significantly more queen cups and swarm queen cells compared to young queens ( $p < 0.01$ ). Noticeably, the fact that we found more number of queen cups and swarm queen cells in colonies headed by old queens than those headed by young queens may reflect the substantial effect of queen's age on the constructions of queen cups and swarm queen cells (Hauser and Lensky, 1994). It has indeed been reported by several studies that colonies with older queens have a higher swarming tendency (Forster, 1969; Hauser and Lensky, 1994; Uzunov, 2013; Uzunov et al., 2014; Winston, 1987). This stronger inclination to swarm in colonies headed by old queens suggests that the age of queen bees affect both their attractiveness (De Hazan et al., 1989) and power to inhibit building of queen cups and swarm queen cells (Lensky and Slabezki, 1981). Other than the queen's age, a strong relationship between brood area, and construction of queen cups and swarm queen cells has been reported under (Lensky and Slabezki, 1981; Simpson, 1960). We analyzed the correlation between the construction of queen cups and swarm queen cells, and that of brood area during the study period. The weak correlation ( $r = 0.21$ ;  $p = 0.09$ ) between brood area, and queen cups and swarm queen cells suggests that brood area has less effect on the construction of queen cups and swarm queen cells in uncongested colonies (Hauser and Lensky, 1994), as our experimental colonies were provided with extra hive volume to prevent swarming, which was achieved by placing additional supers that were only partially occupied by workers. These findings clearly highlight the construction of queen cups and swarm queen cells might be due to the changes occurred in the queen bees with their ages (De Hazan et al., 1989).

**Table 1.** The effects of queen ages on brood production, construction of queen cups and swarm queen cells, brood solidness and honey yield.

Queen's age	Brood area (cm <sup>2</sup> /colony)	Number of queen cups and swarm cells/colony/season	Number of empty cells/100 cells	Honey yield/colony/harvest (kg)
1-year-old	4721.43±511.85 <sup>ab</sup>	0.07±0.07 <sup>b</sup>	1.86±0.38 <sup>b</sup>	13.34± 1.11 <sup>a</sup>
2-year-old	5523.08±490.07 <sup>a</sup>	2.23±0.52 <sup>b</sup>	2.40±1.65 <sup>b</sup>	.
3-year-old	3433.82±413.83 <sup>b</sup>	9.90±1.89 <sup>a</sup>	8.85±2.52 <sup>a</sup>	10.50 ±2.04 <sup>a</sup>

Averages assigned the same letters do not significantly differ at  $p < 0.05$ .

### 3.3 The effect of queen's age on brood pattern

Brood pattern quality, which is meant the degree of worker brood solidity, is one of the general measures that indicates either the queen is not laying eggs well or the developing bees are not surviving to eclosion (Lee et al., 2019; Woyke, 1981). In this study, we found that brood solidity in colonies headed by 1-, and 2-year-old queens were significantly ( $p < 0.01$ ) elevated than those headed by 3-year-old queens (Table 1), confirming that brood pattern quality of young and old queens were different. A better brood pattern, as indicated by solid patches of capped brood in colonies headed by young queens, designates young queens are laying viable eggs which are developing into healthy larval and pupal bees (VanEngelsdorp et al., 2013), which can significantly reduce the risk of colony mortality due to poor brood pattern (Lee et al., 2019). On the contrary, the higher number of empty cells (>7%) in colonies headed by 3-year-old queens is a reliable indicator of poor brood pattern, which suggests that the quality of the queen is falling (Laidlaw, 1979). Our result emphasizes that as age of a queen increases, brood solidity decreases, indicating that brood solidity is among the various metrics to be considered to make management decision whether a queen is judged to be good or failing.

### 3.4 The effect of queen's age on honey yields

During the second year of the experiment, there was a heavy rainfall just three weeks ahead of the expected honey harvesting time for the area. As a result, there was no enough honey yield data collected for 2-year-old queens. The colonies from the first and third year (headed by 1- and 3-year-old queens, respectively) produced average honey yield of 13.34± 1.11 and 10.50 ±2.04 kg/colony/harvest, respectively (Table 1). Differences among the group means of honey yield were not significant ( $p < 0.19$ ). Although there were no significant variations among colonies headed by queens of different ages, colonies headed by 1-year-old queens gave higher amount of honey yield, approximately 27% times more honey than that produced by colonies headed by 3-year-old queens. Regarding honey yield of colonies with young queens, previous studies have provided contradictory results. Some of them have reported that colonies headed by young queens performed better than colonies with old queens (Akyol et al., 2007; Guler and Kaftanoglu, 1999; Hauser and Lensky, 1994; Woyke, 1984), whereas others have found non-significant differences in honey production between such colonies (Kostarelou-Damianidou et al., 1995; Szabo and Lefkovitch, 1989). Our current result is in agreement with what has been concluded by the latter authors. The insignificant variations in honey yields of colonies headed by 1-year-old queens from colonies headed by 3-year-old queens might be attributed to the regular removal of swarm queen cells in the latter group, that might reduce the negative effect of swarm issuing on honey yield (Forster, 1969).

#### 4. Conclusion

The present data provide some insight into the reasons for differences in overall colony performance and honey production among colonies headed with queens of different ages. Colonies with queens 3-year-old produced less brood with relatively poor brood pattern, and more queen cups and swarm queen cells than colonies with queens 1-year-old or 2-year-old, but the honey yield is not significantly different. This suggests that requeening can be effective every three years under Holeta conditions. However, more research may be needed with different races of bees, under different climatic conditions with different food resources to further evaluate this important aspect of honeybee husbandry.

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